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of U. Porto

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# Program

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## *Parallel Oral Sessions I*

**A1** *Biological, Environmental & Health Sciences I*

# Amphibian mortality on country roads in northern Portugal

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This project comes under the master's degree in Ecology, Environment and Planning, Faculty of Science, University of Porto. Here we try to answer questions related to the mortality of amphibians on country roads using tools of geographic information systems (GIS).

In recent years the landscape has been undergoing major changes through urban sprawl and its accesses, leading to an increase in fragmented natural areas. These linear structures (and its factors) interfere with various ecological processes. Collisions with vehicles are the main direct cause of animal mortality on the roads. The real impact on populations is not yet known, but there is evidence that it affects abundance, distribution and specific richness.

Amphibians are the group of animals most commonly affected. These animals show higher biological susceptibility to the impact of roads. Besides their vulnerability and its physiological behavioral variation they depend on environmental spatial complexity to complete their life cycles. Iberian studies have shown that the roads play an important role in the degree of mortality on amphibians, but information regarding spatial patterns on causalities on roads in Northern Portugal is still scarce.

Mitigation measures are necessary, even knowing their use is not always effective. It is important to understand where they should be applied so their real purpose is successfully achieved. Predictive models) are tools that can be used to identify hotspots in existing roads and can be used to prevent the risk of future road kills in upcoming projects. The main goals of this study are (1) to identify the levels of amphibians road-kills in northern Portugal, (2) to analyze their space patterns, (3) to identify road-kill hotspots, (4) relate these patterns with amphibian movements along a permeability matrix and (5) determine patterns of amphibian mortality on country roads in northern Portugal.

The study area was selected within 50 km around the city of Porto, covering a total of 41 counties. This area was considered for logistical reasons but also cover most types of land uses that can be found in northern Portugal. The selection of the study area was performed in three phases: country roads selection, key habitats (CORINE) and land use selection, selection of sample paths and analysis.

We will analyze amphibian species records (recorded as live or road-killed) in order to identify (1) temporal and spatial patterns of occurrence, as well as road-killed hotspots (using GIS clustering tools); (2) amphibian passage routes (using Friction maps); and (3) variables associated with road-killed hotspots (using Ecological Niche Models).

# The evolution of the OPN4x and OPN4m gene family in vertebrates

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Recently, additional photosensitive cells implicated in non-visual light-dependent functions have been identified in the mammalian retina [1]. Melanopsins (OPN4) constitutes a photopigment found in specialized photosensitive ganglion cells involved in the regulation of circadian rhythms and pupillary light reflex [2]. In vertebrates, they have an intrinsic relation with cone and rodopsins, playing an important role in image-forming [3]. In the evolutionary history of the OPN4 gene a duplication event occurred originating the OPN4m and the OPN4x variants [4]. The aim of this study is to characterize the genomic and proteomic features of OPN4 and to distinguish critical structural and functional differences between its variants (OPN4x and OPN4m).

In this study, a detailed bioinformatics approach has been used showing that the genomic structure of the OPN4m and the OPN4x genes is quite variable and different from those of cones and rhodopsins, being reduced the pairwise similarly aminoacid sequence between the OPN4m and OPN4x. The conservation of this molecule is more restricted to the internal residues that are responsible to the structural maintenance of the molecule important structural sites, while by contrast the third intracellular loop shows a great variability (it is responsible for the ligation to the G protein for mediate cellular response). This third intracellular loop shows relatively high selective  $\omega$  (dS/dN) values when compare with the entire molecule, but such values were still below 1. The G protein that mediates the phototransduction of light signal in melanopsins includes at least Gq/11, Gs and Gi/o, showing a great variability of cellular responses.

Thus, the OPN4x and OPN4m photoreceptors are definitively different – they are phylogenetic and molecularly dissimilar. The present analyses show evidence that melanopsin mediates the phototransduction pathway with tree types of G proteins, meaning that melanopsins could led to cellular depo- or hyperpolarization. The variable residues of the third intracellular loop support the idea that melanopsins mediate different responses to light due to the ligation to G protein. These evidences do not exclude the possibility that a new type of G protein could be related with melanopsins function.

[1] Provencio, I. et al.(1998) *Melanopsin: An opsin in melanophores, brain, and eye*. Proceedings of the National Academy of Sciences of the United States of America, 95(1), p.340-345.

[2] Hattar, S. et al. (2002) *Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity*. Science, 295(5557), p.1065-1070.

[3] Dacey, D.M. et al. (2005) *Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN*. Nature, 433(7027), p.749-754.

[4] Bellingham, J. et al. (2006) *Evolution of Melanopsin Photoreceptors: Discovery and Characterization of a New Melanopsin in Nonmammalian Vertebrates* J. Nathans, ed. PLoS Biology, 4(8), p.1.

# Lineage Sequence Discovery - Software to Discover Patterns in Biological Sequences

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Searching for overrepresented motifs across regulatory regions is a way to find new regulatory elements. However, this search for patterns is complicated, mainly due to the fact that most binding site motifs are short and that the possibility exists that the sequence shows some variation but functionality is maintained. This means that false positives may arise during the search and distinguishing false positives from true binding sites is a challenge.

Although there is already available a large number of motif finding tools, like MEME[1], CisModule[2], AlignAce[3], PhyloGibbs[4], Amadeus[5], FIRE[6] and SCOPE[7], Nuno Fonseca developed SigDis[8]. This program uses a different approach, processing two sequence files, unaligned and that may differ in number of sequences, searching for patterns that discriminate the two files and representing those using regular expressions.

Most motif finding tools do not provide a graphical user interface, being only available through a command line or web interfaces that run on foreign servers. The purpose of the Lineage Sequence Discovery program is to provide a simple, accessible to everyone, graphical user interface to quickly visualize and align sequences and search or match patterns to them using free software, like SigDis[8], MEME[1] and T-Coffee[9].

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- [1] Timothy Bailey. The MEME Suite - Motif-based sequence analysis tools. <http://meme.sdsc.edu/meme/meme-intro.html>.
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- [4] Phylogibbs Online. <http://www.phylogibbs.unibas.ch/cgi-bin/phylogibbs.pl>.
- [5] The Amadeus motif discovery platform. <http://acgt.cs.tau.ac.il/amadeus/>.
- [6] Olivier Elemento, Noam Slonim, and Saeed Tavazoie. A universal frame-work for regulatory element discovery across all genomes and data types. *Molecular Cell*, 28(2):337 - 350, 2007.
- [7] SCOPE motif finder. <http://genie.dartmouth.edu/scope/>.
- [8] Nuno Fonseca. SigDis - Signature discovery in biosequences. <http://cracs.fc.up.pt/~nf/projects/sigdis/>.
- [9] PhD. Dr. Cédric Notredame. T-Coffee - Multiple Sequence Alignment Tools. [http://www.tcoffee.org/Projects\\_home\\_page/t\\_coffee\\_home\\_page.html](http://www.tcoffee.org/Projects_home_page/t_coffee_home_page.html).

# Prrxl1 is differentially phosphorylated across nociceptive system development

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Prrxl1 is a paired-like homeodomain (HD) transcription factor specifically expressed in nociceptive neurons of dorsal root ganglia (DRG) and spinal cord dorsal horn (SC) [1]. Evidence suggests that Prrxl1 is crucial in the establishment/maintenance of the nociceptive system, as *Prrxl1*<sup>-/-</sup> mice show neuronal loss, reduced nociception and failure to thrive [2]. Prrxl1 gene encodes for Prrxl1a and Prrxl1b isoforms [3].

When mouse embryonic SC extracts are analysed through immunoblotting (IB), Prrxl1 displays a four-band pattern [4], which results from different phosphorylation states. A detailed IB analysis of Prrxl1a expression in the dorsal SC and DRG at different ages of mouse development shows that, in dorsal SC, Prrxl1a expression evolves from the higher phosphorylated states, at early-development, to the lower phosphorylated forms, at late-development, with a transition phase in between. In addition, the expression of Prrxl1a band-pattern in late-development differs between mouse DRG and SC, indicating that Prrxl1a may be differently regulated in the peripheral and central nervous system. Aiming to identify Prrxl1 phosphorylation domains (PDs), several truncated and mutated forms of Prrxl1a were engineered, expressed in ND7/23 cells and analysed by IB. According to the data collected, Prrxl1a most likely has one PD in the N-terminal HD (the DNA binding domain) and another in the C-terminus, enclosing the OAR domain.

The two PDs identified contain highly conserved amino-acids representing putative kinase phosphorylation sites. LC/ESI-MS analysis is underway, in order to unravel the precise residues where phosphorylation occurs, which will allow future functional analyses of Prrxl1 phosphorylation sites. Since Prrxl1a is differentially phosphorylated across development, this functional analysis will most probably reveal a role for Prrxl1a phosphorylation in the control of Prrxl1a transcriptional program during the establishment of the nociceptive circuitry.

## References:

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- [2] Chen Z.F., Rebelo S., White F., Malmberg A.B., Baba H., Lima D., et al. (2001), *The Paired Homeodomain Protein DRG11 is Required for the Projection of Cutaneous Sensory Afferent Fibers to the dorsal Spinal Cord*, *Neuron*, 31, 59-73
- [3] Rebelo S., Lopes C., Lima D., Reguenga C. (2009) *Expression of a Prrxl1 alternative splice variant during the development of the mouse nociceptive system*. *International Journal of Developmental Biology*, 53(7):1089-95
- [4] Rebelo S., et al. (2007), *DRG11 Immunohistochemical Expression During Embrionic Development in the Mouse*, *Developmental Dynamics*, 236, 2653-2660

# The distinct expression of Prrxl1 5'UTR variants at early-stage of nociceptive neurons development is conferred by differential mRNA stability

**I. Regadas<sup>1,2</sup>, F. Monteiro<sup>1,2</sup>, S. Rebelo<sup>1,2</sup>, D. Lima<sup>1,2</sup> and C. Reguenga<sup>1,2</sup>**

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The transcription factor Prrxl1 (also known as DRG11) has emerged as a crucial molecule in the establishment of the pain-perception circuitry, displaying a preponderant role in the development of the nociceptive peripheral afferent neurons located in the Dorsal Root Ganglia (DRG) and of their synaptic partners located in the dorsal horn spinal cord. Prrxl1 mutant mice embryos present spatio-temporal abnormalities in the morphogenesis of the superficial dorsal horn spinal cord, as well as reduced survival of small DRG neurons, resulting in a diminished sensitivity to various types of noxious stimuli. However, despite Prrxl1's important function, little is known about the genetic programs that regulate the expression of this gene along embryonic development, a pre-requisite for further exploring the mechanisms governing the development of the nociceptive system.

To address this issue, we recently identified three distinct regions displaying promoter activity, which are suggestive of alternative promoter usage as a mechanism of control of Prrxl1 expression. Furthermore, these multiple promoters give rise to at least three Prrxl1 5'-UTR variants, termed 5'UTR-A, 5'UTR-B and 5'UTR-E. To gain insight on the role of these different Prrxl1 transcripts, the expression of these variants along different developmental ages (E11.0 to P21) in DRG and spinal cord was analysed by RT-PCR. From E16.5 to adult, all 5'-UTR variants present the same expression pattern in the DRG and in the spinal cord. However, at earlier stages of spinal cord development, the 5'UTR-B and E precede the 5'-UTR-A expression. The fact that these 5'-UTR variants have no consequences in the Prrxl1 coding region prompted us to determine their mRNA stability and translation efficiency either by the use of vectors carrying distinct 5'-UTR-luciferase fusions under the control of the SV40 promoter to transfect the DRG-derived ND7/23 and non-neuronal HeLa cell lines and by coupled transcription-translation *in vitro* assays of the different Prrxl1 5'-UTR variants. This analysis revealed that the 5'-UTR-A displays neuronal-specific activity, increasing both the rate of Prrxl1 or luciferase translation and the stability of the luciferase mRNA molecule. On the other hand, the 5'-UTR-B variant reduces the *in vitro* translational efficiency of Prrxl1 as well as the luciferase expression both in ND7/23 and HeLa cells.

The collected data suggests that, along with multiple transcriptional mechanisms, Prrxl1 expression could also be differentially regulated by post-transcriptional processes, altogether leading to a tissue-specific modulation during embryonic development.

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# Quality assessment of sperm submitted to technical procedures for Human assisted reproduction

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Infertility is a problem that affects thousands of couples in the world. Male factor is present in about 51% of the cases. In order to address this problem many assisted reproductive techniques have been developed in the last years. The utilization of sperm with high quality is crucial to improve the success of any of these techniques. So, sperm is usually treated using separation methods like density gradients and *swim-up*.

The aim of this work was to optimize these techniques, for that, sperm quality was evaluated in different swim-up media (A – Sperm preparation medium, Medicult; B – Pure sperm wash, Nidacon; C – Sperm medium, COOK e D – Sperm washing media, Irvine) to choose the best. The efficacy of C medium in eliminating genetic anomalies by FISH and TUNEL assays was also evaluated, as they are good indicators of male infertility.

The results obtained in this study showed that combination of density gradients with *swim-up*, deeply improves sperm quality. A significant increase of sperm with high motility and normal morphology in normal, asthenozoospermic, teratozoospermic and asthenoteratozoospermic samples was also observed using the C medium. A, B, C and D media showed identical results in the selection of sperm with normal morphology. However, to chose sperm with good morphology, C medium was slightly better for normal and asthenozoospermic samples, while A medium was slightly better for asthenoteratozoospermic samples.

It was also found that spermatozoa with aneuploidy and DNA fragmentation significantly decrease with C medium treatment.

In conclusion, sperm preparation techniques are important to improve the success of assisted reproduction techniques, and C medium is the most advantageous to increase sperm quality in different parameters.

The improvement of sperm quality is essential in order to achieve not only a pregnancy but also offspring without genetic defects.

# Role of Hydrogen Sulphide in Renal Damage Induced by Gentamicin

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Departaments of <sup>1</sup> Physiology, <sup>2</sup>Pathology and <sup>3</sup>Pharmacology  
School of Medicine of Ribeirão Preto – University of São Paulo

The nephrotoxicity is one of the side effects of gentamicin treatment. Animals models of gentamicin nephrotoxicity show acute tubular necrosis associated with inflammation. The inflammatory process can contribute to intensify the renal damage. Endogenous hydrogen sulphide (H<sub>2</sub>S) is synthesized from L-cysteine and is being recognized as an important signaling molecule in inflammation [1,2]. This study evaluates the effect of DL-Propargylglycine (PAG), an inhibitor of endogenous H<sub>2</sub>S formation, on the renal damage induced by gentamicin. Thirty seven male Wistar rats were injected with gentamicin (40 mg/kg, i.m., twice a day) for 9 days, 19 of them received PAG (10 mg/kg, i.p., once a day) and 18 of them received 0.15 M NaCl solution (i.p., once a day). PAG injection started on day five of gentamicin-treatment. Twelve controls rats were treated with 0.15 M NaCl solution only. Urine and blood samples were collected 2 days after finished these treatments and the animals were killed. The kidneys were removed for H<sub>2</sub>S formation evaluation and immunohistochemical studies. The relative interstitial area was determined by morphometry and the H<sub>2</sub>S formation was quantified in renal tissue.

Compared to the control group, gentamicin-treated rats presented higher plasma urea levels and increase in fractional sodium excretion as well as greater immunostaining for macrophages/monocytes, tubular cell necrosis and increase in relative interstitial area of the renal cortex. These changes were associated with increased H<sub>2</sub>S formation. Treatment with PAG reduced the macrophages infiltration (Fig.1) and the enlargement of the interstitial area. The protective effect of PAG on gentamicin nephrotoxicity was associated with decrease in H<sub>2</sub>S formation, suggesting that this gas has a role in gentamicin nephrotoxicity. However, the reduction of urea plasma levels and fractional sodium excretion provoked by PAG was not significant, probably because this treatment was introduced after the establishment of the renal damage.

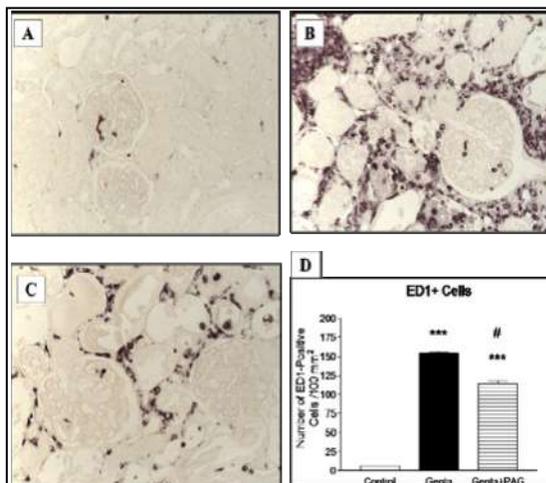


Fig. 1 - Immunolocalization of ED1+ cells in renal cortex of animals from Control (A), Genta (B) and Genta+PAG (C) groups. Number of ED1+ cells (D) in renal cortex of animals from all groups.

## References:

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**U. PORTO**

## *Parallel Oral Sessions I*

**A2** *Biological, Environmental & Health Sciences II*

# Drawing and Digging Cardiac Stem/Progenitor Cell Response(s) to Heart Injury

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Our focus is to elucidate how stem/progenitor cells (SC) engage in regeneration and repair in adult-tissues. Presently, the team works on the paradigm of the heart as an organ endowed with endogenous cardiac stem/progenitor cells (CSC/CPCs) and thus with self-regenerating potential. Putative CSC/CPCs display hallmarks of stemness and differentiate clonally into the main cardiac-cell lineages. The antigenic profile (Sca-1<sup>+</sup>/c-Kit<sup>+</sup>/MDR1<sup>+</sup>) of the myocardial-embedded minute cell-fraction with SC-activity coincides to that of other adult SC. A lack of definitive molecular markers to identify CSCs raises the question as to whether they have their origin confined to the heart or are a result of a continuous replenishment from other organs.

An experimental mouse-model of myocardial infarction (MI) was established and validated, and the stem cell antigen-1 (Sca-1) expressing CSC/CPCs have been elected for further analysis. We report on the implementation of strategies for a detailed portrayal of the Sca-1<sup>+</sup> CSC subset at the cell- and tissue-level. *In situ* examination of the cardiac-tissue aims at illustrating how CSCs distribute throughout the heart, while following the niches dynamics under MI.

Functional and histological validation of the MI model is shown. Briefly, an accentuated decrease of heart systolic function of the left ventricle (LV) following MI was demonstrated by echocardiography, which was confirmed and quantified by analysis of Masson's trichrome heart stained-sections. Furthermore, to obtain a comprehensive picture of the CSC/CPC, their differentiating progeny and supporting cells at the natural niche, the optimal reagents/conditions for *in situ* analysis have been identified. At the cell-level, an improvement is reported on the target Sca-1<sup>+</sup> cell-subset recovery. A majority of Sca-1 purified cells was found to co-express CD31. The antigenic profile indicates a higher cell-surface density of the stem-cell antigen-1 at the Sca-1<sup>+</sup>/CD31<sup>+</sup> as compared to the Sca-1<sup>+</sup>/CD31<sup>-</sup> fraction. Transcriptomic evaluation of the isolated Sca-1<sup>+</sup> cells confirmed the display of early cardiac-affiliation transcription factors and stemness markers. Moreover, aiming at the identification of molecules involved in putative CSC stress-response signaling-pathway(s), and hypothesizing the involvement of Sca-1, expression of the transcript was also evaluated in the cardiac Sca-1<sup>+</sup> cell fraction isolated from non-manipulated, sham-operated and mice subjected to MI.

The herein work constitutes a first draft from an integrative approach towards a molecular blueprint of the CSC/CPC response(s) to MI, which will contribute towards the *in situ* activation and the *ex vivo* amplification of CSC.

## Acknowledgements

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# Mechanisms underlying ischemic diastolic dysfunction in response to acute hemodynamic overload – Role of PKG and actin-myosin interaction

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**Introduction:** Acute myocardial stretch induces an adaptive response both at systolic (immediate and time-dependent increase in contractility) and diastolic levels (time-dependent decrease in myocardial stiffness). Myocardial ischemia leads to hemodynamic overload and, in this setting, the adaptive diastolic response to stretch is impaired [1]. The mechanisms responsible for the failure of diastolic adaptation remain largely unknown. Therefore, we aimed to evaluate the role of PKG and actin-myosin interaction in the acute overloaded heart under basal and ischemic conditions.

**Methods:** Rabbit papillary muscles (0.2 Hz, 30°C) were acutely stretched from 92% to 100% of L<sub>max</sub> (muscle length at which maximal force is developed) in a modified Krebs-Ringer solution in the absence (A) or presence of (B) an inhibitor of Rp-8-Br-PET-cGMPS (an inhibitor of PKG, 10<sup>-6</sup>M, n=7). Group C was stretched during ischemia (without glucose and oxygen) and other protocols were performed in the ischemic setting in the presence of (D) 8-Bromo-cGMP (an agonist of PKG, 10<sup>-5</sup>M, n=7), (E) BDM (an inhibitor of actin-myosin interaction, 3%, n=8) and (F) 8-Bromo-cGMP and BDM (n=7). Immediate and delayed responses to muscle stretch were evaluated. Results are presented as mean±standard error of mean (P<0.05).

**Results:** Under basal conditions, the presence of an inhibitor of PKG attenuated the decrease in passive tension (PT) after myocardial stretch to 26.3±1.1% (15 minutes after stretch) versus 46.2±1.8% in group A. Furthermore, the presence of an agonist of PKG (group D) promoted a decrease in PT of 20.6±3.2% in the 15 minutes after acute overload during ischemia, contrasting with an increase of 16.6±15.7% in group C during the same period. On the other hand, the diastolic response of group E to acute stretch during ischemia was characterized by a decrease in PT of 28.6±1.0% (15 minutes after stretch). When both 8-Bromo-cGMP and BDM were present (group F), there was a decrease in PT of 28,3±1,5% in the 15 minutes following acute overload during ischemia. This decrease in myocardial stiffness observed in group F is significantly greater than the decrease in group D, although it is comparable to group E.

**Conclusion:** Our results suggest that PKG is an active player in the adaptive diastolic response of the acute overloaded heart. During ischemia, the activation of PKG signaling pathway improved the diastolic response to an acute hemodynamic overload, with a decrease in myocardial stiffness, promoting lower filling pressures and decreasing the odds of pulmonary congestion. Furthermore, the modulation of the actin-myosin interaction, an important contributor to the diastolic dysfunction leading to hemodynamic overloading of the ischemic heart, may represent an important therapeutic target in this setting.

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# Angiotensin 1-7 improves the contractile response to isoproterenol after hypoxia-reoxygenation: a Mas receptor and nitric oxide dependent effect

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Angiotensin 1-7 (Ang1-7) is a bioactive peptide of the renin-angiotensin system that has received increased attention for its ability to balance Ang II actions. Ang1-7 attenuates heart failure development after myocardial infarction and reduces the rate of arrhythmias during ischemia-reperfusion[1,2]. In the present study, we aimed to assess Ang1-7 effects on myocardial function in a hypoxia-reoxygenation (HYP-REOX) model.

Ang1-7 effects were evaluated in right ventricular rabbit papillary muscles immersed in a modified Krebs-Ringer solution (35°C; 0.6Hz). Ang1-7 ( $10^{-5}$ M, n=6) or the same volume of vehicle ( $H_2O$ , n=6) was added to the superfusing solution, followed by 30 min of hypoxia (using a gas mixture with 95% $N_2$ +5% $CO_2$ ) and 40 min of reoxygenation. The contractile response to increasing concentrations ( $10^{-8}$ M- $10^{-6}$ M) of isoproterenol (ISO) was then evaluated. The same protocol was performed: in the absence of HYP-REOX (Ang1-7  $10^{-5}$ M, n=7;  $H_2O$ , n=10); in the presence of a selective Mas receptor antagonist (A-779,  $10^{-5}$ M, n=6); and in the presence of a NO synthase inhibitor, NG-L-nitro-arginine (L-NA,  $10^{-5}$ M, n=6). Results presented as mean±standard error; p<0.05 was considered significant.

In the baseline protocol, pretreatment with Ang1-7 improved the inotropic and lusitropic response to ISO after HYP-REOX. For the higher ISO concentration, there was a greater increase in active tension (AT) and  $dT/dt_{min}$  in the Ang1-7 group when compared to vehicle (434%±69% vs 239%±27% for AT; 553%±75% vs 333%±41% for  $dT/dt_{min}$ ). In the absence of HYP-REOX, the inotropic response to ISO was lower both in the presence of Ang1-7 and vehicle, with an increase in AT for the higher concentration of ISO of 144%±16% and 131%±22%. The ISO induced increase in contractility after HYP-REOX in the Ang1-7 group was significantly reduced in the presence of A-779 or L-NA. In the A-779 group, ISO at the higher concentration caused an increase in AT and  $dT/dt_{min}$  of only 228±50% and 294%±68%. In the L-NA group, there was an increase at the higher ISO concentration of only 145%±23% in AT and 233%±47% in  $dT/dt_{min}$ .

In conclusion, Ang1-7 improves the contractile response to beta-adrenergic stimulation after HYP-REOX. This effect is dependent on Mas receptor stimulation and mediated by NO. These results reinforce Ang1-7 role in cardiac function modulation, namely through its ability to improve myocardial inotropic reserve and support a potential beneficial effect on ischemic contractile dysfunction.

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## Screening of bioactivity in seaweeds

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During several decades, the intense study of terrestrial ecosystems resulted in the identification and characterization of new bioactive natural compounds. However, since 80% of the living organisms are found in aquatic ecosystems and 2/3 of the earth is covered by oceans, the scientific community is now interested in this unexplored biodiversity. For instance, seaweeds are able to biosynthesize several secondary metabolites that can mediate a broad range of ecological interactions between marine organisms, including chemical defenses against herbivores. These secondary metabolites are responsible for some bioactivities attributed to seaweeds, such as, antimicrobial, antioxidant, antitumor, anti-inflammatory, antidiabetic and anticholinesterase.

Therefore, a first screening of antioxidant activity against DPPH radical was performed with the aqueous extract of species from Rodophyta (*Osmundea pinnatifida*, *Plocamium cartilagineum*, *Schizimonia dubyi* and *Sphaerococcus coronopifolius*), Chlorophyta (*Codium adhaerens*) and Phaeophyta (*Cystoseira nodicaulis*, *Fucus spiralis* and *Saccorhiza polyschides*). Since *O. pinnatifida* is an edible species consumed in Portugal, its aqueous extract was also subjected to other *in vitro* assays. Concerning the antioxidant activity, this species was very active as a scavenger of  $O_2^{\bullet-}$  ( $EC_{25}=1.00 \mu\text{g/mL}$ , Fig. 1) and  $\cdot\text{NO}$  ( $EC_{25}=0.27 \text{ mg/mL}$ ). Moreover, to a lesser extent, its extract was also active as inhibitor of acetylcholinesterase ( $EC_{10}=0.31 \text{ mg/mL}$ ) and butyrylcholinesterase ( $EC_{10}=3.50 \text{ mg/mL}$ ) activities, which are important in the treatment of Alzheimer's disease. These preliminary results are very promising since they demonstrate that *O. pinnatifida* can be also used to improve human health.

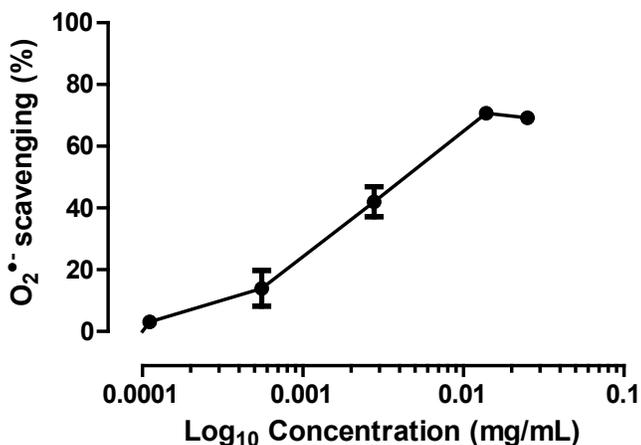


Figure 1: Scavenging activity of the aqueous extract of *O. pinnatifida* against  $O_2^{\bullet-}$ .

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## Growth inhibition of *Chlorella vulgaris* cultures exposed to cyanobacterial cell extracts

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Cyanobacteria produce an array of allelopathic molecules like cyanotoxins. When blooms of cyanobacteria occur these compounds can be present in high concentrations in the aquatic environment and their toxicity may originate negative impacts in the ecosystem. Cyanotoxin effects on phytoplankton are mainly unknown therefore their study is important. Also phytoplankton is responsible for a great part of the oxygen in the atmosphere and is the basic food source for several aquatic species especially bivalves. This may help us to understand what effects cyanotoxins have in the aquatic environment and in the various food chains.

In this study several cultures of the microalga *Chlorella vulgaris* were exposed to cyanobacterial extracts of *Microcystis aeruginosa* and *Aphanizomenon ovalisporum*. The extracts were prepared by disruption of cyanobacterial cells in distilled water. The number of cells used was equivalent to a cellular density of  $1 \times 10^6$  and  $1 \times 10^7$  cells/ml in the exposure cultures. Cultures were grown for 7 days and every day a sample was collected for reading on the spectrophotometer and cell counting on the Neubauer chamber.

The results show that *Chlorella vulgaris* cultures exposed to extracts of *M. aeruginosa* had an initial growth inhibition in the first two days but later the cultures had a significant growth increase in comparison to the controls. The extract equivalent to  $1 \times 10^7$  cell/ml had the highest increase. One explanation for this response is the enrichment of the culture medium with additional nutrients from the cell extracts. *Microcystis* are known to produce microcystins, cyanotoxins that induce cytotoxicity in various aquatic organisms. The initial inhibition may have been caused by these toxins. Nevertheless the subsequent recovery might be attributed to the lost of MC activity. One possibility is the toxin degradation by physical or chemical processes in the culture medium leading to harmless compounds. The cultures exposed to extracts of *A. ovalisporum* had growth inhibition starting from day 1. Highest inhibition was observed with the more concentrated extract (equivalent to  $1 \times 10^7$  cell/ml). *Aphanizomenon* is known to produce cylindrospermopsin, cyanotoxin that induces cytotoxicity like microcystins with different mechanisms of action. Therefore it is possible that growth inhibition was caused by this toxin. The results point that this toxin may be more resistant to degradation in the culture medium and/or more toxic to *Chlorella vulgaris* than microcystins.

# Assessment of the ecological status of the Ferreira river (North of Portugal) in Valongo and Gondomar municipalities

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Small drainage basins situated near urbanized areas are particularly susceptible to anthropogenic impact due to their size and other various pressures to which they are submitted. Both the Ferreira and the Simão rivers belong to the hydrographic sub-basin of Ferreira river located in the North of Portugal which, in turn, belongs to the hydrographic basin of river Douro. The Ferreira river extends for approximately 43 km, with its spring being in Paços de Ferreira. It goes through Valongo and flows into the river Sousa, in the Gondomar municipality; the Simão river, however, stretches out over 7,5 Km and flows into the Ferreira River, precisely where Natura's Network 2000 "Valongo" is situated.

This study's main goal was to evaluate the ecological status and quality of the Ferreira river, in Valongo and Gondomar, through the analysis of biological (benthic macroinvertebrate and aquatic macrophytes), physico-chemical and hydromorphological parameters. Therefore, the method used in this work has highlighted the importance of holistically analyzing the several components of the ecosystems in question, in order to assess their overall quality (water, biota, riparian zone).

This research was performed for 10 months (November 2009 to August 2010), in four distinct sampling spots: the first being in the river Simão (tributary stream of Ferreira river), and the other three being distributed along river Ferreira's ending zone. The sampling included a physical-chemical (monthly), biological (every three months), hydrological (monthly) and morphological (every three months) characterization.

Hence, through the assessment of the physical-chemical water quality, it was established that high levels of organical pollution existed, with a slight aggravation during Summer. The evaluation of the physical habitat which involved the areas in question revealed serious and deep transformations in the course of this study that eventually reflected in the poor ability for the riparian band to function as a tampon to the anthropogenic impacts systems are subjected. Also, the study of the benthonic macro invertebrate community indicated that both systems are somewhere between the slightly polluted and the heavily polluted. The benthonic macro invertebrate diversity was rather small with populations dominated by *taxa* tolerant to pollution, possessing individuals perfectly adapted to the existent conditions. The sampling spot, located near the confluence with the Sousa river, revealed to be the area with the worst ecological quality.

The results obtained were important and may serve as an incentive to the elaboration of future research works that can provide natural resources integrated management solutions. The aim is, indeed, to diminish the scattered and punctual pollution in the entire hydrographic basin of the Ferreira River, along with the rehabilitation of the riparian bands and the creation of proper conditions for the retaining of nutrients, so that a "good" ecological status, commended by the Water Framework Directive, can be achieved.





**U. PORTO**

*Parallel Oral Sessions I*  
**A3** *Literature & Language Sciences*

# **Audiovisual Translation: Subtitling for the d/Deaf and the Hard-of-hearing on all four public television channels in Portugal**

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Although Subtitling for the Deaf and Hard-of-Hearing is available on all four public television channels in Portugal – RTP 1, RTP 2, SIC and TVI – the effectiveness of this service in both quantitative and qualitative terms is questionable.

The aim of this research project was to analyse this issue using three case studies in order to establish a better understanding of the polysystems in which Subtitling for the d/Deaf and Hard-of-Hearing is incorporated.

In addition to a review of existing literature and of the position of Subtitling for the Deaf and Hard-of-Hearing within the scope of Audiovisual Translation and Translation Studies, a case study was conducted to establish a quantitative analysis of the Subtitling for the Deaf and Hard-of-Hearing services offered by the previously mentioned channels. A second case study, more descriptive in nature, analysed existing linguistic standards and practices in this field. Finally, a third case study involved conducting a survey to determine the level of satisfaction reported by the main users of this service.

This study revealed that gaps of both a quantitative and a qualitative nature continue to exist in this area, pointing out an opportunity for technological, technical and human resource investment in this field. Such an investment would lead to the creation of a genuinely useful service for users with a very specific profile and with very specific needs.

**Keywords:** Audiovisual translation, Deaf, Deafness, Subtitling for the Deaf and Hard-of-Hearing.

## « “Exemplary Novels” and «Exemplary Novels»: the paratexts of fiction in prose during the XVII<sup>th</sup> century»

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Paratextual elements in Baroque Literature provide wide literary and historical information and give us a privileged perspective on the relationships between the agents involved in the editorial process and censorship. In order to study this complex subject in portuguese literature we have selected a textual *corpus* of considerably sized fictional narratives in prose, published in the XVII<sup>th</sup> century and early XVIII<sup>th</sup> century, from the publication of *A Primavera* (1601) by Francisco Rodrigues Lobo, to *Roda da Fortuna, e Vida de Alexandre e Jacinta* (1724), by Mateus Ribeiro.

Given the specific characteristics of these texts' contents of production and reception that reveal a complex panorama regarding the prose of Portuguese narrative fiction of the 600's, we firstly considered their social-historic and literary-cultural frameworks.

After this stage, we focused our efforts on the careful reading of the paratexts for the fictional production in prose, giving special attention to the preliminary texts, prologues, dedications, reports and licenses, not only for what they disclose about their authors' intentions and considerations about their censors, but also what they reveal about the conditions of production and literature during those times, in the attempt to identify «clientelar» relationship networks that contribute to understanding the circulation and «readings» throughout the 600's. It is therefore important to emphasise that the orientations of authors, editors, censors and authors' "friends" allows us to identify two types of presuppositions acquired before reading the text, which manifest the knowledge and expectation that would be part of the literary universe of said period. A consequence of the editorial success attained by Cervantes' *Novelas Ejemplares* (1613), is their exemplary character that become a topic that echoes throughout the several paratexts of this narrative genre, be it for the authors to legitimise their works and so protect them from censorship, or to establish new horizons of expectation that captivate readers.

Considering that in question is the lapse of time that, in Portugal, witnessed the rise of several editions of narrative fiction in prose, the exegesis started for the paratexts of these works, in relation to their texts, allowed us to notice that they capture within them forms of legitimisation of the fictional prose, in a context that is often adverse to the point of view of the censorial mechanisms, also contributing to the study of the activity of the censorial institution of the XVII<sup>th</sup> century in Portugal.

## The world as a bad movie: cinema, poetry and politics.

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Following my masters research, that has worked with Poetry and Politics, I propose a study of José Miguel Silva's *Movimentos no Escuro*, published in 2005. In this book, the most part of poems has brought a film title (and its director and year) how your own title.

My idea is so explain how the relationship between cinema and poetry in *Movimentos no Escuro* is worked in a heavy critique of our contemporary society. This way, my presentation will intend to discuss the dialogue between poetry and cinema in *Movimento no Escuro* as a relationship between movie spectator/ world witness: seeing movies, the poet see the reality which, like the movie, is real and unreal in the same time. In face of this, I will work with notion of allegory in contemporaries artistic and theoretical productions, talking about the differences between the visionary modernist and the spectator contemporary poets.

# The Eye of Bensalem: Power in *New Atlantis*

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<sup>1</sup> Holding a Master in Anglo-American Studies and taking part in several projects such as *Eurotopia 2100: Uma Utopia Interactiva* and *Projeções de Futuro: Utopia e Cinema*, Faculty of Letters, University of Porto, Portugal

Preceded by a time where religion was the great matter, when Europe was a place of great religious disputes, and when political despotism prevented thought and enterprise, Sir Francis Bacon (1561-1626) in his ‘unperfected’ work, *New Atlantis*, posthumously published in 1627, describes a postmillennial society, ideal in its perfection, blessed by God with riches, health, peace and knowledge. During the Elizabethan Era, with the triumph of Protestantism, and with the overwhelming destruction of the Armada, there was an increasing vigour of the English Renaissance – anticipation of the Modern England, fact that provided the ideal context for the literary creation of scientific societies, of which *New Atlantis* is a great example.

Throughout this presentation, I hope to prove that *New Atlantis* is not, however, a simple description of a utopian highly developed society, in which ‘science’ is the main ruler. Indeed, parting from theoretical substance by several preeminent academics such as John Amos Comenius (1592–1670), Paul Ricœur (1913–2005), Michel Foucault (1926–1984) and Sir Francis Bacon himself, I expect to show how this, at first glance, ideal society is at its core a highly watched community.

In the final part of the presentation, I will demonstrate, through the symbolism of the ‘eye,’ how the scientists of the Salomon’s House, the most important institution dealt with in *New Atlantis*, is, in fact, the representation of the omnipresence of God as well as the representation of the continuous surveillance of a privileged minority over an entire community.

I owe this work to the so kind sympathy and guidance of Professor Fátima Vieira, who inspired and advised me during a whole semester of hard work, and to my dear friend, Márcia Lemos, who has convinced me to continue researching and whose advice is always so enlightening.

## What hurts is not wanting to erase you absences: Ulixes' figure on greek tradition

Patrícia Lino

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Odisseus, from greek, or Ulixes, from latin, is one of the most important characters on greek & roman mythology. He is, by excellence, Homer's *Odyssey*' leading figure, and he is the inspiration of many other literary works. With Achilles, he represents the maximum exponent of the ancient heroic figure, that later influenced so many other authors, including Virgilius himself. However, unlike Achilles or even Aeneas, Ulixes invites us to other interpretations of his figure, namely if we have the Greek Tragedy (particularly the play *Filoctetes* by Sophocles) in regard, along with some versions that canonical preference has rejected through times. In *Filoctetes* and in these marginal versions, Ulixes isn't another one of Troy's cursed heroes. Instead, he is the portrait of the villain. The claimed king of Ithaca, Penelope's spouse, is, on sophoclean verses, the fictional image of the sophist man, that crosses lies with the truth, and vice-versa, to accomplish his, even if cruel and inhuman, goals.

This little essay, « What hurts is not wanting to erase you absences: Ulixes' figure on greek tradition», proposes itself to prove this very point, starting with a detailed analysis of both *Iliad* and *Odyssey*, and ending with the analysis of the sophoclean tragedies, *Filoctetes* and *Ajax*.

# O lugar sem lugar. Visões da cidade líquida de Luís Quintais e Filipa Leal

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In poetry, ever since the advent of modernity, space and more specifically the new urban centers have been one of its central subject matters; Baudelaire, the original *flâneur*, has established the city as a noble *topos* in lyrical poetry.

In fact, some of the most recent Portuguese poetry written by some of its younger authors seems rather haunted by the city-scape. Whether as a background for the wandering of the poetic I or as a starting point to subjective reflexions, the city is omnipresent. However, in the works of Luís Quintais and Filipa Leal the city has not only spotlight honours, but a quite special characteristic as well: it's liquid.

Using Henri Lefebvre's idea of *space production* [1] as a starting point, and Marc Augé's famous concept of *non-places* [2], this essay aims to interpret the unique vision of the city as a liquid space in Quintais' and Leal's poetry.

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## **“Class report” in the context of processual writing**

**T. Castela**

Department of Portuguese and French studies, Faculty of «Letras», University of Porto, Portugal.

The knowledge of a mother tongue in its written form becomes particularly relevant when the social, professional and personal construction of the individual is considered, mostly because it determines his course. Throughout his school formation it is essential that the individual may acquire the skills required by this field of knowledge so that he will benefit from a balanced development through the course of his life.

In the school context, the transversal character of writing, used as a means of conveying knowledge of the several areas taught in mother tongue, is more than evident. This fact is, in our perspective, a reinforcement of the urgency of working more deeply the writing skill in the teaching frame of a mother tongue. If not, the access to other fields of knowledge will be very compromised. When we consider the foreign languages, a developed knowledge of the mother tongue is a significant advantage. In fact, the domain of mechanisms that regulate the reference language, facilitate the understanding and the acquisition of those, which belong to the language in a process of learning.

If we agree that the development of oral skills both in mother and foreign tongues should be worked, we cannot estimate that the knowledge acquired in this field to be enough to assure a good writing performance. Writing demands a deep understanding of specific knowledge, which requires regular practice throughout the whole formation process. The oral skills are, in fact, the cornerstone for several other learning processes that contribute for a writing domain. However, it is fundamental to establish specific moments dedicated to exercising writing so that students may, eventually dominate its techniques, both in mother and foreign tongues. Through writing, the student will face the difficulties that writing involves, and that he may reread, rewrite, reflect about with or without help, throughout its course. This way, he may transfer to posterior situations the knowledge that resulted from the lived experience that practice will gradually make more perfect.

The analysis we present is based on the production of reports in Portuguese and French language classes, and it aims at verifying to what extent regular practice of a given writing exercise improves students' writing proficiency.

The obtained results allow us to verify some encouraging progresses in the students' domain of sentence and textual structure, both in Portuguese and in French. The clarity and deepness of correction of the detected mistakes proved to be determinant for the students' evolution. The students' progression was more visible in the subject in which the correction of mistakes was made in a more detailed manner.

Beyond the explanation of the rule, which helps avoiding the mistake, spending considerable class time performing this activity, specially, in the beginning of the school year proves to be an important action. Even though it may delay the course of teaching some of the curriculum contents, the final result will overcome that first setback, since the students will make less and less linguist and textual mistakes in their writing. The implemented activity will result in affective evolution, which is determinant for the students' progression, in the immediate school context, and later in the social and professional context they will integrate, and for which school should prepare them.

The “class report” has irrefutable advantages in the school context of teaching mother and foreign tongues. These advantages are far superior to the detected disadvantages. For that reason, we defend that class practices, similar to the ones studied, may spread so that our teaching system may respond directly to the students' needs.



**U. PORTO**

## *Parallel Oral Sessions I*

**A4** *Engineering I*

# Lightweight Roof Structures

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Modern tension structures emerged in the 50s and have a growing presence in the present engineering. The transfer of the concepts associated with the suspension deck to roof structures led to the implementation of systems formed initially by simple cables, evolving into cable grids, and finally to tensile membranes (Fig. 1). From structures like awnings and tents to major structures, like bridges and stadiums, the versatility in shape and single characteristics is evident.



Fig. 1- a) Arène de Nîmes roof, France <sup>1</sup>; b) Expo 67 pavilion, Germany <sup>2</sup>; c) Faro-Loulé stadium, Portugal;

First, general aspects of cable and membrane structures are analysed, regarding materials and joints, and their influence on the structural behaviour and shape [1]. Then, bases of design, security criteria and project actions are referred, focusing on the wind action on stadium structures. In a third section, a brief review of shapes, common structural types and classifications is presented and illustrated with examples. The theoretical formulation of the behaviour of a cable [2], a membrane element and edge cable elements is then introduced, as well as relevant methods of numerical analysis, seldom used, either in form finding, both in static and dynamic analysis. This formulation is used in a parametric study on the sliding effects of cables in a grid structure and in the design of tensile membrane roof structure with edge cables.

Thus, this study aims to synthesize the current state of the art, in order to identify not only advantages and disadvantages of using that cable and membrane structures over conventional ones, but also to focus on the requirements and criteria to adopt in the various phases of the design of a membrane structure, and on the understanding the behaviour of the basic elements that form these structures: cables and membranes.

The European scientific community gives insufficient documentation, although have been motivating interested people in this field with symposiums and workshops.

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<sup>2</sup> Fonte:[http://upload.wikimedia.org/wikipedia/commons/e/e7/Germany\\_Pavilion\\_Expo\\_67\\_-Montreal\\_Quebec.jpg](http://upload.wikimedia.org/wikipedia/commons/e/e7/Germany_Pavilion_Expo_67_-Montreal_Quebec.jpg), 15/04/2010

# Study on the use of rock dust waste in concrete

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Globally, the construction industry deals with a great amount of natural resources through the production processes involved. Concrete is one of the most used building materials in the world. It is estimated that the present consumption of concrete is in the order of 11 billion tonnes per year [1]. The cement industry contributes with over 6% of the global CO<sub>2</sub> emissions, making it a strategic sector for green house gas effect emission mitigation strategies [2]. One such strategy is partial cement replacement materials in concrete with an increased use of additions.

The sector of rock extraction and transformation in Portugal is characterized by the production, and existence, of significant quantities of waste materials, such as rock dust sludge derived from dust control and washing processes. It is estimated that about 0.5% of the total processed rock is wasted in this form. The rock dust sludge can be classified as a polluting residue which needs to be stored in a purposely built landfill, with associated economic costs [3].

In the course of a research project at the Laboratory for Concrete Technology and Structural Behaviour, LABEST, with experimental work being carried out at “Laboratório Ensaio de Materiais de Construção”, LEMC, the use of granite rock dust sludge, PPG, produced in a quarry in the north of Portugal was studied as a possible addition to concrete. The rock dust can potentially be used as filler or even as a partial replacement for cement. For this effect, mineralogy was analyzed, along with parametric studies where the percentage of cement replacement, the fineness of rock dust and heat treatment, were studied. The mechanical properties of fresh and hardened mortar specimens, as well as some properties concerning of durability, were analysed.

Preliminary results show that the use of PPG is possible in conventional concrete, with potential economic benefits and as a contribution to sustainable construction.

Acknowledgements: National Laboratory for Energy and Geology (LNEG); Domingos Silva Teixeira SGPS (DST).

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# Distributed System for Building Evacuation

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In building fire emergency situations, evacuation usually is based on a system of static signs/arrows installed on walls, doors, and stairs to indicate exit paths. Using this static approach, arrows always point to the same paths regardless of whether they are obstructed or not. We propose a dynamic system that can give real-time instructions to safely exit the building, deflecting them from hazardous zones and routing them to the nearest exit. We envision such systems based on Wireless Sensor Networks (WSNs), small, cheap, self-powered devices, capable of exchanging information among themselves using radio communications, enhanced with temperature sensors. By efficiently scattering a number of these devices across the building, we expect them to sense a fire, spread that information across all sensors, and command emergency signs that will direct people towards exits through the safest paths.

However, the efficient and reliable operation of such system faces tremendous engineering challenges. In this particular system, they can be related to three aspects:

- Deployment of the sensors: what is the optimum placement of the sensors that ensures good coverage? Can generic methodologies be applied, or each building is a case by itself? How are the sensors given information about the building topology?
- Their operation in normal circumstances: which routing protocols are best? How frequently can we exchange messages without depleting the battery too fast?
- Their operation during emergencies: what are the best algorithms to compute the routes to leave the building? What happens if one of the sensors gets burnt?

To provide a theoretical framework for the system operation, we modeled the problem using graphs. This approach, which is still on-going research, has enabled us to define rules for the not-so-straightforward topic of node placement and of how to find the best route out of the building, which we hope may reveal themselves generic enough to be applied to any building. Another line of research we are exploring is to make the task of deployment easier. As each sensor must know the topology of the building, the most common approach in literature is to give the map to the sensors *before* they come into operation. We are exploring an alternative in which the sensors can learn the building topology during the deployment stage. Our present work describes an algorithm to create a description of the building topology that is not hard-coded onto to sensor nodes. Our idea is that, whenever a pair of sensors is placed, a special sensor carried by the user will be used to wirelessly associate those two sensors, thus informing the network that a physical passageway exists among those sensors.

Up to this point, we have also built a preliminary proof-of-concept test-bed that we will show at IJUP. In the future, we plan to deploy up to 20 sensors on some corridors of FEUP's ECE Department, with ancillary signalling equipments.

# Wi-Rex: Design of an Integrated Circuit for a Wireless Receiver

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The Wi-Rex project was originally proposed to encourage the involvement of students in extra-curricular activities, to experience methods of research and development. It aims to Electrical and Computer Engineering students, particularly to those who have interests on integrated circuit (IC) design and testing. The project addresses an RF (radio frequency) receiver design, at 2.45-GHz with a 2-MHz intermediate frequency (IF), similar to Bluetooth protocol. The number and students profile involved in this project was quite heterogeneous, with students from the first to last year of the Masters in Electrical and Computers Engineering at the Faculty of Engineering of the University of Porto. This project was developed at the Microelectronics Students' Group.

Due to design complexity involved, the project was divided in two phases. The desired outcome of each phase resulted in an IC with part of Wi-Rex receiver circuits; both were successfully designed using industry standard tools and sent for fabrication, being now ready for testing. The first IC contains a PLL-based frequency synthesizer, and the second comprises a radio-frequency (RF) front-end circuit, namely, LNA (low noise amplifier) a complex filter, two saturators, and a digital demodulator.

The project goals were completely satisfied. Future (short-term) work comprises the ICs testing to validate the design and perform eventual tuning and calibration.

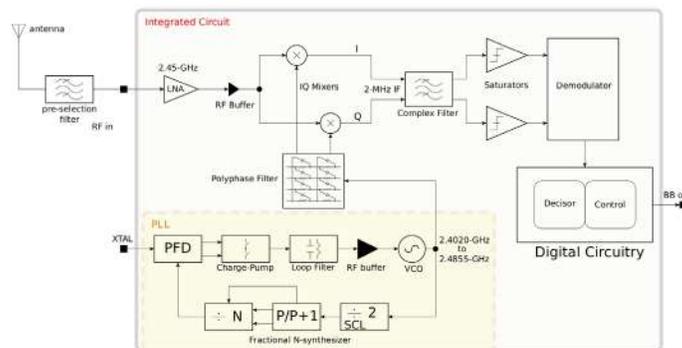


Fig. 1 – Receiver architecture

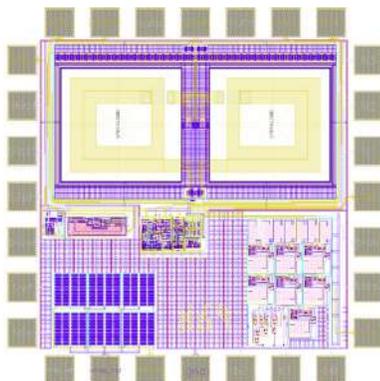


Fig. 2 – 1st IC layout

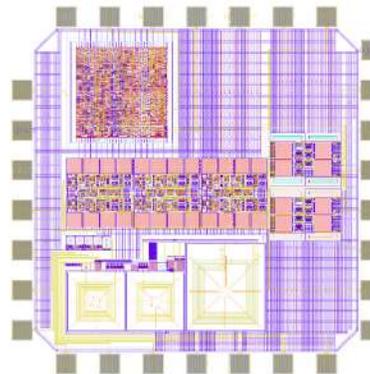


Fig. 3 – 2nd IC layout

# Smartphone's Potentials in Electric Vehicles Applications

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During the last few years there has been a growth in the interest of the electric vehicle (EV) market, with the big groups of the automobile sector introducing several new models into the market, both hybrid or pure electric. The cause for this gradual change is a larger, and growing, pressure both from governmental agencies and the consumer market for cleaner and safer transportation technology. The Electric Vehicles can comply with this requirement as they produce no gas emissions while operating and open new fronts for development of technology that will allow efficient and safer journeys [1].

Although relatively recent, the smartphones emergences had, and still have, a profound impact on the daily life of people. In the automobile sector, and in particular on EVs, the smartphone use is a functionality that may ease the transition to this new electric transportation technology. Contrary to the conventional vehicles, EVs based on batteries face strong constraints on the maximum distance traveled per battery charge. These constraints will introduce new challenges to the traditional driver, forcing him to a greater care in the planning of trips. We can see future phones as the preferred mode of interaction with electric vehicles, because the phone is always available and its improved hardware can provide a better and customizable user interface.

In this context, smartphones, with GPS capabilities, may aid the drivers in the EV management, suggesting travel routes that take in consideration the location of the recharge posts, an important factor in this initial stage of the electric transportation, where the electric recharge possibilities are scarce. Another functionality offered by the smartphone system is the possibility of the driver downloading the EV telemetry and operating data. This data can be important to aid drivers to improve their EV use efficiency: for example, they can check the amount of energy regenerated while braking, analyze the power peaks, and cross that data with the one from GPS to better understand the impact of their driving style in the vehicle efficiency and identify improvement points.

Based on these ideas, we started the development of a smartphone application for the uCar multi-motor electric vehicle prototype [2]. The vehicle has an electronic control unit which manages the vehicle operation and interfaces with the several EV components: the motor controller, the battery manager and charger, the GPS, etc. The smartphone application communicates with the electronic control unit and provides a simple graphical interface for the driver to access important information about the vehicle operation: i) the EV status and diagnostics, ii) the energy management state and preferences and iii) an interface for downloading the vehicle operating data.

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# Micro-Power Generation Based On Electromagnetic Induction

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## **Abstract:**

In this work we propose a system of micro-power generation, based on electromagnetic induction, profiting all of the capabilities of this phenomenon. This concept takes advantage of a type of energy that is constantly devalued, even if the power that can be generated is small. Our approach consists in a small system that takes advantage of little vibrations that occur on a surface and can be efficiently converted in power. Power is generated by means of electromagnetic induction, taking advantage of magnets and coils in a prototype area.

## **Introduction:**

Power generation based on electromagnetic induction is nowadays widely used in energy production, and induction generators can be easily found in wind and hydro power production. The power generated in those locations is mainly used to supply cities and depending on the available grid, it can reach thousands of Watts. The micro production of energy is becoming a fact, and today we can produce almost 100% of the energy that we consume. But why don't we take advantage of all the energy we can afford to produce, even the smallest one? Daily, inadvertently we produce a lot of energy, just by walking, running, jumping and even outside our home driving our car. A system capable of absorb all of these wasted kinetic energy and transform it again in usable electrical energy can be developed and our main goal with this concept is to analyze the idea and gauge all the parameters that characterize it.

## **System Description:**

The system of our concept is based on an oscillator platform that can be simply modeled with springs between the platform and the floor. The hardness of the springs can be adjusted in order to adjust the resonance frequency of the platform. In order to generate power, we conceptualize a system based on a coil and a permanent magnet that oscillates inside the coil. Some studies [1][2] and products are already developed based on this simple concept. We also know that energy conversion is more efficient when a magnet resonates at low frequency above a large area coil, so we pretend to keep this in mind while trying to maximize the production at minimum cost.

## **Conclusions:**

We set out to create an affordable system to generate micro-power that can be used in big, heavily populated areas (such as airports, train stations, shopping malls, etc) or even at our homes, simply replacing the floor by a system based on this concept. Areas where the vibration caused by cars or other vehicles is considerably high (such as city centers or other high traffic places) could also be possible locations for this system, for powering traffic lights or other luminous signs. The power produced can be used in several areas, such as LED illumination, luminous indicators and home applications.

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**U.PORTO**

*Parallel Oral Sessions I*

**A6** *Communication Sciences I*

# Influence of media in humanitarian crises: Case study of Darfur's Conflict

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This paper examines whether the Portuguese and The U.S. press endorses the views of governments in relation to conflicts of humanitarian nature. Thus, we analyze the online news of *Público* and *The New York Times* from August to October 2006 concerning the conflict in Darfur. This work is based on the Indexing Theory of Lance Bennett [1] and proves that the *media* analyzed does index the positions of the respective governments, so in favor the entry of the UN in conflict. There for, we studied the use of the word Genocide, as a label of the conflict, and it is proved that the *media* tend to fiercely criticize the Sudanese leader, Omar Al-Bashir. The methodology used is *Meta-performance analysis* de Rui Novais [2].

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# Entre o Kosovo e o Darfur: Semelhanças e Diferenças na cobertura mediática

## Uma Noticiabilidade Alternativa

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This study examines the differences in media coverage between the Kosovo War and Darfur Conflict in three selected newspapers: New York Times, The Guardian and Público. This article shows how Kosovo war was massively reported and in the other hand how Darfur Genocide was neglected by media. But why? Some answers are difficult to give, because all is influenced of the world development.

This issues are relevant for the discussion of how important are the media in war coverages and humanitarian interventions.

Key words: Kosovo, Darfur, media coverage

# Western Coverage of Rwanda's Genocide

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The massacres carried out in Rwanda in 1994, were not sufficient to draw the attention of international media. However, the genocide in Rwanda is considered to be the most tragic event of the twentieth century, being killed 333 people per hour. This study seeks to assess the media coverage that the West made of the conflict, notably the U.S. and France, two of the key players in the massacres in Rwanda. We study 179 news from *The New York Times* and *Le Monde*, the two leading newspapers in those countries. The analysis period starts in October 1990 and ends in August 1994. The news was selected according to the peak of the events of this conflict.

No international media gives the right attention to, which is probably, one of the biggest massacres in human history. However, the conflict less noticeable ever has not been forgotten and is still analyzed and explored by all those who are interested in the role of media in humanitarian crises worldwide.

After a long and proper review, we came to a research question: What was the trend of Western news coverage of Rwanda's Genocide? Was there conflict journalism instead of denunciation, peace agreements and maintenance?

The three hypothesis we put were:

1 – In the coverage, it is clear the lack of use of the term 'genocide' and the late denomination of the situation in Rwanda.

2 – The news of the case describe the africans as violent, tribal and savages.

3 – In terms of news coverage of Rwanda's genocide, is not fulfilled the role of denouncers that journalists assigned themselves.

To confirm or refute these hypotheses we used the content analysis method. We analyzed the digital files of two Western newspapers, The New York Times and Le Monde, to ensure an effective comparison, knowing that our aim was to analyze trends in Western coverage. In addition, we face two dailies of reference, representing the two sides of the Atlantic: the american by its proeminence and the French because of its intervention.

We developed a table of quantitative and qualitative analysis, taking into account the literature review, the investigation question, our hypothesis and the work of Beaudoin and Thorson. We aim to investigate whether the news content about the conflict is or not excessive; if there are descriptions of events; which patterns of news coverage regarding efforts to maintain peace and agreements and if the coverage patterns differ when the story involves the United States of America and France (Beaudoin and Thorson, 2002: 45)<sup>1</sup>, taking into account our second hypothesis. As expected, the news coverage of the two leading newspapers The New York Times and Le Monde was certainly insufficient. For this reason, and to validate the thesis already defended by Edgar Roskis, Le Monde Diplomatique's journalist, which states that Rwanda was "un sans image genocide - a genocide without images" (1994, cit. For Thompson, 2007: 4)<sup>2</sup>.

In a nutshell, it is easy to see that our three hypotheses were confirmed. In fact, there is no denounce. The news often and improperly rely upon the technicalities and euphemisms, that can lead to misunderstandings in the minds of readers. Finally, our second hypothesis was confirmed, in that the Rwandan violence is news in the speech.

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# O Genocídio no Ruanda: Cobertura mediática do El País e The New York Times

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This study focuses on the analysis of media coverage carried out by two international newspapers on the genocide in Rwanda in 1994. The selected newspapers are El País and The New York Times and the analysis covers the period from 1 April to 31 October 1994, using the technique of ‘month built’. The study is based on the method *metaperformance analysis* proposed by Rui Novais. The four dimensions of analysis in this study are the selection and use of sources of information, terminology, criticism/attacks on government officials and themes present and absent in the news. The results show that the newspapers take different positions on the description of the facts, but both index the positions of Western governments, showing disinterest in further media coverage.

Keywords: Media coverage/Rwanda/Metaperformance analysis/Sources of information

# Iraq War: performance analysis of the publications *The Times* and *El País*

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The media are an important part of international conflicts and strategies of the antagonists that have as objective to influence the evolution of the media narrative of war, trying to impose on the media framing conceptions of reality that serve in the best way their strategic interests. This study aims to reflect about the media's behavior, during the military intervention in Iraq War, by the method of performance analysis, enunciated by McQuail. Thus, we examined the headlines and the editorials of the British journal *The Times* and the Spanish journal *El País*, between March 20, 2003 and April 10, 2003, which is the period that includes the beginning of the invasion and the fall of Saddam Hussein. It is intended that way, heading towards new fields of research. There is a strong predominance of official sources; the publications do not use the editorial as a form of public scrutiny, showing only the visible effects of violence in conflict. Thus, the quality of information is jeopardized.

Keywords: Iraq War, Intervention, Performance Analysis, Press

# Tragédia no Haiti

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This research work tends to understand the media covering in the presence of a certain theme, adapting one of the various communication theories.

Supported in various readings and analyses, the article tries to understand the connection between the tragedy that occurred in Haiti in day 12 of January of the 2010 (when a earthquake of magnitude 7.0 – in Richter Magnitude Scale - provoked the destruction in the whole country) and the Globalization Theory (associated to the sensationalism) based upon the analysis of two Portuguese newspapers (Jornal de Notícias and Correio da Manhã) and a general analysis of an American newspaper, the New York Times and a British daily paper, The Guardian.

Therefore, hypotheses were formulated. Based on these hypotheses, there were adopted qualitative and quantitative methodologies, expressed in the analysis of the newspaper's contents.

Keywords: Haiti, Globalization, Sensationalism





**U. PORTO**

## *Parallel Oral Sessions II*

**A1** *Biological, Environmental & Health Sciences III*

# The Influence of Chitosan and Fibrinogen-Modified Chitosan on the Formation of Osteoclast-like Cells

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A biomaterial, such as chitosan (Ch), must be biocompatible and biodegradable, and this degradation should occur within an appropriate period of time. As Ch may be easily processed into films or porous forms, it has been widely used as temporary templates for *in situ* bone regeneration [1]. Recent reports of osteoclasts (OC) cultured on Ch substrates indicate that Ch *per se* may inhibit OC activity [2], but when Ch was modified with collagen OC activity was enhanced [3]. Thus, OC are attractive candidate cells for promoting modified Ch scaffold degradation, as part of a potential process of bone remodeling *in vivo*. OC are giant multinucleated cells that demineralize bone tissue, resorbing bone, and differentiate from the myeloid-monocyte hematopoietic cell, through a process called osteoclastogenesis [4]. Also, OC produce enzymes that are able to degrade bone matrix, such as Tartrate Resistant Acid Phosphatase (TRAP).

In this context, our aim was to investigate the capacity of fibrinogen-modified Ch to induce the formation of OC-like cells. The use of the pro-inflammatory fibrinogen molecule to modify a scaffold intended for bone regeneration is justified by its crucial role in wound healing and repair [5]. To induce OC differentiation, monocytes were isolated from peripheral blood and cultured in the presence of Receptor Activator Nuclear Factor- $\kappa$ B Ligand (RANKL) and Macrophage-Colony Stimulator Factor (M-CSF). RANKL interacts with RANK, a transmembrane molecule expressed on both OC precursor cells and mature OC, inducing the commitment of monocyte/macrophage precursor cells to the OC lineage, and mature OC activation [6]. The formation of OC-like cells on unmodified or fibrinogen-modified Ch substrates was evaluated by two different approaches: 1 – Staining for TRAP production; 2 – Immunocytochemistry to evaluate the presence of multinucleated cells. Results reveal that cells were TRAP positive. Finally, when OC are cultured on Ch substrates, cells are bigger, exhibit larger actin rings and more nuclei per cell, than cells cultured on tissue culture polystyrene plates, which were used as a control.

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## Natural killer cells responses in presence of biomaterials

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An exacerbated inflammatory response questions biomaterial biocompatibility, but on the other hand inflammation has a central role in regulation of Tissue Regeneration. Therefore, it may be argued that an “ideal” inflammatory response is crucial to achieve efficient tissue repair/regeneration. Natural Killer (NK) cells are one of the first cell populations to arrive at an injury site and it’s evident that NK cells regulate the early onset and resolution of the inflammatory phase. Their unique capacity to lyse target cells, to secrete an immunoregulatory cytokines and to interact with other cells, particularly with Mesenchymal Stem Cells (MSCs), makes them capable of regulating homeostasis of other cell populations. Here, we studied how biomaterials designed to incorporate inflammatory signals affected NK cell behavior and NK cell – MSC interactions. Adsorption of the pro-inflammatory molecule Fibrinogen (Fg) to chitosan films led to a increase of NK cells that adhere to biomaterial when compared with chitosan without adsorbed protein. Also, we consistently found that freshly NK cells produced high amounts of Interferon gamma (IFN- $\gamma$ ), Tumor Necrosis Factor (TNF- $\alpha$ ) and that CD107a expression was up-regulated upon NK cell incubation with MSCs, and modified matrices do not seem to affect cytokine secretion . Most importantly, it was found that NK cells are capable of stimulating human bone marrow MSC invasion. Of significant importance, this NK cell-mediated MSC recruitment was modulated by Fg adsorption. Designing novel biomaterials leading to rational modulation of the inflammatory response is proposed as an alternative to current bone regeneration strategies.

## Modulation of human neutrophil oxidative burst by 3-hydroxyflavones

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Although inflammation underlies of a large number of different diseases, an universal anti-inflammatory preventive or curative therapy has not been achieved. Indeed, the traditional steroidal or non-steroidal anti-inflammatory therapies are either not enough or associated with too many side effects. Thus, further research pursuing the development of new anti-inflammatory agents is clearly endorsed. Neutrophils are the most abundant leukocytes in the blood, and participate actively in the innate host defence response in the event of inflammations. One of the most important functions of these cells is the generation of an array of reactive oxygen species (ROS) and reactive nitrogen species (RNS), in a process known as oxidative burst, to kill invading microorganisms. However, this process can be deleterious to the host in case of over- or sustained-production of ROS and/or RNS.

Considering the antioxidant and anti-inflammatory potential of flavonoids, the aim of the present study was to evaluate the putative modulating effect of an hitherto not studied series of 3-hydroxyflavone derivatives (Fig. 1) on the oxidative burst of human neutrophils, *in vitro*.

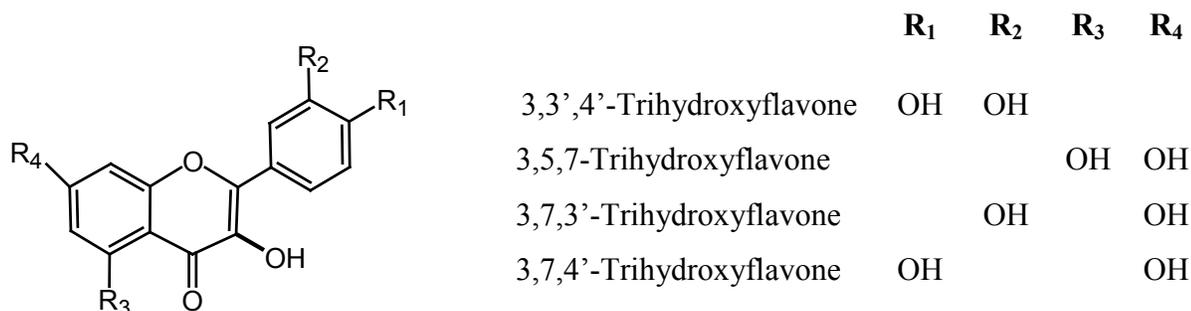


Figure 1. Chemical structure of the studied 3-hydroxyflavones.

For this purpose, isolated human neutrophils were stimulated with phorbol 12-myristate-13-acetate (PMA) with the subsequent measurement of the reactive species produced, using several probes, including luminol, amplex-red, APF and cytochrome c. The obtained results showed that 3,3',4'-trihydroxyflavone was the most active compound, while 3,7,3'-trihydroxyflavone was the less active compound in the majority of the probes tested. These results show that the substituents' position is significant, the catechol moiety having utmost importance for the modulation of human neutrophil oxidative burst.

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## Persistent Organic Pollutants (POPs) levels in human visceral and subcutaneous adipose tissue on an obese Portuguese population- biological implications

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The association between environmental toxicants with hormone-like activity (endocrine disruptors, namely POPs – persistent organic pollutants) and obesity etiology is based on the assumption that compounds that are both persistent and bioaccumulative have the potential to induce adverse effects on human health. Humans, exposed primarily via dietary intake, typically accumulate the highest concentrations, and are unable to metabolize or excrete them. Due to their lipophilicity nature, adipose tissue (AT) is a common site of POPs accumulation. In this regard, evaluate their presence in human AT, especially in an obese population, and subsequently assessing its implication, was consider a priority research.

To address this issue, adipose tissue samples (visceral and subcutaneous) from an obese Portuguese population (body mass index, BMI > 35) were collected during surgery at the Hospital of S. João. The levels of 12 POPs residues (hexachlorobenzenene, ( $\alpha$ ,  $\beta$ ,  $\delta$ ) hexachlorocyclohexane, aldrin, endrin, dieldrin, lindane, endosulpan I, endosulfan II, p,p'-DDD, p,p'-DDE, o,p'-DDT, methoxychlor and TCDD) were determined by gas chromatography-electron-capture detection analysis, with 4-4'-dichlorobenzophenone as an internal standard.

Preliminary results show the presence of POPs in human visceral and subcutaneous adipose tissue of obese individuals in all samples evaluated. Of the quantified compounds, the o,p'-DDT, a pesticide banned for use in several countries, since 70's, was found in 95% of samples of visceral ( $10.47 \pm 0.5$  ng/g fat, n=20) and subcutaneous ( $9.38 \pm 0.9$  ng/g fat, n=20) adipose tissue. Regarding the BMI evolution after surgery, there is a more pronounced decrease in the younger individuals. Curiously, these individuals also had a more balanced visceral/subcutaneous POPs ratio, unlike others that had a greater POPs burden in the subcutaneous AT.

Further determinations will be made with the intention of comparing the presence of POPs in visceral and subcutaneous human AT and correlating with the biochemical determinations follow-up. This evaluation, as seen in BMI evolution, will help to assess metabolic improvement after surgery and correlate with POPs burden.

Acknowledgments/Funding:

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# Treatment of industrial effluents using a vegetal coagulant

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A new type of coagulants of natural source arose recently. Natural polymers such as starch, sodium alginate amylopectin, guar gum, xanthan gum, chitosan and okra mucilage have been reported as flocculants [1]. Other natural products, such as suspensions of *Cactus latifaria* and seeds of *Prosopis juliflora*, have been used successfully for turbidity removal in water [2].

The tree Black Acacia (*Acacia mearnsii*) is very common in Brazil and has a high concentration of tannins. Organic polymers can be extracted from the tannins by polymerization. Earlier studies with the organic polymer Tanfloc [3-4] proved its efficiency in treating surface water, municipal, textile industry and laundry wastewaters.

The present work aimed at evaluating the efficiency of the cationic polymer Flox-QT, extracted from *Acacia mearnsii*, for treating different real industrial effluents. This coagulant (Flox-QT) is extracted from the Black Acacia (*Acacia mearnsii*). The effluents studied were produced in petrochemical, leather, cork stoppers, metalworking, olive oil, glue, paint (printing), textile and paper industries. The parameters analyzed in the effluents before and after treatment were selected according to the type of wastewater and included pH, conductivity, apparent colour, turbidity, total suspended solids (TSS), chemical oxygen demand (COD) and some metals. The coagulant proved to be efficient for almost all effluents tested. The best results were obtained for the paper industry wastewater, with 91% removal of chemical oxygen demand and 95% of total suspended solids removal. The estimated cost of this treatment would be only 0.24 Euro per cubic meter of treated effluent, only regarding the price of the coagulant and the required dosage. The use of this coagulant is also adequate for the valorisation of the sludge obtained, which in this case could be recycled for paper production.

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# Degradation of pesticides Paraquat and Chlorfenvinphos in water by Fenton's reagent

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The advanced oxidation processes (AOP's) are clean technologies and have proven to be highly effective in the degradation of many substances. An example of an AOP is the Fenton's reaction, in which organic matter is degraded by the hydroxyl radicals, generated in the reaction between  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$ , in a non-pressurized reactor and at temperatures close to the room one. This oxidation process converts the pollutants into other compounds (intermediates) and ultimately, the reaction might proceed till total mineralization (i.e., up to formation of  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ). This work intended to study the degradation of some model pollutants in water matrices. The studied pollutants were important pesticides like paraquat and chlorfenvinphos.

Experiments were performed in a batch reactor, with temperature control, collecting samples along the reaction. These samples were subsequently analyzed by an HPLC-DAD (to quantify parent compounds degradation) and also by a TOC (Total Organic Carbon) analyser (in order to measure the degree of mineralization).

It was found that increasing the temperature and the amount of iron salt (catalyst) in the reactor, the rate of the reaction increases. On the other hand, there is an optimal hydrogen peroxide concentration for each pesticide, because above that value parallel and undesired reactions occur (scavenging of the radicals by excess  $\text{H}_2\text{O}_2$ ). It was observed that chlorfenvinphos is degraded faster than paraquat in similar reaction conditions. For an  $\text{H}_2\text{O}_2$  concentration of  $1.5 \times 10^{-2}$  M, conversion of paraquat reaches values above 90% after around 90 minutes, while chlorfenvinphos reaches the same conversion values in less than 10 minutes, under identical conditions.

Acknowledgments:

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**U. PORTO**

## *Parallel Oral Sessions II*

**A2** *Biological, Environmental & Health Sciences IV*

# Synthesis and genotoxicity evaluation of a thioxanthone derivative

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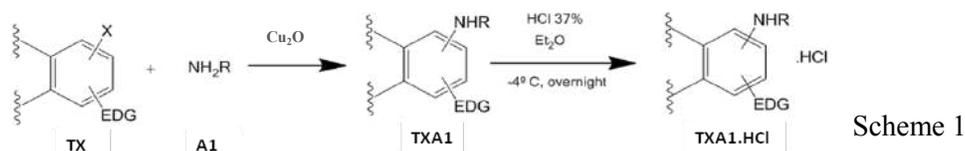
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The need for a continued search of new anticancer drugs is of great importance since many of the current drugs are insufficiently effective, lead to severe side effects, and often lose their efficacy due to the development of tumor resistance. Thioxanthenes have been reported as potential antitumor agents [1]. **TXA1** is a new promising antitumor agent with a thioxanthonic scaffold showing a  $GI_{50} < 10 \mu\text{M}$  against the growth of several tumor cell lines [2]. The aim of this work was to scale-up the synthesis of **TXA1** and to investigate **TXA1** genotoxicity *in vivo*.

**TXA1** was obtained by a copper catalyzed aromatic nucleophilic substitution reaction (Scheme 1). Variation of typical reaction parameters, including solvent and heating source, showed that the best results are achieved by a multimilligram approach with conventional heating at 100°C in closed vessel and MeOH as solvent in the presence of  $K_2CO_3$ . **TXA1** was successfully converted into **TXA1.HCl** ( $\eta=50\%$ ) by the addition of a concentrated aqueous solution of HCl to an organic solution of **TXA1**.



The mutagenicity activity was evaluated by the micronucleus assay in CD1 mice. **TXA1.HCl** revealed a genotoxic effect only at doses higher than 12.5 mg/kg. These findings may suggest a direct DNA reactivity of **TXA1.HCl** and gave a clue to further investigate the antitumor mechanism of action of these derivatives.

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## Rifabutin and analogue: partition, fluorescence and x-ray studies reveal drug-membrane interactions

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Tuberculosis (TB) is still a major concern to public health and the scientific community, since it is an infectious disease caused by *Mycobacterium tuberculosis* (MTb), which is the most common opportunistic pathogen in AIDS patients. Its resurgence and persistence, along with the emergence of multi drug resistant strains (MDR-MTb), are the main reasons behind the development of new drugs against TB.

Rifabutin (RBT) is a second line therapy drug for the tuberculosis treatment and has been useful against some isolates of MDR-MTb. Furthermore, it has been proved to be very effective in prevention and treatment of disseminated atypical MTb infection in AIDS' patients. Hence, the development of RBT analogues, such as N<sup>7</sup>-acetyl-rifabutin (Rifa JC2), is a promising way to reveal new MDR-MTb therapeutics.

In the current work, drug lipophilicity, along with membrane location and ability to change membrane biophysical properties were evaluated since these are essential parameters to understand the mechanism by which antibiotics enter into the cells. Therefore, liposomes made of DMPC (zwitterionic) and DMPG (negatively charged) lipids were used as mimetic models of bacterial membranes and the effects of RBT and Rifa JC2 were evaluated upon interaction with these membranes. Physiologic pH conditions were also guaranteed, in which these drugs are positively charged.

Fluorescence quenching studies and lifetime measurements were made to study drugs' location in the membrane model systems labelled with fluorescent probes. Steady-state anisotropy was also investigated in order to evaluate drug-induced changes in membrane biophysical properties. The application of spectroscopic methods enabled the determination of the partition coefficients ( $K_p$ ). Finally, small and wide angle X-ray scattering (SAXS and WAXS) were used to evaluate the symmetry effects, long and short-range organization and molecular packing of the bilayers with RBT and analogue.

The overall results of this work reveal a higher affinity of RBT and Rifa JC2 to the negatively charged membrane. Membrane location studies allow us to conclude that both drugs are able to permeate the membrane. Regarding this, the mechanism by which these antibiotics permeate through the phospholipid bilayers might include an electrostatic adsorption at the interface region and then its permeation induces also notorious changes into membrane biophysics.

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# Biophysical interactions of diclofenac with phospholipids bilayers

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Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the world's most prescribed drugs for acute and chronic inflammatory diseases. Unfortunately, their use is limited due to cases of gastrointestinal toxicity. This toxicity is hypothetically due to the accumulation of NSAIDs in cells of the gastrointestinal lining, with subsequent mitochondrial impairment [1]. Barrier functions exerted by cellular membranes can prevent or enhance the accumulation of these drugs in or near mitochondria and consequently can control uncoupling. In this context, in the current study, we investigated the effect of diclofenac (DCF), as a representative of the NSAIDs with mitochondrial impairment effects, on membrane biophysical properties. The interaction and distribution of DCF into aqueous and membrane phases was evaluated by a liposome/water system where as the partition coefficient ( $K_p$ ) of DCF was determined by derivative spectroscopy. Hydrophobic fluorophores were used as membrane probes and steady-state, as well as, time-resolved fluorescence measurements have proved useful in monitoring the preferential location of DCF in membranes. Furthermore, the effects in symmetry and in the long and short-range organization of bilayers; in the molecular packing of the bilayers as well as in the chain conformation of the molecules upon the interaction of the bilayers with DCF have been investigated by small and wide angle X-ray scattering (SAXS and WAXS) with synchrotron radiation source.

Results gathered within the biophysical studies performed indicate that DCF is capable of interfering with membrane structure and dynamics, hence influencing membrane-mediated physiological processes.

## Acknowledgments

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# Evaluation of toxicological effects of NSAIDs on the prospects of membrane biophysics

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Non steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation and pain and their action is based on the inhibition of the cyclooxygenase (COX) activity [1]. NSAID have to cross several lipidic barriers to reach their target tissues and inhibit COX. Nowadays, it is known that the sequence of events resulting from COX inhibition does not totally explain the overall gastric toxicity of NSAIDs [2].

Indeed, their biological function is strongly influenced by their lipid affinity and their interaction with the membrane surface can induce changes in the biophysical properties of the membrane. Therefore, the determination of NSAID's partition coefficient ( $K_p$ ) and thermodynamic parameters inherent to the interaction of the indomethacin and nimesulide with the membrane are essential to understand their body distribution, their therapeutic and toxic effects, particularly at the gastrointestinal (GI) level.

The partition of NSAID was determined by calculating their  $K_p$  lipid/water using derivative spectroscopy that allow a better resolution of the overlapped bands and elimination of the light scattering interferences of the spectra. In order to mimic the interaction of indomethacin and nimesulide with the membrane, liposomes of dipalmitoylphosphatidicholine (DPPC) were prepared under physiological (pH 7.4 typical of plasma and cells) and pathological (pH 5.0 typical of inflamed tissues) conditions. The Van't Hoff equation relates the change in temperature ( $T$ ) to the change in the equilibrium constant ( $K$ ). Thus, it was used to determinate the membrane-water variation of enthalpy ( $\Delta H_{w \rightarrow m}$ ), entropy ( $\Delta S_{w \rightarrow m}$ ) and consequently the Gibbs free energy ( $\Delta G_{w \rightarrow m}$ ).

Results have shown that the initial biophysical organization of the membrane (gel or liquid crystalline phase), pH medium and chemical properties of the drug influence both partition and thermodynamic parameters. Furthermore, results indicate a correlation between  $K_p$  value and the efficacy of the drug as anti-inflammatory, namely at pH 5, which is the typical pH of the inflamed cells.

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# **Metalaxyl-exposure of *Solanum nigrum* L. plants effect on guaiacol peroxidase and glutathione-S-transferase activities.**

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Pollution is a major issue to the sustainability of our planet, being the use of pesticides one of the many causes for pollution. Therefore, new forms of effective and more environment friendly remediation techniques are needed. Phytoremediation consists on the use of plants that absorb and detoxify organic and non-organic xenobiotics, from soils, groundwater and sediments [1]. The plants used for this purpose need to have an efficient and active antioxidant system that allows them to be exposed to high concentrations of xenobiotics. For this, plants have several enzymatic mechanisms, such as the guaiacol peroxidase (GPX) and non-enzymatic mechanisms, such as glutathione (GSH) [1]. The GPX acts by transforming the H<sub>2</sub>O<sub>2</sub> into water, thus decreasing the deleterious effects of this highly reactive molecule, and glutathione-S-transferase (GST) acts by conjugating GSH to the organic pollutant, rendering it less toxic, which is then stored in vacuoles or in the apoplast [1]. In this work, *Solanum nigrum* L. plants were used as a model for the study of two possible mechanisms involved in the tolerance of the systemic fungicide metalaxyl: GPX, for its role in the removal of the H<sub>2</sub>O<sub>2</sub> in excess, and GST for its protective role in converting xenobiotics into less toxic compounds. For this purpose, plants grew exposed to 12.5 ppm of metalaxyl, while the control plants were fed with a commercial diluted nutrient solution for one month. Samples of these plants were frozen in liquid N<sub>2</sub> and GPX and GST activities, as well as soluble protein levels, were determined. The results obtained show that both enzymes are more active in the metalaxyl-exposed plants, with the highest activity being found in roots, suggesting that *S. nigrum* can resist in soils contaminated with metalaxyl by storing this organic xenobiotic in their tissues after its conjugation to GSH [1]. The results also suggest that, since these enzymes are more active in roots than in shoots, this organ will be the major processing site of the xenobiotic.

Acknowledgements: The authors gratefully acknowledge the Universidade do Porto for financial support (PhotoWeed), with the contribution of Santander Totta.

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# Fish otoliths as potential chronological biomarkers of environmental aquatic contamination by heavy metals

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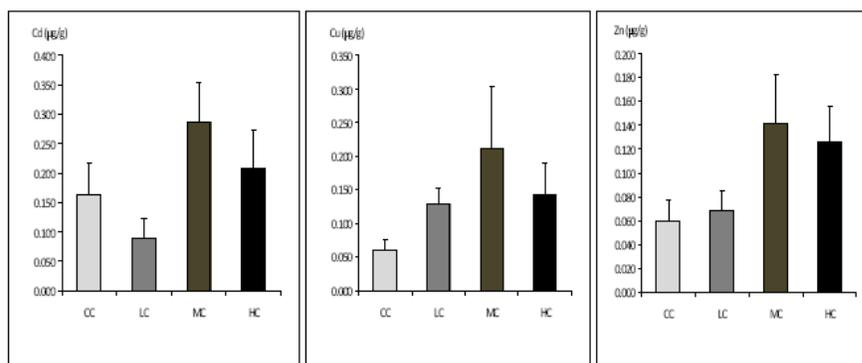
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The European eel, *Anguilla anguilla*, as a facultative catadromic species, presents a complex life cycle. This species habitat goes from salt to freshwater grounds, including also estuarine brackish areas. It usually crosses several habitats, potentially affected by anthropogenic activities. Human activities, like industry, harbour activity or mineral extraction, or nature itself, through particular geological formations, can contaminate aquatic systems with heavy metals. Additionally, the available studies about the potential use of the chronological properties of otoliths as indicator of aquatic contamination by metals are scarce.

In this study we used 180 juveniles (yellow eels) of the European eel (*A. anguilla*) to test the uptake of three important metals (zinc, cadmium and copper) through the water into the otoliths. Simultaneously the otolith growth during the chronic exposure (28 days) was estimated using a fluorescent otolith dye (tetracycline), important to choose the electron beam diameter (ICPMS-LA) used for otolith metal concentrations assessment.

Although our results suggest that the trace metal concentration in otoliths appear to increase from the lower to higher tested concentrations on water (Fig. 1), the obtained differences are not statistically significant. Furthermore there were no significant relationship between the otolith elemental and water concentrations for all the experiments. Our data indicates that water trace metal concentration has no significant effect on incorporation of Cu, Cd and Zn in otoliths of *A. Anguilla*. However these preliminary results should be interpreted with caution, since the obtained data were highly variable, and probably much of this variation is related to poor analytical precision at such low concentrations as consequence of a small ablation spot size.



**Figure 1.** Tested element concentrations in the eel otoliths after 28 days of exposure ( $\mu\text{g g}^{-1}$ ). Error bars represent SE. CC, Concentration Control group; LC, Low Concentration group; MC, Medium Concentration group; HC, High Concentration group

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**U.**PORTO

*Parallel Oral Sessions II*

**A3** *Psychology & Education Sciences I*

# “Brincando com os sons das letras“: Evaluation of a program of phonological awareness development in pre-school context

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The literacy development is a continuous and integrated process, with an early emergency, in what the child's natural environments play a key role. Recent studies have demonstrated that the phonological awareness is a component of emergent literacy with a major role in subsequent learning of reading and writing.

In the current study a program to promote awareness of rhyme was applied in a group of preschool children, with the purpose of developing phonological awareness skills. In the study participate forty nine 5 years old children attending two preschool classes, in a school of Porto. All children were assessed before and after the implementation of the intervention program, using a Battery of Phonological Tests (Silva, A., 2008) [1] and the Peabody Picture Vocabulary Test - Revised Edition (Dunn & Dunn, 1981) [2]. The program was implemented in 11 sessions of 25 minutes each, in one of the preschool classes (experimental group) in their daily routine, with the participation of the teacher.

There is a better performance of the children in the experimental group when compared the control group at the phonological tests, suggesting the effectiveness of the program. These results are supported by the existing literature and also by the positive evaluation of the teacher and children. However, the interpretation of these results must be undertaken with caution due to the small number of participants.

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# Creativity through time – evolutive retrospective of the concept

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In a society that is constantly changing, creativity may prove to be a critical tool for success. If we consider that, since childhood, the human psychological subject is confronted with the necessity of solving problems, being compelled to produce a continuous flow of original ideas, to deal with daily changes and constantly resorting to creative strategies for problem solving, one understands the pertinence that this concept may assume, as well as the influence that its profound understanding may exert, both at the individual and societal levels.

Although the study of creativity goes back to the beginning of the XX<sup>th</sup> century, with authors such as Vygotsky approaching the theme, only in recent years it has been assumed as a prominent subject in the scientific realm. For this progress greatly contributed the studies of Torrance, Mednick, Czsikszentmihalyi, Gardner and Sternberg, who became involved in the production of theoretical frameworks and in the construction of instruments that allow the assessment of a construct that holds as much of complexity as of multidimensionality. This diversity, although constituting one of the most challenging and enriching aspects of creativity, has caused a counterproductive effect at the scientific investigation level, given the difficulty of finding a widely accepted conception by the scientific community. Therefore, we can observe the production of heterogeneous assessment tools that evaluate creativity from manifold perspectives. Consequently we can find instruments that evaluate the process of creativity as divergent thinking (Almeida & Ribeiro, 1992; Guilford, 1958; Torrance, 1966), as the ability to find problems (Getzels & Czsikszentmihalyi, 1976; Sternberg, 1988), as a personality feature, interest and attitude (Covington, 1966; Urban & Gellen, 1995), as a creative product (Amabile, 1983; Archambault & Gubbin, 1980; Reis & Renzulli, 1991; Westberg, 1990), as a self-evaluatable aspect (Colangelo et al. 1992; O’Neil, Abedi & Spielberger, 1994; Richards et al., 1988), and also as a structural element of the lifelong cycle (Schaefer, 197; Amabile, 1989).

With this presentation we hope to draw an evolutive review of the concept and construct, mentioning the most significant contributions in the international panorama but also in the national scene, aiming to establish a sound theoretical base, which will allow a deeper exploration of creativity. Hence, we hope to encourage future research, highlighting the reflexes that a critical and profound understanding of creativity can produce in significant and diverse areas of human psychological functioning and society in general.

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# Family and Work: Coping Strategies and Parenthood Motivation

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Recently, our society has undergone innumerable social, economic, cultural and ideological changes. One of these major changes was women's entry in the labor market. If previously women's main role was in the family domain, with this event they started to have a more active participation in the public sphere. Women's multiple roles (e.g. spouse, mother, worker, and daughter) led to profound changes in the family structure and dynamic. Thus, nowadays, the majority of families are characterized by dual earner couples who postpone parenthood. The main goal of this work is to characterize the strategies used, by families with and without children, to reconcile family and work domains. A second aim is to analyze the motives for and against having children endorsed by these families.

*Sample:* Participants were individuals and couples belonging to dual-earner families. Thus both members of the couple should work at least 15 hours per week ( $n=387$ ). Forty-one percent were men and the majority of the sample was married (75%). Subjects were equally distributed among socioeconomic status and their ages ranged from 21 to 52 years old with a mean age of 33.8 ( $SD = 5.89$ ).

*Instrument:* To the purpose of this work two scales were used. One assessing the work-family (W-F) coping strategies comprised with 5 factors and another one assessing motives for and against having children composed by 4 factors.

*Results:* No significant differences were found concerning sex and the use of work-family coping strategies, neither on the motives for parenthood ( $p > .05$ ). Nevertheless there were differences regarding the parental status of the couple. Coping Strategies: Couples with children adopt more, than couples without children, strategies related to the endorsement of a positive perspective ( $F(1, 366)=6.89, p<.05$ ) and use of personal characteristics to cope with W-F arrangements ( $F(1, 373)=4.84, p<.05$ ). Couples without children favor strategies of partner emotional support ( $F(1, 373)=4.84, p<.05$ ). Parenthood Motivation: Couples with children ( $M=4.25, SD=1.08$ ) are more motivated to have another child than couples without children ( $M=3.83, SD=1.08$ ), highlighting reasons of emotional enrichment ( $F(1, 368)=12.89, p<.001$ ). On the contrary, couples without children highlight the interference in the current life style ( $F(1, 359)=28.76, p<.001$ ) as the main reasons not to have a child.

*Discussion:* It seems that adopting a positive perspective regarding W-F responsibilities and making use of individual traits and characteristics are the main strategies to ease the constraints made by the professional work, especially for couples with children. These families, who already have a child, are also more motivated to have another child. In fact, some studies have shown that the entry of a child in the couple life may produce strain to the relationship. In the case of couples who already have a child these challenges may have been overcome, predisposing them to invest again in another child.

*Conclusion:* The absence of gender differences points out that the reconciliation of work and family is essentially a couple and familial process. Regarding motives for parenthood, the results point out that the experience of parenthood is the best precursor for having another child.

*Acknowledgments:* Anne Marie Fontaine, Marisa Matias and Marina Mendonça.

# Intervention in phonological awareness skills in a group of children at risk: The program "Era uma vez... o mundo dos sons!"

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Promoting emergent literacy skills in pre-school children has proved to be a growing concern at the current picture of child education. Indeed, the early promotion of these skills has proved to increase the subsequent school success of children. Phonological awareness is a fundamental aspect of such skills, being a key component in the decoding process of reading. Children from socially at risk environments, tend to have worse performances in this kind of expertise, benefiting from an intensive and structured intervention at this specific skills level.

This study consisted on developing and implementing a program to promote phonological awareness skills, called "Era uma vez... o mundo dos sons!". The aim was to evaluate the effectiveness of this program in the skills development of children at risk, who have been identified as having more difficulties in terms of emergent literacy skills. Additionally, we also intended to test the suitability of the materials used.

The research study involved 21 children in a preschool classroom at Centro Social Infantil da Cruz de Pau, aged between 5 and 6 years, constituting an experimental group and two control groups. The program was implemented by the researcher over 18 bi-weekly sessions, to a group of eight children identified as having poor emergent literacy skills. All children were assessed on two occasions (pre and post-test), with the Bateria de Provas de Consciência Fonológica (Silva, 2008) [1], the Teste de Vocabulário por Imagens Peabody (TVIP, Dunn, 1986) [2] and the Prova de Leitura de Letras (in Processo de Descodificação da Leitura, Castro, Cary & Gomes, 1998) [3].

The results showed the effectiveness of the program on the development of such skills in the experimental group which showed a greater development in the results of the phonological awareness tests, compared to other groups. These results are corroborated by the existing literature and also by the positive program evaluation by children and teacher. However, the interpretation of these results must be undertaken with caution due to the small number of participants. In addition, it is important to consider that this type of intervention, that focuses on specific skills, should be embedded in preschool settings together with other diversified interventions at the level of emergent literacy.

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## Argumentative Skills in Higher Education – a comparative approach

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Project *Argumentar precisa-se!* was based in the idea that argumentative skills are crucial to the curricular structure of Higher Education. In fact, most of the work students do needs to be conveniently justified. This project intended to find how Curricular Units (UC) refer, demand or promote argumentative thinking and how students use it. Specifically, our objectives were: To analyze assignments produced by students from Faculty of Engineering and Faculty of Psychology and Education Science of the University of Porto, looking for traces of argumentative skills; to study argumentative skills' importance as a students assessment criteria; to identify the argumentative elements used by students in speech construction; to analyze logical patterns of argumentative thinking used by students; to identify utilized pragmatics' arguments; to analyze comparatively how students from different scientific fields produce their thinking/speech; to understand to what extent different assessment instruments influence how students structure and develop their arguments. We've adopted S. Toulmin's definition of argumentation[1] as the process of producing and justifying thesis using evidences. Also, we believe argumentation has a defined structure with various elements included in the two major theoretical lines of thought (Amoussy & Koren) [2].

In order to achieve our goals we first studied the UC programs from the two Faculties, above referred, to identify those which contain argumentative skills and thinking development as goals. Afterwards we collected assessment instruments from those UC (after responsible teachers' permission). We then analyzed qualitative and quantitatively the students work using adequate software, SPSS for quantitative analysis and NVivo for qualitative analysis. Finally we compared the analyzed data with the evaluation method used by teachers.

We concluded that the evaluation methods and assessment instruments influence how and if students use argumentative thinking and how it is elaborated and structured. Therefore, we can affirm that argumentation features depend on assessment conditions used. Also, we concluded that argumentation is, in the analyzed papers, synonymous of referring and adding arguments, so that the organizational and dialectic effort is yet very slight. We also concluded that deductive thinking is a more used thinking type, presented in both Social Sciences and Technical Sciences courses.

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# Mobbing: The Consequences of Empty Desk Syndrome

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*Mobbing* appears to be rooted in organizations around the world, is practiced in the workplace of the victim and entails consequences on their physical and psychological well-being, in particular, increased levels of psychological stress and mental fatigue (Agervold & Mikkelsen, 2004) [1]. Einarsen (1999) [2] illustrates the severity of the consequences declaring that *mobbing* appears to be more destructive to the victims than all of the occupational stress in the world. Thus, the study of the relationship between *burnout* and *mobbing*, understood as an extreme level of occupational stress, is presented as pressing in a scientific context where little or nothing is known of the real consequences of *mobbing*.

Through critical incident technique (Chell, 2004) [3] and content analysis (Sampieri et al., 2006) [4] of 6 cases of workers who were victims of such harassment, we explored the process of *mobbing* in particular, the negative behaviors perpetrated against the victims and the situation that led to course of *mobbing*.

The results show that the start of the climb seems to have a common denominator that lies in the position of the target in relation to proceedings conducted by the management, both ideological, as political level.

Despite observing a wide dispersal of negative behaviors identified by the victims, we can verify that the attacks on the social relations of the target are the most common type, specially emptying function and assign tasks to lower qualifications.

We can conclude that *mobbing* cause serious repercussions on the physical and psychological health of the target, which appear similar to the symptoms described in the literature as a result of *burnout*. In addition to these consequences, expressed in symptoms, the interviewed presents family, social, and economic consequences, resulting from the experience of this type of psychological harassment. Although no strategy proves to be effective in the extinguishment of the course of *mobbing*, we have found *coping* strategies most frequently used, and the "problem solving" strategy proves to be more effective in reducing the perceived consequences for the victims.

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**U.**PORTO

*Parallel Oral Sessions II*

**A4** *Engineering II*

## Agar based edible coatings applied to sliced apples

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Today's consumers are growingly interested in food products that are conveniently available and easy to prepare, preferably "ready-to-eat". At the same time, health concerns are also present, and fresh or minimally processed foods with low nutritional and organoleptic changes are desirable. Parameters such as appearance, texture, flavor, nutritional value and safety are determinant in the acceptability of these products by the consumer [1]. One example of this is 4<sup>th</sup> range fruits (ready-to-eat). However, this type of food has a short shelf life after being opened/sliced. The application of edible coatings can extend its shelf life, reducing changes in its functional and nutritional properties.

The main objective of this work is to obtain information about the behavior of agar coatings when applied to a real food system (sliced apples) and understands its applicability.

Freshly cutted apples were used. Two different coatings were made: the first one with agar and the second one with agar and citric acid (as antioxidant). For each experience, two different tests were made simultaneously: one by dipping the fresh pieces of apple in the agar solution (with or without an antioxidant), while the other (the control) was made by dipping the apple in distilled water using the same conditions as the first one. It was used an agar solution with concentration of 1,20% (w/w) at 41°C and a dipping time of 20 seconds. The evolution of relevant physical-chemical properties of the coated apples and control (texture, using a TA XT2 texturometer from TA Instruments; colour, using a Minolta Chroma Meter CR300 colorimeter measuring with the Lab scale; pH; total weight; moisture content) was monitored over 24 hours (a reasonable time for the shelf life of this kind of products). The differences in firmness between the coated apples and the control were not statistically significant. The weight loss was more significant in the control when compared to the coated apples. L and b were the relevant color parameters, and differences were also more pronounced in the control. The main results obtained shown that the fruit with the agar coating and antioxidant is less oxidized.

With the results obtained, it can be concluded that the presence of an edible coating is benefic to the improvement of shelf life, when compared with no coating, minimizing the weight loss and the oxidation caused by the contact of the fruit with air.

### Acknowledgments

Thanks are due to U.Porto /Santander Totta "Projectos Pluridisciplinares 2010".

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# Application of near infrared technique to urea-formaldehyde resin production

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Current concerns about the effects of formaldehyde on human health have led to constant changes in the synthesis [1] process of urea-formaldehyde (UF) resins. These new formulations are quite susceptible to possible operator error that can significantly influence the final product pretended. The main objective of this project was to implement chemometric techniques for off-line monitoring by near infrared (NIR) of the molar ratio F/U of resins produced by a new process developed by EuroResinas.

NIR measurements need calibration: relation between a spectral data set and known data for a set of samples. So, an extensive test campaign is needed, involving the synthesis and analysis of several resins produced under different conditions. In order to produce samples with different F/U molar ratios, resins were produced and later divided in  $n$  equal portions, to which previously calculated amounts of urea were added in order to achieve F/U molar ratios define previously.

Calibration models for F/U molar ratio were developed taking into account the most important spectral regions for these resins, individually or in combination (7502-6098 and 4601-4246  $\text{cm}^{-1}$ ) [2] and using different preprocessing methods. Take into account the best spectral range, is it important to analyse the statistical parameters of the different models, when the two spectral regions are used simultaneously, shows in Table 1. The preprocessing method that yields the lower values of SEP and SEC and high values of  $R^2_{\text{cal}}$  and  $R^2_{\text{val}}$  is the first derivative with SNV.

When the right spectral range is selected, it is possible to obtain interesting calibration models for UF resins, in this case 7502-6098 and 4601-4246  $\text{cm}^{-1}$ . When a spectral region is chosen, the use of different preprocessings results in different calibration models. The model with the best performance is the one which uses the first derivative with SNV as preprocessing (SEP and SEC equal to 0.00232 and 0.000966, respectively). When applied to the industrial production of UF resins, this technique yielded highly reproducible resins, with chemical properties in the range of interest for the company.

**Table 1** – Statistical parameters for the developed models to analysis the molar ratio of UF resin

Preprocessing method	Validation model		Calibration model	
	$r^2$	SEC	$r^2$	SEP
First derivative	99.96	0.00126	96.54	0.0319
MSC	99.98	0.000815	92.23	0.0183
SNV	99.99	0.00074	95.65	0.0137
First derivative with MSC	99.94	0.00062	97.97	0.00938
First derivative with SNV	99.98	0.000966	99.88	0.00232

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# Elimination of the Pesticide Chlorfenvinphos from Contaminated Drinking Water by Fenton and photo-Fenton Oxidation

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Chlorfenvinphos, 2-chloro-1(2,4-dichlorophenyl)vinyl diethyl phosphate, Figure 1, is an organophosphorus insecticide used in agriculture, and commonly found in water supplies.

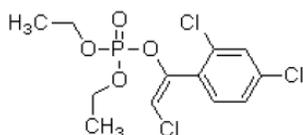


Figure 1: Chemical structure of Chlorfenvinphos.

Due to the fact of being lethal to humans, when present in large amounts, and because of its high solubility in water (145 mg/L), chlorfenvinphos can become a threat public health. Therefore, it is necessary to develop a method of degradation of this pesticide in water matrices.

Fenton's reaction can be briefly described as the reaction between an iron salt and hydrogen peroxide (1), producing hydroxyl radicals species,  $\cdot OH$ , with a high oxidation power ( $E_0 = 2.80$  V).



Hydroxyl radicals react with organic compounds present in water, even at residual concentrations, in a non-selective way. This occurs via a complex mechanism of parallel and consecutive reactions, producing intermediate compounds and ultimately leading to total mineralization (up to formation of CO<sub>2</sub> and water).

Oxidation experiments were performed in a batch reactor, with temperature control. Along the reaction, samples were taken for further analysis using HPLC-UV, to measure the chlorfenvinphos degradation, and using a TOC (Total Organic Carbon) analyzer, to study the degree of mineralization.

Chlorfenvinphos degradation was studied at different conditions: initial concentration of oxidant (H<sub>2</sub>O<sub>2</sub>) and catalyst (Fe<sup>2+</sup>), temperature and initial pH.

Complete degradation of chlorfenvinphos, in a short period of time, is possible to be reached, using moderate conditions. A mineralization of only 30% was achieved after 3 hours. Preliminary experiments in the presence of light (photo-Fenton process) showed however a much faster reaction rate (ca. up to 95% removal of chlorfenvinphos in only 10 min) and much better mineralization degrees (up to 85% in 4 hours).

#### Acknowledgments:

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# LCA Tool for Evaluating the Production of Lyophilized Products via Recombinant Biotechnology

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This work aims to develop a LCA tool that can be used by pharmaceutical companies to analyze their processes by performing an input-output analysis, for evaluating their potential environmental impacts (PEI) and also for identifying opportunities for improvement. The LCA tool was developed using as case study the manufacture of lyophilized products via recombinant biotechnology. Thus it identifies, describes and presents a detailed description of the processes involved in the present case.

This work also includes an overview and short analysis of other tools that have been developed or existing studies assessing the environmental impact of pharmaceutical processes that might be relevant for the present case study [1-3]. In this regard some of the existing software tools to perform LCA studies and evaluate the PEI of products and/or processes, or to assess process potential risks and hazards to the environmental, health and safety (EHS) was listed.

Concerning pharmaceutical manufacturing companies, there is an increasing pressure to ensure that information and data about their processes are accurate and reproducible. Therefore, this work presents a detailed description of the production processes of lyophilized products via recombinant biotechnology, obtained from open literature. This description is an important step to understand the production processes in order to allow for the identification of the relevant inputs and outputs of materials and energy involved in each manufacturing process. For each stage of the primary and secondary processing, i.e. of the Active Pharmaceutical Ingredient (API) synthesis and of the lyophilized product manufacture, respectively, some of the main inputs and outputs were identified, which have been further used in the LCA-tool development. Some inventory data obtained from literature and from a Spanish pharmaceutical company was later used to calculate the most relevant PEI.

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# Optimization of nickel biosorption on surface modified algae

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Biosorption process has been recognized as one of the economical and efficient methods for the remediation of metal bearing wastewater [1]. In the present work, *Pelvetia canaliculata*, commonly found brown algae in the north coast of Portugal [2], has been examined for its sorption potential for nickel. Various forms of algae were prepared after different chemical modifications in order to know the best form of algae for the maximum uptake of nickel. Protonated form of algae was prepared by stirring it with 0.2 M HNO<sub>3</sub>, while different ionic forms e.g. Ca-, Mg-, K- and Na- forms (with or without protonation) were prepared by treating the algae with corresponding metal chloride salts.

The kinetics of the process was studied in 1 L capacity perfectly mixed sorbers, operating in batch mode. 0.5 g of algae prepared by different methods was added to 1 L of the nickel solution (50 mg/L, pH ~ 4). 10 mL aliquots were collected at different time intervals. Samples were analyzed for residual metal concentration by atomic absorption spectrometry (AAS). The prepared biosorbents exhibited an initial rapid uptake of nickel followed by a slower removal rate that gradually reached to an equilibrium condition. The equilibrium was achieved within 2 h. Among the various forms of prepared algae, Na-algae prepared directly from virgin algae (without protonation) showed highest uptake value of nickel (~ 44 mg/g). Further research is under progress at LSRE, FEUP.

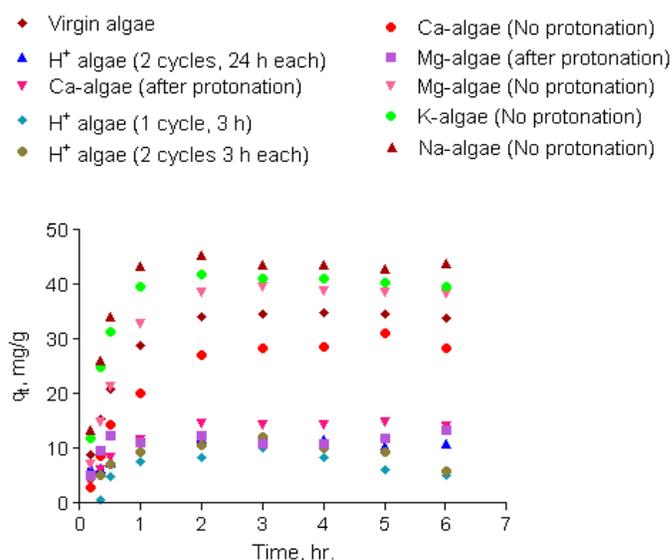


Figure-1: Biosorption kinetic studies with nickel on various forms of algae.

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## Paper consumption at six higher education schools

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This work includes an environmental assessment focusing on paper consumption used to photocopy and print in six higher education schools at Porto. In focus are three institutions from the University of Porto (UP), namely, the Faculty of Sports (FADEUP), the Faculty of Engineering (FEUP) and the Faculty of Humanities (FLUP). Two institutions of the Polytechnic Institute of Porto (IPP), namely the Accounting and Administration school (ISCAP) and the school for Education (ESE). The Nursing school (ESEP) is also here included.

It was gathered and analysed information from all the above-mentioned institutions from 2008 and 2009. The information includes the number of students, teachers, institution's employees and also the amount of paper sheets used in photocopies and printing facilities. The paper grammage used was not disregarded. For each institution it was calculated an indicator expressing the annual amount of paper sheets consumed per person.

The results allow drawing conclusions associated to the changes in consumption for the two years analysed and for each institution. In addition, the largest and the smallest consumer (total consumption and consumption per person) were identified.

It was observed that the consumption rises, by comparison to all other months, at the beginning of the academic year (September) and at the beginning of the examination periods (January and June). It was also observed that the total paper consumption increased in 2009 for the analysed institutions, namely, FEUP (increase of 19 %), FADEUP (increase of 11 %), FLUP (increase of 9 %) and ESEP (increase of 3 %). The exceptions are calculated for ESE (calculated a reduction of 13 %) and ISCAP that showed a reduction of 5 % in the total paper consumption. The Faculty of Engineering (FEUP) is, by comparison, the largest consumer of paper sheets. During 2008, it was consumed 5,5 million paper sheets and in 2009, the consumption raised to 7 million sheets. The Nursing school (ESEP) has the lowest consumption, consuming only about 200 thousand copies during the two years.

In conclusion and regarding the paper consumption per person, the Nursing school (ESEP) is the institution presenting the smallest variation for the two years. Moreover, ESEP has the smallest environmental performance indicator, i.e., each person consumed around 120 paper sheets per year. In contrast, the Faculty of Humanities (FLUP) is the institution presenting the largest consumption, i.e., each person consumed more than 900 paper sheets per year.

Final remarks:

The work here presented results from an assignment performed for the curricular unit *Environmental Decision Support Tools* (2009/2010) of the Integrated Master Program in Environmental Engineering at the Faculty of Engineering, University of Porto.





**U.PORTO**

## *Parallel Oral Sessions II*

**A5** *Applied Physics*

# Nanowires in Optical Fibers

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The main objective of this work is to explore nanowires based on suspended-core optical fibers for the development of new optical devices with nanometric dimensions. As for applications, the intention is to produce optical sensors for the detection of environmental parameters such as temperature, refractive index or gas sensing.

Nanowires in optical fiber can be produced using several chemical or physical techniques. The nanowire is made by stretching a heated fiber, forming a structure comprising a narrow filament. Only in 2003 was it possible to reach sub-micrometric dimensions through the optimization of technological equipment [1]. This fabrication provides longer, more uniform and robust nanowires with transversal dimensions between 100 and 600 nm. Due to residual surface roughness associated with the high homogeneity that nanowires in optical fibers exhibit, optical losses are low, allowing the use of nanowires in a wide range of new applications for communications, sensors, lasers, biology, and chemistry. The nanowires exhibit excellent optical and mechanical properties, including a substantial evanescent field, high nonlinearity, strong confinement and low loss coupling between optical fibers. The nanowires are manufactured in an adiabatic way, preserving the original dimensions of the optical fiber at the entrance and at the exit, allowing easy splices with other optical devices [2].

However, nanowires need some external protection. One possible solution is to protect them in silica tubes or manufacturing them inside a suspended-core fiber (Fig. 1). The suspended core fibers appeared in 2001 and are a particular class of fibers where the microstructured core with small diameter ( $\sim 1$  micrometer) is surrounded by large alveoli, and is fixed to the cladding by very thin membranes of silica, so the observation of the fiber cross section gives the idea that the core is suspended [3]. Recently, it was possible to design suspended twin or multi-core fibers. These fibers have special physical properties, such as high birefringence and intermodal interference making them very interesting to manufacture fiber optic interferometers.

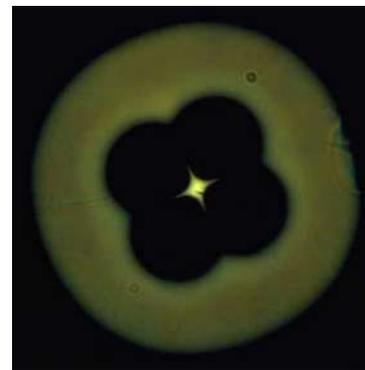


Fig. 2 - Example of a suspended-core optical fibre

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# Strain Sensor based on Four-Wave Mixing using Raman Fiber Bragg Grating Laser Sensor with Cooperative Rayleigh Scattering

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Fiber Bragg grating (FBG) sensors can be used as sensing head for the measurement of strain, temperature or others physical parameters [1]. The variation of these parameters induces changes of the central Bragg wavelength, which can also be converted in optical power variation through a linear filter [1]. The Rayleigh scattering growth can be a problem for specific applications namely in optical communications but can be used as a distributed mirror to enhance the generation of Brillouin Stokes combs [2], multiwavelength generation [3] and distributed lasers [4]. The Four-Wave Mixing (FWM) for wavelength conversion has advantages that include large spectral and dynamic range as well as providing strict bit rate and modulation format transparency. In optical sensing, this effect is underexplored. Only in 2007 was demonstrated a fiber ring laser sensor for strain-temperature discrimination [5]. In this work, the authors present two possibilities for an interrogation system based on FWM effect obtained by two Raman fiber Bragg grating laser sensors with cooperative Rayleigh scattering. Due to the transfer function of the FWM efficiency, it is possible to obtain a temperature-independent strain sensor when the power of the converted signal is used. The second possibility is to use the difference between the signal sensor wavelength and the converted signal wavelength to obtain a temperature-independent strain sensor [6].

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# Tomographic Optical Extinction Coefficient Measurement

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Optical Coherence Tomography (OCT) is a high spatial resolution imaging technique based on White Light Interferometry [1], established in the 1990s. It uses low-coherence optical sources to attain imaging depth selectivity, generating very narrow and well resolved optical sections of the imaged object. Being a non-invasive technique, it allows for depth imaging of biological samples in vivo and in real time [1]. There are a number of applications for this technique, the most evident being medical imaging. One can also use the depth scanning capabilities of OCT to characterize structural integrity of any kind of material, which is always an important task in industry-related applications.

In this work the development of devoted software to compute micrometric sized voxel resolved extinction coefficients of OCT three dimensional data is presented. It is well known from the Beer-Lambert's law that radiation propagating through a medium with absorption and scattering will experience a reduction in its amplitude as  $\propto e^{-\mu z}$ , where  $\mu$  is the extinction coefficient [2]. By taking the natural logarithm of the data and iteratively performing linear fits over it, we are able to determine  $\mu$  for a given region of the sample.

An algorithm was implemented in a LabVIEW<sup>TM</sup> environment, whose mode of operation is illustrated in Figure 1. The program iterates over the entire original data matrix and computes a matrix with the spatially resolved extinction coefficients.

This new information allows gaining some insight over the internal structures of materials and biological tissues, and will allow segmenting the OCT volume into regions of space sharing common extinction coefficients, and thus identifying structures or layers in the samples. The algorithm was tested on 3D OCT data acquired by imaging wood, cork, micro-machined and laser processed light waveguides samples.

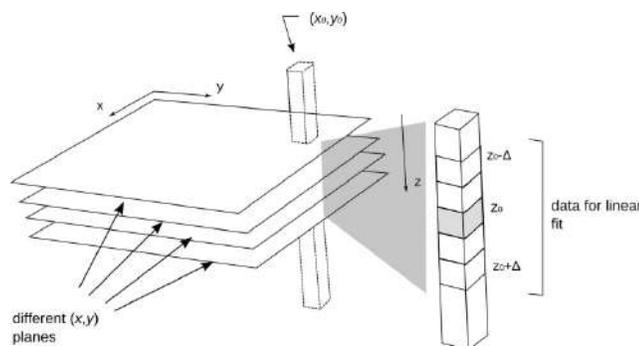


Figure 1 – Representation of the algorithm developed.

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# Magnetocaloric Effect Based Micro-Refrigeration

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Current integrated circuit (IC) technology has reached an extremely high density of nanometric-sized components, all of which require efficient refrigeration to maintain functionality. The most common solution for this problem is a simple heat sink in contact with the IC to provide a larger contact area with the environment and a fan to renew the air and thus keep a temperature difference between the sink and the air around it.

Here, we alternatively present two devices that use materials with a large magnetocaloric effect (MCE) as a way to actively reduce the IC temperature. The MCE is a refrigeration method [1-2] in which a large temperature variation is achieved upon the application/removal of magnetic fields (H) on materials such as  $Gd_5Si_2Ge_2$ ,  $La(Fe_{0.88}Si_{0.12})_{13}$  or  $La_{0.66}Sr_{0.33}MnO_2$  [1], i.e. the temperature of these materials increases when H is applied and decreases when it is removed.

Our first device uses a fluid in microchannels to dispose of the heat in an external radiator. We found water to be the best cooling fluid in most situations but, if corrosion is not a technical issue, the use of gallium increases the cooling power of a device by one order of magnitude. The second device is a solid state device and uses yet another effect the application of H can induce on materials to further increase heat transfer efficiency: the variation of heat conductivity of some materials (e.g.  $LaMnO_3$  [3]). Our simulations suggest that these devices can achieve a temperature difference of approximately 12,5K between the IC and the environment (Fig. 1).

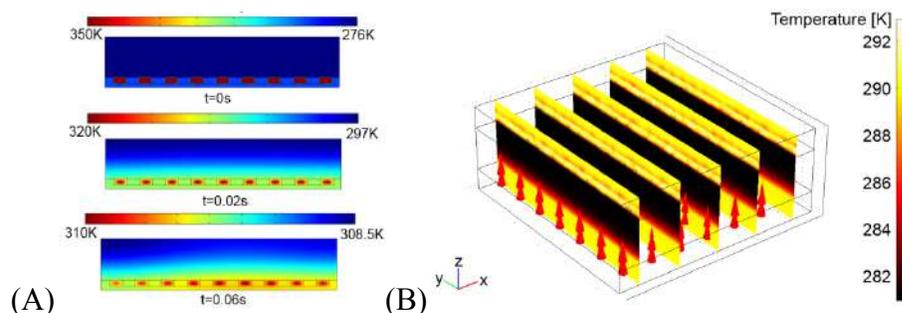


Fig. 1 – (A) Numerical simulation of heat transfer in a magnetic refrigeration device at different stages of fluid cooling and (B) temperature gradient in a solid state device after H is applied.

Acknowledgements: Funding through “Projectos Pluri-disciplinares da Universidade do Porto” (Micro-Cooling units using Magnetocaloric materials) is acknowledged.

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## Growth control of TiO<sub>2</sub> nanotubes for dye-sensitized solar cell

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The Dye-sensitized solar cell (DSCs) provides a technically and economically credible alternative to present day based on p-n junction photovoltaic devices [1]. In a DSC the light is absorbed by a sensitizer which is anchored to the surface of a wide band semiconductor that acts as the charge carrier, in contrast to the conventional silicon systems where the semiconductor assumes both tasks. At the interface sensitizer/semiconductor occurs photo-induced electron injection from the dye into the conduction band of the semiconductor. These electrons are transported to the charge collector. The use of sensitizers having a broad absorption band in conjunction with oxide films of nanostructured morphology permits to harvest a large fraction of sunlight. Nanostructured semiconductors increase the contact area with the sensitizer leading to an increase of efficiency. Titanium dioxide (TiO<sub>2</sub>) nanotube arrays obtained by potentiostatic anodization due to their high-aspect-ratio morphology have been widely used for applications in DSCs.

In this work, we studied the potentiostatic anodization of Ti foils to obtain TiO<sub>2</sub> nanotubes [2]. We report the conditions which the nanotubes layer detaches from the Ti foil, this property enables its easier use in DSCs. Moreover we also verified that it provides a mean of obtaining highly ordered TiO<sub>2</sub> nanotubes arrays by performing of a second anodization of the Titanium foil. Furthermore, we also present the calibration of the TiO<sub>2</sub> nanotubes growth rate by anodization. The growth rate decrease with anodization time was observed, due to the loss of the electrolyte properties and electrochemical dissolution on the top of the tubes. However, the growth rate as a function of the charge to anodize the Ti foil shows a linear trend as a function of the nanotube length, that was used to calibrate the growth of the tubes during the anodization process. TiO<sub>2</sub> nanotubes with different tube diameter were also obtained changing the anodization potential.

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# Synthesis of $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> nanowires arrays by pulsed electrodeposition of Fe in alumina templates

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Developments in nanotechnology made the fabrication of nanostructures possible by disposing a wide variety of techniques and methods that allows a very precise control over the morphology of such structures at the nanoscale. In the past years, high aspect ratio nanoparticles, such as nanowires (NWs), have become increasingly important in a wide range of applications, from electronic devices, solar energy and even bio-medical applications, for their unique electrical, thermoelectrical, optical, magnetic and chemical properties. In particular, beyond its metallic properties, Fe NWs fabrication rouse interest for its oxide application in photoelectrochemical cells as a photoanode with improved properties. Photoelectrochemical cells for splitting of water into H<sub>2</sub> are one of the most promising ways for converting solar energy into chemical energy [1-3].

Many different approaches can be considered to fabricate NW arrays. Nanoporous alumina template (NATs) assisted method is extensively used due to their several unique structural properties. This technique is very versatile since the pore size, pore density and height can be easy and readily controlled by the electrolyte type, anodising temperature, voltage and time. Electrodeposition is a well controllable method to fabricate uniform and metallic or semiconducting NWs by filling alumina templates. Particularly, the pulsed electrodeposition (PED) is a simple, inexpensive and industrial applicable method for high aspect ratio nanostructures deposition.

In this work we present an optimization process of PED for Fe NWs. The Fe Electrodeposition was performed on an alumina template, after a two-step anodization for higher pore organization and dendrites formation to reduce the alumina barrier layer in the pore bottom. Aiming at the maximum fill ratio of alumina template with Fe NWs, the deposition temperature, current density and electrolyte molar concentration were optimized. We report a successful approach to the fabrication of highly ordered Fe NWs with lengths ranging from 1 to 10  $\mu$ m and with a degree of pore filling of 99%. The Fe NWs were then thermally oxidized in an O<sub>2</sub> atmosphere to convert it to  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>. All of the samples were structural, morphological and magnetically characterized using Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDS), X-Ray Diffraction (XRD) and Superconducting Quantum Interference Device (SQUID) measurements.

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**U. PORTO**

## *Parallel Oral Sessions III*

**A1** *Biological, Environmental & Health Sciences V*

# Salinity effect on organic sulfur compounds decomposition and on the denitrification pathway in estuarine sediments

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Dimethylsulfoniumpropionate (DMSP) and methionine are major precursors of the volatile organic sulfur compounds (VOSC) dimethyl sulphide (DMS) and methanethiol (MeSH). In addition, previous studies demonstrated that DMSP and methionine degradation products interfere with the nitrous oxide (N<sub>2</sub>O) reductase step of denitrification [1,2], potentially leading to the accumulation of potent greenhouse gases in the atmosphere. Here we investigated the regulatory effect of salinity on the DMS and MeSH production during microbial DMSP and methionine decomposition and its subsequent interaction with the last step of the denitrification process. This was achieved by monitoring DMS, MeSH and N<sub>2</sub>O accumulation in sediment slurries from a temperate estuary (Ave, NW Portugal), after additions of DMSP (0 – 1.5 mM) and methionine (0 – 1.5 mM) at different salinities (0, 15 and 30 ppm). Results showed a clear interference of salinity on DMS and MeSH accumulation during DMSP and methionine degradation. The N<sub>2</sub>O accumulation during the denitrification process also increased at the lowest salinity (0 ppm) in treatments with DMSP, and at higher salinities (15, 30 ppm), in treatments amended with methionine. Overall, results suggested that salinity may have an important regulatory role in previously identified interactions [1,2] between the marine biogeochemical nitrogen and organic sulfur cycles.

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# Molecular detection and impact of multiple-sired litters in the domestic pig

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The domestic pig (*Sus scrofa*), is among the most economically important species in Europe and East Asian regions. During the past years, the advances of scientific research in the domestic pig resulted in more efficient and better animal welfare and farming practices, though they are only valid for intensive production systems of commercial and highly selected breeds. Many important pieces of knowledge are lacking; such as whether it is possible that domestic sows use polyandry (a mating system that often results in multiple-sired litters) as a reproductive strategy, one that can be neglected in intensive pig farming as artificial insemination is generally used, but in more extensive farming systems, such as the Iberian pig production, there is a large probability of polyandry as both males and females live most of their lives in the fields. The detection and quantification of this phenomenon is of extreme importance, as misidentification of the pedigree can wrongly influence the estimates of genetic parameters and breeding values for those animals [1].

In this study, we conduct paternity testing in the *Alentejano* Pig, which is raised in traditional extensive woodland farming system, to verify whether we can detect multiple-sired litters by genotyping autosomal DNA markers (*i.e.* microsatellites) [2, 3]. In domestic pigs, each group of mother/piglets and the putative sires will be genotyped for the same set of markers to detect the occurrence of more than one father per litter. We expect to analyze over 70 independent family groups (mother/piglets and putative sires) from the region of Alentejo. The sows were selected based on their age/number of pregnancies to test for age/pregnancy number associations and the sires by age intervals. Data obtained will be analyzed to verify whether multiple-sired litters occur, and if they occur how often and in what patterns. For this we will estimate (1) the percentage and the impact of multi-sired litter events per population of the domestic pigs (2) the degree of association between multiple sired litters and the age/number of pregnancies of the mother (3) mean number of different sires per litter and (4) sexual ratio in the multiple-sired litter and comparisons with single-sired litter. This study will advance scientific knowledge by understanding (1) the social behavior of wild pig and its family structure, and (2) it will have immediate practical application to improve pig breeding by taking into account the potential bias in the genetic value estimates and response to selection.

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# Synthesis and Physicochemical Properties of Xanthone Derivatives with Potential Antitumor Activity

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Xanthenes are a group of *O*-heterocyclic compounds which are well known for their important biological activities. Among a myriad of biological activities described for xanthenes, the *in vitro* growth inhibitory activity on tumor cell lines, especially for prenylated derivatives, appears to be quite remarkable [1].

Our group has been focusing on the synthesis and biological activities of a collection of open-chain and fused prenylated xanthone derivatives. From our library a compound (XP13) has emerged with promising antitumor activity [2]. In order to obtain new compounds with balanced activity and pharmacokinetic properties, seven XP13 analogues (Fig. 1) were synthesized and their lipophilicity evaluated using biomembrane-based models (micelles and liposomes). This methodology is more accurate than the classical *n*-octanol/water model since it takes into account not only the hydrophobic but also the electrostatic interactions [3].

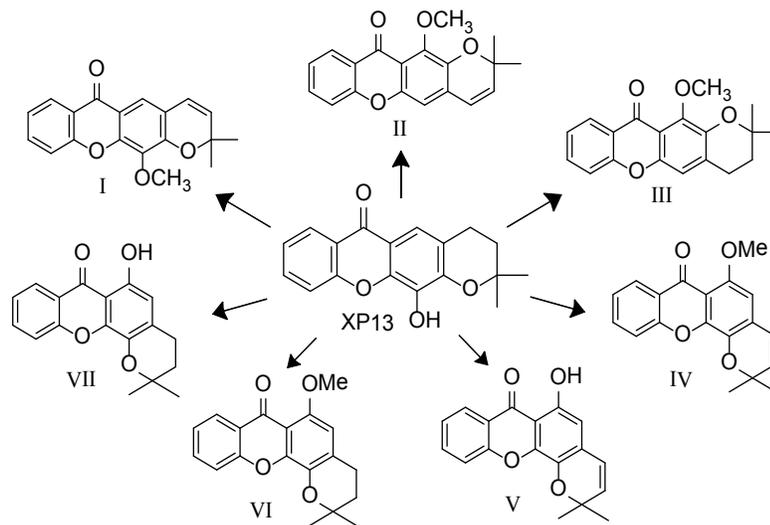


Fig. 1 -Analogues of XP13 synthesized

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# Efficient Method for Evaluation of the Enantiomeric Purity of Chiral Xanthone Derivatives

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The increasing demand for the production of enantiomerically pure biologically active compounds is of great importance in Medicinal Chemistry [1]. In this context, High Performance Liquid Chromatography (HPLC) with chiral stationary phases (CSP) has become a powerful and essential tool for the resolution of racemates and evaluation of the enantiomeric purity [2]. In our group HPLC methods using carbamate chiral stationary phases of polysaccharide derivatives already demonstrated to be efficient for resolution of xanthonolignoids [3]. This work reports the evaluation of the enantiomeric purity of a small library of chiral xanthone derivatives (CXD), using four polysaccharide based CSP, under multimodal elution. These CSP consist of cellulose *tris*-3,5-dimethylphenyl carbamate (CSP1), amylose *tris*-3,5-dimethylphenyl carbamate (CSP2), amylose *tris*-3,5-dimethoxyphenyl carbamate (CSP3) and amylose *tris*-(S)-1-phenylethylcarbamate (CSP4) coated onto APS-Nucleosil. CSP2 demonstrated to be the most efficient, showing excellent enantioselectivity and resolution for five xanthonic racemates (Table 1). The chiral HPLC method was successfully employed for determination of the enantiomeric purity of the CXD.

Table 1: The optimized chromatographic conditions that allowed the determination of the enantiomeric purity (Column CSP2; flow rate of 0.5mL.min<sup>-1</sup>, UV detection at 254 nm).

CXD	Mobile Phase (V:V)	$k_1$	$\alpha$	$R_s$	e.e.
1	EtOH:ACN (5:5)	0.8	5.2	6.89	> 99%
2	EtOH:ACN (5:5)	0.6	5.2	6.36	> 99%
3	EtOH:ACN (5:5)	0.8	4.4	7.76	> 99%
4	Hex:2-PrOH (5:5)	4.2	1.5	2.80	> 99%
5	EtOH:ACN (5:5)	0.7	5.2	6.89	> 97%

Hex = hexane, EtOH = ethanol, ACN = acetonitrile, 2-PrOH = 2-propanol; e.e. = enantiomeric excess

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# Studies on the anticarcinogenic effect of clotrimazole at the intestinal epithelial level: investigation of the putative effect in metabolic substrate cellular uptake

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Colorectal cancer (CRC) is one of the most common solid tumours worldwide. Butyrate (BT) is one of the main end products of anaerobic bacterial fermentation of dietary fibre in the human colon and is an important metabolic substrate in normal colonic epithelial cells. BT becomes less essential for growth of neoplastic cells, which show an increase in the rate of glucose uptake and glycolysis, producing excessive lactic acid [1, 2]. Clotrimazole (CTZ) is an antifungal drug that has demonstrated anticancer activity by inducing detachment of some glycolytic enzymes from cytoskeleton, thus inhibiting glycolysis [1]. The aim of this study was to investigate: (a) the anticarcinogenic effect of CTZ on tumoral and non-tumoral intestinal epithelial cell lines (Caco-2 and IEC-6 cells, respectively), by investigating its effect upon cell proliferation, viability and differentiation, alone or in conjunction with an inhibitor of mitochondrial oxidative phosphorylation (rhodamine123), and (b) the possibility of inhibition of the apical uptake of glucose or BT as a mechanism contributing to the anticarcinogenic effect of CTZ in Caco-2 cells.

In Caco-2 cells, CTZ showed anticarcinogenic activity, decreasing cellular viability and proliferation, and increasing cell differentiation. The effect of CTZ upon cell proliferation and viability was greatly potentiated in the presence of rhodamine123. In IEC-6 cells, CTZ also decreased cellular viability and proliferation, but increased cellular DNA synthesis rate and had no effect on cell differentiation. Exposure of Caco-2 cells to CTZ (10  $\mu$ M) for 1 and 7 days increased (by 20-30%) the uptake of the glucose analogs <sup>3</sup>H-deoxyglucose (<sup>3</sup>H-DG) and <sup>3</sup>H-O-methylglucose (<sup>3</sup>H-OMG), respectively, but had no effect on the uptake of <sup>14</sup>C-BT. The effect of CTZ upon <sup>3</sup>H-DG and <sup>3</sup>H-OMG uptake showed concentration-dependency and was maximal at 10  $\mu$ M. CTZ did not alter the pharmacological characteristics of <sup>3</sup>H-DG and <sup>3</sup>H-OMG transport, but produced significant changes at the level of mRNA expression (evaluated by qRT-PCR) of facilitative glucose transporter 2 (GLUT2) and Na<sup>+</sup>-dependent glucose co-transporter (SGLT1).

In conclusion, CTZ exhibits anticarcinogenic effect both in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines. In Caco-2 cells, the anticarcinogenic effect of CTZ was strongly potentiated in the presence of rhodamine123. Moreover, stimulation of glucose membrane uptake might be a compensation mechanism in response to the inhibition of glycolysis induced by CTZ.

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# The primary bile salt chenodeoxycholic acid inhibits butyrate uptake in a non-tumoral intestinal epithelial cell line

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Colorectal cancer (CRC) is one of the most common solid tumours worldwide. Although the causes of CRC are multifactorial, a diet high in dietary fibre and low in saturated fats is associated with a reduced risk of CRC [1]. Butyrate (BT) is one of the main end products of anaerobic bacterial fermentation of dietary fibre in the human colon and is known to play a key role in colonic epithelium homeostasis, being able to prevent/inhibit colon carcinogenesis [2]. BT is transported into colonic epithelial cells by two specific carrier-mediated transport systems, the monocarboxylate transporter 1 (MCT1) and the Na<sup>+</sup>-coupled monocarboxylate transporter 1 (SMCT1), both being proposed to function as tumour suppressors. Epidemiological and experimental studies (both *in vitro* and *in vivo*) suggest that fecal bile acids may play a role in the etiology of CRC [3]. A significantly higher fecal concentration of the primary bile salt chenodeoxycholic acid (CDCA) was seen in patients with CRC/adenoma [3], and CDCA was also shown to be tumour promoting in animal studies [4].

So, our aim was characterize the effect of CDCA upon <sup>14</sup>C-BT uptake in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines. Comparison between the effect of CDCA in Caco-2 and IEC-6 cells seemed interesting in the context of a possible distinct effect in these two cell lines.

A 2-day exposure to CDCA did not affect <sup>14</sup>C-BT uptake in Caco-2 cells. In contrast, it markedly inhibited <sup>14</sup>C-BT uptake by IEC-6 cells (to a maximum of 18% of control (150 μM CDCA)). The effect of CDCA was concentration-dependent (IC<sub>50</sub>=104.2 (96.4-112.5) μM), and quantitatively similar from 1 to 7 days of exposure. CDCA acted as a competitive inhibitor of <sup>14</sup>C-BT uptake, as it significantly increased the Km, while having no effect on the Vmax. CDCA up to 100 μM caused no significant effect upon cell proliferation (SRB assay) and viability (MTT and LDH assays); however, at 200 μM, it significantly decreased cell proliferation and viability.

In conclusion, CDCA is an effective inhibitor of <sup>14</sup>C-BT uptake in non-tumoral (IEC-6) but not in tumoral (Caco-2) intestinal epithelial cells. Given the role played by BT in the intestine, this mechanism may contribute to the pro-carcinogenic effect of CDCA at this level.

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**U.**PORTO

## *Parallel Oral Sessions III*

**A2** *Biological, Environmental & Health Sciences VI*

# Quality Evaluation of Meals served at a Portuguese Hospital

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**Introduction:** Hospital Food Service must provide food and nutritional support to patients, supplying balanced meals, nutritional counselling and food education.

**Objective:** The purpose of this study was to characterize sensory and safety and adequacy of meals served at a Hospital Center in the north of Portugal which has a private catering company responsible for the Food Service.

**Methodology:** A total of 40 meals of regular Diet were evaluated, 10 lunches and 10 dinners in each ward. To collect the information a Check List was drawn. Time of meals' arrival and beginning were registered.

**Results:** Food Mealtime has never been respected; soup and desert were the primary components which were not according to the menu; transport inadequacy was due to lack of isolation; temperatures were often inadequate; capitations were inadequate in most of the meals evaluated; the main source of protein was red meat; carbohydrate was rice; the supply of legumes and vegetables was very low; the major cooking methods were stewed and fried; the supply of cooked fruit was excessive and the variety of fruit was reduced; meals presentation was satisfactory and acceptance of meals was considered good in general.

**Conclusions:** The Food Service of this Hospital Centre needs substantially improvements, since the number of non conformities found was significant. The concession of the Food Service to a specialized company with no legal contract and without a regular monitoring has negative implications on the quality of the service. Special concern with meals' presentation is necessary since it greatly influences the acceptance. Periodic evaluations developed by a nutritionist or a specialized technician focusing the degree of satisfaction with meals and service, are necessary to maximize meals acceptance, contributing for a better nutritional status.

Key Words

Food Service, qualitative evaluation of meals, acceptance

# Consumer's food preferences at a Hospital food service unit

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**Introduction:** All successful companies have a common target: consumer's expectations. Satisfaction generates consumer's loyalty and this must be the first goal of a company.

The profile of consumers of food service units in Portugal is not well known. This knowledge is important to identify food preferences in order to adequate food options and menus, enabling catering companies to attract more clients. Considering the number of people using food service units these have been achieving increased role on Public Health. In Portugal the two main catering companies distribute approximately 250000 meals daily.

**Objective:** The purpose of this pilot study was to know consumer's food preferences at a food service unit of Oporto Hospital Centre.

**Methods:** A questionnaire was developed including 36 close ended questions. The preliminary version of the questionnaire was applied to several employees followed by interview, in order to refine its format. Data collection was applied on June 2010. Besides socio demographic characterization, the main variables evaluated, were preferences by food groups, cooking methods and ways of food presentation. A convenience sample was used and it was composed by individuals that volunteered to answer the questionnaire available at the canteen. Statistical analysis was developed using *Statistical Package for the Social Sciences (SPSS®)* version 17.0 e *Microsoft Excel Professional edition 2003®*.

**Results:** Grilled meals were the preferred by respondents. In the group of meat, the preference was for sirloin and turkey steak and the preferred fish specimen was cod. For the side dish, consumers choose rice and the most appreciated of the bread types was the mixed grain bread. Vegetables were less appreciated than salads and, concerning fruit; consumers prefer it raw and shelled. The favourite seasoning was olive oil. The favourite dishes mostly belong to the traditional Portuguese cuisine.

**Discussion and conclusion:** In general the results obtained are in agreement with the evolution of Portuguese Food Balance Sheets; a correlation was found between preferences and food availability. Future work is necessary to analyse food preferences of specific groups of populations and evaluate potential relationship with food intake, aiming to identify the determinants of food consumption in adults.

## Job satisfaction of Food Handlers

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Job satisfaction is widely studied at the level of psychology, sociology, economics and science. This concept is often related to the performance of workers, physical well-being, psychological and satisfaction with life in general. Some authors have correlated job satisfaction with several weaknesses in food service area, as the high rate of absenteeism and worker's turnover.

The purpose of this study was to evaluate the overall job satisfaction of food handlers of Oporto University Food Units.

To achieve this goal a socio-demographic characterization was developed and these data was related to working conditions and job satisfaction. A questionnaire including socio-demographic characteristics and a satisfaction scale based on Overall Job Satisfaction was applied for data collecting.

The respondents were overall satisfied with their work and correlated activities, showing greater dissatisfaction with salary and promotion perspectives. The age, number of years working in food service area and the cashier function were the demographic variables that mostly affected overall satisfaction.

Physical conditions of work, work performed, job stability, the immediate superior, the organization and operation of the food units and recognition of work by supervisors were the characteristics that showed more influence in predicting overall satisfaction

According to our results we may conclude that it is not enough to analyze job satisfaction from a single item, as workers respond differently to different issues related to their work, depending on individual feelings and expectations.

# Effects of metalaxyl on biomarkers of oxidative stress in *Solanum nigrum* L. cell suspension cultures

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Phytoremediation is an innovative technology that uses plants and their associated microbes to clean up toxic elements such heavy metals and persistent organic compounds from contaminated areas (soils, groundwater and sediments) [1]. Metalaxyl is a systemic fungicide with protective and curative action used to control diseases caused by fungi of the order Peronosporales and therefore widely used in agriculture. This fungicide is stable to a wide range of pH, light, temperature and therefore it has a tendency to accumulate in soil and groundwater representing a serial concern not only to the environment but also to public health [2]. Previous studies reported plant tissue cultures of *S. nigrum* as a model applied in phytoremediation research [3,4]. In the present study physiological parameters related to lipid peroxidation, proline and hydrogen peroxide accumulation were evaluated to provide knowledge for the use of cell suspensions as a model research system. Pale green and friable calli were used to establish cell suspensions in Murashige & Skoog medium (1962) supplemented with 2 mg/dm<sup>3</sup> 2,4-D and 0.5 mg/dm<sup>3</sup> BA, at pH 5.7. Cells were propagated on a rotary shaker (120 rpm), in the dark, at 25 °C. Subcultures were performed every 14 days, for approximately 1 month after induction, thus providing biological material for further studies. To evaluate the effect of metalaxyl on the referred stress biomarkers, cells were transferred to identical MS medium supplemented with 0 µM, 71.60 µM and 143.20 µM of metalaxyl and cell samples were collected at 0 h and after 5 h, 7 and 14 days. The data obtained in this study reveals that the fungicide caused a significant increase in the accumulation of hydrogen peroxide and free proline. Preliminary results also showed an increase of lipid peroxides in metalaxyl treated cells. Globally, the results showed that the presence of the fungicide in the culture medium affected all the evaluated stress biomarkers on a concentration-dependent manner over time. So, it was observed that both metalaxyl concentrations tested induced oxidative stress, with more pronounced effects at the higher concentration. In conclusion, these preliminary results strongly suggest that *S. nigrum* cell suspensions cultures can be an useful and good model for studying plant stress metabolism and can be used to investigate the antioxidant responses of plants to metalaxyl. In order to deepen the knowledge about the usefulness and reliability of *in vitro* cultures in phytoremediation research for *S. nigrum* further studies will be performed to evaluate its enzymatic antioxidant system.

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## ***Solanum nigrum* L. plants as a post-remediation tool for photocatalytic-treated effluents**

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Phytoremediation has recently been raising public and scientific interest as an effective and low-cost technology to be used in the clean up of contaminated and polluted soils [1]. This study comes in the context of a larger project that seeks to investigate an innovative strategy to combine the phytoextraction properties of *Solanum nigrum* L. plants with a solar-driven engineering approach (photo-Fenton). In this work, the integration of the photocatalytic treatment with the biological process was studied in order to determine the effects that intermediates (Fe salts) formed by the chemical route could have on *S. nigrum* plants, and to assess to what extent could these plants retain and/or extract the released iron species (harmful for the environment) and eliminate the inorganic nitrogen species also released with the treated effluent [2]. Firstly, *S. nigrum* seed germination was studied in the presence of high exogenous Fe concentrations (up to 56 ppm) added to the Hoagland solution, and subsequent biometric parameters were measured to assess for possible effects on seedling development. Plant growth was also monitored by exposing seedlings to increasing Fe concentrations (up to 4 ppm) in containers, in a growth chamber, with a mixture of vermiculite:perlite (2:1) with Hoagland solution. Lastly, the biochemical parameters chlorophyll and carotenoids content, and Guaiacol Peroxidase (GPX) activity were assayed. The results obtained from the seedling biometry parameters point at 11 ppm as the limit Fe concentration supported with less negative effects on growth. Plant growth assays revealed a significant increase in shoot and root length and no significant variation regarding fresh and dry weight in all treatments. The data retrieved from the biochemical determinations seem to corroborate these results reinforcing the idea that these plants are able to tolerate increasing iron concentrations, at least at the concerning values. All the biological tests showed that plants were able to tolerate Fe concentrations up to 4 ppm (equal to the optimal loads used in the photo-Fenton process) and, furthermore, also able to extract Fe from the solution, thus revealing *S. nigrum* as perfect for being used downstream the photo-Fenton treatment in this integrative remediation approach.

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## Cardosins A and B expression in *Arabidopsis thaliana*: a comparative view

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Cardosins are aspartic proteases (APs) abundant in *Cynara cardunculus* (cardoon) flowers. Cardosins A and B have been extensively characterized, as they are considered to be good models to study plant aspartic proteases, namely in what concerns protein intracellular sorting and trafficking [1,2]. *Arabidopsis thaliana* was already used as an heterologous system to study cardosins A and B expression and accumulation in seeds and proved to be effective and trustworthy [3]. However, we raised the hypothesis that the ectopic/overexpression of cardosins directed by 35-S promoter in Arabidopsis could lead to phenotypic alterations affecting plant viability. To answer this question, we designed this work using *A. thaliana* stable lines expressing cardosins A or B directed by 35-S promoter. The possible effects of cardosins in plant phenotype were analyzed, by measuring the length of the roots, the number of lateral roots and the number of flowers. The results showed that, when compared to *wild type* plants, the transgenic lines showed a delayed development, considering the above referred parameters. Additionally, differences between cardosin A and B Arabidopsis lines were still detected. After 12 days of growth, roots of plants expressing cardosin A were longer than roots of plants expressing cardosin B, although the medium number of lateral roots was the same in both lines. The number of flowers in plants expressing cardosin A was higher than in plants expressing cardosin B. Generally, plants expressing cardosin B presented delayed growth and were shorter than plants expressing cardosin A. These results showed that ectopic/overexpression of cardosins directed by 35-S promoter, while reducing growth, does not affect viability of transgenic lines of Arabidopsis.

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## *Parallel Oral Sessions III*

### **A3** *Geography, Sociology & Economics I*

# Organising Capacity for Sustainable Urban Planning

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For almost 25 years now *sustainable development* has been a trendy concept, whose real meaning and implications are still under discussion. Namely in cities, it is widely accepted that *sustainability* refers to dealing simultaneously with economic, social and environmental issues [1], and that it requires strong organisational and integrative skills from urban planners [2]. In the realm of urban studies, van den Berg et al. (1997) developed a framework with a combination of elements “designed to respond to fundamental developments and create conditions for sustainable development”: strategic networks, leadership, vision, political support, societal support and communication [3]. However, sustainability can be placed on a *weak-strong continuum*: “the degree to which individuals are required to change their lifestyle and behaviours” [4]. If sustainability notions among urban planners (and stakeholders) differ along this continuum, different organisational needs and outcomes may be expected. Moreover, sustainability notions may vary over the duration of a project. Thus, for planning theory and practice, it is an important task to assess whether and how the notion of organising capacity is appropriate enough to deal with different sustainability understandings.

Hence, the main objective of this ongoing study is to better understand the relationship between weak/strong sustainability notions and organising capacity. To do so, and after unfolding a theoretical framework combining these different literatures, this study will test a set of hypothesis in a case study about the Riverfront project in Coimbra, namely: (1) organising capacity’s framework is appropriate to deal with weak sustainability but not with strong sustainability; (2) improvements in organising capacity through time are associated with stronger sustainability notions; (3) organising capacity speeds up a project’s implementation, but is associated with weaker sustainability notions (4) the evolution from weaker to stronger sustainability is associated with improved roles of communication and societal support. This project will be analysed over a ten year period, triangulating different data sources. The methodology adopted for this time comparative analysis is based on the *rounds model* [5], each round comprising a problem-solution combination and certain participating actors.

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# Modelling urban sprawl scenarios to support regional land-use planning and management

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In recent years, urban and regional planning has progressively relied on dynamic models based on increasingly sophisticated technique to generate multiple scenarios decision support for planning and sustainable land use management, allowing anticipating scenarios, minimize problems and maximize gains [1].

With the development of geocomputing, there was an increase in the dissemination of spatial analysis methods that combine approaches recurrent from Geographic Information Systems with other emergents, such as the use of modeling with cellular automata [2]. These models have assumed an important role in contributing to the reading and interpretation of spatial dynamics, allowing it to model and simulate "behaviors" of evolution and reaction of the territories.

After our initial approach [3], this research [4] focused on two key issues: the first on issues of assessment (intervention *versus* resultants) of spatial planning in the municipality of Cabeceiras de Basto (Northern Portugal) and its influence on urban growth, the second associated to this one, passed by the test of methodologies for assessing trends, by simulating scenarios of urban sprawl, using the modeling with cellular automata through SLEUTH model, which allows measurement of trend-use change and land use in view of the contribution for better definition of territorial intervention instruments.

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# Urban sprawl and agricultural land fragmentation in the dairy region of Entre-Douro-e-Minho (*Porto metapolis*)

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Since the end of World War II, the Western World has witnessed a particularly fast and intensive process of urban expansion. Meanwhile, the variety of suburbanization models has been acquiring an increasingly scattered and uncontrollable outline, referred to as “urban sprawl”.

This specific model of urban expansion (or dispersion) is essentially characterized by its low density, great discontinuity or fragmentation, and insufficient planning. Consequently, it implies the occupation of large tracts of land and induces numerous effects at an environmental level, as well as in the planning and management of natural resources.

The recent urbanization of the Portuguese territory has undergone different rhythms and intensities due to the growing drift to coastal areas and sprawl around its two major cities, Lisbon and Porto. In the Portuguese northwest, however, the urbanization process was more scattered and fragmented, resting on the existing network of middle-sized towns and on the historically discontinuous population patterns which characterize this particular region.

The rapid expansion of Porto’s Metropolitan Area has increased the pressure on rural areas, including those presenting strong land-use-capacity in the Entre-Douro-e-Minho region. Here lies the largest "dairy basin" in the country, which is characterized by a large spatial concentration of farmsteads and by intensive, mechanized and dairy-specialized agriculture.

This study’s empirical approach was based on the following key tasks: the analysis of the dynamics in land use/land cover changes using *CORINE Land Cover* (1990, 2000 and 2006); the application of several landscape metrics in order to measure the fragmentation degree of the Agricultural Land Reserve (ALR) areas; and the georeferencing, statistical and spatial analysis of the processes presented for non-agricultural use in the ALR in two of the most representative counties in the dairy basin (Barcelos and Vila do Conde).

Furthermore, it highlights the crucial role of Geographic Information Systems (GIS) and Landscape Ecology metrics in supporting Territorial Management policies and instruments and, specifically, in the study and evaluation of conversion and transformation dynamics of land uses, of urban pressure on natural areas, as well as in the reflection, implementation and monitoring of protective measures regarding agricultural lands.

# A Core-Periphery model

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This project analysed an analytically solvable version of Krugman's Core-Periphery model - Forslid and Ottaviano (2003), which study, in general ways, the effect of cost transportations on the location of skilled agents, with two regions. Consequently, the aim is to understand how migrations would occur when transport costs increased, thus the question we try to answer is: where will the first worker move when the transport cost rises, if skilled agents are in the *sustain point*, which leads to concentration of agents in a single region?

So, the first part of this project generalised the model for “R” regions and then analysed a special case for 4 regions.

We reach a generalized equation for the skilled workers wage (Eq.1):

$$w_i = \frac{\mu}{\sigma} \sum_{j=1}^R \left[ \frac{Y_i \tau_{ij}^{1-\sigma}}{\sum_{k=1}^R H_k \phi_{jk}} \right]$$

Eq. 1

Then, considering 4 regions display in a circumference (Fig.1) it is analysed how skilled workers, who will be concentrate on region 1, move to others regions and to which one(s), analysing their wages.

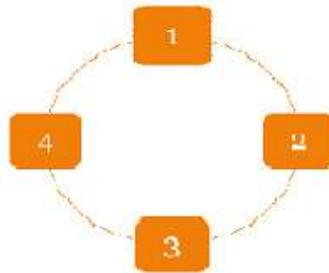


Fig 1

Then can be concluded that: the wage on region 1 do not depend on the transportations costs (only on: the weight of consumption of manufactures (produce by the skilled workers) on the utility; and on the elasticity of demand of any variety of manufactures) and in the wages of region 2, 3 and 4: both the weight of consumption of manufactures on the utility and the elasticity of demand of any variety of manufactures have a small impact on them, specially when transportation cost are higher. And finally and most important, when the transportation cost rises, the agents will go from region 1 to region 3.

References:

[1] Forslid, R., Ottaviano, G. I. P. (2003) *An analytically solvable core-periphery model*. Journal of Economic Geography, 3: 229–240.

# URBAN DYNAMICS AND HISTORICAL CENTER: Oporto case

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With this study it is intended to contribute for the analysis of the applicability of politics, of instruments and programs for historical centers, with special reference to the Oporto case.

For the effect, we explore the theme from theoretical framework diachronic evolution of the concept of historic center, as well as several special approaches. Concerning this case study, the research relies on identification, description and analysis of interventions that occurred in the historical center of Oporto, since 70's decade of twentieth century till the present.

Currently, the historical centers are presented as a key policy of the city, and they intersect with the scale of land use, which show multiple strands of research. The historical centers, place of origin of many cities today, focus on themselves a set of problems and opportunities in terms of planning.

It is intended by the analysis of past realities to under stand how to integrate urban policy in the concerned area, the same main limitations and results, contributing to an unbiased discussion of new policy instruments, which allow the rehabilitation policies, preservations and revitalization of historical centers.



Fig. 3- Mapa da área de estudo \_ Centro Histórico do Porto

Keywords: Historic Center, Oporto, Programs, Policies, Urban Rehabilitation.

# Oporto – Gaia, the river banks of Douro in urban historic area

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This project work aims to contribute to the study of urban territory of Porto - Gaia, built on the banks of the Douro River and adjacent historic areas. It is about investigating its dynamics and impact of the current state of urban regeneration in the areas adjoining the waterfront on the borders of the river Douro, including the river itself as a network.

The study will make a temporal approach in three stages: In the first, more descriptive, it will generally define the historic thickness of this area; then it will analyze the changes over the last 50 years, starting with the construction of the Arrábida Bridge in 1960; a third and final stage will examine the field data collected in 2010 on the use of some public spaces in this area.

The territory under study consists of the waterfront and the urban fabric on the borders of the River Douro, which is strongly marked by the spaces of the historic Centre of Porto, which for the purpose of this study is considered the area between the bridges of Freixo and Arrábida.

The main goal is to understand the dynamics of this area by examining the appropriation of the public spaces that structured it. For this purpose, we will analyze how the public spaces at the local level, in its physical social and economic dimensions, tend to influence the dynamics of the urban regeneration of such territories. Another goal is to better understand the quality and type of the use of public spaces and show how a GIS, as a tool and analytical process, can monitor the variation of intensity and the type of use of these places, in pursuit of a better management.

Mapa da Área de Estudo  
Porto-Vila Nova de Gaia



Fig. 1 – Map of study area Oporto – Vila Nova de Gaia 2

Keywords: historic center, Oporto, public space, Vila Nova de Gaia, waterfronts.





**U.**PORTO

## *Parallel Oral Sessions III*

**A4** *Engineering III*

# Hand Geometry Based Recognition System – A New Method with no Constraints on Image Acquisition

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Hand recognition systems are among the oldest biometric tools used for automatic person authentication. Access control devices have been manufactured and commercialized since the late 1970s. Several patents have already been issued for hand recognition devices and live applications have been launched and used in nuclear plants, airports and hotels over the last 30 years. Hand-based recognition is reliable, low-cost, intuitive and non-invasive. It is a viable solution for a range of access control applications.

However, recognition systems based on hand geometry are still far from the favouritism that other types of systems already possess. This unpopularity is related with the restrictions imposed on acquisition of images to use on the Recognition or Identification phase. Despite of the performances stated by the state-of-the-art, there are still many limitations on the hand positioning upon the acquisition. In some systems pegs are used to force the user to place his hand in a certain position [1]. However, in addition to being uncomfortable, these pegs cause deformations on hand, which subsequently will influence the feature extraction. Other systems use a peg-free methodology [2], which allows a little more of freedom to the user, but at the same time it is imposed to the user a predetermined hand position.

In this work we propose to design a system capable of showing an acceptable performance for a bigger universe of users and also eliminate all or, at least, most of the restrictions imposed on the acquisition. The exclusion of restrictions on the acquisition will enable a significant advance evolution of these systems, mainly because they will become simpler to use, with a considerable improvement in the interaction with user.

Thus, we developed a new algorithm that uses the skeleton of the hand binary image to detect the 5 fingertip's points. For detection of the 4 valleys between fingers, this algorithm uses an edge following algorithm. This method has reached a rate of 97% accuracy on the detection of the desired points, and was applied to images without restrictions on the acquisition. After point detection, 21 features are extracted and then a recognition algorithm, based on feature-matching, is applied. This system presents a Genuine Acceptance Rate of 73% with a False Acceptance Rate of only 8%.

Within this work, a new image database was also created with images acquired without restrictions to the hand positioning. Besides being public and without antecedents, this database also allows a greater motivation on the study of such systems and their constraints.

References:

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- [2] Jugurta Montalvão, Lucas Molina and Jânio Canuto (2010), *Robust hand image processing for biometric application*, Pattern Analysis & Applications, Volume 13, Number 4, 397-407.

# Biometric Analysis of the Human Ear

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Over the last decade, person identification using ear analysis has garnered increasing attention in the biometrics field, offering several advantages over other methods, based on other human characteristics. One of the most important strong points of this method is that, unlike other features like voice or hands, ears age in a predictable pattern. Also, studies have shown that ear biometrics has high receptability among potential users, who consider methods like retina scanning more invasive.

In this paper, a simple recognition system using ear biometrics was implemented. The method chosen relied on a small set of features of easy extraction, so that complex image processing techniques and strict conditions for sample collection wouldn't be necessary.

Since this work required a very specific kind of samples, no online image database that met our requirements was found, so a few samples were collected, taking a few ear photographs (using common digital cameras, with flash on) of 8 different subjects. A white cardboard was used to achieve better results in segmentation, since the ear detection (without shadows or hair to obstruct a clear view of it) was outside the scope our project. No other scene conditioning measures were taken, including distance from the object since our final implementation will be scale-invariant.

The characteristics used to trace a profile of each individual ear were the proportions between the longest line contained within the ear and the lines that are perpendicular to it (measured from the intersection with the longest line to the outer contour of the ear). Through the method described, a vector of ratios for each ear in the database is obtained. The matching criteria the algorithm uses to establish correspondence between a sample and an individual in the database is the minimum distance between these feature vectors.

The results data show that our algorithm is invariant to scale or rotation variation (rotation tested for  $-15^\circ$ ,  $-5^\circ$ ,  $5^\circ$  and  $15^\circ$ ). Although having a rather high mismatch rate (20%) as of the writing of this abstract, several improvements are being tested with very promising results.

Although the amount of data used does not suffice for robust solution, at this stage the project allowed us to collect useful information about the implemented method of analysis. It can be concluded that through a small number of easily extracted features of the human ear, a fairly singular profile of the ear can be obtained, which attests to the convenience of ear biometrics.

[1] Yan P. and Bowyer, K.. Empirical Evaluation of Ear Biometrics.

[2] Shailaja, D..[2006] A Simple Geometric Approach for Ear Recognition.

# Braille to Latin characters Converter

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## Abstract:

In this work, we present software that can change a Braille text image into Latin characters, designed to help people who aren't visually handicapped but have a relation of sociability with those who have and can't, by some reason, learn how to read/write in Braille.

## Introduction:

For certain people, living and working with blind people is an every day requirement. This software is intended for those who, due to lack of transport, money, time or even due to the non-existence of a place to learn, cannot learn how to read or write in Braille, being unable to properly communicate with the visually handicapped around them.

## System description:

This system is divided in based on image segmentation and is divided into five modules: filtering, rotation, segmentation, conversion and user interface.

Filtering consists in removing all undesired from the background, leaving an unicolour background with dots of a different color (the Braille characters dots).

Assuming the image can be rotated by an undetermined angle, it becomes necessary to create a module which will approximate the rotation of the image and correct it to zero degrees if needed.

Segmentation will implement the binarization of the filtered and correctly orientated image, leaving black dots on white background.

Conversion will use the segmented image to separate each line and each character, converting them into a Latin character text.

All this is made through an user interface, which permits the choice of the input image, its pre-visualization and the output text.

## Conclusion:

This work has been quite successful in its results. All the images considered were correctly translated, provided the right codification is known and adequate. In the future, the software should be expanded in a first stage to identify the maximum number of codifications possible and in a second stage, expand its input image characteristics, making the software considerably more flexible.

## References:

[1] Gonzalez, Rafael C. and Woods, Richard E. (2002), *Digital Image Processing*, Prentice Hall, New Jersey

# Image Analysis increase efficiency in Heavy Media Separation

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ENEAS Project (European Network for Environmental Assessment and Services) recently acquired a last generation image analysis system, intended to study the physical properties of particle populations at the micro and nano ranges of size. The system combines the high quality images with statistically significant particle shape and particle size measurements. Although intended primarily for the study of soil flow phenomena after wild fires and to the analysis of behaviour of atmospheric sprays it conforms to 21CFR Part 11 requirements of the Code of Federal Regulations of FDA (General Principles of Software Validation).

For that purpose the equipment contains an integrated dry powder disperser and its measurements have real statistical significance, as they are accurate and repeatable. However for a good operation and reproducibility of measures it is mandatory to establish a prior very strict SOP (Standard Operation Procedures).

To acquire the desired sensitivity to the operations we decided first to set up an SOP over a well known industrial process: a Heavy Media Separation was chosen. This unitary separation process is located in a mineral processing plant and uses as heavy media a suspension of ferrosilicon particles. We analysed population particles coming from two separate suppliers with different known industrial behaviours.

High viscosity separation media are undesirable as they reduce the velocity of the particles being separated thus increasing the chance of particle misplacement and reducing recovery (possible loss of ore). On the other side, low densities are inefficient as they allow undesired particles to be placed in the concentrate (low grade separation). Thus a very delicate tradeoff follows where the properties of the heavy media are paramount.

Knowledge and understanding of particle size, shape and density are essential to develop and control this type of industrial processes. However, particle shape and size depend on various basic parameters such as: circularity, convexity, elongation, intensity. These parameters analyses give us like a “fingerprint” of the particle and relevant statistic data about the particle population. And the image analysis equipment is able to produce a very useful description of these parameters.

We were pleased to see that, besides a previous training on the establishment of a SOP, we were able to produce a valuable industrial report. It states a very clear distinction between the populations in several basic parameters that influence decisively the stability and viscosity of the suspension taken as a separation medium. It was possible to produce a first report able to advise about the desired composition in size, shape and roughness of the ferrosilicon population of particles and in this way to contribute to a more stable and efficient separation process.

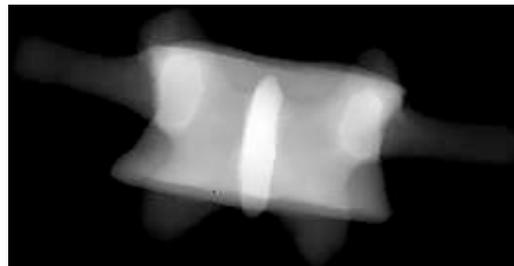
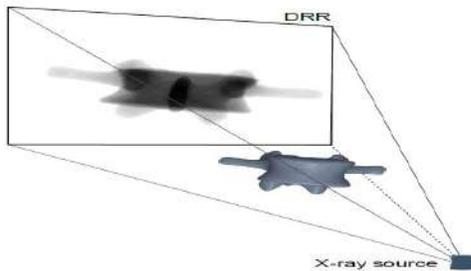
# Generation of Planar Radiographs from 3D Anatomical Models Using the GPU

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Our work describes the attempt to produce parallel, high performance algorithms for extraction of Digitally Reconstructed Radiographs (DRRs) out of 3D vertebrae models, using the Graphics Processing Unit (GPU). This problem has, inherently, huge amounts of data parallelism, which makes it suitable for acceleration under parallel architectures such as the GPU. The developed algorithms will be implemented utilizing NVIDIA CUDA platform [1], a parallel computing architecture that enables the programmer to take advantage of the GPU functions.



This research is motivated by a recent work by Moura, D. *et al* aiming to attain a process to recover the shape of the human spine of *scoliosis* patients using two planar radiographs [2]. The former encompasses solving 2D/3D non-rigid image registration problems, involving the creation of DRRs, hundreds of times per second. For this reason, it demands DRR extraction methods with higher throughputs than the ones a CPU can meet.

Using an approach based on the *multi-depth test* reported by Liu, F. *et al* [3] and Bresenham line algorithm [4] we were able to devise a novel approach to extract DRRs from vertebrae CAD models in one geometry pass, while avoiding the emergence of image artifacts and further post-processing steps. Though it is still soon to report on benchmarks, initial tests exhibit good performances while providing desirable image quality levels.

References:

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- [4] Bresenham, J.E., *Algorithm for computer control of a digital plotter*, IBM Systems Journal, 4(1):25 – 30, 1965.



**U. PORTO**

*Parallel Oral Sessions III*

**A5** *Law & Criminological Sciences*

# Private Entities with Administrative Powers and Desirable Return of the Administrative Law

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In this paper, we will dedicate to the study of private entities that integrate the Public Administration, either by the institutional path, the case of the private administrative entities (public foundations of the private law e.g., University of Porto and University of Aveiro), or by the functional path, the case of the private entities with public powers, and that through this same integration could become exposed to the incidence of administrative law.

In the first point, we have private entities that integrate the Administration, using a functional path, in that, these entities are genuine private and that through the exercise of administrative functions that were invested, integrate the administration. This transfer of responsibility for the implementation of public tasks to private entities has been called by doctrine as public administration conceded or delegated, inserting, in a process of organic material privatization.

In the second point, we have private administrative entities, organisms in the form legal - private, created by public entities, under the dominant influence of these, in order to accomplish tasks of public interest, in the context of formal organic privatization, and has been called by doctrine as "public administration in private form"<sup>3</sup>. The integration of these private administrative entities in administration is achieved in the institutional plane, because although they are coated a private form, are entities that are under the dominant influence of a public entity, thus entities administrative. In the case of UP, translates dominant influence – as soon the power to the government appoint the Curators Council.

The escape to private law by the administration, projects not only on relationships that the private administrative entity will establish with others (relations *ad extra*), where administrative law intervenes exceptionally, but is also reflected (here more troubling) in the relationship between the private administrative entity and public entity that has created it (relations *ad intra*).

What seems somewhat inconceivable, bearing in mind the provisions of Article 267 paragraph 2 of the Constitution of Republic Portuguese, which states that the forms of deconcentration may not affect the unity of action by the administration nor the powers of oversight and supervision of the competent organisms. Therefore, in this sense, public entities should have the power to intervene, control and exert external influence, with public administrative nature over the private entity, independent of the dominant internal influence (which shall always develop by private law). This leads us to desirable "return of administrative law"<sup>4</sup> to a time when the administration in private form is under *pubblico comando*<sup>5</sup>.

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[<sup>3</sup>] Gonçalves, Pedro Costa, (2008) *Entidades Privadas com Poderes Públicos*, Almedina, pp.396

[<sup>4</sup>] Napolitano, G., *Sogetti Privati "Enti Pubblici?" Dir. Amn.*, pp.802 *apud* Pedro Gonçalves, ob. Cit., pp.415

[<sup>5</sup>] Cassese, Sabino, (2003) *Trattato Di Diritto Amministrativo*, pp.325

# Empowering Citizenship: Agency Theory applied to the Relationship Between Citizens and Politicians

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In modern history, all democratic political systems have been based upon the idea of popular sovereignty; uphold by a concept of citizenship in which the individual is the ultimate source of political legitimacy, and power; sustained by the notion that governments and rulers exist only to protect individual freedom and personal well-being. Politicians have the crucial role of being the agents of society chosen to serve its best interests. Citizens trust them with their property and freedom, so they can run public business, in a very similar way stakeholders trust managers their money, so they can run private businesses.

Evidence from agency theory shows us why managers and politicians alike have an incentive not to run public business according to society's best interests. With low voter turnouts, and a great delusion about the political process citizens it's important for democracy not only to restate its founding principles, but, above all, for it to find new and rediscover old ways to empower citizenship.

In this paper we compile and briefly analyze a series of mechanisms that might be used to align interests between agents -- that is politicians -- and principals -- that is citizens -- reestablishing the center of power in the relationship where it belongs. This can hardly be done without redoubled vigilance and regained interest from society in political issues, setting a new paradigm to participatory democracy.

# Fear of crime and Physical Space: a systematic review

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This systematic review sustains the research project – Experimental Security – which is taking place in the School of Criminology of University of Porto, under the supervision of Carla Cardoso and Cândido Agra.

This presentation aims to explore the relevant research that has contributed to the study of the emotional reactions to some places and, most fundamentally, to certain features of the urban environment. In particular, the physical cues that could constitute relevant elements of what has been called of *hotspots of fear of crime*. According to some authors, the literature has overlooked the contextual variables of fear of crime, focusing mostly in the individual ones. Thus, individual variables such as gender, age and socio-economic status have been associated with fear of crime. In fact, women, elderly subjects and individuals with low socio-economic status are considered the most fearful ones. On the contrary, the relationship between fear and victimization is not so linear (for a review see Hale, 1996). Since we propose to study the emotional reactions to physical space, it is important to answer the following question: what are the cues in the environment that can trigger negative and positive feelings?

Research suggests that fear of victimization has an uneven spatial and temporal distribution. This can be analyzed in different levels: from macro to micro level. In fact, individuals are more fearful in certain countries (macro level), neighborhoods (meso level) and in specific places or streets (micro level). Temporal patterns of fear intensity also suggest that significant differences between day and night exist. So, investigators have suggested a set of physical cues that contribute to fear spots: limited prospect, hiding places for would-be offenders and blocked escape (e.g. Fisher and Nasar, 1992); social and physical incivilities (e.g. Wilson & Kelling, 1982) and lack of luminosity or darkness.

Altogether, these physical aspects of urban space associated with insecurity feelings will be analyzed in this presentation. Finally, an ongoing experimental research project of the School of Criminology will be presented.

[1] Hale, C. (1996). Fear of crime: a review of the literature. *International Review of Victimology*, 4(2): 79-150.

[2] Fisher, B. & Nasar, JL. (1992). Fear in relation to three site features: prospect, refuge and escape. *Environment and Behavior*, 24: 35-62.

[3] Wilson, JW. & Kelling, G. (1982). Broken Windows. *Atlantic Monthly*, 249(3): 29-38.

# The Implementation of Electronic Monitoring

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“Electronic monitoring (EM) is the constant (active) or intermittent (passive) surveillance of a client-offender through telephonic or radio/microwave transmissions interfaced with computer system(s)” (Rush, 1988, p. 222)<sup>6</sup>. EM appeared in the 80’s as an alternative penal measure in the Justice System in response to the prison overcrowding.

In Portugal, during the 80’s and 90’s, the prison’s population showed an increase and this was no exception to the European trend. As a result, in the mid 90’s, prisional’s maintenance system was seriously compromised. So, EM was launched in 1998, after the review of the article 201º of the Code of Criminal Procedure. Distance control methods were adopted to make an efficient accurate home detention curfew. The main goal was the achievement of another option towards traditional measures, mainly in the pre-trial stage. After 2007, this type of decision was extended also to post trial measures<sup>7</sup>.

The measure is still recent, however, it is important to assess EM’s implementation in Portugal. There is little theoretical work in Portugal about this issue.

There is a partnership between the School of Criminology and DGRS (Direcção-Geral de Reinserção Social) to promote an evaluation study of Portugal’s EM implementation. The research focuses in three main guidelines: i) the empirical; ii) the legal; iii) the economic. We will start with the empirical line, studying the individual’s perceptions about EM (e.g. prisoners - with or without EM -, public, probation officers). Based on international research, we will try to understand the opinion and the cognitions of individuals about this modern way of justice.

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<sup>6</sup> Rush, G. E. (1988). Electronic surveillance: An alternative to incarceration – an overview of The San Diego County Program. *AJ CJ*, 12(2), 219-242.

<sup>7</sup> Caiado, N. F. (2008). Vigilância electrónica em Portugal. Contributos para a história do primeiro ciclo da vigilância electrónica (2002-2005). *Ousar integrar – Revista de Inserção Social e Prova*, 1, 79-95.

# Conflict Mediation among Juveniles in Institutional Context: Breaking the cycle of deviance

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This presentation is a description of an experiment of conflict mediation among juveniles conducted in a youth centre in Oporto, taking Restorative Justice as theoretical framework.

This centre is a shelter for young males, aged between 12 and 18 who are under Law 147/99 of September 1st (*Lei de Promoção e Protecção de Jovens em Perigo*). This Centre, hence, handles situations of young people who are in danger.

Many of these youngsters present anti-social behaviour and even delinquent behaviour, and some of these adolescents are already involved in judicial processes.

Quite often, institutions with the above mentioned structural profile have not developed adequate strategies to deal with these “troubling adolescents”, who cause problems to the internal dynamics of the institution and also conflicts amongst their peers and caretakers.

In order to face these institutional difficulties, an action-research study was developed to be implemented in the centre, aiming to deal with the conflicts among juveniles through mediation.

The core of this project was the conception of a mediation program that takes into account the characteristics of youngsters, their developmental level and the institutional context.

This experiment was sustained by some instruments created and adapted through time to the characteristics of the youngsters and the type of offenses that have occurred in the youth centre.

Conclusions and adaptations were made regarding the characteristics, advantages and dangers in the institutional context on the subject of the implementation of Restorative Justice as a model of intervention.

# History of Criminology in Portugal

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Usually, the History of Criminology is taught to the students based on its developments in English speaking [1] and Francophone countries [2]. However, it is important to supplement this information with the History of Criminology in other countries where there is a scientific tradition in this field. This work is an attempt to discover how Criminology developed as an area of scientific interest in Portugal. The period of analysis goes from the end of the nineteenth century to the first quarter of the twentieth century.

There are several elements that can be explored when trying to analyze the History of Criminology, such as the formation of scientific knowledge and its emergence, maintenance and improvement conditions (e.g., institutional, social, political). We will explore only the first point of analysis, namely, the scientific knowledge first produced, in Portugal, about the crime and the criminal.

The method employed was the analysis of historical documents published by the main authors of criminological thinking during the period under review.

We concluded that Criminology, in our country, followed the international criminological movement. In fact, as a scientific field, Criminology has emerge due to authors who defended the postulates of the Italian Positivist School, such as Basílio Freire (1886; 1889), Maria de Sena (1888) and Júlio de Matos (1893). These authors argued that offenders show signs of physical and moral degeneration and atavism and, for this reason, were determined to commit crimes. 2

However, while these authors diffused this line of thought, other authors like Mendes Corrêa (1931) and Ferreira Deusdado (1889) began to criticize it and its principles. Mendes Corrêa focused the analysis on psychological factors, stressing their importance in explaining criminal behavior. Ferreira Deusdado argued that social factors, such as education, would be the most important ones in explaining criminal behavior, though not the only ones. Besides that, Mendes Corrêa e Deusdado defended the free will typical of human nature.

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**U.**PORTO

## *Parallel Oral Sessions IV*

**A1** *Biological, Environmental & Health Sciences VII*

# Mps1 regulates SAC through BubR1 phosphorylation

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The spindle assembly checkpoint (SAC) monitors kinetochore-microtubule attachment, preventing APC<sup>Cdc20</sup>-mediated anaphase onset until chromosome bi-orientation is achieved. This ensures the correct segregation of replicated chromosomes, thus preventing aneuploidy, a hallmark of many solid tumors. In addition to conserved Mad and Bub proteins, Mps1 is reported to be a crucial player in SAC signaling in species ranging from yeast to human. However the precise molecular mechanisms, by which this last protein operates, are still poorly understood. With the present work, we pretended to dissect the SAC signaling function of Mps1 kinase in *Drosophila*.

Mps1 was efficiently depleted from *Drosophila* S2 cells by specific dsRNAi as evaluated by Western blot (WB) and immunofluorescence (IF) analysis. Mps1 role on SAC function was assessed by quantification of the mitotic index upon cell cultures treatment with microtubule poison, colchicine. The occurrence of precocious sister chromatid separation and anaphase defects were also evaluated by means of IF analysis. Furthermore the effect of Mps1 depletion on mitotic timing was analyzed through time-lapse videomicroscopy using an S2 cell line co-expressing EGFP-tubulin and mCherry-CID. We co-immunoprecipitated Mps1 and BubR1 and analyzed the latter's phosphorylation status through WB. Lastly we constructed a S2 cell line co-expressing EGFP-Mps1 and mCherry-tubulin that allowed us to induce Mps1 overexpression.

Mps1 depleted cells lost their ability to maintain a colchicine-induced mitotic arrest and proved to separate sister chromatids precociously roughly five times more often than control cells. In addition, cultures treated with Mps1 dsRNAi display a higher percentage of cells that exit mitosis with anaphase defects and a faster mitotic timing when compared to control cultures. These results indicate that Mps1 is absolutely required for SAC function and proper mitotic timing. To our surprise, kinetochore localization of other known SAC proteins remained unaffected by Mps1 depletion. However, IF analysis revealed that Mps1 and BubR1 kinases colocalize at kinetochores, and, using specific antibodies, these two proteins were able to co-immunoprecipitate with each other, strongly suggesting that they interact at the kinetochore. Furthermore Mps1 was shown to be necessary at kinetochores for BubR1 phosphorylation, as well as for Cdc20 kinetochore recruitment and formation of Cdc20-BubR1 and Cdc20-Mad2 APC/C inhibitory complexes. Accordingly, Mps1 overexpression resulted in BubR1 hyperphosphorylation and in an increase of Cdc20 levels at kinetochores when compared to non-induced cells. Moreover, cells overexpressing Mps1 were unable to undergo mitotic exit, even upon correct congression of chromosomes.

Our results suggest that Mps1 may contribute to SAC signaling through the regulation of BubR1 phosphorylation, and hereby control Cdc20 recruitment onto kinetochores, a prerequisite for a functional SAC response and tumorigenesis prevention.

## Effect of resveratrol in stress-induced premature senescent human fibroblasts

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Fibroblasts in culture have been used as models for the study of aging. Under standard culture conditions or after the action of known stressors as oxidants, cells exhibit several features of which cell cycle arrest is the loss-of-function hallmark. Resveratrol, shown to prevent age-related effects in different organisms, is currently under active study and is employed in cell ageing models. Early passage WI-38 fibroblasts grown in medium with or without resveratrol were submitted to subcytotoxic levels of H<sub>2</sub>O<sub>2</sub>. Cell proliferation, measured by MTT assays for day 2 upon to day 5, exhibited a dose dependent reduction, which was intensified by H<sub>2</sub>O<sub>2</sub>. Similarly, senescence associated - $\beta$  - galactosidase (SA- $\beta$ -gal) positively stained cells increased. Resveratrol attenuated H<sub>2</sub>O<sub>2</sub> cytotoxic effect but enhanced sirtuin 1 expression, manganese superoxide dismutase, p21 and Cyclin D1. Exposure to H<sub>2</sub>O<sub>2</sub> resulted in SIRT1 expression decrease but the effect was reverted in cells pre-treated with resveratrol. In H<sub>2</sub>O<sub>2</sub>-induced SIPS, resveratrol blunted MnSOD, p21 and Cyclin D expression. The results show that resveratrol results in the expression of features resembling replicative senescence, which are enhanced by the exposition to H<sub>2</sub>O<sub>2</sub>.

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## The role of ceramide pathway in yeast apoptosis induced by acetic acid

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The yeast *Saccharomyces cerevisiae* can undergo programmed cell death in response to different stimuli showing typical apoptotic markers, such as DNA fragmentation, chromatin condensation, cytochrome c release from mitochondria and production of reactive oxygen species (ROS). Acetic acid, a normal end product of the yeast fermentation, was already described as an agent capable to induce mitochondrial dependent apoptosis.

Changes in sphingolipids levels have been linked to apoptosis and oxidative stress in yeast and mammalian cells. Ceramide accumulates upon diverse stress treatments and increases the permeability of the mitochondria to cytochrome c, leading to the generation of ROS.

To characterize the relative contribution of de novo biosynthesis versus catabolism of ceramide in apoptotic cell death induced by acetic acid, yeast mutant cells were used: *lag1Δ* and *lac1Δ* (unable to generate ceramide by de novo synthesis), *isc1Δ* (unable to generate ceramide by degradation of complex sphingolipids) and *ydc1Δ* and *ypc1Δ* (unable to breakdown ceramide). *isc1Δ* and *lag1Δ* mutants exhibited a higher resistance to acetic acid that was correlated with lower levels of mitochondrial ROS production. However, acetic acid induced cell death was not associated with protein oxidation, nor even with cell cycle arrest. Silencing of *YDC1* in *ypc1Δ* cells and of *LAC1* in *lag1Δ* cells, by using an antisense gene expression plasmid, did not affect stress resistance.

Structurally, the deletion of *ISCI* induced a severe alteration in lipid rafts distribution throughout the plasma membrane. Acetic acid also induces a rearrangement of these membrane microdomains. Acetic acid treatment decreased the rafts levels of Pma1p, the major membrane ATPase. In untreated cells, Pma1p was less abundant in the *isc1Δ* mutant than in the wild type strain. However, after treatment with acetic acid, the decrease in Pma1p levels of *isc1Δ* cells wasn't so severe.

The *isc1Δ* cells also showed higher levels of phosphorylated (active) Hog1p. This mitogen activated protein kinase phosphorylates Fps1p, an aquaglyceroporine that also facilitates the entrance of acetic acid to the cell, and triggers its degradation.

The overall results suggest that ceramide production contributes to cell death induced by acetic acid especially through hydrolysis of complex sphingolipids catalyzed by Isc1p. The increase in acetic acid resistance of *isc1Δ* cells was correlated with the stabilization of the Pma1p ATPase in lipid rafts and higher levels of active Hog1p that probably decrease acetic acid transport.

# The effect of green spaces' (bio) structure on the bioclimatic comfort

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The presentation of this project arises from the opportunity to expose our work in the context of climatology allied with the landscape architecture.

The project focuses on the importance of green spaces in urban areas, as elements of mitigation on climate change. Among its many functions (environmental, ecological, social and economical), the perspective of green spaces as climate regulators is a central issue. In this context, they have varied properties, such as thermo-regularization and control of soil and air humidity, solar radiation and atmosphere purification. Therefore, they contribute towards to the mitigation of urban heat islands, improving urban environmental quality and urban life quality, increasing bioclimatic comfort.

This study looks forward to improve knowledge about the influence of green spaces' morphology and the organization of vegetation in the bioclimatic comfort in its users, as well as understand its influence in the climatic context of the geographical environment, through fixed measurements and itinerant measurements of distinctive factors (which influence the bioclimatic comfort) and surveys to users looking forward to perceive climate comfort.

On Porto, Serralves' gardens seem a good laboratory study. They have a considerable size, access to public and spaces with different bioclimatic contexts and potentiality for different active and passive recreation. Moreover, they are inserted in a highly urbanized context wherefore it allow us to gauge their influence on the local climatic context.

Some techniques applied to geographical information systems, climatological statistical analysis, inquiries analysis and the calculation of bioclimatic indexes will be discussed as a method for analysis, synthesis, and conclusive results of the project.

Note: This project has financial support from Porto University | Santander Totta (multidisciplinary projects – 2010).

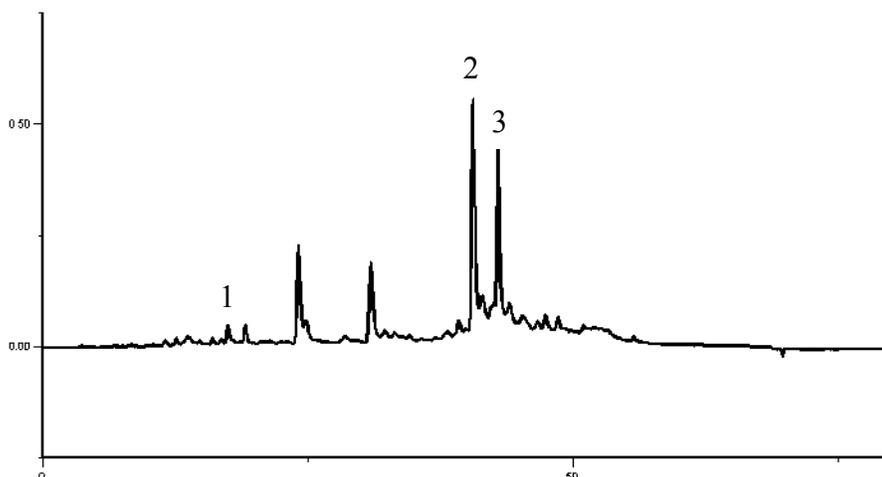
# Metabolic profiling and bioactivity of *Lithospermum diffusum*

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*Lithospermum diffusum* Lag is an herbal species belonging to the Boraginaceae family that grows spontaneously in the Iberian Peninsula and Norwest Africa. It has been used in traditional medicine for common cold and urinary diseases due to its diaphoretic and depurative properties. Within the *Lithospermum* genus, several properties have been reported, such as TSH and prolactin suppressing activity and antigonadotropic activity. Some studies have described the presence of naphthoquinones, such as shikonin and its derivatives, and pyrrolizidine alkaloids.

In this study phenolics and organic acids profiles of *L. diffusum* aqueous extract were characterized by HPLC/DAD and HPLC/UV, respectively. Three phenolic compounds (caffeic, lithospermic and rosmarinic acids) were determined (Fig. 1). Five organic acids (citric, pyruvic, malic, lactic and fumaric acids) were also identified and quantified. Caffeic and fumaric acids were revealed to be the main compounds in this infusion.



**Fig. 1.** HPLC phenolic profile of *Lithospermum diffusum* infusion. Detection at 320 nm. Peaks: (1) caffeic acid; (2) rosmarinic acid; (3) lithospermic acid.

The capacity of *L. diffusum* aqueous extract to act as a scavenger of DPPH radical and reactive oxygen and nitrogen species was investigated. The extract exhibited a scavenging effect against DPPH, nitric oxide and superoxide radicals in a concentration-dependent way. No activity was found against hypochlorous acid. Additionally, acetylcholinesterase and butyrylcholinesterase inhibitory ability was evaluated, but no effect was observed. As far as we know, this is the first study on the chemical composition and biological potential of *L. diffusum*.

# Morphological, biochemical and molecular analysis of *Olea europaea* pollen

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Olive tree (*Olea europaea* L.) pollen is a major contributor to seasonal respiratory allergies in the countries of the Mediterranean basin, affecting about 20% of the population in areas where it is intensively cultivated [1]. The olive tree has an important socioeconomic role. In the Portuguese territory coexists at least twenty five cultivars, of which fifteen are considered autochthonous. The pollen grain of *Olea europaea* has a complex allergenic profile, from which eleven allergens have been isolated and identified (Ole e 1 to Ole e 11) [2, 3]. No information regarding the presence of these allergens in Portuguese olive cultivars is available to date.

This work carried out a multidisciplinary approach in order to deepen the knowledge about the pollen allergenic characteristics of nine cultivars of *Olea europaea*: Carrasquenha, Cobrançosa, Galega, Maçanilha de Almendral, Maçanilha de Tavira, Negrinha, Redondil, Verdeal de Serpa and Verdeal de Trás-os-Montes.

The morphology of the pollen was studied by scanning electron microscopy, and pollen fertility was assessed by viability tests (fluorescein diacetate and *Trypan* blue) and *in vitro* germination. The protein extracts from nine cultivars were analyzed by SDS-PAGE and their allergenic profiles visualized by *immunoblot* using sera from patients sensitized to *Olea europaea* and antibodies against Ole e 1, Ole e 2, Ole e 5, and Ole e 9. The expression polymorphism of Ole e 1 was analyzed in some cultivars by RT-PCR and subsequent cloning was performed.

Pollen fertility results showed differences amongst the nine cultivars studied, being Carrasquenha the cultivar with the highest germination and viability values. *Immunoblot* studies with patient sera and specific antibodies allowed the evaluation of the allergenic content: Verdeal de Serpa had the highest content of Ole e 1, Ole e 2, and Ole e 5, while Verdeal de Trás-os-Montes had a higher content of Ole e 9. These results led to the conclusion that these cultivars could be the most allergenic. The results of expression polymorphism analysis of Ole e 1, although preliminary, indicated the existence of polymorphism among the cultivars analyzed.

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# Identification of *Fusarium* spp. isolated from damaged kernels of corn based on the partial sequence of the TEF gene

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The differentiation of *Fusarium* species based on morphology is not simple, since these are somehow variable. For this reason, species-specific primers were developed for rapid classification based on the amplification of genomic sequences using PCR. In addition to these primers, the gene sequences are also used for classification purposes, such as the TEF gene, which encodes the elongation factor 1-alpha [1].

The objective was to compare the classification of *Fusarium* spp. obtained by amplification with species-specific oligos from the literature with those obtained by sequencing the gene TEF.

For this research seventy seven strains of *Fusarium* spp. isolated from damaged kernels of corn were previously classified to the species level based on PCR reactions using specific primers [2-3]. For this study, DNA was extracted from these strains and the gene (TEF) was amplified by a PCR with primers EF1(5'-ATGGGTAAGGA(A/G)GACAAGAC-3') and EF2(5'-GGA(G/A)GTACCAGT(G/C)ATATGTT-3'). For sequencing reactions we used the same primer EF1 reaction of gene amplification, and the use of internal primer EF22(5'-AGGAACCCTTACCGAGCTC-

3') [1]. The sequences were compared to sequences deposited in the bank FUSARIUM- ID [4], aligned with the software ClustalW and subjected to distance analysis by Neighbour Joining method using the software Mega 4.

The classification of strains based on the sequence of the TEF gene agreed with the classification performed [2] based on species-specific oligos proposed by [3]. Cluster analysis corroborated the results of identification of strains at species level based on the comparison of the sequence of the gene TEF and also based on the use of species-specific primers.

The results showed that *Fusarium verticillioides* is the predominant species in burned grains in Brazil. Classification at the species level can be effected by PCR using specific primers developed by [3] once the prior classification of the strains used in this study with these primers agreed with the results of classification based on gene sequence of TEF.

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## *Parallel Oral Sessions IV*

### **A2** *History, Visual & Cultural Studies I*

# “The Sense of the Imagery” – Methodological Ways to the History of Art

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Through this oral communication we aim to present some of the results of our master's thesis in Portuguese Art History, presented on November last, entitled “The Meaning of Imagery. The Retable of the Souls of Santa Clara's Church, Oporto”. Having the retable as a study object, we have focused on its original structure which, to this day, remains its material witness. In order to better understand this structure, as accurately as possible, a methodological synchronization was needed. Thus, what we've proposed was the union of the various Social Sciences under historiography's wing. Having an object of study, which is usually connected to Art History, as a starting point, we hope to get from it more information than we usually would through the latter.

The Retable of the Souls of Santa Clara's Church in Oporto is a typical baroque structure, and its iconography appeals to the attention of the spectator. The central figure is Our Lady of Carmel, surrounded by the Purgatory's souls (of which she's patron). Flanking the central image are two exotic representations (considering the occidental culture's values) of black saints – Saint Ifigénia and Saint Elesbão.

The memory of these images is in some measure lost, as nowadays we hardly know anything more than their iconographic identification. So we proposed to revive their memory, through the understanding of their original functions. An image is never created exempt of a function, be it a practical or a symbolic one, and it's in the unravelling of those functions that we find their true meaning.

Thus, we've been able to divide this study in two vertexes which converge in one idea – the image as a devotional icon that appeases a collective fear. Accordingly, the great *post-mortem* fear of Purgatory (which articulately focuses on its two dimensions – Time and Power –, which according to Pierre Bourdieu lead to social cohesion, through their inherent characteristics of expectation and hope) becomes a generator of associated collective fears, which need appeasing. It's in the attempt to erase these fears that resorting to a divine, maternal figure (as the Our Lady of Carmel) plays a fundamental part, where the apotropaic (scapular) symbols work as a way to salvation.

The religious brotherhoods that ordered this altarpiece also had an important role. They encouraged praying to the souls, in a gift, counter-gift play which coerced the prayers, maintaining this Purgatory system. At last, we focus on the other vector – the black saints, Saint Elesbão and Saint Ifigénia. We've investigated how they were worshipped then, for they are virtually unknown now. We've thus realised the big role of the apologetic text and the image – through an anthropological analysis of the latter, where we find the caucasian features of the saints to be of the utmost importance.

These aspects lead us to realise how important the rhetoric reading of the retable is. It was through that reconstruction that we were able to understand how these saints were, most likely, psychopomp, helpers for the post-mortem, and so for the Purgatory's souls. This retable appears at last as a vehicle for the sublimation of this individual fear, turned collective.

# Complexism and Randomness in Contemporary Arts

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The Complexism concept is already a usual term to consider in Contemporary Arts. The randomness, being one of the Complexism's parameters is used in order to complete artistic works.

My interest in this essay is to question the use of this parameter in the music and visual arts' field, exploring the fascination of the artist, giving up, in a certain degree, the full control of the final result,

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# Programming and Algorithm: a sensorial vocabulary in artwork

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The computer may be interfering as an operative form of language and, paradoxically, sustaining a programmatic predetermined relation that involves non deterministic variables and random events. The decentralized time / space self in a dizzying chase / update of cyberspace as an extension of the body and multisensory model.

Since the introduction of mechanisms in weaving instruments during the 19<sup>th</sup> century that automation is gradually extended to the social fabric. Like an amplified microscopic effect that burst out “with the arrival of electric technology, man extended, or set outside himself, a live model of the central nervous system itself”.

The act of programming and the possibility of interaction between viewer and the artwork generates a limited factor of unpredictability since the structure is established by the artist and usually is dominated by details of some social, psychological, physical or mathematical contingency. There may not be an absolute randomness but formally it is clear a new level of abstraction.

It seems reasonable enough to establish a parallel or a sense of continuity between basic operations Cut, Copy and Paste allowed by digital media and the relations of production propelled in the 21<sup>st</sup> century. The appropriation of anything even in a virtual metaphorical sense is the main catalyst from creation. The intent of the artist is attracted in the direction of the creative process itself and not of the manufacturing of an object. In broad sense, the act of creation is the insertion of an object (real or not) into a new scenario considering it as an integral element of a narrative. Take as an example before the use of digital tools the mustache that Duchamp scribbled on the Mona Lisa.

It was recently public to the world the development in laboratory of the first living cell controlled exclusively by synthetic DNA. Scientists used computer software to break the genetic code of a bacterial cell, copying it and building a new chemical synthetic chromosome afterwards. Is this a scenario familiar of a science fiction movie? The manifestation of Isaac Asimov, Arthur C. Clarke and William Gibson world's seems to be more than mere episodes of the extension / implosion of mankind, sustaining therefore a framework for a broader future of sensorial awareness.

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# The Cinema seen by History of Art. The relevance and importance of its study. An essay on different approaches.

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Although Ricciotto Canudo has classified the Cinema as the “Seventh Art” almost a century ago, its study as an integrated part of History of Art’s (HA) *curricula* is still very limited. One finds the Cinema’s technical and production aspects as the subject of various academic studies and criticism and one also finds the films themselves used in other Social and Human Sciences studies (like History or Philosophy) but, the direct approach of HA, with its inherent advantages, is still very limited.

Aiming to counter this tendency, we essayed a series of possible HA’s approaches to the study of a film, that, in the light of its specificities, can act as a valuing tool to the artistic object (the films) and the creative processes inherent (all the network of *organisms* and *knowledge* needed to a film’s creation). The basis of these approaches will be the scientific and solid study of the films and its creation processes, with the aid of previous work made by academic areas like the History of Cinema, its Criticism, etc.

Taking into consideration that film making was often the work of artists (painters, architects, photographers, etc.), or that their direct contribution was notorious to the process, we also aimed to distance ourselves from the “Cinema as Art” quarrel and let the films *speak by themselves to the researcher*, analyzing them as containers *per se* of qualities in the field of artistic production.

One can find in Rino Lupo’s *Mulheres da Beira* (1921), the film which forms the basis and starting point of this essay, the union of two tendencies that, along the History of Motion Picture, can also act as two antagonistic approaches: shooting outdoors and shooting inside the studio. This dichotomy of practices has its parallel in the conceptual principles inherent to a film’s creation. We can divide them between the director’s appropriation of “Nature’s qualities”, transformed by his eye and by cinematographic processes, and Nature’s recreation and reinvention in the studio. Therefore, one should not forget the film as a “recording mechanism” and the benefits, still virtually unexplored, that its study as a source can bring to HA’s and Heritage researchers.

## Conclusions

The Cinema is a process by which reality is revealed and transformed through the eyes of its creator (or creators) and the camera’s mechanical eye. The works result from the overcoming of the medium’s limitations and these can also be used as an advantage to a film’s creation. The director’s (as well as other members of the production team’s) artistic training constitutes a major contribution to the *plastic (pictorial) qualities* of a film’s images and to the approaches chosen to produce them. The experiences of the twenties will be pursued, being abandoned or experiencing processes of maturation in line with the technological advances and giving rise to new problems. A film can be a source for one to know and study the Heritage’s (both natural and manmade) state at the time of its filming, as well as the view that a culture has of another one (or of itself) and of its *cultural identity*. Consequently, the Cinema constitutes a subject of study and also a source and resource for the History of Art.

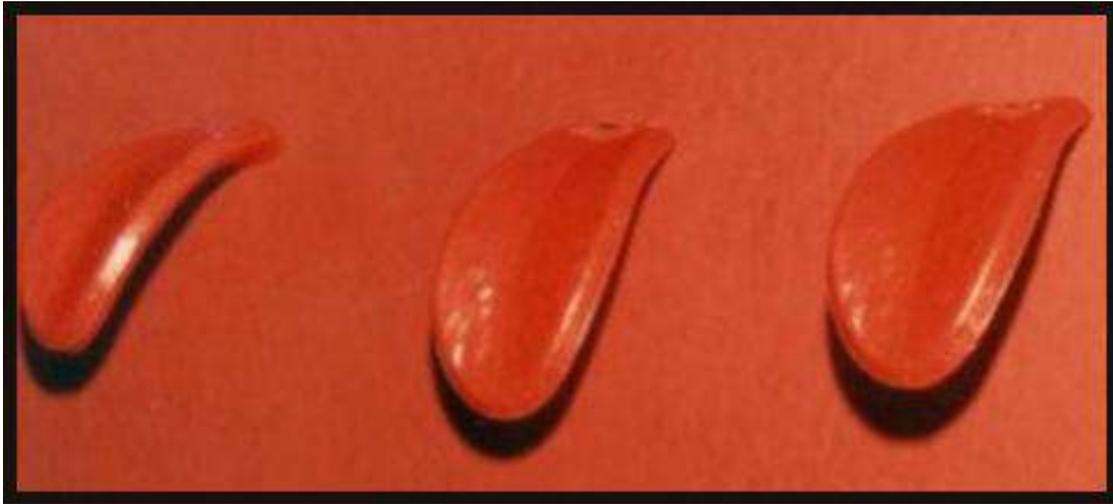
# DISCURSOS CRÍTICOS ATRAVÉS DA POÉTICA VISUAL DE MÁRCIA X.

Tales Frey

Faculdade de Belas Artes, Universidade do Porto

Through the visual poetics of the Brazilian artist Márcia X. (1959 - 2005), this study conducts a critical analysis of her production achieved between 1980 and 2005, the year that she died prematurely.

Besides looking at the history of performance art in Brazil and in the world, as a way of affirming that the artist chooses as her main strategy of creation, the existing intersections in Nietzsche's thought, in what Lyotard called for *post-modernity*, in feminism artistic expressions, in concepts by Duchamp and Warhol, in kitsch like esthetic option, in Brazilian art, in other words, crossings about all these subjects come like alternative for to analyze the main manifests by Márcia X.



**Key words:** art criticism, contemporary art in Brazil, performance art.



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## *Parallel Oral Sessions IV*

### **A3** *Communication Sciences II*

# Black Flood

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This study examines the *agenda-setting* theory applied to the approach done by three different online press publications (The New York Times, The Guardian and Público) in a time frame between the 21<sup>st</sup> of April and the 30<sup>th</sup> of October concerning the explosions in the Gulf of Mexico, at Deepwater Horizon oil rig, on the 20<sup>th</sup> of April in that same year. The comparative variations of the occurrences and its insertion in thematic areas, by each studied publication, lead us to perceive if the published articles are more relevant in economic or environmental areas, as well as other aspects of articles and the growth and fall of its importance during this long period of time (Fig.1). The results observation clearly demonstrates that the prevalent information *framing* has been environment and economy, when compared to others, through similar number of occurrences, thus establishing an evident correlation to an *agenda-setting* treatment. This approach is, mainly, determined by news agencies (e.g. Associated Press) that establish and organize how the subject is presented by media. The analysis of scientific articles in distinguished press (like The National Geographic) reveals the diffuse kind of information published by newspapers and some absence on approaching other worst events like the Delta's Niger, showing a clear prevalence of volatile issues when compared to serious news. Upon this, remains the possibility for media to achieve some undesired disclosures like the audience's perception about their oblivion or intentional elimination.

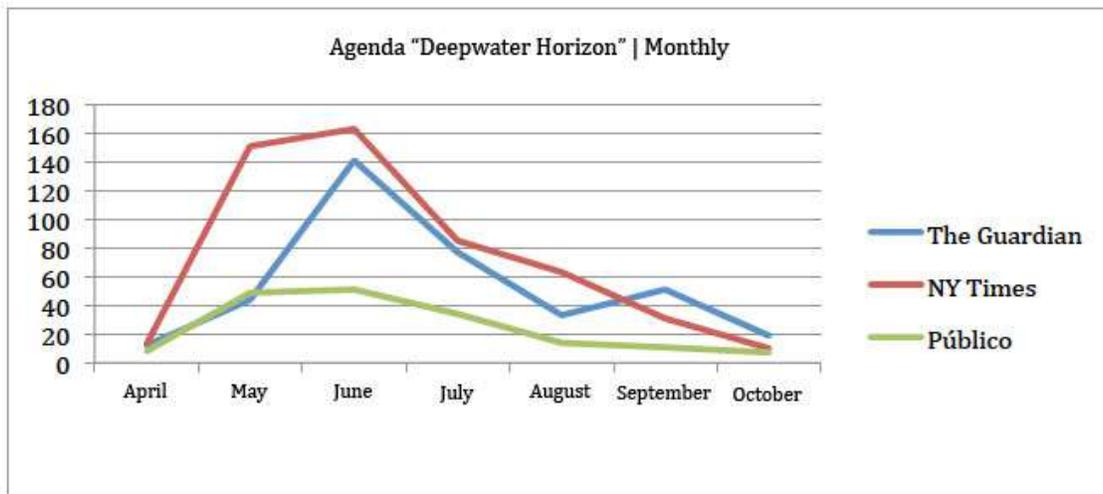


Figure 1

# O Fenómeno tablóide no tratamento noticioso das “Explosões da Plataforma Golfo do México: (maior desastre ambiental da História dos EUA Deepwater Horizon – 20 Abril de 2010)”

M<sup>a</sup> Anabela Jacinto, Joana Loureiro

Faculdade de Letras da Universidade do Porto

Our article will explore the tabloidization phenomenon in the journalistic cover of the oil spill disaster occurred in the Gulf of Mexico. In that way, we will analyse news from six different online newspapers, three Portuguese and other three international – with or without tabloid nature. The period of analysis is the next six weeks from the accident. Assuming that the case study is a natural disaster with major repercussion, its pertinence reverts in the absence of tabloidization in the treatment of news information. Still, *infotainment* is present in every studied newspaper, which leads us to question about the relevance of tabloid elements in the spill.

Our article intends to dismitify tabloidization, or the miss of it, as a phenomenon in clear rising.

**Key-words:** Gulf of Mexico, oil spill disaster, tabloidization, international/national media.



# Portuguese Press Coverage of the Kosovo Crisis

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**Abstract:** This article examines how the news coverage of the Kosovo's crisis was made in two Portuguese newspapers – Público (a quality paper) and Jornal de Notícias (a popular paper) – during a two-week period (24-30 March and 5-11 June 1999). Using the metaperformance analysis, proposed by Rui Novais [1], we wanted to see what the framing of the Portuguese coverage is, which sources are the most consulted and if there are significant differences between the coverage of the popular paper and the quality paper. The results show that the newspapers take the NATO's side and diabolize Milosevic, even though, sometimes, they criticize NATO as well.

**Keywords:** Kosovo/ Metaperformance analysis/ Media Coverage/ Sources of Information

[1] Novais, R.A. (2007), *National Influences in Foreign News: British and Portuguese Press Coverage of the Dili Massacre in East Timor*, International Communication Gazette

# **Análise da cobertura mediática à intervenção da UNAMET em Timor-Leste (11 de Junho a 25 de Outubro de 1999)**

**Ana Leite**

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This article explores the media coverage of the work carried out by the UNAMET (United Nations Mission in East Timor) done by *Diário de Notícias* (DN) and *The New York Times* (NYT). The question we ask is: Do DN and NYT have opposite positions about the impact of the media agenda in the UNAMET's intervention in East Timor?

To answer this question we raised two hypotheses: There is more news about the UNAMET's intervention in *Diário de Notícias* because East Timor was a Portuguese colony and therefore the Portuguese media has more interest in this matter; Under the theory of Daniel Hallin's three "Spheres of Public Discourse", we expected to find the two newspapers in different spheres: *The New York Times* in the Sphere of Consensus, conveying UNAMET's and the United State's version and not questioning their actions; and *Diário de Notícias* between the Sphere of Legitimate Controversy and the Sphere of Deviance, transmitting thoroughly UNAMET's actions.

We analysed the five months of the intervention (from June 11<sup>th</sup> to October 25<sup>th</sup> of 1999) through a constructed week sample (one constructed week per month which led to a total of five constructed weeks). The method used was "Meta-Performance Analysis" (Novais, 2007) which explores four dimensions: selection and use of the sources; terminology; critics or attacks to authorities; and present and absent themes.

Our question was partially confirmed because the two newspapers weren't in complete opposition. The two hypotheses were confirmed: DN had more news than NYT; and DN was in the Sphere of Legitimate Controversy because of its more neutral coverage, while NYT, with a more positive vision, was in the Sphere of Consensus. It is also important to state that there was a preponderance of official sources and that there were few critics or attacks to the authorities. The most present theme was the violence in East Timor and the absent themes were the hidden interests of certain countries about the Timor matter.

## Tragédia na Madeira

### A Semana Noticiosa de 21 a 28 de Fevereiro de 2010

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<sup>1</sup>Faculdade de Letras da Universidade do Porto

The present article consists in the analysis of all the news of the week between 21st to 28th of February, the week after the tragedy in the island of Madeira. We based our study in five different national newspapers, three diary newspapers – Diário de Notícias, Público and Correio da Manhã – and two weeklies newspaper- Expresso and Sol. Based in this five newspapers, we tried to detect tabloidization in the news of Madeira. Different newspapers use different journalism styles, but were there evidences of tabloidization?

**Keywords:** Tragedy, Madeira, Destruction, Tabloidization and Sensationalism.

# Testing the Globalization Theory – Haiti’s Earthquake 12th January 2010

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As a response to this challenge, we didn’t have doubts about choosing the treatment media gave to the tragedy occurred in Haiti on 12<sup>th</sup> January 2010. The reasons that lead us to choose this subject were mainly two: the notorious spread of information by the media in a global scale, leading to a significant mediatization of the case and due to the involvement of several material and human means worldwide (emergency of a global conscience), with one only aim: to rehabilitate Haiti.

This way, we intend to investigate the mediatization of the case itself in diverse countries, analyzing news from four different papers, with the purpose of testing the Globalization Theory: Jornal de Notícias, The Guardian, The New York Times and Le Figaro, with the objective of verifying the most focused aspects in the set of newspapers and in each one of them; this way, we plan to find evidences of Globalization.

We consider that the qualitative thematic analysis and the quantitative analysis will prove (or not) the existence of Globalization, allowing us to fulfill our aim. The analysis will be particular (group of news from each newspaper) and general (analysis and comparison of the four newspapers).

This way, it is possible to infer some conclusions that point in the direction of the existence of Globalization. However, as it is expressed in the article’s results, the spread of information and the main subjects approached on the papers weren’t completely similar in the four newspapers analyzed (an example of this is Fig.1, above, where the same subject was slightly different explored by each newspaper).



Fig.1

To sum up, the definition of Globalization used in the article to describe the theory we pretend to test allows us to conclude, based on data analyzed, that this phenomenon occurred: nevertheless, it is not verified a complete and typical Globalization.





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## *Parallel Oral Sessions IV*

**A4** *Engineering IV*

## Interactions of leukocytes and platelets to immobilized poly(lysine/leucine) onto tetra(ethylene glycol) – terminated self assembled-monolayers

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Surfaces that bind heparin are important for biomaterials for blood deheparinization. During extracorporeal blood circulation that is used in many surgical procedures including cardiopulmonary bypass, haemodialysis, blood oxygenation and implantation of artificial organs, systemic heparinization is required to prevent the initiation of blood clotting [1]. However, the accumulation of the anticoagulant heparin has been associated with bleeding complications in many patients [2].

In our recent work, it was demonstrated that the polypeptide composed of L-lysine and L-leucine (pKL), after immobilization onto tetra(ethylene glycol)-terminated self-assembled monolayers (EG4-SAMs), can bind heparin from blood plasma in a selective, concentration-dependent way [3]. During this work, the effect of this peptide on platelet adhesion and activation and leukocyte adhesion was studied. The surface charge of these nanostructured surfaces was evaluated in order to correlate the effect of positive charged amine groups and hydrophobic methyl groups on the behavior of platelets and leukocyte adhesion.

Results demonstrated that the presence of pKL decreased leukocyte adhesion to EG4-SAMs at all concentrations used. This effect is even more pronounced when surfaces were pre-immersed in heparinized plasma. Oppositely, there is an increase of platelet adhesion and activation with increased percentage of immobilized pKL. This effect is enhanced when surfaces were pre-immersed in heparinized plasma. However, adsorbed pKL in very low amounts does not induce platelet adhesion and activation when compared to EG4, even when pre-immersed in plasma. Since only low pKL amounts are necessary to induce heparin selectivity, these results are promising for the development of heparin-binding biomaterials for blood deheparinization.

### References:

- [1] Dailey, J. (1998), *Blood*. second ed. Ipswich, MA, Medical Consulting Group, USA.
- [2] (2002) *Section V: Chronic intermittent haemodialysis and prevention of clotting in the extracorporeal system*. Nephrology Dialysis Transplantation, 17, pp. 63-71.
- [3] Martins, M.C., Curtin, S.A., Freitas, S.C., Salgueiro P., Ratner B. D., Barbosa M. A., (2009), *Molecularly designed surfaces for blood deheparinization using an immobilized heparin binding peptide*, J Biomed Mater Res 88,162-73

# Development of Sheet Metal Stamping Dies using Finite Element Simulation

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Sheet metal forming of components represents an important production process, being commonly applied in the automobile industry. To process these components one requires the manufacturing of tools, which in turn will incorporate the needed steps to process the part successfully, thus assuring no breakages or other defects. Nowadays, life cycles of products tend to decrease, new materials are used and difficult geometries need to be produced. For these reasons tool designers may not rely on conventional methods of design by using previous experience and empirical formulas, due to different behaviors of new materials as well as complexities and challenges of new components. The use of finite element method when performing the simulation of metal forming processes is providing an efficient approach when developing a new component and the corresponding stamping dies needed to its processing.

In this work it is presented the use of finite element simulation, during the development stage of the stamping dies, for a selected component. When performing first simulations, results have shown a variety of problems, like excessive strain, which would create difficulties obtaining the part with success. Such results were the basis for changing and improving the steps to processing the component. Included in the development of the process, the optimization of geometry and blank dimensions were also considered by using special functions of the code. In this work, experimental results will also be included, which will allow the validation of presented simulation results.

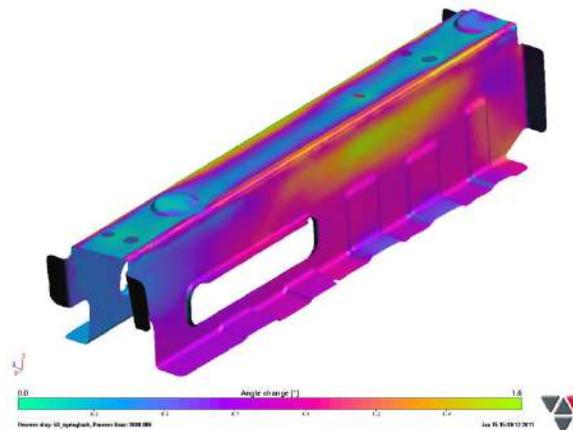


Fig. 1 – Results of an automobile component, showing prediction of geometry changes, due to springback behavior, after stamping of the part and removal of stamping dies.

## References:

- [1] T. Schönbach, (2010), *Simulation techniques for robust process layout of hot forming processes*, International Deep Drawing Research Group Conference – IDDRG 2010, , May 31 – June 02, Graz, Austria.
- [2] AutoForm Engineering GmbH (2010), "AutoForm Plus R2 Software Manual".

# Investigation on the Causes of Failure in Progressive Stamping Tools for Sheet Metal Forming Processes

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Due to the increasing demands towards vehicle safety and weight reduction, there has been a trend in using high strength and ultra high strength steels in structural automotive components, which are processed by sheet metal forming technology. Using these high strength materials in the corresponding forming tools causes higher active loads in the cutting tools, which in turn will decrease their performance by some failure mechanisms. For this usage, besides a need of increased hardness, tools require higher levels of toughness so that premature failure is avoided.

In this work it is presented a study for a progressive type of stamping tool, in which is analyzed the causes of punch failures. A procedure is defined to associate and investigate such failures with every possible variable, including tooling, process and press variables. Besides process parameters such as clearances, edge radii, angles, geometries, evaluation tests are also considered for tool steel selection, press to be used, feeding systems, sheet material, tool dynamics and the stamping process. Finally, a group of control procedures for tools is presented and suggested when processing high demanding components, which shall reduce the possibilities of process failure during the industrialization of such components.

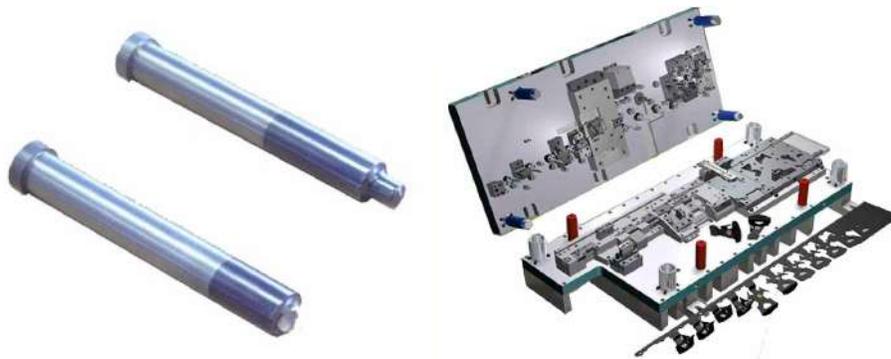


Fig. 1 – Punch failure and safe punch (left); progressive tool and corresponding processed sheet (right).

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[2] Axel Maurer, *Extending Tool Life Using Simulation-Based Wear Prediction*, International Deep Drawing Research Group Conference – IDDRG 2010, pp.315-322, May 31 – June 02, Graz, Austria.

## LipoTool - Mechanical design of a new Calliper

**A. Monteiro<sup>1</sup>, T. Andrade<sup>1</sup>, T. Restivo<sup>1</sup>, M. R. Quintas<sup>1</sup>, C. M. Silva<sup>1</sup> and T. Amaral<sup>2</sup>**

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The Measurement of body composition is highly relevant in health. For instance, it is a current procedure in the assessment of individual nutritional status, in the evaluation of body growth, in the analysis of the impact of disease and in body monitoring in sports.

However, the existing calipers have experienced insufficient progress, suffering of different limitations and shortcomings, namely inconsistency in the data collecting process. In addition, since many reference calipers still require manual data recording, their operation is time consuming and may demand a second technician, even increasing the evaluation error.

This novel wireless skinfold caliper was developed in order to address these issues. The device is based on the use of low cost and portable equipment.



The developed prototype uses the general working principle of a typical skinfold calliper, integrating important additional elements for improving working characteristics, accuracy and measuring range. It comprises housing with a cylindrical part and a handle extension, one structure with two jaws with hinged end tips and a lever.

The housing includes all the force transmission mechanical elements from the constant force actuator to the jaws, the sensing element, a power supply and the electronic system which is responsible for the management of the whole device and for establishing the communication with the software application residing in the remote station where data is recorded in an integrated database.

This device has a novel mechanical design distinct from those available in the market. It uses a constant force actuator within the housing handle, which imposes a constant contact pressure between the end tip faces and the skinfold under measurement. It also offers an opening limit increase of the end tips. Electronic and mechanical features make it able of performing dynamic measurements. The device prototype was calibrated at CATIM and evaluated in health environment under UPorto protocol. This novelty justifies a patent submissions at National[1] and International[2] levels and has been also the reason for two awards in 2010 – 1<sup>st</sup> place Innovation in Nutrition Awards 2010 and FEUP Colheita 75.

References:

[1] Patent submission PT 105187, pending.

[2] Patent submission PCT/IB2010/055701, pending.

## SeepTool – Small scale studies of groundwater flow

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Groundwater flow or seepage, the underground movement of water through soil and rock, is a physical phenomenon of great importance to Civil Engineering, which is usually covered in introductory Soil Mechanics courses. Students tend to have some difficulty in mastering the basic concepts, mostly due to their lack of feeling for the underlying physics. This has provided the motivation for the development of an experimental tool for small scale studies of seepage.

The apparatus comprises a large, two-meter long acrylic tank, a supporting structure and a closed hydraulic circuit with a submersible pump (Fig.1). It has been used for performing small scale studies of seepage around a sheet-pile wall, into a trench and through a homogeneous earth dam [1]. The setup provides qualitative and quantitative data, such as the visualization of flow lines and of total head loss, the values of the flow rate and those of the total head in 48 pressure tap points. The experimental data may be used for sketching the flow net and also for comparative purposes with numerical results obtained from finite element modelling. The activity of the setup can be transmitted to the lecture theatre by means of an IP network camera. This facility enhances the substantial pedagogic value of this teaching tool.

The equipment features have been further developed and it has been successfully applied to the experimental study of various earth dam cross sections in order to highlight the operating principle and the complementarity between several seepage control devices that are frequently used in the design of real structures of this type, namely drainage blankets, toe drains, chimney drains and low permeability central cores (Fig.1) [2]. A good match was achieved between experimental and numerical results.

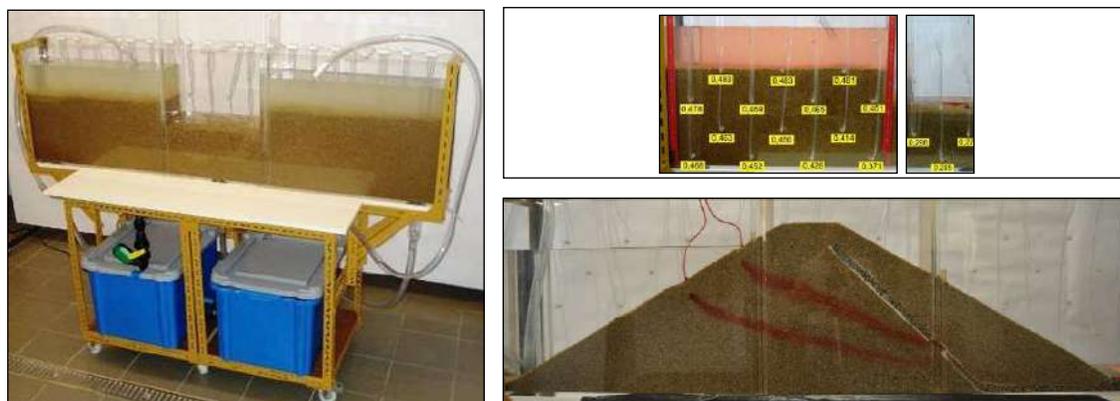


Figure 1 – Overall view (left). Total head value comparison (top right). Flow line visualization.

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# Using ultrasound technology to analyse cement based materials.

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This report presents the research work done by civil engineer graduate student André Vilaça Moreira, part of a research grant, BII, given by the *Fundação para Ciência e Tecnologia, FCT*, carried out at the Laboratory for the Concrete Technology and Structural Behavior, LABEST, of *Faculdade de Engenharia da Universidade do Porto, FEUP*, from October 2009 until October 2010, under the supervision of Dr<sup>o</sup> João Rio.

The research focused on the non-destructive analysis of cement based materials such as concrete, mainly through the use of ultrasound technology on mortar and grout specimens. The main objective was to relate ultrasound pulse wave velocity with the mechanical properties of material, such as its Young modulus, mechanical resistance, porosity and others.

The tests were done with an ultrasound pulse wave generator, which send pulses from the emissary to the receptor, through the analyzed material, and measure the travel time of the wave.

The work was divided into the following three parts: 1 - study of existing of ultrasound technology with theoretic foundations and practice tests; 2 - study of mortar and grout mixes; 3 - parametric experimental program. There was also the use of electric resistivity technology, because these tests allow the determination of mechanical properties comparable to the ones obtained by the use of ultrasound tests.

In spite of some changes of the experimental program during the work, the main results were as expected and enough to satisfy an initial research work. It was clear that some correlations between the measured and the calculated properties of the specimens were possible.

Key-Words: cement based materials, concrete, ultrasounds, Young's modulus, Mechanical strength, porosity.





**U.PORTO**

*Parallel Oral Sessions IV*

**A5** *Engineering V*

# Vanillin from Kraft Lignin production in paper pulp mill industry

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This work was developed within “Product Engineering” discipline, which has as purpose the creation of a new product based in chemical product design steps [1], and its main goal is to create a package process to enable the utilization of lignin presented in waste black liquor to produce vanillin by the kraft process in paper pulp mills.

Vanillin is the major flavour constituent of vanilla. It has applications in food industry as a flavour agent and in perfumery as an additive. Natural vanilla beans cannot fulfill the market needs so it is also produced synthetically.

For synthetic vanillin there are different chemical routes like guaiacol, sulphite or Kraft process. Guaiacol is petrol based process but still is the most commonly used; about 85% of vanillin is produced by this process and only 15% from lignin. However, the lignin process is a valuable one because it allows the recycle of waste liquors. Vanillin market demand is of 16 000 tons/year and costs near 15 \$/kg.

Lignin is a complex hydrocarbon molecule which can be found in high concentrations within the wastes of paper pulp mills. The main current use of black liquor is energy generation by incineration and conversion to electricity.

Based on previous laboratory studies [2] it was investigated the possibility of production of vanillin by paper pulp mills. It would take two main steps: Reaction and Separation.

Black liquor enters in a continuous bubble structured packed reactor, where it is processed and follows into an ultrafiltration system to separate the vanillin. The permeate product stream from the previous unit involves sodium vanillate which needs an ion exchange process to lose the sodium and receive a proton in return. Then a reverse osmosis unit is needed to extract excess water. Finally the product stream enters in a vacuum continuous crystallizer associated to a filter press and pure vanillin is obtained.

A packaged process was designed concerning the steps described above and all the machinery and manpower needed for its execution.

Economic evaluation was done regarding different business plans according to different paper pulp mills’ needs.

Even with the low conversion of lignin to vanillin, with an efficient separation process, vanillin production from black liquor in a conventional paper pulp mill appears to be possible and also feasible.

## References:

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# New sources of renewable energy: High Altitude Wind

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Wind energy derives from the sun radiation over the earth. Wind is created by displacement of air masses giving rise to kinetic energy and has its origin in the differential heating/bounciness of the air. This kinetic energy can be transformed into mechanical energy and then in electricity.<sup>[1]</sup>

Over thousands of years, wind energy was used to power the sailboats, grind grain in the traditional windmills and pumping water. The great development of modern wind turbines used for generating electricity on a large scale begun on 1980s.<sup>[2]</sup>

Portugal has a great potential for producing electricity from wind, since it is a mountainous windy country. However, the wind is not regular and often at around 4 o'clock in the morning it stops for restarting again in the morning. This is not the only problem of the conventional wind turbines, as one would expect the potential sites for placing windmills to become scarce. So how to increase the installed capacity without increasing the installation costs and in order to capture as much wind as possible?

The offshore systems are now being proposed since they are expected to capture more regular and stronger winds. However, they are only economically viable for shallow waters. At around 10 km of altitude winds blow regular and strong and researchers are now studying how to take advantage of this energy. One of the proposals considers a flying turbine bonded to the place using special high strength low weight electrical cables. Systems such as Sky-Windpowers, Magenn System (MARS), Rotokite, Kitegen Kite and Glider are examples of these new technologies. [3].

The high-altitude wind energy is still in prototype stage, has not yet been implemented and it is impossible to predict how effective or how well will run in the real world, and their costs remain speculative.

This work describes MARS technology, giving details about its advantages and weakness.

**Keywords:** Wind, Offshore, High altitude.

[1][http://www.energiasrenovaveis.com/Area.asp?ID\\_area=3](http://www.energiasrenovaveis.com/Area.asp?ID_area=3)

[2]<http://www.worldchanging.com/archives/011530.html>

[3] <http://www.theepochtimes.com/n2/content/view/41694/>

# Bio-ethanol Production from Brewery Spent Grains

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This work aims to study the process of bio-ethanol production from brewery spent grains (SG) or crushed malt. These are mainly composed by barley grain husks, some lipids and proteins that are retained in the process of wort filtration after the barley malting and mashing processes for the brewing of beer. This residue is the major by-product of brewing that can represent more than 60 thousand tons a year in one industrial process. Given its high content of polysaccharides and cellulose, the usual destination of SG has been the manufacture of animal feed [1].

Spent grains are considered a lignin-cellulosic material, comprising approximately 20 % protein and 70 % fiber [2, 3], including cellulose, arabinoxylans (hemicelluloses) and lignin, but also have a considerable amount of lipids. Since cellulose is an essential raw material for the production of fermentable sugars to ethanol, it is possible to perform an enzymatic hydrolysis of cellulose contained in the spent grains and then perform the fermentation of sugars for producing bio-ethanol. The advantage of this biofuel is that it is not competitive with the human food and provides an alternative and environmentally acceptable destination for this brewery by-product.

The experimental procedure involved testing different steps such as the acid and basic pretreatment, hydrolysis with different enzymes, fermentation of sugars and determinations of the conversion of hemicellulose and cellulose fractions to glucose, xylose and arabinose and the conversion of these sugars to ethanol.

Results of this work show the best pretreatment (acid or alkali) of SG for obtaining higher amount of sugars after hydrolysis. The sugar profile was determined by HPLC. The hydrolysis and fermentation performances were also optimized using *Viscozyme*<sup>®</sup> L, *Ultraflo*<sup>®</sup> L, *Glucanex*<sup>®</sup> 100 G, *Novozym*<sup>®</sup> 50010, *Novozym*<sup>®</sup> 50013, *BAN*<sup>®</sup> 480 L and the yeast *Saccharomyces cerevisiae*.

## Acknowledgments:

Financial support of UNICER is acknowledged.

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# Biodiesel Production and Treatment of Brewery Wastewater by Microalgae

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Using microalgae for wastewater treatment seems to be quite promising for their growth combined with biological cleaning [1]. At the same time microalgae provide a means of expanding and exploring photosynthesis getting a way to produce biomass that can be used for biofuel production, as feed in aquaculture, or as fertilizer in agriculture. Microalgae show several advantages over conventional activated sludge processes for which there is associated high energy consumption for aeration and high costs for the subsequent sludge management processes. Also, by removing nitrogen, phosphorus and carbon from water, microalgae can help reducing the eutrophication in the aquatic environment [2].

This work aims to use microalgae for the treatment of a brewery wastewater, having in mind their utilization as feedstock for biodiesel production. For this purpose three microalgae species were studied and cultivated under mixotrophic growth conditions in a synthetic brewery effluent (used as culture medium): *Chlorella vulgaris*, *Scenedesmus obliquus* and *Haematococcus pluvialis*. The growth rate of these algae was determined through biomass dry weight concentration and the corresponding culture's absorbance variation in time. Also, since one objective is the wastewater treatment by removal of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{PO}_4^{3-}$  by making algae to grow using these water contaminants as nutrients, the variation in the amount of these components and also of chemical oxygen demand (COD) have been followed in time. After growth the microalgal cells were harvested by centrifugation and the total lipids were extracted from the fresh biomass using a modified method of Bligh and Dyer [3]. Then, the total lipids content was determined. After extraction, the fatty acids were analysed using a modified method of Lepage and Roy [4] and the resulting methyl esters of fatty acids were analysed with a gas chromatograph.

As a result of this work we have selected which of the three microalgae species is the best one to treat this kind of effluent and produce biodiesel.

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# On the optimization of a wind tunnel by CFD

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Clothing consumers are becoming more demanding about products quality and associated comfort. In order to meet this demand it is important to improve techniques and methods to evaluate the performance of products in representative conditions.

The clothing thermal performance can be evaluated using thermal manikins and climate chambers (Fig. 1). The former allows the monitoring of heat losses across the garments whereas the latter allows the precise control of the test conditions (temperature, humidity and air speed).

The purpose of this project is to adapt the geometry of an existing climatic chamber to allow the imposition of wide range of air speeds [1-2]. Therefore, a computational tool (COMSOL Multiphysics 3.5) was used to simulate numerically the fluid flow inside the chamber, in order to identify ways to homogenise the velocity profile inside a “wind tunnel” to be placed in the chamber interior.

Several parameters were studied, namely the test zone position, the shape of the climate chamber corners and the wind tunnel geometry. The wind tunnel was found to assure more symmetrical velocity profiles when placed at the chamber centre, whereas the chamber corners were found to have negligible influence on the velocity profiles at the test zone and. The shape of the wind tunnel contraction was optimized (Fig. 2).

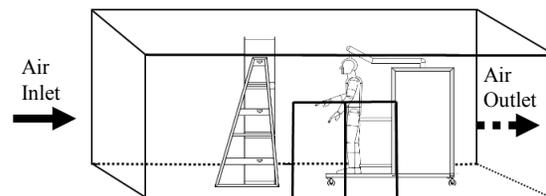


Figure 2 - Climatic chamber, dummy and fans

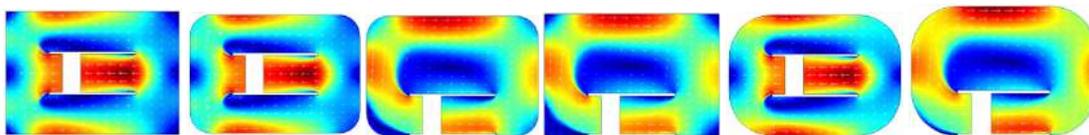


Figure 3 – Influence of the climatic chamber’s geometry and wind tunnel position on the flow characteristics (velocity distribution on the climatic chamber - transversal cut)

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# Numerical study on the heat and mass transfer through a sock

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Textile fabrics perform differently when submitted to different conditions, for instance regarding the exposure to high relative humidity that induces water sorption in the fabric.

A numerical investigation was carried out using COMSOL Multiphysics 3.5a to characterise the heat and mass transfer across a textile sock, exposed to a moist air layer (Fig. 1a). The description of the sock properties was based on a volume average method (Fig. 1b). The sock domain includes textile fibres, bounded water and gas phase within the pores. Heat transfer by conduction and convection were considered whereas mass transfer was implemented based on diffusion and convection mechanisms.

The influence of such parameters as velocity, temperature and relative humidity of the air layer on the heat and mass transfer across the sock was studied. The effect of some fabric properties, such as its density, thermal conductivity, dry solid fraction and tortuosity were also investigated. Both the dry solid fraction and tortuosity were found to have the most significant influences. A decrease of 82% was observed in the mass removal from the skin, for the dry solid fractions considered in the study (in the range 0,25- 0,45). For tortuosity values in the range 1.5- 4, a reduction of 85% was obtained in the mass removal from the skin.

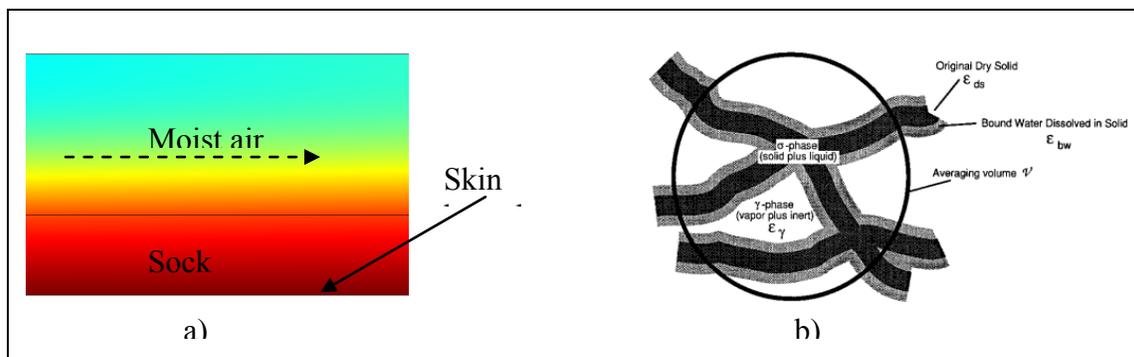


Fig. 4: a) Temperature distribution along the sock and moist air and b) representation of volume average method approach [1]

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# Can footwear moisture management performance be assessed in a reproducible way?

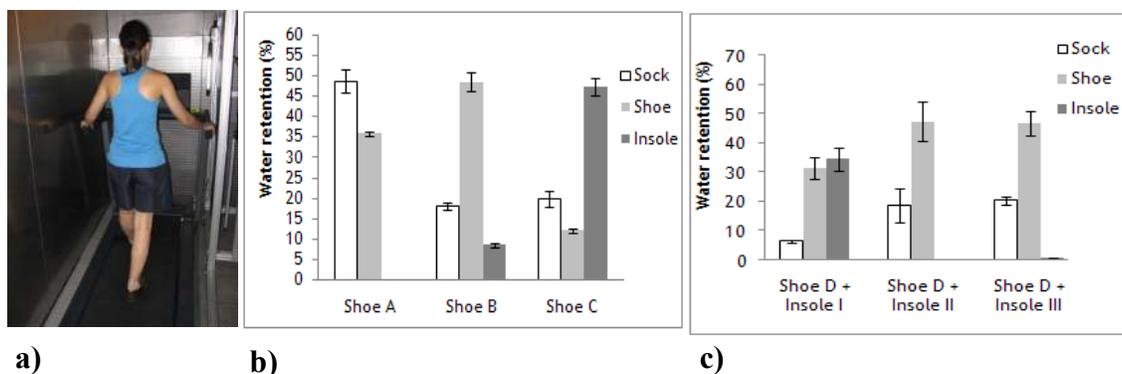
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Nowadays the consumers are increasingly demanding about the need to have very comfortable shoes. Foot comfort is determined by the interaction of socks, soles and shoes. The comfort of the footwear is the result of a complex interplay between various factors affecting the function of the foot during human activity. One of the main factors to consider is the presence of moisture in the shoe and its moisture management performance. The moisture management performance of footwear is related to its ability to transport water from the inside to the outside. Given that the user movement enhances the heat and mass transfer inside the footwear, it is important that the assessment of the footwear performance regarding moisture management be done in dynamic conditions [1-2].

In this project, an experimental (wear-trial-based, Fig. 1a) procedure was devised to compare the performance of different types of footwear regarding moisture management. Several parameters were taken into consideration, which resulted in a set of indexes characterizing the ability of the footwear to absorb, transfer and evaporate moisture. The experimental procedure was found to produce reproducible data on the mentioned parameters. The method can thus be used to compare different types of shoes (Fig. 1b) and insoles (Fig. 1c).



**Figure 1-** a) Image during one test; b) Water retention (%) at the sock, shoe and insole for 3 types of shoes and the confidence interval ( $\alpha = 0,05$ ); c) Water retention (%) at the sock the shoe and insole for 3 types of insoles used on the same shoe and associated confidence interval ( $\alpha = 0,05$ ).

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**U. PORTO**

*Parallel Oral Sessions IV*

**A6** *Architecture*

# To Invent the Future and/or Rediscover the Past

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Keywords: architecture, ecology, sustainability, technology, vernacular

It is not the strongest of the species that survives, not the most intelligent that survives. It is the one that is most adaptable to change. [1]

The aim of this reflection is to discuss the framework in the field of thought and contemporary architectural project, to highlight the dichotomy between the technological emphasis of architectural production and the nature of vernacular architecture, and to take the perspective of sustainability as a factor in cultural development.

Nowadays, few words are more fashionable than *sustainability*. We are used to listen *it* in a political speech or in an economic report, but it is worthless if the concept is not expressed. The approach to sustainability in architectural design is still delicate and can be a very controversial issue (because of all the implications) in terms of conceptual and methodological approaches.

John Ruskin thought about human identity in a world dominated and destroyed by the machine, Frank Lloyd Wright worked on the reconciliation of nature and architecture, and Walter Gropius was involved in streamlining the construction process. ¿To develop an ecologically sustainable architecture will be necessary to *Invent the Future or Rediscover the Past?*

Clearly sophisticated technology is created in a number of countries on the basis of the already existing scientific and industrial infrastructure, in order to deal with specific climate conditions. However, low level energy design techniques can learn from the traditional architecture and a great number of techniques used in the past can be improved with actual knowledge and advanced technology of today.

The second part is the analysis of case studies of projects and architectural objects that stand out positively in relation to the theme, their strategy, intent of the concepts and of the proposed materials.

I expect the reflection and the promotion of drawing tools, which allow to the architect to know where to get and how to use the applications of energy principles.

[1] Darwin, C., *The Origin of Species*.

# Computer Application for Architectural Competitions: an opportunity for public participation in the Information Society

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Architectural competitions are an opportunity to promote critical thinking in the discipline and interaction between the players involved in the process, namely: the client, the architect and the general public. Only with a proper communication can this interaction foster a better quality of the built environment.

In our society the networks and the computers are essential for allowing the universality of information in all disciplines and in social, economic and political activities. The Internet with its ability to handle numerous types of interactive multimedia content, and the increasing capabilities of computers and mobile devices are enhancing the desire for global communication and user-generated content. These technologies can and should be used to allow a better understanding of the architects' designs by a public less capable of reading the traditional representations of architecture.

Our work tries to highlight this context of opportunity and, by studying and experiencing existing applications in the field (from both user and developer perspectives) to point ways for the future development of our application.

Our main objectives were: a) to define an analytical framework comprised of several themes and indicators that emerged from our literature review and to translate it into a matrix; b) to fill that matrix with variables coming from the study of existing applications in order to inform the application's requirements specification.

We've been able to identify, besides other things, the existence of four essential themes (users, structure, interface, contents) and their respective indicators, as described below:

- 1) In "users" we have determined the benefits of a community-driven platform in order to promote interaction and the sense of belonging; the need to structure those users into groups to facilitate communication and information flow; the integration of several authentication methods to offer both immediate and responsible ways of interaction.
- 2) In "structure" we have realized the need to create a modular system providing the users with the flexibility to turn on/off features and functions while maintaining the application's coherence as a whole and reducing its maintenance efforts.
- 3) In "interface" we have concluded on the need to offer a simple yet customizable interface and the need to offer a coherent and accessible interface in order to reach a wider population.
- 4) In "contents", we determined the need to communicate different and complementary multimedia contents in order to promote a better communication of the designs' ideas and forms, and the ability to provide flexible search mechanisms.

Finally, by analyzing the variables we've obtained, we have also concluded the existence of several "types" of applications for architectural competitions, ranging from those more informative/less interactive to those more interactive/communicative.

# Spatial and Functional Analysis of Asprela Campus: A Space Syntax approach

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The present research, titled “Spatial and functional analysis of Asprela campus: A space syntax approach”, will lead to a dissertation[1] of the “Mestrado integrado em Arquitectura”.

The aim of this project is to assess, in spatial and functional terms, the performance of buildings and urban plan of "Pólo da Asprela", one of the three campuses of University of Porto. The space analysis is conducted using the theoretical and analytical framework known as Space Syntax, developed in the 70's by Bill Hillier and Julienne Hanson's Bartlett School of Architecture, University College of London.

Firstly, the project will define the spatial context and current functioning of the areas in question, identifying characteristics, main purposes and existing dysfunctions. Afterwards, the configurational analysis will be carried out on Space Syntax software and the research will focus in local structures but also in the global integration of the campus within the urban fabric. The results obtained by this method will be correlated with architects and planners purposes and statistical investigation such as movement flows, intending to express the benefits arising from the use of this technique as a support for urban and architectural design.

The goal of syntactic theory is the creation of knowledge about space, demonstrating the use by societies of configurational properties as their primary tool to organise themselves. Current concerns about architectural and urban space morphology appear as insufficient for the creation of a framework capable of creating a procedural methodology aware of social and behavioural impacts of urban plans and architectural projects. In this context, space syntax appears as a theory that allows spatial analysis through rigorous values, enabling a correlative and empirical study on social behaviour and spatial morphology. In this technique, space is perceived through gaps and limits that restrict movements or visual fields, these properties are then analysed by mathematical procedures. This method is not based on apparent traditional features such as geometric or metric values, but extracts configurational space data considered the "genotype" of space.

It is intended that the findings and conclusions of the analysis will allow an intensification of present uses, and fix any malfunctions identified in the study area, in order to promote their optimisation. It is expected that these evaluations will be useful for both understanding of the current experience in the area as for the weighting of the potential for transformation.

## **PP N°97: Architectural modeling of components in composite materials for temporary shelter facilities**

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<sup>1</sup> Faculty of Engineering - MDI, <sup>2</sup> Faculty of Architecture, <sup>3</sup> INEGI, University of Porto

The PP97 had, for its object and title, the "architectural modeling of components in composite materials for temporary shelter facilities", and its development comprised several disciplines (architecture, design, engineering, etc.). The results and targets included a computer modeling and a prototype with its components, as well the strategies outlined for their manufacture and distribution. Overall, the study was structured into three areas or components - "project", "manufacture" and "analysis / dissemination" - which were developed interactively in two phases.

The area of research "project" involved the conception / designing of installation for temporary shelters facilities, its components, configurations and conditions of assembly by the user. The relevance of the proposed solution was thus identified and in order to be used for instance in the context of social action. The concept of the product, based on the idea that the construction materials could reuse abundant and wasted materials for the production of the temporary shelters by applying expeditious construction techniques was also defined. The study was carried out by, at first, a formal and geometric speculation, followed by the accomplishment of designs with all the necessary detail, and later, studies with 3D computer modeling.

This was preceded by the accomplishment and coordination of computational models with 3D CAM in the area of research "manufacture". Production techniques with composite materials, which enabled collaboration with INEGI, were studied, and several series of experiments were performed. These were later and more swiftly continued at the FAUP. For their relevance, composite materials consisting of waste (rubble, etc.) and binders (polymers, etc.), as well as additive and subtractive techniques of rapid prototyping with milling and "MakerBot" machinery's model / formwork were selected. In accordance with what was expected, prototypes of the shelter facility and its components were accomplished, which tried to simulate the conditions existing in-situ for the manufacture and assembly. Collaboration with the workshop of printing techniques of FBAUP has been also developed, exploring the graphic treatment of the installation components and envisioning ways of appropriation by the user.

The final stage consisted of a brief "analysis" functional and aesthetic, which, for example, enabled collaboration with designer Dirk Lloyens of ESAD / UM, comprising an environmental analysis of light and heat. In parallel, strategies related to the "spread" of the investigation of PP97 and its products were outlined. On one hand, the discussion of an "application" that uses the channels as a means of social or marketing, etc. On the other hand the "advertisement" in both scientific and general contexts. This fostered, in the first case, collaboration with the Porto GAS group, including the carrying out of investigations and the outlining of lines of action. In the second case, presentations and an exhibition at "Galeria dos Leões" were carried out (RUP).

In short, the investigation conducted allowed for the projection of a versatile installation for temporary shelter facilities, composed of several architectural components, modeled with the computer support of CAD-CAM. The established process for manufacturing these components included mold / formwork and rapid prototyping, as well as composite materials made with matrix and specific binders. Finally we have developed an analysis of the product and outlined the strategies for its spread and hypothetical commercialization.

# Interactive Architectural Surfaces, Modelled in Composites Materials

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The Multidisciplinary Project no. 99 (PP99), entitled *Interactive Architectural Surfaces Modelled in Composites Materials*, will explore built surfaces that enable and encourage a multi-sensory interaction with the inhabitant of the architectural space. The PP99 has been developed by a team comprising by people from FAUP, INEGI, IPL, FCTUC and among others, myself. In this communication, I wish to share some aspects related to my experience and participation as a junior researcher in PP99.

It is important to mention that, in this project, more than discovering materials and singular systems, the main goal is to apply the already existent resources, which allow the exponentiation of the idea of a more and interactive architectonic. At the end, we expect to obtain a prototype of a section of that area or architectonic vestment, which is preferably modular in order to be adapted to different contexts and spatial configurations.

Despite the very recent beginning of the PP99, it has already developed the study of different existing interactive projects, as well as a compilation of a bibliographic ‘state of art’. The first point to take into account is that most existing projects are mainly related to the visual sense, neglecting all others. However, it is known that other senses are equally important to our sensorial perception. For example, the sense of smell arouses emotions, and the auditory and tactile senses are very important in the seizure of a space. The architect Juhani Pallasmaa, even defends that “when experiencing a multi-sensory architecture, the qualities of matter, space and scale are measured at the same time by the eyes, ears, nose, skin, tongue, skeleton and muscles”. The PP99 wishes to develop itself in the previous referred to perspective. On the other hand, one such a goal will require some applications and developments – including hardware, software and materials – targeted to build the structure and the interactive interface of the surface.

The referred structure will be built with composites materials and its project and modelling will call upon some CAD software in articulation with CAM, as well as several tools and Plug-ins.

As far as the stipulation of the interactive interface system is concerned, so far, only the phase of collecting and surveying the existing potentialities and the capable use and interest for the PP99 was held.

We will study software, hardware, programming languages and Plug-ins likely to eventual use, in a universe that, for the time being, includes: tracking, Eye tracking, SynthEyes, ActionScript, Processing, C, C++, ReacTable, Lightwave, Ableton, etc. In parallel, it will be important to study specific computational-electronic devices, capable of any application within a universe that, for the time being, includes: sensors (light, sound, temperature, presence, motion and proximity), arduino, cameras, projectors, mechanical structures, lamps, leds cell end plate, flip dots, kinects and motors, etc.

A very comprehensive survey has been conducted, (it will not be necessary, in the future, to employ all the examples shown), which will soon be the matter of an assertive selection. As it was initially mentioned, along the project’s run an architectural surface will be explored, modelled and projected in composites materials. This should have aspects of interaction “out(put)” and “in(put)” with the user, and enable experiments that involve multiple senses beyond the visual one.

[1] Pallasmaa, J. (2005) *The Eyes of the Skin: Architecture and the Senses*, (p. 25).

# Technical and scientific analysis of Brasiliana Guita and José Mindlin Library building

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**Objectives** | The objective of this research is to elaborate a didactic information of the stages of idealization, creation and construction the Brasiliana Guita and José Mindlin Library for use in classrooms at architecture and engineering schools. Besides registering the various stages of infrastructure construction and some technical details, it contains essential references related to the entire work. For this, we have collected information from the professionals involved in the project, merging their testimonials with images of work in progress. Thus, it is possible that the results of this research will also be of interest to other public areas.

**Methodology** | The methodology of this project consists of the revision of the literature on the constructive processes involved in the Brasiliana | USP, through the work reports. We made a technical approach on the project stages, from the conception to the program of necessities, the activity of leveling the ground, the architectural project, the structural project and the construction innovations involved. This process was accompanied by interviews with the professionals involved. All material obtained had the support of the Laboratory Video FAU-USP whose technical team has extensive experience in the production of documentaries on topics related to Architecture, Urbanism, Art and Environment. Brasiliana | USP has an important role on Architecture in Brazil due to its symbolic architecture that applies modern but simple techniques on the construction process. Its implementation, the use of classification, homogenization, interaction-project work, that all makes the project a model to be studied and disseminated.

**Partial Results** | The interviews were conducted with architects responsible for the architectural project (Arch. Rodrigo Loeb and Arch. Eduardo de Almeida), the collection donator (José Mindlin – in memoriam), the professional in charge of the program of necessities (Arch. Milton Braga), the coordinator and creator of the Brasiliana | USP (Dr. Istvan - in memoriam), the responsible for structural project (Eng Heloisa Maringoni) and the responsible for the project of fire safety (Prof. Rosaria Ono). These interviews were transcribed and the film it, and are currently being edited to make, along with images of the work, the DVD of the research project in question. The work, which is currently in the process of coverage, have been performed and recorded the stages of foundation, levelling of the ground, concrete-to structures and erection of steel structure for receiving the metal roofs.

**Conclusions** | The construction of the building has very particular aspects of project and implementation that should be recorded for dissemination among professionals and students and serve as a model for future projects with similar objectives. Therefore, besides the immense contribution to the Brazilian literary world with the access to BRASILIANA | USP, the project also brings with it an immense collaboration to the technical-scientific community that, with its conclusion, can be disseminated among universities.





**U. PORTO**

## *Parallel Oral Sessions V*

**A1** *Biological, Environmental & Health Sciences VIII*

# Leap of Faith: Understanding the Relationships between *BRCA1* and Estrogen Receptor in Hereditary Breast Cancer

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Hereditary breast cancers due to genetic predisposition account for 5 to 10%. They were shown to be essentially related to germline mutations in one of the cancer susceptibility genes, *BRCA1* [1]. Although *BRCA1* was cloned over 10 years ago, it is only in the last few years that significant progress has been made towards understanding its role in cells (DNA repair, cell cycle) [2, 3]. The loss of heterozygosity is systematically observed in the tumors. Understanding this mechanism and its consequences is essential to determine its role in tumor appearance and progression. Moreover, it is known that the estrogen-receptor (ER) signaling pathway has also a main role in the genesis and progression of breast tumors, so interfering with this pathway is becoming an attractive therapeutic approach [4].

As the tumors due to loss of heterozygosity of *BRCA1* are usually negative for ER expression, the main goal of this study was to investigate a possible interaction between *BRCA1* loss and ER pathway. Some estradiol (E2) stimulations were done to see the effects on the cell lines. We also performed the knockdown of *BRCA1* in different cell lines to characterize eventual changes (loss of ER, cell proliferation, cell viability).

To start this work, MCF-7 (breast cancer cell line) were cultured in medium without phenol red supplemented with 10% charcoal-dextran treated serum (free from estrogens). This removes any kind of stimulation of the ER signaling pathway. We used the pS2 expression as a reporter because it is an estrogen-induced protein. After testing different concentrations of E2, we found that 10nM were sufficient to induce pS2 expression. When MCF-7 were cultured in the free-estrogen media for 72h, we showed by immunoblotting that pS2 protein was gone. When we stimulated by E2 for 24h, we were able to restore it. Interestingly, we showed that when pS2 was back, a decrease in ER expression was observed.

Then, we used MCF-7 and HeLa (cervical cell line) to follow the consequences of *BRCA1* knockdown. We optimized the transfection efficiency, and observed that more than 50% of the cells have lost *BRCA1* expression at 48h post-transfection. The next step was to measure the effects of *BRCA1* depletion for 6 days by immunofluorescence, immunoblotting and cell counting to evaluate cell proliferation and viability. The results showed that transfected MCF-7 stopped proliferating while HeLa cells grew normally, even in the absence of BRCA1 protein. The cell viability was also decreased for MCF-7 after transfection in contrary to HeLa cells.

It will be interesting to see by immunoblotting if we have any decrease of ER expression after BRCA1 knockdown. And, as BRCA1 seems to be essential for the growth and survival of MCF-7, we will need to do the same experiment while we are stimulating by E2 and determine if the consequences are the same or not. This study will help drawing conclusions about what is happening in the tumor genesis to understand the order of events between loss of BRCA1 expression and loss of ER expression in the breast submitted continuously to hormone stimulations.

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# Effect of Quercetin and Epigallocatechin Gallate upon Glucose Transport in a Breast Cancer Cell Line: Implications on Cell Metabolism and Survival

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Breast cancer is the most common type of cancer amongst women in Western Europe and North America and is the second leading cause of cancer deaths in this population [1]. Tumour cells, when compared with normal differentiated cells, have higher glucose uptake and metabolism which are positively correlated with malignancy and tumour cell invasiveness [2]. Nonetheless, knowledge on glucose uptake and metabolism in breast cancer cells is still largely unexplored. Considering that the flavonoids quercetin (QUE) and epigallocatechin-3-gallate (EGCG) inhibit glucose uptake by placental cells [3], the aims of this work were to characterize glucose uptake by the human breast cancer cell line MCF7 and to investigate the effect of the flavonoids QUE and EGCG upon glucose uptake, metabolism and glucose transporter 1 (GLUT1) gene expression and upon MCF7 cell viability and proliferation. To do this, we characterized <sup>3</sup>H-2-deoxy-D-glucose (<sup>3</sup>H-DG) transport in MCF7 cells. Then, we investigated the effect of QUE and EGCG upon <sup>3</sup>H-DG uptake, GLUT1 gene expression, glucose metabolism, cell viability and cell proliferation. Our results show that <sup>3</sup>H-DG uptake by MCF7 cells was: a) time-dependent, b) saturable, c) sodium-independent, d) inhibited by the GLUT inhibitor cytochalasin B, and e) partially stimulated by insulin. Our results also demonstrate that <sup>3</sup>H-DG uptake by MCF7 cells was markedly inhibited by QUE and EGCG in a concentration dependent manner. Moreover, both flavonoids acted as competitive inhibitors of <sup>3</sup>H-DG uptake, in the short-term (26 min) exposure. Unexpectedly, a 4h-exposure to either flavonoid increased GLUT1 expression in MCF7 cells. With respect to glucose metabolism, both compounds markedly reduced lactate production by MCF7 cells, both after short- and long-exposure. In addition, a 4 h-exposure to QUE or EGCG was shown to decrease MCF7 cell viability and proliferation more markedly when glucose was available in the extracellular medium. We can conclude that <sup>3</sup>H-DG uptake by MCF-7 cells is mediated by members of the GLUT family of glucose transporters. Also, QUE and EGCG potently impair <sup>3</sup>H-DG uptake and metabolism by this cancer cell line and these effects may contribute to the negative impact of these compounds on MCF7 cell viability and proliferation rates.

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# Genetic alterations in Papillary Thyroid Carcinoma and its association with clinicopathologic features

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Thyroid cancer is the most frequent endocrine neoplasia, and papillary thyroid carcinoma (PTC) the most common type (85-90%). Presents a good rate of survival at 10 years (90-95%), but 5-20% of patients will experience recurrences and metastasis [1,2].

BRAF mutations can be found in PTC with a prevalence of 36 to 69%; this mutation does not co-exist with RAS mutation. However, the role of BRAF mutations in metastatic potential of PTC has not been proven. Is also described that patients with carcinomas with BRAF mutations were significantly older than patients with tumors without mutations in BRAF [3,4,5].

The high prevalence of BRAF mutations, as well as the involvement of RAS mutations and RET rearrangements in the majority of PTC, suggesting a fundamental function of alterations in RET/RAS/BRAF/MAPK signal transduction pathway in the etiopathogenesis of PTC.

In this study, we intended to evaluate the prevalence of genetic mutations in BRAF and NRAS in a series of PTC and their nodal metastases and verify the association between the presence of mutations in these genes in primary tumor and its metastasis.

The series was composed of 58 cases of thyroid carcinoma and their nodal metastases. We performed DNA extraction from paraffin material and gene regions of interest of BRAF and NRAS were separately amplified by PCR, were then subject to automated sequencing.

We found that the observed frequencies for the BRAF mutation were consistent with those previously described (40%) as the low prevalence of NRAS mutations observed in this series (3,8%). In this study, we conclude that the existence of BRAF and NRAS mutations is not related to the dimensions of the primary tumor. Regarding the age of the patients it was found that mutations in the BRAF gene were present in older individuals. It was observed that 25% patients had discordance between the presence/absence of BRAF mutations in the tumor and its metastasis. To emphasize the discovery of a mutation in BRAF was not described in the thyroid: a *missense* mutation L597Q.

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# The Role of Mitochondrial Dysfunction in the Acquisition of the “Warburg Effect” in Tumour Cells: insights into cancer metabolism

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The “Warburg effect”, also known as aerobic glycolysis was initially described by Otto Warburg who reported a high consume of glucose and release of lactate by malignant cells comparing to non-neoplastic cells in presence of oxygen. Warburg hypothesized that this aerobic glycolysis was irreversible and due to an impairment of mitochondrial respiration [1]. The causal relationship between this property of cancer cells with cancer progression remains unclear. Many models have been advanced and one proposes that mutations in oxidative phosphorylation (OXPHOS) genes lead to mitochondrial dysfunction, being responsible for the acquisition of the “Warburg effect” [2]. However, the latter hypothesis has not been consistently evaluated.

The aim of this work was to establish cellular models of mitochondrial dysfunction caused by mutations in the mitochondrial DNA (mtDNA). We also wanted to demonstrate that these models present the “Warburg effect” and this is essential for the progression of tumorigenesis. We made use of the cybrid (transmitochondrial hybrid) technology, that allows the analysis of mtDNA mutations against a common nuclear background (143B osteosarcoma cells) [3]. We have constructed a cybrid cell line harbouring a mutation in the mtDNA gene for tRNA<sup>Leu</sup>(UUR).

We analysed the metabolic phenotype of these cell lines, by assessing the fluxes of some metabolites across the plasma membrane and the expression of some key enzymes in bioenergetic pathway. We have concluded that the mtDNA mutation alters the cellular metabolism, increasing glycolysis. To assess the influence of the mtDNA mutation and the “Warburg effect” in tumourigenesis, these cybrids were analyzed for markers associated to tumourigenesis such as cell growth, cell death, and motility and migration. Although mutant cybrids showed no differences in the population doubling time, they displayed more resistance to apoptosis and more motility and migration ability. Finally, by injecting both wild-type and mtDNA-mutated cybrids in nude mice, we saw tumours formation only in mice injected with mtDNA mutated cybrids; in addition some mice already displayed invasion and metastasis, clearly demonstrating the tumourigenic potential of this specific mutation in vivo.

Although these results are yet superficial, they show that mitochondrial dysfunction caused by mtDNA mutation results in altered cellular metabolism and is associated with an increased tumourigenic potential.

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## Folate availability effect on adipocyte life cycle and metabolism

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The role of folate in obesity and metabolic syndrome is beginning to be investigated but is far from being fully understood. Many studies have drawn attention to the association of folate status and plasma homocysteine levels [1], an established independent risk factor for cardiovascular disease development. Nevertheless, epidemiologic data had also recently shown an inverse association between serum folate and body mass index [2]. However, there is no straight evidence about the effects of folate on adipose tissue, therefore, the aim of this study was to investigate the effect of cellular folate availability on preadipocyte proliferation and differentiation.

Proliferation of 3T3-L1 preadipocytes was evaluated by sulforhodamine B staining and methyl-<sup>3</sup>H-thymidine incorporation, after 24h or 48h of treatment with methotrexate (MTX, 0.1 and 10  $\mu$ M), an inducer of a low cellular folate status. Differentiation of preadipocytes was induced in the presence or absence of MTX, and adipogenesis was determined by measuring lipid accumulation after staining with oil red O [3].

Preadipocytes treated with MTX for 24 and 48h presented reduced culture protein content and showed a decrease on methyl-<sup>3</sup>H-thymidine incorporation, in a time and concentration-dependent manner ( $P < 0.05$ ). MTX treatment, in adipocytes, increased lipid accumulation and the effect was much more pronounced for the highest concentration of MTX used.

In conclusion, our results showed that, when folate availability was compromised, there was a stimulation of preadipocyte differentiation, but this stimulation was accompanied by a decrease in preadipocyte number. This suggests that cellular folate deprivation can interfere with adipocyte proliferation, differentiation and metabolism and promote the hypertrophic growth of adipocytes, which may contribute to the development of obesity complications and the metabolic syndrome.

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## Cellular effects and signalling pathways induced by MLK3 mutations

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Mixed lineage kinase 3 (MLK3) is a serine/threonine kinase widely expressed and involved in different physiological functions, such as proliferation, apoptosis, cell survival and cell migration. Responding to mitogens and stress stimuli it regulates MAPK signalling through activation of ERK, JNK, p38 pathways. Its kinase domain is crucial to phosphorylate and thus activate downstream targets. However, MLK3 activation is not always required to MLK-induced modulation of some cellular functions. MLK3 may indeed function as a scaffold protein, linking different signalling pathways.

MLK3 mutations were reported, for the first time, in 2010 by our group. Missense MLK3 mutations were found to be able to harbour transforming and tumourigenic potential. Two of the mutations (P252H and R799C) were found to show an invasive behaviour. Taking this data into account we generated HEK293 stable cell lines harbouring these mutations in order to identify associated cellular effects and evaluate which molecular targets were dependent of these two MLK3 mutants. We found that a mutation in MLK3 kinase domain (P252H) confers different properties when compared to a one localized in the proline-rich domain. P252H mutation seems to induce increased cell migration, while R799C seems to interfere with cell differentiation. Furthermore, we verified that both mutations regulate in distinct manner molecules previously appointed to belong to the WNT pathway. In summary, we showed that two of the MLK3 mutations previously described interfere with crucial pathways of colorectal development and in this way contribute to colorectal tumourigenesis.





**U.PORTO**

*Parallel Oral Sessions V*

**A2** *History, Visual & Cultural Studies II*

## Art through hands and wires – Tapestry in Portugal and world wide

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Art has not always been what we think it is today. An object regarded as art today may not have been perceived as such when it was first made, and even the concept of artist appeared only in 15th and 16th century in Italy. But the concept of beauty, if we can speak of it as a concept, sometimes created by the freedom of thought, other times by imposition, varies throughout history.<sup>[1]</sup> To experience the beautiful, man sets himself as the measure of perfection. This meeting is always an unique experience because it is an experience live in first instance and if the feeling is felt it becomes a exemplary feeling.<sup>[2]</sup> The history of art is also rich in its various times, from the ancient classical art to modern art and contemporary art, whose some subdivisions are difficult to perceive or feel for the common mortal little connoisseur of the genre.

What type of work of art do we desire? What type of art do we want to spend money on?

Portuguese poet Fernando Pessoa wrote: "The essence of art is to express", and what expresses a piece of arte better than a tapestry? With over one million points of embroidery derived of long hours of handicraft work, with huge traditional values. The work of craftsmanship produces pieces of unique value, created by anonymous artists, taking all the rooms they are in to an engrossing historical richness through the power of a single peace. But we still insist on filling our rooms with avant-garde that we fail to understand.

The present work aims to promote the traditional tapestry in Portugal not as an ancient art, but as an artistic modern force, full of potential.

In Europe, the carpet fever started in the 15th century, when it was the symbol of refined gentleman and an assertion of status. Carpets were the one Islamic art form known as well in the West as in the East. They were part of the material cultures of both worlds at the same time. But while the Arabs, in general, were using them on the ground, Europeans put them on all sides except where it was meant to be. Because of the increase of search for this objects, parallel businesses opened, like carpet renting shops and even black markets full of what we call now piracy of carpets. This carpets where usual made with wool and with natural dyes.<sup>[3]</sup>

The techniques of tapestry in Portugal have been developed in such a way that today they are recognized internationally. The techniques are culturally associated with the region of the country they came from. Examples are Arraiolos, Portalegre and Castelo Branco, Portugal, locations known by the name they gave to these techniques.

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## The treatises in shaping the artistic profile of a figure from Rococo bracarense

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Early Modern Europe was marked by a technical invention that would revolutionize the production and reproduction of the book: the press. The written culture has spread with great rapidity in the first half of the sixteenth century, reaching a wide geographic area. The book, true vehicle transmitter of thought, knowledge, culture and language, played the same role when he took the aspect of technical book, such as treaty. The theoretic and visual information conveyed through its pages of text and / or images contributed greatly to the construction of the artistic culture of different countries at different times, becoming accessible as a substrate for the artists and the conception of their works.

Following the trend in Europe, Portugal was one of the countries from the sixteenth century, that acquired a number of treaties from main publishing centers in Europe. This action has intensified especially during the reign of King John V (1706-1750), with increasing interest for the information conveyed by books and engravings. Some of these copies appeared in the nuclei of monastic libraries that the Monastery of Saint Martin of Tibães' library was one of the best examples at national level. Other repositories were revealed were outnumbered, but no less significant: the private libraries, some of which with copies of the best technical books that circulated in Europe. Among the private libraries emphasis to the Frei José de Santo António Ferreira Vilaça's library remarkable figure of Rococo.

The study of the bibliographic acquis of this monk artist is an exceptional situation, possible by his personal diary. The technical books that were part of it, some of which acquired in a trip to Lisbon (1774), reveal an artist persevering in updating their knowledge and artistic language but also in updating their shaping and information. At the same time reveals a knowledgeable artist of the better and more updated European treatises. A careful analysis of their achievements in the art of carving, especially the run from the 70's of the eighteenth century, shows the intersection of Italian treatises, that Frei Vilaça had only one specimen, and the French treatises, in considerable number, especially Parisians treatises edited in the first half of the eighteenth century. If the language of the Italian Baroque is present mainly in the structural elements, the language of French Rococo reveals itself more in decoration.

Thus, the artistic profile of Vilaça Frei seems to us to be strongly influenced by Baroque and Rocaille artistic forms supplied by Italian and French treatises, which the artist knew how to interpret, cross and shape in the works masterfully created, highlighting the excellence of their artistic and creative genius.

# Entrepasto Frigorífico do Peixe de Massarelos: a reflection on the possibilities of intervention in industrial heritage

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The Entrepasto Frigorífico do Peixe de Massarelos is a work of avant-garde by the architect Januário Godinho (1910-1990). It is located in the parish of Massarelos, Porto, near the right bank of the Douro River and is classified as "Property of Public Interest" since 1977. This is one of the most modern buildings of its era, both in terms of architecture, the use of materials and spaces, and of various fields of engineering (civil, electronics and food engineering), reflecting the ideological principles of the Modern Movement and industrial architecture, but it is especially a historical document. However, the historical and artistic importance of the Entrepasto Frigorífico do Peixe, even in international terms, and the fact of being covered by legal protection, have not been sufficient factors to ensure its preservation. There are currently major changes and structural problems that have led to its degradation.

This work is a reflection about the industrial heritage and possible interventions in the Entrepasto Frigorífico do Peixe de Massarelos. The investigation was conducted by consulting books, catalogs, old photographs, academic thesis and licensing and classification processes found at the archives of the city of Oporto. Several visits were made to the building with the intention of shooting or studying and confronting it with the sources used. It is intended to alert to the importance of the property, and to identify the main causes of damage and possible forms of intervention and rehabilitation of this building and other industrial structures in the city of Porto.

As there are no uniform or pre-defined solutions for the recovery of industrial heritage, and specifically for this building, it becomes necessary to consider, in addition to its value, the needs of the local community so that it adheres and collaborates on a project to the building's preservation and requalification. Considering the studied building, we chose to address the interventions by "museumification", the "reuse" and "reconversion", since they are currently the three most appropriate and comprehensive types of intervention in industrial heritage. It is not intended to indicate the best intervention in this building or do a practical analysis to each case. Rather, it is intended to indicate the main advantages and disadvantages of each of these forms, to be considered in future interventions.

# University of Porto: One Hundred Years of Architectural Heritage. A Digital Platform of Scientific Knowledge.

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The project presented is integrated in the program of the course “Seminário de Projecto I” of the graduation in Art History. It aims to develop the method and the practice of scientific research and the application of new technologies in this field of knowledge.

Being this the year of the commemoration of the first centenary of the University of Porto (1911-2011), it was chose to address scientifically the architectural heritage of this institution, with the aim of valorizing and raising awareness among the academic community and the general public. The architectural heritage of University of Porto (UP) consists of several types: educational buildings, laboratories, museums, observatories, gardens, etc. Also different are its chronology (between XIX and XXI century) and producers. It’s an objective to demonstrate the diversity of the architectural heritage of UP and its formation in time, highlighting the role of creators an artistic languages.

The first step in this research consists of identifying all the organizations belonging to UP, their history and architectural heritage. Following is the survey of the available data relating to each building, in order to establish the “State of the Art.” Completed these steps, using new technologies, we seek a means of divulgation and access to the scientific content acquired, as a concrete implementation of knowledge.

Thus, we developed a digital platform of scientific knowledge, named “University of Porto: One Hundred Years of Architectural Heritage”, based on the applications: Google Earth™ (platform), Google Sketchup™ e Adobe Photoshop™ (editor), among others. Accessing Google Earth, are marked the three poles of UP, in satellite images. Then it’s possible to click on the buildings and have access to their 3D view and a brief synopsis about them. To deepen this knowledge, there will be available a link to a website, where will be the scientific content produced by all the students in the “Seminário de Projecto I” course.

The architectural heritage in question has artistic and historical value, in the evolution of this institution, constantly changing, and the history of Architecture in Porto (4% of the buildings are classified as National Monuments or Property of the Public Interest).

The solid and thorough scientific knowledge about this subject, its organization and easy access, through a digital platform of scientific knowledge, is an asset for UP. It provides a better understanding of this century-old institution and an appreciation of its heritage.

# The museums of contemporary art .The role of the innovative architecture of museums

## Casa das Histórias da Paula Rego

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Considering that the museums of contemporary art constitute emblematic works in the 21<sup>th</sup> century architecture scene, this research aims to analyze the main questions related to this type of architecture and exposition presentation [1].

This work starts from an international framework, focusing themes, trends and reference projects, in order to contextualize the study of a representative set of museums and centers of contemporary art in Portugal [2-4], regarding situations of Casa das Histórias da Paula Rego, Cascais [5]. The study of this institution comprehends several scales, from an urban approach to the design of exhibition space, with different contents and displays. At the same time, each building is observed as part of the architect Souto de Moura [6] creative process, identifying aspects of innovation, continuity or synthesis in relation to previous projects. The study of this museum is complemented with a global interpretation, in order to evaluate the relevance of this building in the framework of Portuguese contemporary architecture [7], and its contribution to the renovation of the country's cultural heritage.

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**U.PORTO**

*Parallel Oral Sessions V*

**A3** *Psychology & Education Sciences II*

# Cognitive effects of depression: Valence evaluation, decision times, focal attention, recall, priming and repetition effects

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Depression is a serious mental health problem characterized by a number of maladaptive cognitive phenomena. Despite the growing study of the mechanisms involved, there is still insufficient knowledge on how depression affects cognitive abilities. The aim of this study was to explore the association between the level of depression in a nonclinical sample and the performance in tasks of valence evaluation, decision, focal attention, explicit and implicit memory and the repetition effects in the valence evaluation task.

The study involved 240 college students (207 females and 33 males) aged between 18 and 27 years ( $M = 20:18$ ,  $SD = 1.83$ ) and with average education of 14.09 years ( $SD = 1.07$ ). Participants were allocated to four conditions, each one corresponding to different sequences of tasks. The distribution was random and balanced by gender, age and course. Independent variables were the experimental condition to which participants were allocated and the level of depression, as measured by the Inventory of Clinical Assessment of Depression. The stimuli used were positive, neutral and negative words, controlled in terms of grammatical features, length and familiarity.

There was no effect of depression on attention and recall, but there appears to be a trend toward worse performance in terms of implicit memory in participants with higher levels of depression. Trends were also found towards that more depressed participants evaluated stimuli with emotional valence more neutrally and presented greater repetition effects to the same stimuli.

The results were interpreted as showing the involvement of executive functions and other cognitive deficits associated with depression, the preferential processing of emotional stimuli congruent with the level of depression and its secondary activation by stimuli of opposite valence.

# **(Re)Dimensioning Actions and Directions: A Vocational Guidance Psychological Intervention Project for Young People and High Functioning Autism Disturbances**

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Attending to the reconstructive exploration of the vocational investment model (Campos & Coimbra, 1991), the current study aims to reach the preparation of the project proposal for psychological intervention in vocational orientation for pre-adolescents with Asperger Syndrome and High Functioning Autism. Based on the content of interviews to parents and professionals that intervene daily with these population problems, it was carried out an assessment of the needs, abilities and attributes that characterize these young people, and a survey of intervention strategies most frequently used by technicians in this population. Thus, aiming to promote more independence and autonomy in the construction of a formative and professional way leading to a higher quality of life, this project aims to focus the pre-adolescents in the exploration of the capacities, competences, values and interests that they were developing or that they can optimize those and making them able to relate these dimensions with the world of the qualification and professions.

Despite the existence of specific programs that have as goal the inclusion of individuals with educational special needs in the work market in protected conditions, these programs do not consider the Asperger Syndrome or the High Functioning Autism individuals. In such circumstances, the phenomenon of social exclusion that affects this population in adulthood, according to the results of this study, frightens the parents of these adolescents when they perspective their future.

This was the motivating and powerful reason of this study realization, which intents to promote a deeper reflection about the resources that in the field of vocational guidance can be available to these young people to provide them with meaningful experiences at the perspective of their relationship with the social world in general and with the world work in particular.

# Humour in Portuguese as a Foreign Language lessons - contributions to its learning

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In the context of a globalised world and with the proliferation of multicultural environments it is important to promote interculture and inter-understanding as a basis of (co)existence and, in the case of teaching a Foreign Language (FL), as the learning basis.

Therefore, and because (Portuguese) FL lessons are not only a place of declarative learning, but also a rich and privileged space where cultures and languages meet, it is essential to develop teaching and learning techniques that promote, besides all else, knowing the Other. This was the goal of the current work which was developed within the scope of the Final Report of the Internship of the Master's Degree in Portuguese as a Second/Foreign Language.

Hence, humour can be a very important tool, if not a unique one, when it comes to teaching linguistic, cultural and discursive aspects of a target language. The very same stance is highlighted by Schmitz (2002) when he states that “*classroom exposure to humor prepares students to understand and react to this pervasive and authentic element of discourse during real communicative language interactions. [...] This, [...], is rarely if ever employed as an explicit pedagogical tool in the mind of the teacher, nor as an explicit learning tool in the mind of the student. Rather, it represents the natural occurrence of humor as a part of the human condition just as it emphasizes its importance to comprehensive language learning*”[1].

While providing humour with the prominence that it deserves in Portuguese as a Foreign Language (PFL) lessons, we also resorted to humour as the core of didactic activities and proposals, particularly for level C.

Regarding the methodology, the observation protocol was systematic in nature. The observation carried out was general in nature and covered descriptive, training, evaluation, heuristic and verification aspects. In relation to observation, the adopted position was that of a participant. Moreover, in relation to the nature of the observed object, the observation was narrative and varied between internal and external observation. According to the situation criterion, the observation was natural, molar and molecular. Despite all the aforementioned observations, we also resorted to documentary observation.

Naturally, one should not draw general conclusions from this case study due its scope and occasional nature. However, we believe these activities and their results may be a good starting point for practical, pedagogical and didactic reflections.

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## **A post-nuclear scenery: definitions of parenthood and family in a sample of future psychologists**

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The family is a concept in the midst of a transition. The traditional nuclear family coexists nowadays with other familial configurations such as adoptive, blended or same-sex families. Many of these novel configurations provide the forming of strong bonds between people who don't share any genetic linkage or whose status is not legally recognized. However, in a world whose dominant social and cultural family model is still that of a mother and a father, legally married, with their children (usually biologically related), it's not surprising to find that the common language does not account for new familial arrangements. One hopes, however, a greater openness on the part of future professionals, whose task will precisely be to deal with all kinds of families, conventional or otherwise. This article's main purpose is to identify the definitions of family and parenthood conveyed by a group of soon-to-be psychologists from several Portuguese institutions of higher education.

Three hundred twenty six students were asked to name the features or conditions they considered important to be a good mother or father, and to define the term "family". Resorting to the program NVivo-8, it was found that the features associated with "good parenting" were, in most, cases, identical for mother and father. These included, amongst others, motivation and investment in parenthood, educational abilities, a liberal educational style, the capacity to serve as role-model and convey values, a high self-esteem, and some personality traits like pleasantness or extroversion. Where the definition of "family" is concerned, very few participants considered the essence of family to be necessarily dependent on biological ties. On the contrary, most participants considered the family to be based on the presence of affective bonds between its members.

The results of this article are expected to contribute towards outlining a portrait of the way family and parenthood are faced nowadays, notably by future professionals in the field of psychology.

# The teacher-child verbal interaction in Preschool context

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The main purpose of this present investigation is describe and learn more about the characteristics of linguistic environment in a portuguese preschool context. The aim of this study was explore and answer the follow questions 1) What kind of teacher-child verbal interaction exists in preschool classrooms? 2) The teacher-child verbal interaction differs as result of the type of developed activities? 3) The characteristics of the verbal interaction vary as result of children's age? 4) What kind of limitations and opportunities the children's participation in verbal interaction with their teacher are? To answer these questions were naturally observed activities of three preschool classrooms in Gaia's educational context.

Were observed 69 children (19 of 3 years old classroom; 22 of 4 years old classroom and 26 of 5 years old classroom) in verbal interaction with their teachers, during their daily activities. The procedure of naturalistic observation follows two moments: in the first, were observed target-children interacting with their teacher in different activities and in the second, were observed teacher-child verbal interactions in three types of activities: welcome; structural activities and free play. These activities were also selected as result of the potential linguistic wealth.

The analysis of the naturalistic records showed that the teacher-child verbal interactions not differ significantly as result of the type of activities and also as result of the children's age. The teacher's communication determines significantly the children's participation in verbal interaction as their linguistic development. The teacher-child conversations are characterized by a question-and-answer shape, with the child's role mainly confined to answering questions. The questions are often close, and the open ones are often transformed in close questions. The teacher doesn't give enough time for child to answer and give few verbal helps. The teacher's cognitive demands are usually low, because the content of verbal information has a low level of abstraction.

The results of this investigation can't be generalized to other educational contexts, although this data are relevant to understand more detailed the nature of oral communication in preschool classrooms.

## **Students older than 23 years: Why do they apply for and integrate themselves in the University?**

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At a time when employers are increasingly paying attention to the quality of training of candidates for a certain job and that the experiences and non-formal and informal learning take a prominent place in the individual curriculum, becomes important to reflect about the need demonstrated by the growing adult population in updating their knowledge and having formally recognized the skills acquired throughout their lives. This research aims at analyzing and understanding the access process into Higher Education (ES) of adults older than 23 years (M23). Based in the principles of lifelong learning as well as in the contributions of development and adult learning theories, this study aims to the clarification of this issue through (1) the characterization of M23 students entering the ES, (2) the exploration of their motivations for entering and their integration process at University, (3) the identification of common and distinguishing factors between these students and students who enter University through the regular system (AR). 18 interviews were conducted with students from the 1.st and 2.nd years of the Master's degree in Psychology (MIP) at Faculty of Psychology and Educational Sciences, University of Porto (n = 9 M23 and 9 AR). Results point to the existence of a constellation of motivational factors from the personal, social and professional realms for both M23 and AR students in the origin of their will to enter into ES, while personal factors and previous experiences appear as compelling in the choice of the Psychology course. Interviewed participants highlight positive aspects and underline the key role of family and teachers' support in the adaptation/integration process. There is also a positive perception of both M23 and AR students about the arrangements for M23's access, and it was found evidence of a positive relationship between these two categories of students. Taking a questioning position about the divergent opinions on the subject, it is assumed a critical attitude with respect to this special access conditions reflecting on the repercussions and challenges that M23 students face when joining Higher Education as well as identifying clues for future research in this area and suggesting psychological intervention initiatives to support M23 in their integration process.

# Reader's Diary: a teaching pedagogical instrument used for a reading and writing pedagogy in the Portuguese and French subjects

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The reader's diary "Diário do leitor (DL) / Journal du Lecteur (JDL)" is a teaching pedagogical instrument that is used for a reading and writing pedagogy in the Portuguese and French subjects. At first, this instrument tried to develop the pleasure for reading and writing in our classes/students. Secondly, we changed a few aspects that were based on values such as Freedom, Trust, Confidence, Responsibility, Commitment and Cooperation. These changes consisted in: the creation of a readers/ writers community; the creation of several writing instructions that students could choose; and the application of self-regulation and metacognition grids. The writing results in the DL/JDL in both subjects were generally positive.



**U.**PORTO

*Parallel Oral Sessions V*

**A4** *Engineering VI*

# SecurEmail: Email Security Using Cryptography Based on Computer Security Principles

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Currently, email is one of the most used common forms of communication. It is globally used, whether in formal and informal conversations or by sending public or private information. Though, this sort of communication, in its simple form, lacks of guaranteeing the 3 main principles in computer security: integrity, confidentiality and authenticity.

The ease occurrence of attacks while sending or receiving an email as well as simulating an user while sending it, breaches all principles set out above. These principles are fundamental for this type of communication, assuring that no message/information is changed (integrity), that a specific information only is accessed by authorized individuals (confidentiality) and that all senders are valid and truthful (authenticity).

Thus, it is crucial creating a tool that enables any user to use email as preferred form of communication, but with actual security guarantees.

SecurEmail[1] is a tool intended web-based email clients, which enables the performance of a special security module, with the use of cryptography. Therefore, any user with low cryptographic knowledge, could send or receive his messages, being able to encrypt/decrypt or sign/verify those messages, as well as generating and storing its keys that enables this process. These keys can be seen as passwords, divided in two categories (public and private), in which the public ones are generally known and the private ones are always in the possession of its owner. Another module implemented in SecurEmail is a public key server [2], which allows users to search other users' keys for future use.

References:

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[2] SecurEmail – Public Key server, <http://getsecuremail.fe.up.pt/>.

# Security Vulnerability in U. Porto Academic Information System's (SIGARRA) Cookie Implementation

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Web-based services and applications are becoming ubiquitous in our every-day life, so the security of their use is of paramount importance. One of the most useful features of web-based services is the possibility that the site you are visiting knows who you are and keeps information of your past visiting history and actions. Think of "shopping carts" and the way Google "knows" your browsing preferences. One of the techniques for enabling those features rely on "cookies"! We have studied their use and security challenges on our work in a unit course we are taking for our Informatics Engineering degree.

Cookies are a simple mechanism that enables us to maintain a state in, otherwise, stateless systems, while interacting with those systems. The basic data structure (the "cookie" itself) is a part of the HTTP request and response headers and allows the storing of sessions (the mentioned shopping carts and users preferences) between requests to a Web server.

Although the initial objective of our work was to study cookie systems in general and how to render their theft useless, we quickly shifted our focus to studying our own FEUP academic information system (SIGARRA) and its cookie's implementation, trying to understand their specific structure, how the system used them in order to find vulnerabilities and exploit them. As a first step, our work consisted on studying the group of cookies that were normally generated and identifying the ones that are mandatory to maintain a session in SIGARRA. After that analysis we repeatedly started sessions using an automated script to store the generated cookies' individual data and then analyzed all the collected data trying to find patterns and possible vulnerabilities.

As a result of this work, we were able to identify a vulnerability in our faculty SIGARRA's cookie implementation, successfully exploiting it and even replicating this exploit in different versions of the system in several U.Porto faculties, such as FMUP and FCUP!

# Development of Services and Applications for Set-Top Boxes in Ambient Assisted Living Environments

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General world population is getting older [1]. This fact has severe consequences. One is that retired people are slowly replacing working people, thus overloading public retirement programs created by governments, like social security. The other is that hospitals and care sites will become overcrowded, because with age, health issues come that require constant monitoring of one's health conditions.

However, we are also experiencing an era of technological (r)evolution. Technology has evolved to take an important part in every day-to-day situation, aiding elderly, or physically debilitated, people. This concept is called Ambient Assisted Living (AAL).

To prepare itself for a future to come, the European Union created the AAL Joint Programme [4]. In the context of this programme, and succeeding the CAALYX project, the eCAALYX [3] project was funded. The latest consists on various tools that are able to monitor, indoors and outdoors, a person's health conditions 24/7. Through numerous sensors and devices, one's health condition is monitored and then transmitted to a remote server, where healthcare services will analyze it, withdrawing the need of a physical debilitated person to leave their home and go to the hospital, sometimes just for a routine examination.

Part of the eCAALYX project is the interaction of the subject being monitored with a television. Several services are being deployed into a set-top box that will guide and aid its users through the process of living alone at home, by interacting with the channel being developed, named Personal Health Channel (PHC) [2].

Parallel to the PHC development is this project we are presenting. It aims at developing from scratch all of PHC features, this time using the Google TV™ as the base framework, including services such as an health agenda, an health status monitoring screen, questionnaires, reminders and video conference. Google TV™ is a recently developed product by Google™ that fits perfectly in the needs of the PHC, because of its powerful integration with television, the support that it will receive through Google™, television manufacturers, and open communities, and the fact that there are already in the market some devices powered by Google TV, including some television sets and set-top boxes with this service built in.

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# A Visual Debugger for Efficient Software Fault Localization

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Locating components which are responsible for observed failures is the most expensive, error-prone phase in the software development life cycle [7]. Automated diagnosis of software faults (aka bugs) can improve the efficiency of the debugging process, and is therefore an important process for the development of dependable software.

In the past, we have presented a toolset for automatic fault localization, dubbed Zoltar, which adopts a fault localization technique based on abstractions of program traces [1,6]. The toolset [2] provides the infrastructure to automatically instrument the source code to produce runtime data, which is subsequently analyzed to return a ranked list of potential faulty locations. Using a thread-based example program as well as a large, realistic program, we show the applicability of the proposed toolset in [2].

Although its output is deemed useful [1,6], Zoltar's debugging potential has been limited by the lack of a visualization tool that provides intuitive feedback about the defect distribution over the code base, and easy access to the faulty locations. To help unleash that potential, we propose exploring two visualization techniques - treemap and sunburst - aimed at aiding the developer to acquire a broad sense of the error distribution, and find faults quickly (see Figure 1).

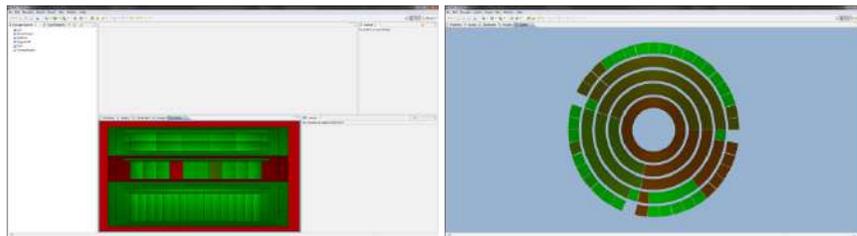


Figure 1 – Eclipse Plugin GZoltar: Treemap view (left) and Sunburst view (right).

The visualizations are implemented as an Eclipse [5] plugin, dubbed GZoltar, allowing direct access from the visualization tool to the faulty locations. Eclipse has been chosen because it is a popular integrated development environment. Compared to other visualization techniques, such as [2,4,6], our tool shows the relevant information for the debugging process in a more intuitive way. Experiments with human developers show that the technique has indeed the potential to aid in locating software faults.

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# Anonymity in Wireless Networks Under Traffic Analysis Attacks

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In wireless multi-hop networks, communication sessions between nodes are established by using intermediate nodes to relay traffic from a source to its intended destinations. However, the broadcast nature of the wireless media allows for malicious nodes to obtain information from different nodes' transmissions. While end-to-end security mechanisms can provide some level of confidentiality, authentication, and integrity of the transmitted data, other valuable information, such as the active sessions among communicating entities, may be easily determined via a traffic analysis attack. Network-based anonymity techniques may allow us to hide this information from malicious nodes. Previous work on this area has focused on providing anonymity for source, destination or both, using different mechanisms and eavesdropper capabilities. An example of a destination anonymity protocol is [1], where the source of a packet is known but the destination is hidden because the packet is transmitted to a set of nodes within an anonymity zone. Mechanisms to provide source and destination anonymity have considered, for example, 1) introducing randomness on the multi-hop path of each packet, 2) transmitting innocuous ("dummy") packets to mask actual transmissions [2], 3) using layers of encryption [3], and 4) using mixers, intermediate nodes that change the timing of packets by mixing data packets coming from multiple sources [4].

The main focus of this work is on providing session anonymity against traffic analysis attacks in multi-hop wireless networks by using scheduling mechanisms, in particular, in the face of a global eavesdropper, i.e., an eavesdropper that detects all transmissions, but not their contents. In this setting, perfect information-theoretic anonymity is achieved if sessions are indistinguishable from one another based on the transmission patterns of the network. One of the mechanisms to achieve this condition is the injection of innocuous dummy transmissions by the different nodes in order to comply with the transmission pattern. The injection of dummy transmissions comes at an additional cost in energy, delay, and throughput to the active sessions. We study transmission schedules and patterns that guarantee perfect anonymity. We also analyse explicit trade-offs between session anonymity patterns and energy, delay or throughput. Finally, we show gains from using network coding in wireless networks to reduce the cost of anonymous sessions. While preserving perfect anonymity, network coding is shown to reduce energy consumption by factor of  $2 - 2/N$  and to double the throughput for a line network with  $N$  nodes.

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# Facebook's hidden feature: User Models and Interest Maps

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<sup>2</sup> Research Grant (BII) at GECAD, research unit of Institute of Engineering - Polytechnic of Porto (ISEP/IPP), Portugal

User profiles have become ubiquitous in the age of Facebook. The popular social networking website has gathered more than 500 million profiles from people all over the world. Facebook is popular for its social features, connecting friends and sharing media, but its most impressive feature lies in the wealth of user information. Each profile details the user's interests in music, television, movies and books as well as community pages, which can be about anything. Recommender systems, such as those used for online shopping in product recommendation, usually rely on information specific to their application domain. For example, shopping websites generally use your shopping history to recommend your next purchase. What Facebook allows is a sort of broad-spectrum information extraction that effectively models the person, and not the user whose context is specific to the domain of the application.

This project goes beyond simply extracting the information from the profile. The information is used to build a variation on an InterestMap[1] - a network style view of the space of interconnecting interests and identities. This work augments the information in the profile with information from Wikipedia. This information is accessed through Dbpedia[2] which extracts structured information from Wikipedia and provides its own formal ontology, used exclusively in this project. Furthermore, semantic relatedness is maintained by creating weights between connections that are calculated by querying Google for the result count of a search about the elements in connection.

Pairing broad-spectrum information from Facebook with multi-domain information from Dbpedia seems like a natural match. This approach is leaner in sources and more formal in ontology making it more reliable than the original InterestMaps, which used a multitude of sources and compiled their own ontology.

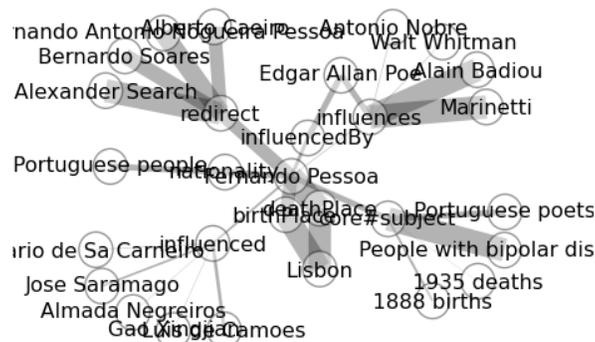


Fig. 1: InterestMap built for “Fernando Pessoa” as found on the interests of a profile. Intermediate nodes are ontology attributes and edge thickness represents relevance.

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**U.PORTO**

*Parallel Oral Sessions V*

**A5** *Sport Sciences I*

# Changes in cross-sectional area per myonucleus on mice *soleus* muscle during one week of hindlimb suspension reinforce the concept of myonuclear domain

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The skeletal muscle fiber is a syncytium where various myonucleus share their products. This particularity, associated with the fact that in single nuclear cells the nucleus is controlling the surrounding cytoplasm, has led to the concept of myonuclear domain (MND), which is the theoretical volume of cytoplasm within the myofibre regulated by the gene products of a single myonucleus [1, 2]. The effects of hindlimb unweighting by tail suspension on mice *soleus* muscle cross-sectional area (CSA) and myonuclei content were studied. Five groups of male Charles River CD 1 mice (n=10 per group) were assigned attending to the hindlimb suspension (HS) period: control (cont), 1 (1HS), 2 (2HS), 3 (3HS) and 8 (8HS) days. Immediately after the suspension period, the *soleus* muscles were removed in order to assess morphometry data. The CSA and number of myonuclei were determined in a total of 5011 fibres (1098, 793, 1363, 1281, 1276 fibres for the cont, 1HS, 2HS, 3HS, 8HS groups, respectively) from different areas in the mid-portion region of the *soleus* muscle, and the CSA *per* myonucleus ratio was calculated. The results showed that the removing of weight bearing had a negative impact on muscle fibre CSA ( $1686 \pm 516 \mu\text{m}^2$ ,  $1506 \pm 470 \mu\text{m}^2$ ,  $1235 \pm 340 \mu\text{m}^2$ ,  $1277 \pm 412 \mu\text{m}^2$  and  $1075 \pm 356 \mu\text{m}^2$  in the cont, 1HS, 2HS, 3HS and 8HS groups, respectively) and myonuclei number *per* fibre ( $1.91 \pm 1.17$ ,  $1.94 \pm 1.30$ ,  $1.55 \pm 1.01$ ,  $1.48 \pm 1.00$  and  $0.83 \pm 0.76$  in the cont, 1HS, 2HS, 3HS and 8HS groups, respectively). The CSA *per* myonucleus ratio was  $993 \pm 522 \mu\text{m}^2$ ,  $854 \pm 443 \mu\text{m}^2$ ,  $815 \pm 374 \mu\text{m}^2$ ,  $881 \pm 442 \mu\text{m}^2$  and  $957 \pm 367 \mu\text{m}^2$  in the cont, 1HS, 2HS, 3HS and 8HS groups, respectively. Thus, there were no significant differences between the cont and 8HS groups. The morphological data is consistent with the hypothesis that, despite the muscle fibre atrophy induced by HS, the CSA *per* myonucleus ratio is apparently maintained, reinforcing the concept of myonuclear domain.

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## Exercise training blunts cardiac hypertrophy and neurohumoral activation in an experimental model of Pulmonary Hypertension

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Exercise training as a therapeutic and/or preventive strategy is gaining interest in pulmonary arterial hypertension (PAH). We aimed to study the influence of endurance exercise training in monocrotaline (MCT) induced PH.

Male Wistar rats were randomly divided according the following groups: i) sedentary with MCT (60 mg/kg sc) or vehicle (SED+MCT and SED+V), ii) 4 week-exercise training before MCT or vehicle injection (EX+MCT and EX+V) and iii) 4 week-exercise training after MCT or vehicle injection (MCT+EX and V+MCT). Hemodynamic bi-ventricular instrumentation with conductance catheters was assessed at 4 weeks after MCT or vehicle injection. After cardiac instrumentation, morphometric, histological and molecular biology analysis was performed according to regular procedures.

In SED+MCT group, MCT induced body and muscular cachexia, RV hypertrophy, collagen deposition and RV peak systolic pressure increase, and RV relaxation disturbances. These alterations were prevented in both EX+MCT and MCT+EX. Regarding gene expression, SED+MCT presented increased levels of interleukin-6 (IL-6) mRNA in the lungs and b-type natriuretic peptide (BNP) and endothelin-1 (ET-1) mRNA in the RV. On its turn, while MCT+EX showed attenuated mRNA levels of IL-6, BNP and ET-1, EX+MCT only avoided IL-6 and ET-1 mRNA upregulation. There were no significant differences among groups injected with vehicle. Exercise significantly reduced mortality rate after 4 weeks of MCT injection.

Our results allow us to conclude that exercise training has protective effects on MCT model of PAH, by protecting cardiac function, attenuating cardiac hypertrophy, extracellular remodeling and attenuating neurohumoral activation.

# The impact of water, sports drink and sports drink with caffeine intake on physic, skill and cognitive performance before a roller hockey game

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**Purpose:** To determine the differences between water, sports drink and sports drink with caffeine in the hydration status, physical, skill and cognitive performance after a simulated roller hockey game.

**Methods:** 12 male participants aged between 18 and 32, completed a cross-over design study, in 3 different conditions during a 2h30 trial. An intake of 1 x 5ml/kg (beginning), 4 x 2ml/kg (15'-15') and 3 x 2ml/kg (20'-20'),, of water (Ag), sports drink with 7% of carbohydrates (BD) and sports drink with added caffeine (25mg/100ml) (BDC). Each session was composed by a simulated game ( $\approx 56\%VO_2max \Delta_{t1h}$ ), a set of drills ( $\approx 32\%VO_2max \Delta_{t1h}$ ) like the muscular strength, velocity, agility, dribble, shoot, pace, and YoYoIET L2 ( $\approx 56\%VO_2max \Delta_{t8m}$ ). The sweat rate, the urine color, the rating of perceived exertion (RPE) and the mood states (BRUMS) were determinated.

**Results:** The Squat Jump was significantly higher at the BDC condition than Ag (+11,5%  $p=0,033$ ), and agility and reaction too (+6,5%  $p=0,002$ ). The RPE was higher when the game was over ( $p=0,021$ ), at the conditions Ag ( $13,75 \pm 1,06$ ), BD ( $12,75 \pm 1,85$ ) or BDC ( $11,83 \pm 1,4$ ). The BRUMS scale reported lower values of cognitive fatigue ( $p=0,03$ ), at the BDC condition ( $-0,67$ ), then BD ( $+0,08$ ) and finally Ag ( $+0,83$ ). There were no significant differences between hydration, urine color and skill performance.

# How much resistance should be applied in a *Wingate* test performed by pre-pubertal swimmers?

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Wingate test is probably the most used test for anaerobic performance evaluation. As initially described [1], it is still typically performed in cycloergometer. When mechanically beaked ergometers are used, the weight resistance is  $0.075\text{g}\cdot\text{kg}^{-1}$  of body weight [1]. Some studies have already showed that this standard value could not be accurate for different sports players [2]. The use of resistance charges higher or lower than the accurate one leads to the underestimation of maximum power output of the subject tested. Our group have already carried some pre-tests in order to determine the best resistance to apply in the 30s Wingate test [3]. The purpose of this study was to find the best weight resistance to apply during a Wingate test performed by pre-pubertal swimmers of both genders.

The study protocol consisted on a pre-test of nx10s maximal cycloergometer pedal exercise (*Monark<sup>TM</sup>*, Sports Medicine Industries, Inc.), applied to 15 male and female pre-pubertal swimmers. This test allowed the determination of the weight resistance theoretically more able to induce the higher maximal power value in the 30s Wingate test (ideal resistance). The ideal resistance chosen was the one who induced the higher maximal power output in the 10 s maximal pedal. A 3x30s Wingate test was then performed. Resistances applied were the ideal resistance, and the ideal resistance addicted and subtracted in  $0.005\text{ g}\cdot\text{kg}^{-1}$  of body weight.

As could be observed in Fig.1 results of the nx10s pre-test suggested that the ideal resistance that should be applied in 30s Wingate tests performed by pre-pubertal swimmers is higher than the usual  $0.075\text{ g}\cdot\text{kg}^{-1}$  [1]. But, when ideal resistance was applied, a large number of swimmers doesn't finish the 30s Wingate test. In conclusion, this proposed pre-test seemed to not be accurate for determine the resistance to apply in 30s Wingate test performed by pre-pubertal swimmers, even that the same pre-test was showed to be accurate for adult swimmers [3].

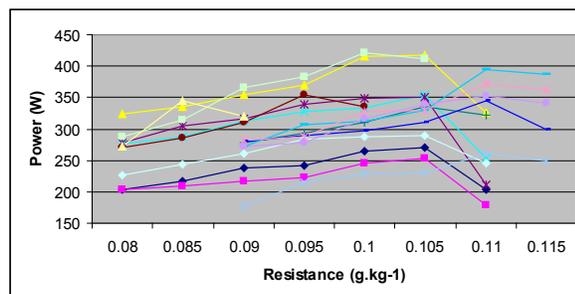


Fig. 1 – Results of nx10s maximal pedal pre-test.

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# Comparison between different swimming incremental protocols for anaerobic threshold assessment

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The anaerobic threshold (AnT) is considered the highest possible workload without an exponential increase in blood lactate concentration values ([La]), being an important predictor in the diagnosis of aerobic performance in swimming [1]. Our aim was to compare the following physiological and functional parameters assessed in three variants of incremental intermittent protocol with different step durations and in the maximal lactate steady state test (MaxLass): (i) maximal [La] ([La]<sub>max</sub>), and [La] and velocity corresponding to AnT ([La]<sub>AnT</sub> and v<sub>AnT</sub>); (ii) maximal heart rate (HR<sub>max</sub>) and heart rate corresponding to AnT (HR<sub>AnT</sub>).

Seventeen triathletes (1.75 ± 0.51 m and 67.1 ± 5.7 kg) performed three variants of a swimming intermittent incremental protocol until exhaustion (cf. [2]) and a continuous MaxLass test (cf. [3]) used as “gold standard” to assess AnT.

The [La]<sub>AnT</sub> found in nx200, nx300 and nx400 protocols, as well as in the MaxLass, were much lower than the traditionally used 4 mmol/l value (Table 1). Equally, the different v<sub>AnT</sub> were considerable lower than velocity corresponding to 4 mmol/l (v4). In addition, differences were observed in the [La]<sub>AnT</sub> between nx300 and nx400 protocols, and between MaxLass and all the incremental protocols. The v<sub>AnT</sub> obtained in the intermittent protocols were similar to that obtained in the MaxLass.

Table 1: Mean ± SD values of the variables assessed in the intermittent incremental tests and in the continuous one.

	nx200	nx300	nx400	MaxLass
[La] <sub>AnT</sub> (mmol.l <sup>-1</sup> )	2.1±0.1	2.2±0.2 <sup>c</sup>	1.8±0.1 <sup>b</sup>	2.9 ± 1.2 <sup>a,b,c</sup>
[La] <sub>max</sub> (mmol.l <sup>-1</sup> )	7.28±2.03	7.39±2.02	6.89±2.06	---
v <sub>AnT</sub> (m.s <sup>-1</sup> )	1.10±0.04	1.09±0.04	1.07±0.04	1.09 ± 0.14
HR <sub>max</sub> (b/m)	173.2±11.6	176.7±11.8	174.2±10.9	---
HR <sub>AnT</sub> (b/m)	145.4±14.7 <sup>c</sup>	147.4±14.2 <sup>c</sup>	142.5±15.6 <sup>c</sup>	156.0±16.3 <sup>a,b,c</sup>

<sup>a,b,c,d</sup> differences between nx200, nx300, nx400 and MaxLass, respectively (p≤0.05).

It is possible to conclude that: (i) intermittent incremental protocols of 200 and 300 m step durations seem to be valid procedures for AnT assessment; (ii) the use of 400 m distance seems overestimate [La]<sub>AnT</sub>; (iii) the velocity corresponding to AnT obtained in intermittent incremental protocols are similar to velocity obtained in MaxLass; (iv) the HR<sub>max</sub> and HR<sub>AnT</sub> values attained in all intermittent incremental protocols were similar.

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## Intra-cycle velocity variations of the centre of mass during submaximal and maximal 100-m butterfly

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In the swimming scientific and coaching communities it is commonly accepted that the assessment and research of intra-cycle velocity variations (IVV) is quite relevant to enhance the swimming performance and to better understand the factors that constrains the technical evolution [1-2]. Nonetheless, butterfly specific training series are conducted in different intensities zones and few is known about the behavior of IVV at different training regimens. We aimed to compare the IVV of the centre of mass during a 100-m (between first and fourth laps) at three swimming intensities: two submaximal and one maximal.

Seven female trained swimmers (mean  $\pm$  SD: 17.57  $\pm$  1.96 years old, 1.66  $\pm$  0.06 m, 10.8  $\pm$  2.1 years of training background and 66.36  $\pm$  4.07 s at the long course 100-m butterfly), performed a protocol of 3x100-m butterfly at submaximal (60 and 80%, v60 and v80, respectively) and at maximal velocities (v100), with a 30 min interval. One above and one underwater camera, positioned in the sagittal plane, collected the kinematic data. ApaSystem was used to determine the IVV of the centre of mass. The butterfly stroke was divided into four phases (adapted from(cf. [3]): entry/catch, pull, push and recovery. The phases' duration were normalized by the total time duration of the stroke cycle. Kinematic analysis was conducted at the first and fourth lap of each 100-m test. Significance level was considered for  $p < 0.05$ .

Comparing the IVV obtained at v60, no differences were observed between first and fourth lap (24.16  $\pm$  3.25 vs 21.34  $\pm$  5.87,  $p = 0.37$ ). Regarding v80, first lap showed a lower IVV than fourth (17.11  $\pm$  2.13 vs 21.16  $\pm$  1.15,  $p = 0.02$ ). At v100, first lap registered lower IVV than fourth (16.67  $\pm$  10.43 vs 24.64  $\pm$  0.36,  $p < 0.01$ ).

The values of IVV obtained in the present study showed that swimmers experienced more facility to sustain the effort at v60, which was evidenced by the maintenance of constant IVV. However at v80 and v100, as the swim progress from the first to the fourth lap, swimmers appeared more fatigued, and as consequence, less mechanically efficient due to an increase in IVV.

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# Biomechanical analysis of backstroke start: comparison between immersed and emerged feet positioning

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Since Hay's study [1] it is objectively known that swimming starts represent ~0.5% to 11% of the total competition time. Specifically, the backstroke swimming start has been estimated to represent up to 30% of the 50 m competition time [2]. Apparently, no study has yet deal with the technical adjustments allowed by the new rules endorsed by the Federation Internationale de Natation. The aim of this study was to describe and compare two actually used start variants for backstroke events, one with the feet parallel and entirely immersed (BSFI) and one with the feet parallel and entirely emerged (BSFE).

Six male high level swimmers performed two sets of four 15 m trials in a randomized order of each start variant. Trials were monitored with synchronized dual-media image, underwater platform and handgrip with a load cell, and electromyographic (EMG) signal of *Deltoideus Anterior*, *Biceps Brachii*, *Triceps Brachii*, *Erector Spinae Longissimus*, *Gluteus Maximus*, *Rectus Femoris* and *Gastrocnemius Medialis* muscles. The backstroke start was breakdown into five phases: (i) hands-off; (ii) take-off, (iii) flight, (iv) entry and (v) glide. Significance level was set at  $p < .05$ .

The main kinematic and kinetic data showed that BSFI registered shorter time from the starting signal to the hands reaching the 5 m ( $2.03 \pm 0.19$  s vs  $2.14 \pm 0.36$  s), greater hands horizontal water reach at flight phase ( $1.75 \pm 0.18$  m vs  $1.59 \pm 0.22$  m) and greater upper limbs average normalized horizontal force ( $0.38 \pm 0.03$  N/bw vs  $0.33 \pm 0.08$  N/bw) than BSFE. Regarding BSFE, it was observed longer time at take-off phase ( $0.18 \pm 0.09$  s vs  $0.16 \pm 0.08$  s), greater centre of mass resultant velocity ( $3.31 \pm 0.54$  m.s<sup>-1</sup> vs  $3.64 \pm 0.61$  m.s<sup>-1</sup>) and greater lower limbs horizontal impulse at take-off instant ( $0.54 \pm 0.04$  N.s/bw vs  $0.71 \pm 0.05$  N.s/bw) than BSFI. Electromyographic data revealed greater integrated EMG (iEMG) of the *Gastrocnemius Medialis* during take-off phase ( $0.16 \pm 0.02$  vs  $0.12 \pm 0.05$ ) for BSFI than BSFE. When analyzed BSFE, *Deltoideus Anterior* ( $0.07 \pm 0.05$  vs  $0.02 \pm 0.01$ ), *Biceps Brachii* ( $0.19 \pm 0.10$  vs  $0.10 \pm 0.05$ ) and *Triceps Brachii* ( $0.13 \pm 0.06$  vs  $0.08 \pm 0.03$ ) registered greater activity at hands-off phase than BSFI.

The main conclusion is that BSFI, being faster than the BSFE should be preferred for competitive use. Conversely, BSFE seems to be a more complex movement, requiring special attention during training sessions.

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# Effects of warm-up and a football match on knee joint position sense

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**Introduction:** Lower limb neuromuscular control can be affected both by warm-up and prolonged fatiguing exercise. These exercise-related effects can be mediated by changes in joint proprioception. In football athletes, the study of the warm-up and match-induced muscle fatigue effects on proprioception, specifically on knee joint position sense is a matter of interest, not solely in terms of impact on performance but also in preventing injuries. **Objectives:** The main purposes of this study were to describe and compare the knee joint position sense (JPS) assessed at rest, immediately after warm-up and after an official football match. **Methodology:** Sixteen male adult football players aged  $25,8 \pm 4,6$  years old participated in the study. The rate of perceived exertion using the Borg scale was evaluated at rest, immediately after warm-up and the football match. Knee proprioception was assessed by measuring JPS (absolute angular error) using the technique of active repositioning in open kinetic chain of a ipsilateral position passively determined. All data was analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL) statistical software. Variables were tested for normal distribution with the Shapiro-Wilk test. Means and standard deviations were calculated. A repeated measures ANOVA was performed to compare the mean differences between JPS acuity prior to and after the warm-up and football match. Bonferroni post-hoc tests were used for multiple comparisons. Statistical significance was set at  $p < 0,05$ . **Results:** Perceived exertion increased after warm-up and even more after the match comparatively to rest (data not shown). Mean absolute angular errors significantly decreased after warm-up comparatively to rest, and increased significantly after the match comparatively both rest and after warm-up (Table 1).

Table 1. Knee joint absolute angular errors (degrees) at rest and immediately after the warm-up and football match (values are mean  $\pm$  SD)

Rest	4,1 $\pm$ 2,0*
After warm-up	2,0 $\pm$ 1,0 <sup>#</sup>
After football match	8,7 $\pm$ 3,8

\* statistical significant difference rest *versus* after warm-up and football match;  $p < 0,05$

<sup>#</sup> statistical significant difference rest *versus* after football match;  $p < 0,05$

**Conclusions:** Warm-up improves proprioception acuity, while football match induced-fatigue has a deleterious effect on knee joint position sense.





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*Parallel Oral Sessions V*

**A6** *Agronomy & Chemistry I*

# Quantification of patulin in organic and conventional apples and identification of producing moulds

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Patulin is one of the several secondary metabolites produced by the fungi belonging to the genera *Penicillium*, *Aspergillus* and *Byssochlamys*. It has been demonstrated that the presence of patulin in foodstuffs, such as apples or processed apple products, can be a health concern because this mycotoxin may be responsible for severe acute (e.g. convulsions, nausea, ulceration) and chronic (e.g. carcinogenic, genotoxic, immunotoxic) hazardous effects in humans [1]. Patulin, mainly in apples and apple juices, was also described in the brown rot of other fruits such as pears and quince [2]. A maximum of 50µg/L for patulin in apple juice is considered acceptable by the Codex Alimentarius Commission [3]. The European Union adopted the same maximum level for apple juice. Moreover, the maximum level allowed for apple products for infants and young children consumption is 10µg/kg [4].

The aim of this work was to determine if agriculture practices (conventional or organic) have any effect on the occurrence and on the level of patulin. The presence of mycobiota was investigated in different rotten area percentages (about 75% of brown area or not visible rot) of three varieties (golden, reineta, fuji) obtained from conventional and organic agriculture, as well as, in apple juices from commercialized brands in Portugal. A previously validated GC-MS method [2] was utilized for patulin identification and quantification.

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# A novel HPLC-UV-FLD method for the determination of the sugar content in pectin extracts

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Pectins are a family of hydrocolloids, with a complex internal structural organization playing an important role as primary constituents in cell walls of plants. They are anionic biopolymers consisting of partially esterified  $\alpha$ -(1-4)-D-galacturonic acid residues, containing varying amounts of neutral sugars such as D-galactose, D-glucose, L-rhamnose, L-arabinose, L-fucose, D-mannose and D-xylose. Some of these sugars are constituents of side chains to the galacturonan backbone [1]. This soluble fiber is used as a gelling and stabilizing agent in the food industry with multiple positive effects on human health including lowering cholesterol levels and reducing the risk of different type of cancers [2].

The developed methodology is based on a previous one, developed by our research group [3], consisting in the initial chemical and enzymatic hydrolysis of the pectin chain, followed by derivatization with 4-aminobenzoic acid (4-AMBA) and determination by high performance liquid chromatography with ultraviolet and fluorimetric detection (HPLC-UV-FLD).

The separation of sugars was performed on a C<sub>18</sub> column with a polar endcapping. In this study a two steps method was developed, by using two different elution conditions. It was thus possible to achieve an efficient separation between mannose and xylose in the first run (ammonium acetate buffer, pH 5.5) and between galacturonic acid and glucuronic acid in the second run (sodium phosphate buffer, pH 3.5 with tetrabutylammonium hydrogensulfate as ion-pair).

The method here described proves to be simple and provides good separation and resolution of analytes. The pH of the eluent was shown to be a determining factor for efficient separation of all neutral sugars and the use of ion pair proved to be important for the successful resolution of uronic acids in study.

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# Optimization of SPE extraction of polycyclic aromatic hydrocarbon in barbequed muscle foods

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Polycyclic aromatic hydrocarbons (PAHs) are an important class of toxicological compounds formed during the incomplete combustion of organic matter. They are widely spread in the environment and human exposure to PAHs is unavoidable. Their presence in the environment contribute to accidental contamination of many raw foods, additionally, the traditional preservation practice of food with wood smoke or food cooked over an open flame induces PAHs generation and their presence in foodstuffs. Consequently, the major source of human exposure is attributable to the diet. The presence of PAHs in food is a matter of concern and requires continuous monitoring.

However, extraction and quantification of PAHs in thermally processed meat and fish is difficult because these compounds occur in food at microtrace levels, i.e. ppb or ppt levels and many other organic components are co-extracted from the matrix. Additionally, most PAHs are structurally similar and occur as isomers, which make identification more difficult. Studies concerning the levels of grilled or barbecued food are scarce or quantified only benzo[a]pyrene alone or more few PAHs.

In the present study we proposed the optimization of an extraction method of 16 PAHs from two frequent consumed barbecued muscle food, beef and salmon by solid-phase extraction (SPE) and analysis by HPLC with fluorescence detection.

The extraction method consists in extraction/sonication of an appropriate amount of lyophilized sample in *n*-hexane. After evaporation until dryness the residue was re-dissolved in 3 mL of *n*-hexane and then loading onto a 5 g silica cartridge (manually packed with deactivated silica gel or purchased disposable cartridge silica gel). The cartridge was eluted with a mixture of *n*-hexane/dichloromethane 70:30 (v/v), the optimum volume used for PAHs fraction was dependent of the fat amount loaded.

Different barbecued muscle samples need an adjustment in the PAHs extraction, to use higher amount of sample and minimum elution volumes. The conditions for beef were 2 g of lyophilized meat, and the optimum elution with purchased columns was 10 mL of PAHs fraction after having discharged the first 8 mL, and for salmon 1,5 g of lyophilized fish was the amount selected and the best elution with the purchased columns was 10 ml of PAHs fraction after having discharged the first 10 mL. When manually packed cartridges are used the PAHs fraction needs higher elution volumes to obtain reproducible results.

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# Determination of riboflavin in milk using molecularly imprinted polymers for selective extraction

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Molecularly imprinted materials (MIPs) have been receiving increasing attention as solid phases for selective extraction and preconcentration of target analytes existing in complex matrices. They are generally composed by two monomers (a functional and a cross-linking monomer), which have been previously associate to a template molecule that creates a cavity in the polymer with high, specific affinity to the target analyte. Several MIPs are currently commercially available, including a MIP selective towards riboflavin. For this MIP, selectivity towards riboflavin is due to tailored polymer composition by selection of template (riboflavin tetraacetate) and functional monomer (2,6-bis(acrylamido)pyridine), which controls the binding site, and also selection of cross-linking monomer (pentaerythritol triacrylate), which influences the polarity and functionality of the polymer matrix [1].

In the present work, a new protocol for determination of riboflavin in milks is proposed, where the target analyte is retained in a commercial cartridge filled with MIP, eluted using acetonitrile + 1% acetic acid and further determined by direct fluorimetry in a microplate format. Initially, the microplate determination conditions were studied (resorting to riboflavin standards) in order to evaluate the influence of total well volume (50 to 300  $\mu\text{L}$ ), the influence of solvent (water, 2.4% acetic acid, 3 mM HCl, eluent solution, among others) and the application of direct UV or fluorimetric detection. After selection of fluorimetric detection with the maximum possible volume (300  $\mu\text{L}$ ), the determination was carried out using eluate from MIP cartridge diluted 1:10 in 1% acetic acid. The methodology was applied to the standard reference material SRM NIST 1846 (milk based infant formula) and a z-score value  $< 1$  was attained, indicating that the proposed methodology provided a result in agreement to those obtained in the interlaboratory study applied in the sample validation.

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# Gas-diffusion microextraction for HPLC-UV analysis of volatile aldehydes in alcoholic beverages

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Aldehydes are compounds widely distributed in foods and beverages. Some of them are volatile and have significant sensory properties that can impart pleasant or unpleasant notes to different products [1]. Factors such as yeast strain, temperature, pH of must, O<sub>2</sub> levels in juice, added SO<sub>2</sub> levels, and nutrient availability are among the variables that can influence production of aldehydes (the most important is acetaldehyde) during fermentation [2].

The purpose of this study is to develop a rapid and easy extraction procedure for HPLC-UV analysis of volatile aldehydes in alcoholic beverages by gas-diffusion microextraction (GDME). GDME is an innovative technique that combines the advantages of membrane aided gas-diffusion with microextraction concepts [3,4]. GDME makes use of a novel portable and low-cost device that comprises a small, commercially available, semi-permeable membrane. Volatile and semi-volatile compounds released by the sample can permeate the membrane pores and are collected by an adequate solution. The main advantages of this extraction system are: the experimental simplicity of the sample preparation process and the use of a disposable small sized membrane [4].

The methodology is based on the extraction of volatile aldehydes from a heated sample through the membrane, and their entrapment by an appropriate solution placed in the extraction module. Such solution is 2,4-dinitrophenylhydrazine (2,4-DNPH) that reacts with aldehydes originating 2,4-dinitrophenylhydrazones, detected by UV spectrometry [5]. Several extraction parameters were studied: temperature, time, membrane repeatability and SO<sub>2</sub> influence. Finally, aldehydes were quantified in wine and beer samples.

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# Nutritional characterization of lettuce cultivated on soil containing fresh spent-coffee grounds

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Coffee is one of the most popular beverages in the world, currently with a global production around seven thousand million tons/year [1]. This progressive market expansion leads to the accumulation of organic residues, such as spent-coffee grounds, presenting a huge diversity of compounds, some still bioactive like caffeine or chlorogenic acids. Over the past few years, attempts have been made to use coffee residues for mushroom, biogas and compost/vermicompost production [2].

The use of spent-coffee grounds in domestic agriculture is a popular practice, despite the absence of scientific evidence upon its effectiveness or even its safety. In this study a few preliminary assays about the influence of spent-coffee ground mixture in the culture medium (0-20% w/w) on lettuce (*Lactuca sativa* var. *capitata*, cultivar “butterhead”) physical, chemical and biological features were performed. Toxicological analysis of the lettuce and the environment, due to the potential caffeine contamination from the spent-coffee grounds, are under development.

Several analytical methods were applied to the nutritional characterization, based on official analysis methods, including total protein content, mineral and carbohydrate contents. The remaining caffeine in spent-coffee grounds and drained waters was efficiently quantified by HPLC – DAD, whereas GC – MS is being used to evaluate the reduced caffeine amount in post-harvest soil mixture and lettuce, having both methods been validated for the purpose.

Preliminary results indicate that the presence of low amounts of fresh coffee grounds in the culture medium (up to 10%) is not responsible for physical alterations in the plants (number, size of both leaves and roots) but cannot be regarded as an efficient soil fertilizer, although there seems to be a slight improvement in the presence of low amounts of fresh coffee grounds (2.5% to 10%). The variations in the nutritional constituents were also reduced or inexistent. Regarding lettuce bioactive properties, the results on chlorophylls and carotenoids evaluation seem to indicate some content reduction, which requires advanced analysis.

## Acknowledgment

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**U.PORTO**

## *Parallel Oral Sessions VI*

**A1** *Biological, Environmental & Health Sciences IX*

## Neuregulin attenuates right ventricular hypertrophy and dysfunction in an experimental model of pulmonary hypertension.

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Neuregulin (NRG)-1 belongs to the epidermal growth factor family and has important implications in the maintenance of structural and functional integrity of the adult heart. Recent studies have shown the role of NRG-1 in the pathophysiology of left ventricular heart failure (HF) [1]. Nevertheless, to date no studies concerning the NRG-1 effects on pulmonary arterial hypertension (PAH) and right ventricular HF have been published. The goal of this study was to determine the cardiac functional and structural effects of NRG-1 chronic treatment in an animal model of PAH and HF.

Male Wistar rats (180-200g) randomly received either a subcutaneous injection of monocrotaline (MCT, 60mg/Kg) or an equal volume of vehicle. After 14 days, animals from these groups were randomly assigned to receive treatment with either NRG-1 (4ug/Kg/day, ip) or vehicle. The chronic study resulted in 4 groups: control (CTRL, n=10); CTRL+NRG (n=10); MCT (n=10); MCT+NRG (n=10). Right ventricle (RV) hemodynamic evaluation and sample collection for morphometric and molecular analysis were performed 25 to 28 days after MCT administration. Only significant results (mean±SEM, p<0.05) are given.

MCT group developed PAH, as shown by the increase in RV maximum pressure (Pmax) (MCT vs CTRL: 63.1±2.6 vs 34.1±2.9mmHg) which was attenuated in the MCT+NRG group (53.1±3.3mmHg).

Regarding morphometric analysis, animals from the MCT group developed RV hypertrophy evaluated by the RV/tibia length ratio (MCT vs CTRL: 0.076±0.002 vs 0.045±0.003 g/cm) and pulmonary congestion evaluated by the lung weight/tibia length ratio (MCT vs CTRL: 0.74±0.03 vs 0.42±0.03g/cm). Both changes were minimized by the NRG-1 chronic treatment (0.057±0.002 g/cm and 0.61±0.03 g/cm, respectively).

We consistently observed an increased expression of brain natriuretic peptide and endothelin-1 in the RV of MCT group animals (17.5 and 5.0 times vs CTRL, respectively). These changes were however attenuated or reversed in the RV from the MCT-NRG group (the increase in brain natriuretic peptide expression was only 5.6 times vs CTRL, and regarding ET-1 no increase in expression was found).

NRG-1 chronic treatment significantly reduced the severity of PAH and RV hypertrophy, as well as the expression of genes associated with overload and ventricular hypertrophy. These findings suggest that the NRG-1 pathway has a relevant role on the pathophysiology of PAH and right ventricular HF, representing a potential therapeutically target.

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# Ghrelin decreases the intraocular pressure in an acute glaucoma model

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**Introduction:** Ghrelin is an acylated, 28-amino-acid peptide that promotes the release of growth hormone (GH) by the anterior pituitary. It is the natural ligand of the GHSR-1a receptor. During adult life ghrelin is synthesized mainly in the gastric oxyntic mucosa in the X/A cells, although it can also be found in several other organs/systems.

Recently there have been described some important actions of ghrelin in the ocular physiology. Ghrelin's mRNA was identified mainly in the posterior surface of the iris and in the non-pigmented ciliary epithelium. In the eye ghrelin induces the relaxation of the iris sphincter and dilator muscles, through a prostaglandins dependent pathway. The presence of ghrelin was demonstrated in human aqueous humour and its level is decreased in patients with glaucoma.

The aim of this project is to study the effect of ghrelin in the elevated intraocular pressure (IOP) using a new model of acute glaucoma.

**Methods:** We established a correlation between the real IOP and the pressure given by the TONOVET® rebound tonometer in 14 out of 18 eyes of New Zealand albino rabbits. In order to do that, a needle connected to a water reservoir and a pressure transducer was introduced in the anterior chamber of the eye. Afterwards, different pressures were obtained by changing the level of the water reservoir and recorded using both TONOVET® and the transducer.

Concerning the acute glaucoma model, 100 µL of sodium hyaluronate (10mg/mL) were injected in the anterior chamber of 30 eyes to induce an elevation in the IOP. After 30 minutes of sustained elevation of the IOP, an injection of either ghrelin (200 µL of  $10^{-5}$ M,  $n = 8$ ) or vehicle solution (200 µL,  $n = 7$ ) was performed into the posterior segment of the eye. We did not include in the statistical analysis the eyes in which there was leakage of sodium hyaluronate during the injection of ghrelin or vehicle in the posterior segment.

**Results:** There is a linear correlation between the real IOP and the pressure given by the rebound tonometer with a correlation coefficient of 0,933. The formula obtained was  $y = 0.750x + 0.331$  (y being the IOP measured by the tonometer and x being the real pressure).

The injection of sodium hyaluronate has promoted an increase of the IOP from  $11.62 \pm 3.74$  mmHg to  $51.70 \pm 7.58$  mmHg. The injection of ghrelin in the posterior segment of the eye promoted a statistically significant decrease (25.4%) in the IOP 30 minutes injection (from  $53.17 \pm 6.78$  mmHg to  $27.76 \pm 10.41$  mmHg) when compared to the control group (from  $49.73 \pm 8.74$  mmHg to  $38.22 \pm 11.20$  mmHg).

**Conclusion:** This model has proven to successfully increase the IOP in an acute manner. Ghrelin decreases the IOP in this acute angle glaucoma model.

## Defective retinal angiogenesis in isoxanthohumol-treated mice

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Angiogenesis is a biological process that controls the formation of new blood vessels from an established vascular network and occurs in many physiological conditions, such as wound healing or pregnancy. Pathological angiogenesis, conversely, is associated with several diseases, like cancer, diabetes or ischemia. Identification and characterization of dietary compounds, able to modulate angiogenesis and inflammation may constitute an important strategy for prevention of these pathologies.

Isoxanthohumol (IXN), a flavonoid of beer, is one of the main xanthohumol metabolites, previously studied by our group. We showed that IXN has anti-angiogenic properties, demonstrated by reduced proliferation, invasion, cord-like structures formation and increased apoptosis of endothelial cells (EC) in culture. Furthermore, we noticed a reduced number of vessels in the mouse matrigel plug and rat skin wound-healing assays and anti-inflammatory effects *in vivo*.

To further evaluate the angiogenic modulation by IXN, the neonatal retinal neovascularization model was used. In the developing retina, endothelial sprouting and organization of vascular networks occur in a reproducible temporal and spatial pattern and can be easily imaged to study the interactions of EC with growth factors, astrocytes and mural cells and to analyse several angiogenic steps, namely EC survival, proliferation, migration and vascular pruning and normalization.

C57BL/6 mice pups were injected intraperitoneally with IXN or control (PBS), daily, until post-natal day (P)4. Then, pups were euthanised and eyes enucleated, fixed in 4% p-formaldehyde overnight, and retinas were dissected out and stained with FITC-conjugated isolectin. Finally, retinal endothelium was visualized using fluorescent and confocal microscopy, photographed and vascular quantification measurements were performed.

At P4, the IXN group displayed a significant decrease in the vascular length and increase in the width of the capillary free zone of main arteries and no differences in the number of artery side branches. Furthermore, it was observed a decline in the number of endothelial tip cells and filopodia and decreased filopodia length in the IXN-treated mice. Taken together these results suggest that IXN negatively modulates EC migration (tip cells and filopodia) and vessel sprouting (vascular length) and endothelial cell survival (with of capillary free areas).

Further analysis on later stages of retinal angiogenesis are being carried out in P5 and P7 pups and are expected to deeply elucidate the role of this polyphenol in tip cells behaviour and vessel morphology. Nevertheless, the accumulated evidence proposes IXN as an interesting anti-angiogenic compound that might have therapeutic and preventive applications in angiogenic and inflammation associated diseases.

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# ADENOSINE A<sub>1</sub> AND A<sub>2A</sub> RECEPTORS MODULATION OF CALCIUM UPTAKE BY SYNAPTOSOMES OF THE HIPPOCAMPUS AND NEOCORTEX OF MESIAL TEMPORAL LOBE EPILEPSY (MTLE) PATIENTS

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Adenosine (ADO) is considered a ubiquitous homeostatic substance acting as an “endogenous anticonvulsant” in the brain, via the activation of inhibitory A<sub>1</sub> receptors that down-regulate excitatory glutamatergic synapses. ADO can also activate A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> adenosine receptors that are usually less abundant in the brain. Unbalanced ADO modulation is implicated in pathological situations resulting from excessive function of glutamatergic pathways, such as epilepsy. Controversy exists, however, on the role of ADO on epileptic hippocampi perhaps because ADO neuromodulation in this region might result from a balance between inhibitory A<sub>1</sub> and excitatory A<sub>2A</sub> receptors.

Here, we addressed the modulatory role of ADO on veratridine (VT, 10 μM)-induced calcium influx ([Ca<sup>2+</sup>]<sub>i</sub>) and exocytosis from synaptosomes of the hippocampus and adjacent neocortex of cadaveric controls, non-MTLE or drug-resistant MTLE patients submitted to surgery. All the procedures were approved by the Ethics Committees of Centro Hospitalar do Porto and ICBAS-UP. Synaptosomes incubated with calcium-sensitive (Fluo4-NW) and exocytosis-indicating (acridine orange) fluorescent dyes were tested in a microplate reader (BioTek™ HT) to measure fluorescence signals.

The A<sub>1</sub> receptor agonist, R-PIA (100 nM), decreased (~15%) [Ca<sup>2+</sup>]<sub>i</sub> accumulation by synaptosomes of cadaveric hippocampus and non-MTLE neocortex depolarized with VT (10 μM). R-PIA (100 nM) was devoid of effect on VT-depolarized synaptosomes of the neocortex and hippocampus of MTLE patients; under these conditions, the inhibitory effect of R-PIA on [Ca<sup>2+</sup>]<sub>i</sub> accumulation and transmitter exocytosis became apparent only after increasing the concentration of the agonist to 300 nM. Blockade of A<sub>1</sub> receptors with DPCPX (10 nM) prevented inhibition caused by R-PIA (100 nM). In the neocortex of MTLE patients, DPCPX (10 nM) transformed inhibition by R-PIA (100 nM) into a facilitatory effect (~15%); the A<sub>2A</sub> receptor agonist, CGS21680C (10 nM) raised [Ca<sup>2+</sup>]<sub>i</sub> accumulation by about 26%. The facilitatory effect of CGS21680C was antagonized by the A<sub>2A</sub> receptor antagonist, ZM241385 (10 nM). CGS21680C (10 nM) slightly decreased [Ca<sup>2+</sup>]<sub>i</sub> accumulation in cadaveric hippocampus and non-MTLE neocortex, but this effect was unaffected by ZM241385 (10 nM).

Data suggest that the ability of adenosine A<sub>1</sub> receptors to control Ca<sup>2+</sup> influx and transmitter exocytosis in the hippocampus and neocortex of patients with MTLE is significantly impaired. Furthermore, [Ca<sup>2+</sup>]<sub>i</sub> accumulation inside nerve terminals of MTLE hippocampus and adjacent neocortex may be increased by A<sub>2A</sub> receptors activation. Thus, nerve terminals from hippocampus and neocortex of MTLE patients exhibit significant impairment of adenosine A<sub>1</sub>-receptor-mediated anti-epileptic action leading to increased excitability, which might be exaggerated by compensatory activation of excitatory A<sub>2A</sub> receptors.

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# Interstitial cells of cajal regulate cholinergic neurotransmission in the rat myenteric plexus: On the role of endogenous adenosine

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Interstitial cells of Cajal (ICC) coordinate gastrointestinal motility by regulating neuromuscular transmission in the tripartite myenteric synapse. The way ICCs use to integrate cholinergic nerve inputs to smooth fibers is not fully understood. Typically, the muscarinic M<sub>3</sub> receptor is localized in smooth muscle cells, where they mediate muscle contraction. The M<sub>3</sub> receptor is also detected on myenteric ICC (ICC-IM). Previous findings from our group demonstrate that muscarinic M<sub>3</sub> receptor activation facilitates acetylcholine (ACh) release from stimulated myenteric neurons, through a mechanism that depends on adenosine outflow leading to activation of excitatory A<sub>2A</sub> receptors (Vieira *et al.*, 2009, *Neurogastroenterol. Motil.* **21**:1118-95). Therefore, we aimed at investigating the role of adenosine released from ICC-IM on the muscarinic M<sub>3</sub> receptor-mediated facilitation of neuromuscular transmission in longitudinal muscle-myenteric plexus (LM-MP) preparations of the rat ileum.

Stimulation-evoked (5Hz, 200-3000 pulses) release of [<sup>3</sup>H]-ACh and adenine nucleosides (INO+ADO) were measured by liquid scintillation spectrometry and HPLC, respectively (see Vieira *et al.*, 2009). Muscarinic-induced smooth muscle contractions were continuously monitored via a PowerLab data acquisition system.

Using confocal microscopy, we showed that ICC-IM of the rat ileum exhibit high immunoreactivity against muscarinic M<sub>3</sub> receptors, whereas A<sub>2A</sub> receptors are predominantly localized in VAcHT-positive cholinergic nerve terminals. The muscarinic agonist, oxotremorine (Oxo) concentration-dependently increased both the release of [<sup>3</sup>H]-ACh and the outflow of ADO from the stimulated LM-MP. Blockade of muscarinic M<sub>3</sub> receptors with J104129 (6 nM) prevented Oxo (300 μM)-induced facilitation of [<sup>3</sup>H]-ACh and ADO release and competitively antagonized Oxo (0.003-300 μM)-induced contractions of the LM-MP. The adenosine A<sub>2A</sub> receptor antagonist, ZM241385 (50 nM), prevented Oxo-induced facilitation of [<sup>3</sup>H]-ACh, but kept unchanged the outflow of ADO. Blockade of nerve action potentials generation and smooth muscle contractions, respectively with tetrodotoxin (1 μM) and nifedipine (1 μM), failed to modify Oxo (300 μM)-induced facilitation of ADO outflow. Implication of ICC-IM on M<sub>3</sub>-receptor mediated facilitation of ADO outflow was confirmed, since mibefradil (3 μM, a blocker of T-type Ca<sup>2+</sup> channels located predominantly in ICC-IM) substantially (97±5%, n=3) decreased Oxo-induced facilitation of ADO release. Mibefradil (3 μM) attenuated (by 60%, n=4) the spontaneous motor activity and the maximal tension produced by Oxo (0.003-300 μM) in the LM-MP. Mibefradil (3 μM) also decreased Oxo (300 μM)-induced facilitation of [<sup>3</sup>H]-ACh release by a similar amount (22±4%, n=4) to that observed with J104129 (6 nM) and ZM241385 (50 nM).

Data suggest that M<sub>3</sub> receptors activation on ICC-IM mediate a positive feedback mechanism on evoked ACh release by increasing the outflow of ADO to concentrations high enough to those required to activate neuronal facilitatory A<sub>2A</sub> receptors.

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**U. PORTO**

*Parallel Oral Sessions VI*

**A2** *Communication Sciences III*

# French Romani repatriation - Agenda-Setting Theory: a content analysis of the three major French newspapers

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The aim of this article is to prove the existence of the Agenda Setting theory in the covering of the polemic case of the repatriation of the gipsy community in France.

After being formulated the analytic methodologies, as much quantitative as qualitative (that is formed by the Framing) and objectifying the period, in which our investigation would fall upon, we tried to prove the hypothesis and expectations that has been created a priori. The hypothesis that has suggested the agreement of our three aim publications of study of his known and assumed politic orientations, had tended to be corroborated, exposing, in this way, a well-conditioned and partial well- informed covering.

The concern that newspapers suggest in the treatment of news, with different criterions according to the lines of the situation, is demonstrative of the dimension of media and their influence in the public opinion of one society.

**Keywords:** Agenda-Setting, Framing, Gypsies

# The youth's vocational guidance mediated by media - The influence of criminal television series in young academic choices

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The youth's vocational guidance, despite belonging to oneself, is affected by external factors such as family, friends, school and media. In today's society, as the mass media, particularly television, take great relevance when it comes to shaping youngsters minds and new challenges arise for the vocational building. It is therefore pertinent to examine the relevance of criminal television series when it comes to young academic choices. So this investigation wants to understand if the CSI Effect extends itself to the motivations that lead students to choose a graduation in criminology area. The study began by a literature research on factors influencing the vocational choices. Authors stand out as Magalhães, Redivo [1] and Sibere [2], among others. After identifying the main determinants for choosing a profession, we analyzed the influence of television. It became important to study the involvement of criminal television series due to its huge success in Portugal. To this end, it deepened the literature review using american investigations on the subject. Podlas [3], Smith, Stinson and Patry [4] are some examples of authors that report the "CSI effect". Then it was important to understand whether this "CSI effect" extends to the motivations that lead students to choose a degree in the field of Criminology.

So, to support the hypothesis that the projected image of the forensic work on the criminal television series leads young people to engage in this area we used the interview technique via e-mail with U.S. authors Schweitzer and Smith. From this hypothesis we conducted a survey of students in the 1st and 4th years of the Criminology Course, Faculty of Law, University of Porto. Finally, to validate the results in Portugal, we used the interview technique with José Pinto da Costa.

In short, this investigation confirmed the premise of this work, that is, the most significant external factor for students to choose Criminology is the media (28%). Pinto da Costa reinforces the idea that such series have an influence on the students. As they are exposed to perceptions of reality conveyed by the forensic crime series, the students admit they were influenced by media in their choice. The media stand out, yet, in the role of disclosure since students admit that they were alerted to the existence of the course through the media. However, this study does not determine a direct relationship between the viewing of programs like "CSI" and the choice of Criminology course, since that percentage is not very expressive. The 1st year's students of Criminology have a higher influence of mass media in the perception they have on Criminology, while the 4th year's show a more realistic perception. This can be explained by learning throughout the course.

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# The Standardization of News: The Case Study of Sport News on Twitter

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«Whether they are reporting about it, finding sources on it or urging viewers, listeners and readers to follow them on it, journalists just can't seem to get enough of the social networking service. Just how effective is it as a journalism tool?» [1]

“In the beginning was the Word”, then the chat rooms, the news publications online, the blogs, the backpack journalism, the citizen journalist, the disinformation and more recently the social networks and micro blogs.

Those social networks such as Facebook or micro blogs such as Twitter are now, more than ever, part of our lives. Throughout personal pages, contact webs, simple navigation, source of information this new ways of communication have surely come to stay.

With this in mind we wonder how the journalists work got affected. Twitter is quick, instantaneous and provides information from various sources but...is it efficient? Does the news we see on Twitter provides us different and meaningful information? Or is there standardization on the news process? And by standardization we mean are the tweets, actors and signification of the news the same.

This work is relevant because it tests, if not the quality, at least the range and difference on the news coverage on Twitter and can be used as guide line of what-to-do and what-not-to-do. Besides it's a pioneer work on the area and presents a new question and new results.

To accomplish this we followed 4 Twitters accounts (O Jogo, A Bola, Record e Maisfutebol) during a weekend of sports activities (17 – 20 December 2010). This resulted on 686 tweets. Then, content and discursive analyses were taken to deconstruct the tweets.

The results of the study indicate that there is, in fact, standardization. Not total standardization on all the tweets, but significant enough to be noticed. Roughly, 75% of the tweets analyzed present some kind of standardization, even if it is just on the tweet itself, on the news actors or in the meaning of the tweet.

This proves, among other, two essential things: in first place Twitter gives us a lot of information, quick and instantaneously but not too differentiated. In second that newspapers are losing their identities when they go to Twitter.

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# Wikipedia, the freely edited online encyclopaedia: a comparison between areas of study

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There is still a widespread concern about the credibility of the information found in Wikipedia, despite all efforts made by the community to control and verify it. However, a study has shown that its articles related to scientific areas are as accurate as the ones in *Encyclopaedia Britannica*. [1] Others related to history or politics, for instance, are much more subject to errors, since “emotion and ideology may affect the article’s objectivity and precision”. [2]

Our ongoing research aims to prove whether or not students of science and students of humanities use this tool differently. The main hypothesis is that the formers tend to mistrust Wikipedia and look for more certified sources, given the rigour required by their type of degree; on the other hand, students of humanities rely on this encyclopaedia, even though the kind of entries they consult is more likely to contain false or at least biased statements.

For this purpose, a survey involving the faculties of Engineering and Arts at the University of Porto has been carried out. The respective results are now under analysis.

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# The practice of citizenship in extension projects

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The Extension Project Social Management Citizenship aim to expand spaces socials of discussion and citizen problematization, especially when its about themes such as citizenship, participation, social management and public policy. Thus, the project constitutes an educational space to qualify for the participation of civil society in the process of shaping the public agenda, at the monitoring of policy, evaluation of performance and accountability of agents of public sector. The main actions developed by the project are: informative and dynamic electronic portal, weekly radio program, database with socioeconomics, demographic, tourism, politics informations, seminars and workshops for qualification.

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# Social networks and Cyberjournalism

## Som à Letra: a case study

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Multimedia nowadays is one of the keypoints (the main one?) to a new way of journalism, close to readers. Know how to work with different components (images, audio, video, graphics, animations) is be able to inform in a enriched and direct way. The readers feel as they make part of a family. And isn't that the correct position?

Social Network, as twitter and facebook become truly important to get close to readers. A new function, profession is created: **community host**. *The job of engaging with those formerly known as “the audience” is in some ways becoming a new online “beat”—one in search of a simple moniker to describe what it is, the skills required, and the tasks entailed.* [1] Nathasha Lim works as a community host at TBD. *That's what I say when people ask what I do. Hearing this, they smile, sort of, and nod their heads, and then they ask again what it is I really do. By now, this routine is all too familiar—but I can appreciate why. Until I started this job, I hadn't heard of a community host either. Unlike the previous positions I've held—reporter, producer, video journalist—this one was unfamiliar, with responsibilities undefined and always evolving* [2].

Som à Letra is a cyberjournal created in 2009, with social networks support. Results: the audience increases each day.

It's social networks a new way of converging audience? Or just another tool to journalists?

After nine months counting visits from our blogger we found that facebook is definitely an important tool to an efficient work. (Table 1)

### Sites de referência

<a href="http://www.google.com.br">www.google.com.br</a>	9 695	
<a href="http://www.google.pt">www.google.pt</a>	5 304	
<a href="http://www.facebook.com">www.facebook.com</a>	3 303	
<a href="http://www.google.com">www.google.com</a>	1 085	
<a href="http://www.google.fr">www.google.fr</a>	159	
<a href="http://images.google.com">images.google.com</a>	103	
<a href="http://www.blogger.com">www.blogger.com</a>	103	
<a href="http://www.blogcatalog.com">www.blogcatalog.com</a>	94	
<a href="http://www.google.co.uk">www.google.co.uk</a>	71	
<a href="http://www.google.it">www.google.it</a>	42	

Table 1

**Keywords:** Social Network, Facebook, Twitter, Cyberjournalism, Community Host

### References:

[1]Community Host: An Emerging Newsroom ‘Beat’ Without a Guid

<http://www.nieman.harvard.edu/reports/article/102521/Community-Host-An-Emerging-Newsroom-Beat-Without-a-Guide.aspx>, accessed at 22/11/10

[2]Community Host: An Emerging Newsroom ‘Beat’ Without a Guid

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**U. PORTO**

## *Parallel Oral Sessions VI*

### **A3** *Agronomy & Chemistry II*

## Creation of a condiment for seasoning meat before cooking to minimize the formation of carcinogenic compounds

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Cooking meat has clear beneficial impact, as the microbial content decreases, the digestibility increases and the flavor and texture improves. However, amino acids, creatine and sugars naturally present can react and generate carcinogens under household conditions. When basic human diets include meat dishes, it is impossible to avoid the risk of exposure to the heat generated contaminants and it is important to have a clearer picture of levels of different contaminants in the meat. Minimization strategies of their formation are also of great importance from the viewpoint of food safety, with satisfactory acceptance of consumers.

Mutagenic compounds in heated meat and fish products include heterocyclic amines (HAs). The antioxidant effect on this reaction is well described in model systems; however the effect in real meat samples is not so clear. Marinating with natural antioxidant is a common practice in meat dishes, with possible advantages in food safety [1,2].

There are several factors involved in the inhibition of HAs by meat ingredients, though it is apparent that antioxidant-containing juices/spices/herbs play a role in this process. This study proposed to understand the contribution of the antioxidant ingredients, including different marinade liquid (vehicle) alone and liquid and the mixture of spices/herbs all together, in the formation and/or inhibition of HAs.

Pilson beer (B-beer, BH-beer and herbs) and white wine (W-wine, WH-wine and herbs) were tested as the marinade vehicles, the herbs/spices used were ginger, garlic, rosemary, thyme, red chili pepper. The marinating of small pieces of beef was performed during 4 hours before grilling the meat. The meat was cooked until medium done level. Radical scavenging activity was tested for all the marinades in 30 min periods along 4 hours, using DPPH assay.

Radical scavenging activity was highest for W (64% of inhibition); WH presented lower activity (60% inhibition), without significant differences. BH presented higher radical scavenging activity than B (33,5 and 26% inhibition, respectively), and the variations recorded along 4 hours were more significant, with higher activity at 90 minutes (42% inhibition). W activity was stable, although a small decrease was noted along the 4 hours of the assay. Despite the lower antioxidant activity of B and BH marinades their contribution for reduction of HAs formation was greater than the W and WH marinades.

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# Anthocyanins and Anthocyanidins in Port Wine

**A.I. Couto, C. Pinho and I.M.P.L.V.O. Ferreira**

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The vineyards of the Douro Demarcated Region in Northern Portugal are the birthplace of the wellknown Port wine, produced from grapes of Touriga Nacional, Tinta Barroca, Tinta Roriz, Touriga Francesa, Tinto Cão and Tinta Amarela varieties. One important family of polyphenolic compounds present in grapes, which influence the final wine quality, are the anthocyanins. In addition, these pigments are responsible for the characteristic color of red wine. Grape skins are amongst the best natural sources of anthocyanins and they occur exclusively as 3-O-monoglucosides and the 3-O-acylated monoglucosides of five main anthocyanidins (aglycone forms) – delphinidin, cyanidin, petunidin, peonidin and malvidin. Individual anthocyanins are differentiated by their degree of hydroxylation and methylation, and by the position and nature of their glycosylating structures [1].

The anthocyanins and anthocyanidins content in Port wine can vary depending on climatic factors, grape ripeness, winemaking process and aging. The aim of the present work was to provide some new insights into the understanding of the content in anthocyanin and anthocyanidin compounds in Tawny, Ruby and LBV Port wines. For this purpose, Port wine worts and different types of Port wine samples were analyzed by high performance liquid chromatography with Diode Array detection (HPLC/DAD).

Port wine worts produced from *Vitis vinifera* (var. Touriga Nacional, Touriga Francesa, Tinta Roriz and Tinta Barroca) grapes from “Quinta da Roeda”- Croft were analysed at different states of the fermentation process (after 2, 4 and 6 days). Commercial Port wine samples including Tawny (Reserve, 10 and 20 years), Ruby and Late Bottled Vintage – LBV were also analysed. Extraction and hydrolysis of anthocyanins was performed in acidic medium [2].

The HPLC analysis of Port wine wort methanolic extracts revealed similar qualitative patterns. However, owing to the continuous extraction of anthocyanins from grape skins, there was a quantitative increase of these pigments during wort fermentation. Lower amounts of anthocyanins and anthocyanidins were observed in Ruby samples when compared with Port wine worts due to the addition of brandy at the end of fermentation. Tawny samples presented a completely different profile since no anthocyanins were detected.

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## Electrochemical studies on xanthohumol

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Xanthohumol (XN) is a prenylated chalcone that belongs to the family of phenolic compounds. Among other phenolic compounds XN has been exhibiting a positive response in studies related to the prevention of lifestyle-related diseases such as diabetes, osteoporosis, coronary heart disease, neurodegenerative disorders (dementia, Alzheimer, Parkinson), hypertension, atherosclerosis, and many types of cancer [1, 2]. Within such promising health promoting effects this compound is of the major interest to food industries (as a food additive, per example) and to pharmaceutical companies. Although large scientific work about its biological benefits has been published on recent years, information about its electrochemical behavior is still scarce. In the present work a mechanism of XN reduction has been proposed using cyclic voltammetry (CV) on a hanging mercury drop electrode (HMDE). The reduction of XN seemed to be an overall irreversible process and a mixed adsorptive and diffusion response was obtained [3, 4]. This study provided a double reduction profile depending on the proton concentration. As so the electrochemical stoichiometric was found to be  $-3H^+$ ,  $-2e^-$  in acidic conditions contrasting to a typical  $-2H^+$ ,  $-2e^-$  in basic pH. Fig. 1 shows the proposed electrochemical reduction mechanism of XN at  $pH > 6$ .

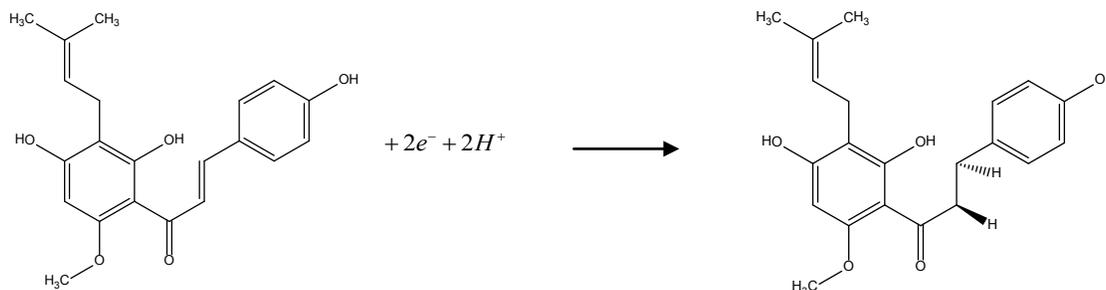


Fig. 1 - Electrochemical reduction of XN.

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# L-asparaginase II: Computational Studies on a New Chemotherapeutic Enzyme

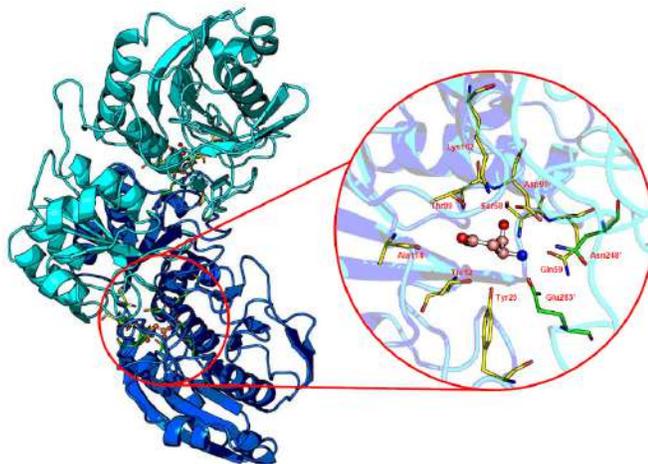
D. S. Gesto<sup>1</sup>, NMFSA Cerqueira<sup>1</sup>, P.A. Fernandes<sup>1</sup> and M.J. Ramos<sup>1</sup>

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L-asparaginase (fig. 1) is an enzyme which catalyzes the hydrolysis of L-asparagine to aspartate and NH<sub>3</sub>. This enzyme can be found in a large number of organisms, from bacteria to mammals, although, until today it was not found in humans or primate [1].

Nowadays, L-asparaginase has several applications, especially in cancer therapy, where it is used as a chemotherapeutic agent against acute lymphoblastic leukemia and some other types of cancer [2,3], and in food industry, as a mean of reducing the formation of acrylamide in starch rich foods. Despite having this and some other applications, the reaction mechanism of L-asparaginase is still unknown.

The aim of this study was to investigate the catalytic mechanism of L-asparaginase using hybrid methodologies (namely ONIOM). The results have shown that the hydrolysis of asparagine to aspartic acid is highly governed by a catalytic quintet formed by Thr89, Thr12, Tyr25, Lys162 and Glu283. The reaction involves 7 sequential steps, contrarily to what was previously thought, and requires the participation of a water molecule. The rate-limiting step is the fifth step, which involves the bonding of the water molecule to the acyl-enzyme intermediate.



**Figure 1** – Representation of the *E. coli* dimer of L-asparaginase II (PDB: 3ECA). In detail, we can see the residues that compose the active site, as well as the reaction product.

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# Unveiling the internal aldimine formation in Ornithine Decarboxylase by computational methodologies

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Ornithine Decarboxylase (ODC) is a vitamin B6 - pyridoxal 5'-phosphate (PLP) dependent enzyme that catalyzes the first committed step in the biosynthesis of polyamines. Since the polyamines are a family of aliphatic amines that fulfill multiple and essential physiological roles [1,2], this enzyme is a cornerstone in many biological processes, such as cell growth. Polyamines have long been associated with cancer and increased polyamine levels are a frequent hallmark of cancer cells. Furthermore, it has been shown that polyamines are required for neoplastic transformation and tumor development. This means that ODC may provide an important target for the design of new compounds that prevent carcinogenesis and tumor growth [3].

Transition state analogues are potent inhibitors of enzymatic activity. Taking that into account, the aim of this study was to investigate the catalytic mechanism of ODC using quantum mechanical DFT/semi-empirical AM-1 computational methods (Figure 1). So far the obtained results provide a complete description about the activation of ODC, i.e, the formation of the internal aldimine from vitamin B6 (Figure 1).

As all PLP dependent enzymes require the binding of vitamin B6 to an active site conserved Lysine, this mechanism should be general for all PLP dependent enzymes that require vitamin B6 as a cofactor.

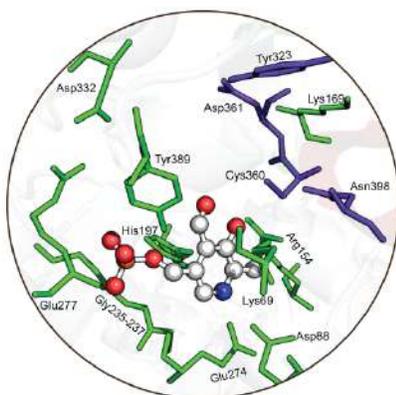


Figure 4 – Representation of the active site model of ODC that was used to study the formation of the internal aldimine by computational means.

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# Density Functional Theory Investigation of the pH effect on the Firefly Bioluminescence Colour

**L. Pinto da Silva and J.C.G. Esteves da Silva**

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Firefly luciferase (EC 1.13.12.7, Luc) is an enzyme that catalyzes the oxidation of firefly luciferin ( $\text{LH}_2$ ), giving rise to light in a two-step reaction. The most remarkable characteristics of this system are the large quantum yields of light production and its pH-dependent multicolor bioluminescence (in the range of 530-640 nm). Due to these advantageous characteristics this system has been used as a sensitive tool in molecular biology, as a gene reporter and in detection of environmental contamination [1].

The origin of the multicolor variation is still one of the major mysteries in this research area. Several aspects have been highlighted thus far: keto-enol tautomerism of the light emitter oxyluciferin ( $\text{OxyLH}_2$ ), different conformations of Luc active site, resonance-based charge delocalization, the polarity of the active site and interaction of  $\text{OxyLH}_2$  with a protonated moiety [2].

The objective of this work was the prediction of  $\text{OxyLH}_2$  species  $\text{pK}$  and  $\text{pK}^*$ , in the open and closed conformations of Luc, and their distribution in the pH range and the study of the structural and electronic properties of the possible emitters [3].

Our calculations concluded that the anionic keto-form is the sole light emitter (Fig. 1) and the hypotheses formulated to date do not suffice for explaining the multicolor variation. The changes of emitted light can then be explained by the interactions of the emitter with active site molecules, which effects on light emission are modulated by the internal polarity of the different conformations. These interactions can suffer also from rearrangement due to entry of external solvent and changes in the protonation state of some active site molecules.

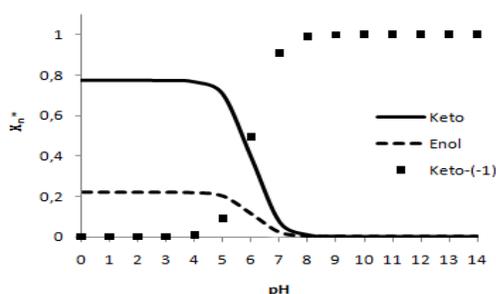


Fig. 1) Computed distribution diagram for the first excited

Acknowledgments: Financial support from Fundação para a Ciência e Tecnologia (FCT, Lisbon) (FSE-FEDER) (Project PTDC/QUI/71366/2006) is acknowledge.

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**U.**PORTO

*Parallel Oral Sessions VI*

**A4** *Geography, Sociology & Economics II*

## Valuation of fresh produce at Local Markets – consumer’s perspectives

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**Introduction:** There has been an increasing consumer’s interest on fresh products from a local origin, even when prices are higher. To preserve consumer’s trust and confidence and to enlarge this consumer segment it is essential that producers and retailers know and recognize the fresh produce quality indicators valued by consumers at these points of purchase.

**Objectives:** To identify the major benefits and barriers associated to fresh produce at Traditional Food Markets.

**Material and Methods:** Based on focus groups discussion and on preliminary studies made by the research team, the questionnaire was designed. Questionnaires were applied between November and December 2010 to regular and non-regular users of five Traditional Food Markets in the North of Portugal (Bolhão, Bonsucesso, Matosinhos, Mercado da Foz and Braga). A convenience sample of approximately 230 respondents was used.

**Results:** The majority of respondents were female and living in households with three persons (33 %). The products more frequently bought at Traditional Food Markets were fruit (87 %) and vegetables (68 %). A greater agreement of respondents was found towards sentences referring to a more restrained shopping basket when shopping at Traditional Food Markets, freshness and origin of products sold there. The disagreement was more noticeable on sentences related to food safety and hygiene at food markets. It was found a greater degree of agreement on sentences related to quality of hygiene and cleaning. The major discrepancy was found about the origin and familiarity of produce sold at traditional Food Markets.

**Conclusions:** A predominance of women shopping at food markets was confirmed nevertheless presence of younger shoppers was also identified. It is important to notice the perception that shopping at Traditional Food Markets are perceived as more controlled by the shopper than the ones at supermarkets, showing less tendency to buy by impulse in traditional shopping places. This aspect is of great relevance for food market operators indicating the possibility of developing strategies to allow consumers to feel more comfortable at the Food Market and possibly shopping larger quantities. Some of the changes valued by consumers are the parking facilities and the improvement of sanitary and hygienic conditions. The results from this study may contribute to different stakeholders focus their communication in a more efficient way aiming to attract more clients to the Traditional Food Markets.

Acknowledgments: authors acknowledge financial support given by University of Porto and Santander through: “Projectos Iniciação à investigação 2009”

# **Social Tourism: reality, opportunity. A study Case: INATEL**

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The purpose of this paper is to apply a social sciences approach to study a subject which, as a result of the economical, social and political transformations, has effects in all social dimensions, and therefore, can be approached by several sciences – Social Tourism.

The geographical approach allows us to acquire a multidimensional perspective, understand the dynamics under the subject, their causes and consequences.

The specific goal is to elaborate patterns of evolution of the Social Tourism activities in our country, particularly focusing on the programs undertaken, types of activity, as well as in the effects of such activities, in social, economical and land planning matters.

To achieve these goals, we apply the social sciences research method, knowing that there is not only one but several ways to knowledge construction, and therefore we must apply a continuous evaluation and reevaluation of the research options.

From the research question “Which is the role of INATEL in the evolution of Social Tourism in Portugal”, we could follow a various set of research lines.

The expected outcomes of the ongoing empirical study are:

- To Identify the legal documents that regulate the social tourism activities
- To analyze the evolution of Demand and Supply dynamics of Social Tourism (infrastructures; activities; programs, geographical distribution)
- The role of the State and the Municipalities in the promotion of Social Tourism programs

# The Cycle of Flax in the Municipality of Penafiel

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In this communication I propose to present the study of the Cycle of Linen in the Municipality of Penafiel, dissertation topic held for completion of the Master in Archaeology (FLUP-2010).

Initially we observed, in field work, the remaining artisanal production of flax in Penafiel and Vale do Sousa and confront the historical attempts to industrialization. We describe the cultivation of flax as traditional agricultural activity through a brief analysis of their annual cycle, characterizing the type of land tenure and social relations that favored their continuance.

In the following, we present the flax as plant, its varieties, the choice of land, field preparation for sowing, irrigation system, weeding, harvesting and separation of the grain (ripping) “ripagem”, which the next year will restart the cycle.

Once obtained the raw material, we look at the fiber preparation followed by “enlagamento” (retting) or “curtimento” (tanning), and “maçagem” (break and crush the flax’s husk) manual or machine-driven. Here we have looked more carefully at these important facilities, arising from the second half of the nineteenth century and analyze the previous technique of “maçagem”, without the use of the machine. We contextualize the introduction of the mechanical device and its advantages, characterizing the animal traction driven and the hydraulic driven mechanical devices in its different operation and components, remembering the architecture of the buildings, where they are inserted, the forms of ownership and operation, as well as payment of service.

The “espadelagem” (scotching) and “assedagem” (heckling), still in preparation of the fiber, are the latest stages of the process conducted in the farmer's house.

With spinning, performed at home or handed off, the manufacture of yarn begins, the weaver woman (home or abroad) will be responsible for making the cloth. Looking at the part of the cycle where the warp is prepared and its assembly on the loom, and the stage of weaving, with particular attention to textures and points to contribute to a classification of regional productions.

We also analyze the linen cycle activities that take place in party atmosphere, an identifiable and inseparable feature of rural identity.

# 'Poor' housing: social and material conditions that underlie the construction of everyday life - the case of *ilhas* at Oporto

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With this presentation we intend to present the main results of a research conducted under the master's degree in Sociology, about *the central status of domestic space in the construction of everyday life* in the case study of Oporto's *ilhas*.

The theoretical framework lays upon the inclusion of the axes of time and space for the configuration of the *social space's* concept as for its objective manifestation into the physical space's structures. This dynamics produce varying and changeable representations and perceptions of places. Housing is then perceived by the different social groups as an object of unequal access, and, as such, triggered as a domination tool. Then, in this analytical framework we aim to lay down a set of propositions which could give us a wide comprehension of the *ilhas* city sociogenesis. After that, and anchored on the specific case-study of the *ilhas* - *Grande* and *Padeiro* in S. Vítor Street and in the neighborhood/*bairro* *Herculano* – we seek to identify the modes of daily life organization and the perceptions of the space perceived by its inhabitants. This analytical set contains, as a main objective, a comprehensive framing at the residence issue in *ilhas*, taking in account the wider processes of change in the city, especially their place for solving the “housing issue” as a particular one that assaults the city since its industrialization.

From the standpoint of a qualitative strategy of inquiry and operatory methods, such as direct observation and interview, we look to identify the multiple residential and social trajectories of the agents living at *ilhas*.

In those analyzed cases, is seen a commonly identifiable weak set of social and economic resources that leads to the reification of precarious condition. This condition translates itself in a particular representation of the city where it underlies the immobilization of agents in both the physical and social space of the city. Our discussion will focus on a reflection between theory and practice, and how we can use scientific tools from multiple research fields, such as Spatial Anthropology and Architecture, besides Sociology among others.

# The Professional Transition of the Graduates in Sociology from FLUP: new results from 2006

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This communication takes as a general theme the employability of the graduates in sociology, already addressed in a summarized and exploratory approach during the 2<sup>nd</sup> IJUP's edition, in 2009. At this present moment, we seek to consider and reflect about the importance that a superior education degree in sociology holds in the labor market.

This matter has become particularly relevant following the deep on-going changes occurring in the economic and social framework. Under these circumstances, the transition of young graduates in the labor market has gained increasingly insecure and unstable patterns, with diverse manifestations, both in its social and professional experienced terms, in their situation of access to the employment and, also, by the timings and shapes outlining the unemployment.

Therefore, in continuity with the two previous studies carried out in 1998 and 2003, the main purposes of this communication are to identify and discuss the insecure and unstable professional transition features of the graduates in sociology from the Faculty of Arts of University of Porto, with the data collected in 2006 under Employment Observatory of Graduates in Sociology, headed by the Institute of Sociology of University of Porto

# The importance of natural and anthropic characteristics in the urban climate - Study the in historic centre of Porto, Vitoria

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The present work represents an attempt to analyze temperature parameters assessed during an entire night measuring process and pre-defined in one of the oldest districts of the historic centre of Porto, Vitoria.

The monitored route was covered by car, during different synoptic situations days, with a total length of 4km including 79 measuring points reached in 20 minutes time-frame. The measurements have been realized between March and May of 2010, with a Delta OM thermometer.

Defining this itinerary and choosing the 79 evaluating points intended to enclose different existing urban metabolisms in the Vitoria district, as well as urban design and morphological differences. For selecting this route different sky view factors, solar exposure, declivity and proximity to green areas were considered. The largest number possible of control parameters for local energetic balance was pursued.

The analysis of gathered data as well as of the different factors that finally cause the alterations, was backed up by the analysis of the synoptic maps and satellite images collected by UKMO.

The distance of the measured route, the time interval and the definition of the recording points seek above all to analyze the weight of each feature, both natural and anthropic, in order to explain the different results that were obtained.

The combined analysis of the registered temperature parameters and the classification of different spots of urban metabolism in this city area, allowed us mainly to improve the knowledge of the impact of these various urban puzzle pieces, in tangible, in the local energetic balance. Therefore, we shall provide the decision makers with more and better information in order to evaluate the relative weight of some of their decisions on the territory for the success of the implementation of their sustainable development policies for the urban space of medium dimensions as the city of Porto.





**U. PORTO**

*Parallel Oral Sessions VI*

**A5** *Sports Science II*

# The (re)construction of Professional Identity: a study with a practicum group of FADEUP. Presentation of a research Project

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Nowadays, we assist in Portugal to a change in the processes of teaching education, particularly at the initial training of Physical Education (PE) teachers, which is associated with a new paradigm in higher education resulting from the implementation of the Process of Bolonha (PB). Cumulatively, the teaching profession is in a period marked by increased bureaucratization, increased forms of managerialism, and greater accountability [1]. Thus, not only how the school is experienced has changed, but also emerges a professional identity (PI) with a new twist, for the reason that the PI is historical, situational and relational [2]. Changes are also visible in the international context, and advocate for a new vision for school science, comprising principles, standards, and practices that are grounded on research in the learning sciences [3]. Thus, the research about the (re)construction of the PI is of great importance, the outcome of an interface between the personal experiences of teachers and the social, cultural, and institutional context in which they function on a daily basis [4]. The main goal of this paper is to present a project's framework regarding how pre-service teachers experience the impact with the real teaching experience on the school context and, simultaneously, how they (re)construct their PI and the roles inherent to it. Additionally, we are trying to understand how cooperating teachers, while supervisors of the professional practicum process, (re)construct their PI. The participants on the study will be pre-service teachers and cooperating teachers of the Faculty of Sport of the University of Porto of the school year 2010/2011. The questions surrounding this study are directed to the way how the experience of being trainee and supervisor is lived and interpreted, as well as the role of the professional practicum on the acquisition and (re)construction of PI by the actors. In this way, we will develop two studies of interpretative nature, in order to understand the practicum process in the (re)construction of PI of the pre-service teachers and of the cooperating teachers. Additionally, another study of interpretative nature will be conducted, which goal is to understand the functioning of the group practicum practice, starting from the understanding that it represents a community of practice.

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## The professor interaction as a way to (re)construct the Professional Identity. Written and visual reports.

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Becoming a teacher involves, in essence, the (trans)formation of the teacher identity, a process described as being open, negotiated and shifting that occurs along of all the teacher career [1]. The purpose of this study was to understand how the physical education teacher interacts with students in the classroom context and to identify how they perceive their own actions. Two complementary methods were used, namely, the board Diary and Visual ethnography. The data collection has been made for eight consecutive days. The participants in this study were one experienced teacher and three pre-service teachers. For data analysis we used the content analysis of the written documents and images captured, sustained by the qualitative analysis software, NVivo 8. The categories were defined *a posteriori* and are described as: a) *characteristics of the personal self*, b) *particulars of professional self*, c) *participation in education* and d) *actions for education*. The results indicate that: a) being teacher is a profession deeply related to personal experiences, b) the conviction, objectivity, motivation, individuality, persistence and leadership emerge as positive traits in winning the attention of students, c) planning, reflection and decision proved to be important measures in managing and teaching quality, and how the teacher manages the classroom intervention affects other tasks, d) the moments shared among teachers are valued as well as the continuing professional development.

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# Handball teaching at School – a Sport Education Model approach

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This study was a research action concerning the usage of the Sport Education Model in handball teaching at school, whose goal is to educate students to be knowledgeable and enthusiastic in sports [1]. The study was done during the Practicum (Master degree in Physical Education in Faculty of Sport, University of Porto). Twenty-four students (12 male and 12 female) from 10<sup>th</sup> grade (15-16 years old) were involved, in the school year 2009-10, from Porto District School. This experiment comprised 12 teaching sessions, of 90 min each. A pre-service teacher was in charge of the class.

The goal of this study was to evaluate the impact of using the Sport Education Model in handball teaching in various domains of student learning (social, cognitive and motor). In addition we tried to analyze the way the pre-service teacher experienced and felt the model, and the student's perception of its effectiveness. We used a learning constructivist approach, based on simplified forms of the handball game, to promote not only motor skill learning but also responsibility, autonomy and cooperation between students.

The analyzed data was extracted from the written documentation of the pre-service teacher (class planning and teacher board diary), from the classes' video, from a declarative test and from a socio-affective questionnaire, both directed to the students.

The results indicated that the usage of this model resulted in significant increase in students learning in various domains (social, cognitive and motor). The fields related with autonomy, responsibility, cooperation and competitive spirit, were those with a major impact in students. In general, the students considered the usage of the model very positive, because it increased the engagement between students and improved the student's involvement in the class activities, as well as their game skills level. In summary, we want to emphasize the value of this approach both in the pre-service teacher and students' development.

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# Pilates Training Effects on Ballet Dancers' Performance

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Dance, as an artistic and athletic activity, includes a set of aesthetic, technical and physical requirements. Concerning to physical requirement, optimal levels of muscular strength, flexibility and balance are associated to remarkable performances [1]. So the, purpose of the present study was to assess the effects of a Pilates training program on muscular strength, flexibility and balance.

The sample consisted of 15 Ballet students who were divided into 2 groups: experimental (n=7) and control (n=8). Both groups were assessed in the beginning and in the end of the study. Muscular strength was assessed measuring the time supported in the technical skills *penché* and *developpé* (front, side, back). To asses flexibility, it was measured the angle between limbs in the technical skills *arabesque*, *developpé* (front, side, back) and *cambré* forward. Balance was evaluated using a Bertec force plate (4060-15), and the migration area of the centre of pressure was calculated in the first position and *attitude derrière* skills. After the first moment of evaluation, the experimental group performed a Mat-Based Pilates Exercise during 11 weeks (2 sessions per week of 60 minutes).

The statistic analyses showed significant differences ( $p \leq 0,05$ ) on muscular strength and flexibility measurements between groups after the Pilates training program in all technical skills evaluated, except in the *cambré*. Probably, the experimental group had not improved this technical skill due to the weak stimulation of dorsal and lumbar muscles elicited by the Pilates exercises used. No significant differences were obtained between groups at the level of the migration area of the centre of pressure, since both improved their balance. This fact might be related to the excessive use of the point shoe by both groups during the last 3 weeks of Pilates training, which provided balance improvements in both groups.

The present data demonstrated that Pilates training can significant improve Ballet dancers' muscular strength and flexibility. As Pilates exercises emphasizes movements with some kinetic similarity to ballet technique, we believe that this type of training is suitable for dancers to improve muscular strength and flexibility, with positive effects on technical performance. Yet, we cannot state if Pilates training benefit ballet dancers' balance.

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# Effect of Age on Manual Dexterity and Functional Motor Asymmetry. Comparative study between elderly and children.

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The hand is a creative tool, an extension of the intellect, a way of nonverbal communication and an extremely important tactile sense organ. The quality of the performance in most of the daily life activities is mostly determined by the function and manual dexterity, since the hand is the most active and most important part of the upper limb (Carmeli et al., 2003). This investigation aimed to evaluate the effect of age on manual dexterity of the preferred hand (PH), non-preferred hand (NPH) and the functional motor asymmetry (FMA). The sample encompassed 24 right-handed females, 12 elderly (aged 65 to 75 years) and 12 children (between 7 and 9 years-old), all practiced dance and swimming. It was used the Soda Pop Test (AAPHERD, 1996) in the assessment of manual dexterity and the *Statistical Package for Social Sciences* (SPSS) version 18.0 to analyze the data, using the descriptive and inferential statistics. The significance level was set at  $p \leq 0.05$ . It was revealed, either for PH or NPH, the absence of statistically significant differences between groups. However, regarding the NPH, there was a tendency to obtain a better performance by children. The analyses of the FMA revealed that children are significantly less asymmetrical. In future investigations it would be interesting to study the effect of a training program aimed to manual function in the elderly and children, to observe the behavior of both in each member and the FMA.

**Key Words:** Manual dexterity; functional motor asymmetry; elderly; children.

# The Dynamic Equilibrium Structural Organization on the Offensive Football

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The Soccer assumes itself as a complex phenomenon. Thus, we consider the relevance of the systemic approach to the game, seeking an intelligibility of the complexity of [inter] actions of the players at the local and global, referring to the mutual influence of microscopic structures in the macro, also being ensured by systemic fractality. The coach plays a key role in the operationalization Specific indicators find that it takes the team to relate in Organization Offensive «ar-ti-cu-la-te-ly». The hyphens that «ar-ti-cu-tion» represent the importance of intervening in training to consider the «relations of relationships» of an Organization Structure. This intervention also leads to considerations that take place at the level of «sensitivity» of the coach which will require the team behavior patterns that express the rigidity and plasticity of the team towards greater adaptability to the environment, and better adjusted when there is a «Dynamic Balance» in Moments of the Game and the «circumstances» that require tailored actions. Under a specific focus led to the «game speed own» the player and the team, the protests and evolves in ever more complex by approaching the «border of chaos», emphasizing the relevance of understanding systemic interaction between the players, play Team Soccer Game in that «dissipates» but with «some closure» that preserves the self-organizing structure. These conditions «chaos» in the Game Model manifest themselves in favor of a qualitative and harmonic playing, because it proposes a wider range of actions to do all the interacting parts that «behavioral fractality» keep the wealth and the shape of pre Structure -conceived and under a «specific language» developed by the Team's «Principles of Game» Specific feature a "Dynamic Balance» supporting 'effective' action of the Team's under chaotic storm of the Soccer Game and they thus, maintain high levels of performance.

**KEY - WORDS:** FOOTBALL, TACTIC PERIODIZATION, COMPLEX SYSTEMS, MODEL OF THE GAME, PRINCIPLES OF PLAY, COMMUNICATION

## Physical activity, cardiorespiratory fitness and the metabolic syndrome in youth from Calanga, Mozambique

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Sedentarism and obesity are correlated to increasing metabolic risks. When clustered it is called metabolic syndrome (MS). The association between MS, physical activity (PA) and cardiorespiratory fitness (CRF) has been well studied in developed countries, but in developing countries, namely in Africa, there is a gap of information.

The aims of this study were: (1) to estimate the prevalence of metabolic risk factors and MS, PA and CRF levels in a school-age sample from Calanga (Mozambique), and (2) to examine the association between PA, CRF and MS.

Sample size comprised 209 children and adolescents (93 boys and 116 girls) from Calanga, aged 7 to 15 years old. Height and weight were measured according to Lohman et al. [1], and body mass index (BMI) was calculated. PA was estimated with a questionnaire developed for Mozambican population [2]. CRF was estimated by 1-mile run test. Metabolic risk indicators were determined using blood sample analysis and blood pressure was measured with standardized protocols. Cutoff points suggested by Cook et al. [3], Cole et al. [4] and Brazilian Society of Cardiology [5] were used to define the MS in children and adolescents. Descriptive statistics, independent *t* test and ANOVA were computed in SPSS 18.0, and the significance level was set at 5%.

Results showed that children and adolescents from Calanga have high levels of PA and CRF, and an almost inexistent MS prevalence (<1%). On the other hand, it was observed a high prevalence of elevated systolic blood pressure (26,8%) and triglycerides (18,7%). It was found a trend to a negative relationship between PA, CRF and MS among children aged 7 to 12 years, especially in girls; however, among older youngsters (13 – 15 years) it was found a positive relationship between PA and MS.

In conclusion, (1) youth from Calanga are physically active and reported high CRF; (2) they are predisponent to develop hypertension and hypertriglyceridemia; and (3) the lifestyle of youth from Calanga has a strong protective effect against MS.

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## **A multilevel modeling approach to the study of physical activity levels in Portuguese nuclear families**

**T. Gomes, F. dos Santos, D. Santos, R. Chaves, M. Souza, A. Seabra, R. Garganta, and J. Maia**

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The family is an important social support for the physical activity (PA) of its members, and many studies have focused their attention in its understanding. However, these studies do not consider the family as a hierarchical structure, organized in dyads and where all its members are mutually dependent. This fact reduces the knowledge that can be obtained when analyzing families.

The aims of this study were: (1) to investigate predictors of PA levels in nuclear families using multilevel modeling, and (2) to analyze if socioeconomic status (SES) is related to dyadic relationships and PA levels in families.

The sample was composed by 2661 Portuguese nuclear families, from central and northern region, with a total of 10644 subjects. PA was estimated with the Baecke et al. questionnaire, and information about parents' job was obtained to determine the family SES. Height and weight were measured in children, and parents reported their values. Descriptive statistics were computed in SPSS 17.0, and hierarchical analysis was computed in HLM 6.0. Significance level was set at 5%.

Results showed that intragenerational similarities are higher than intergenerational. SES was not related to dyadic relationship or PA levels, but gender ( $\beta = 0.307$ ,  $t = 20.107$  for male), age ( $\beta = -1.193$ ,  $t = -5.930$  for fathers) and BMI ( $\beta = 0.031$ ,  $t = 3.092$  for mother and  $\beta = -0.024$ ,  $t = -2.388$  for siblings) had different effects on individuals' PA.

In conclusion, results (1) indicated a strong dyadic resemblance on PA in Portuguese families, (2) showed different effects of gender, age and BMI on individuals' PA and (3) demonstrated that multilevel modeling is an interesting strategy of data analysis to study PA in families.





**U. PORTO**

*Posters I*

*Thursday, February 17<sup>th</sup>*

## A study of the antitumour potential of three Portuguese Wild Mushrooms

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Natural matrixes such as mushrooms represent a rich source of biologically active compounds with recognized potential in drug discovery and development [1, 2]. Indeed, many pre-clinical studies have been conducted in human tumour cell lines and in some cases, a number of compounds extracted from mushrooms have entered clinical trials [3]. Our previous results showed that extracts from *Agaricus arvensis*, *Suillus collinitus* and *Clitocybe alexandri* are promising sources of low molecular weight bioactive compounds [4]. The aim of the present work was to study the antitumour potential of the extracts and isolated compounds from three Portuguese wild mushrooms by verifying their effect on various human tumour cell lines in what concerns effect on cell growth, cell cycle profile and programmed cell death. Wild mushrooms were collected from the Northeast of Portugal and classified as *Agaricus arvensis*, *Suillus collinitus* and *Clitocybe alexandri*. Phenolic (methanolic and ethanolic) and polysaccharidic extracts were prepared. The effect of the extracts on tumour cell growth inhibition was verified with the SRB assay and the GI<sub>50</sub> of each extract was determined for each of the cell lines studied (NCI-H460, MCF-7, AGS and HCT-15). Our preliminary results revealed that all the extracts from *Clitocybe alexandri* are capable of causing cell growth inhibition and provided GI<sub>50</sub> concentrations below 60µg/ml in all the cell lines tested [4]. Regarding the effect of the *Agaricus arvensis* extracts, they all caused an inhibition of cell growth in all cell lines, particularly the methanolic extract which revealed to be a very potent inhibitor of cell growth, especially in the MCF-7 cell line. The evaluation of the effect of the *Suillus collinitus* extracts will be carried out as well as cell cycle and apoptosis analyses, by flow cytometry. Finally, the isolation and characterization of compounds from these extracts will also be carried out, using HPLC-DAD or HPLC-RI. The structures of the compounds will be established by NMR spectral analysis (<sup>1</sup>H, <sup>13</sup>C, DEPT, COSY, HSQC and HMBC).

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## A yeast model for LRRK2: biological studies and screening for small-molecule inhibitors

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Parkinson disease (PD) is the second most prevalent neurodegenerative disease among the elderly population. Recently, it was discovered that mutations in the *PARK8* locus, leucine-rich repeat kinase 2 (LRRK2), are the most common monogenetic cause of PD, responsible for 5-10% of the familial PD and also for a significant proportion of apparently sporadic cases [1]. The most frequent LRRK2 mutation associated with PD is G2019S. This mutation, found in the kinase domain, causes an increase in the kinase activity when compared with wild-type (wt) LRRK2 [2]. However, little is known about their regulation and physiological substrates. Furthermore, no pharmacological inhibitors of these proteins have been described so far.

Yeast has been successfully used as a simpler eukaryotic cell system in the study and high-throughput screening of small-molecule modulators of human proteins [3]. In order to understand the biology of wt and mutant (G2019S) LRRK2 and also to search for selective small-molecule inhibitors, human wt and mutant LRRK2 were individually expressed in the yeast *Saccharomyces cerevisiae*. The G2019S mutation was obtained by site-directed mutagenesis from wt LRRK2. High copy expression of human wt or mutant LRRK2 in yeast caused significant growth inhibition, more pronounced for the PD-causing mutant G2019S. Furthermore, wt and mutant LRRK2-mediated cytotoxicity was rescued in yeast cells without *ATG5*, an essential component of autophagy. This suggests the involvement of an autophagic pathway in wt and mutant LRRK2-mediated cytotoxicity. In order to study LRRK2 dynamics in yeast, wt and mutant LRRK2-EYFP fusions were further created. Both wt and mutant LRRK2 were predominantly found in large pericentriolar cytoplasmic aggregates. As a whole, this work represents the first attempt to express wt and mutant LRRK2 in yeast. The yeast system developed may help uncover basic aspects of both normal and pathogenic LRRK2 biology and may be further used as a first-line drug screening approach to search for selective pharmacological inhibitors with promising therapeutic applications in neurodegenerative disorders, such as PD.

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## Activation of adenosine receptors alters angiotensin II formation in mesangial cells cultured on high-glucose medium

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Diabetic nephropathy (DN) is a frequent complication of diabetes, with hyperglycemia being one of the key factors for its development and progression [1]. Activation of the renin-angiotensin system (RAS), in particular intracellularly, also contributes to the deterioration of renal function [2][3]. Considering the inhibitory role of adenosine on renin release, we aimed at assessing the effect of adenosine receptor agonists in the production of intracellular and extracellular angiotensin II (AngII) by rat mesangial cells in primary culture, in the presence of a normal (10 mM; NG) or high (30 mM; HG) concentration of glucose.

For the quantification of the Ang II an immunoenzymatic method (ELISA) was performed using a commercially available kit.

Both with NG and HG medium, intracellular AngII production was higher than extracellular one. In the presence of NG, activation of adenosine A<sub>1</sub>, A<sub>2A</sub> and A<sub>3</sub> receptors increased the intracellular AngII concentration, as did activation of adenosine A<sub>2A</sub> and A<sub>3</sub> receptors for extracellular AngII concentration. In the presence of HG, activation of adenosine A<sub>1</sub> receptors increases both intra and extracellular AngII production, activation of adenosine A<sub>2A</sub> receptors decreases intracellular AngII production and activation of adenosine A<sub>3</sub> receptors decreases both intra and extracellular AngII production.

Future experiments need to be done in order to confirm these results. However, these preliminary results suggest that the adenosine A<sub>2A</sub> and A<sub>3</sub> receptors can regulate AngII production in mesangial cells, namely intracellularly, in high-glucose conditions. This might constitute a new target for the development of new therapeutic approaches to ND.

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# Acute effect of saturated fatty acids on CA cellular content and secretion from adrenomedullary cells

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High intake of saturated fatty acids (SFA) has been implicated as an important dietary risk factor for cardiovascular disease [1]. The catecholamines (CA) adrenaline (AD) and noradrenaline (NA) are the main mediators of the sympathoadrenomedullary system, playing crucial roles in the regulation of metabolic and cardiovascular homeostasis.

The aim of our work was to test the effect of a short-, a medium- and a long-chain saturated fatty acid upon CA handling by primary cultures of bovine adrenomedullary cells.

After 4 days in culture, the cells were incubated for 60 minutes with ethanol (control), butyric acid (4:0; 100  $\mu$ M-1mM; n=6-8), capric acid (10:0; 50-200  $\mu$ M; n=6-8) or palmitic acid (16:0; 50-200  $\mu$ M; n=6-8). For studies on CA release, cells were preincubated for 10 min, and then incubated for 15 min in the absence (control) or presence of KCl. CA were determined by means of high pressure liquid chromatography with electrochemical detection (HPLC-ED) [2]. The cell viability was evaluated by the MTT assay [3]. For comparison between two groups, Student's *t*-test was used. Differences were considered to be significant when  $P < 0.05$ .

MTT assays showed that there was no loss of cell viability after acute exposure to any of the three fatty acids. All fatty acids tested, regardless of their chain-length, behave in much the same way, inducing a significant reduction of both AD and NA cellular content, as well as of total CA production. This significantly lower CA cellular content observed after 60 minutes exposure of cells to any of the three SFA was accompanied by a higher CA basal release and a lower CA release induced by KCl stimulation. Altogether the data are compatible with a reduction of the vesicular CA transporter function due to the acute-term exposure to any of these SFA.

In conclusion, our data suggest that SFA influence CA handling by adrenomedullary cells. Further studies will be needed to clarify underlying mechanisms of our findings.

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## Adenine dinucleotides induce $[Ca^{2+}]_i$ transients and operate proliferation of rat fibroblasts through $p2y_{13}$ receptor activation

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Pain related to the musculoskeletal system is very common. Purines, such as ATP, are involved in nociception via a number of distinct signaling mechanisms. In this study, we aimed at investigating whether purines, via P2 purinoceptors activation, could excite and/or sensitize myofascial nociceptors to probe their role as pharmacological targets for chronic painful conditions (*e.g.* fibromyalgia). The extent of the autocrine/paracrine activity mediated by ATP (and related purines) may be influenced by membrane-bound ectonucleotidases, which sequentially metabolize ATP to ADP, AMP, adenosine (ADO), inosine (INO) and hypoxanthine. We, therefore, evaluated the kinetics of the extracellular metabolism of adenine nucleotides on fibroblast cell cultures isolated from the rat subcutaneous connective tissue. Experiments were performed in the first subculture of Wistar rat fibroblasts. The kinetics of the extracellular catabolism of adenine nucleotides was performed by HPLC. Intracellular  $[Ca^{2+}]_i$  oscillations was tested using a microplate reader after loading the cells with Fluo-4NW (2.5  $\mu$ M, 45 min at 37°C). Cell viability/proliferation (Live/Dead and MTT assay) experiments were performed maintaining the cells in culture for up to 21 days. In rat fibroblast cultures, ATP (3  $\mu$ M) was metabolized with a half-degradation time of 10 min (n=2); the metabolites detected in the bath were ADP, ADO and INO. ADP transiently accumulated in the cultures; the half-degradation time of ADP (3  $\mu$ M) was 52 min (n=2). In contrast, AMP was hardly detected as a product of ATP catabolism in the cultures (half-degradation time of AMP 3  $\mu$ M was 5 min, n=2), unless ecto-5'-nucleotidase activity was inhibited with concanavalin A (0.1 mg/mL). Incubation of the fibroblasts with the stable ADP analogue, ADP $\beta$ S (100  $\mu$ M), caused a transient rise of intracellular  $[Ca^{2+}]_i$  (n=21). The effect of ADP $\beta$ S (100  $\mu$ M) was significantly ( $P<0.05$ ) attenuated by the selective P2Y<sub>13</sub> antagonist, MRS 2211 (10  $\mu$ M, n=8), but not by MRS2179 (0.3  $\mu$ M, n=6) and AR-C66096 (0.1  $\mu$ M, n=8), which block respectively ADP-sensitive P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors. Selective activation of the P2Y<sub>1</sub> receptor with MRS2365 (10 nM, n=6) was virtually devoid of effect on  $[Ca^{2+}]_i$  accumulation. In addition, ADP $\beta$ S (100  $\mu$ M, n=5) enhanced the proliferation of fibroblasts in culture. The proliferative effect of ADP $\beta$ S (100  $\mu$ M) was also prevented by MRS2211 (10  $\mu$ M, n=5). Data suggest that fibroblast exhibit high ecto-NTPDase 1 activity converting ATP directly into AMP, which is quickly dephosphorylated into ADO by ecto-5'-nucleotidase. We also demonstrated that ADP derived from the hydrolysis of ATP accumulates in fibroblast cell cultures, where it might be physiologically relevant by inducing  $[Ca^{2+}]_i$  oscillations and cell proliferation through the activation of P2Y<sub>13</sub> receptors.

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# Adenosine deaminase activity regulates the adenosine $A_1/A_{2A}$ receptor activation balance on rat motor nerve terminals

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At the rat neuromuscular junction, the adenosine  $A_1 / A_{2A}$  receptor activation balance depends on the synaptic concentration of the nucleoside, which seems to be tightly regulated by the nucleoside formation and inactivation mechanisms (Correia-de-Sá & Ribeiro, 1996, *Neuroscience* **73**:85-92). While adenosine (ADO) acts predominantly as an inhibitory signal (via  $A_1$  receptors) under resting conditions, amplification of neuromuscular transmission due to  $A_{2A}$  receptor activation becomes evident at high levels of synaptic ADO due to intense neuronal activity. These prompted us to evaluate the role of ADO inactivation mechanisms, via the nucleoside transport system and adenosine deaminase (ADA), on  $A_1/A_{2A}$  receptors activation balance upon increasing the nucleoside concentration at the rat motor endplate with exogenous ADO.

The experiments were performed at 37°C on rat phrenic nerve-hemidiaphragm preparations loaded with [ $^3$ H]-choline (2.5  $\mu$ Ci/ml). The preparations were superfused with Tyrode's solution and were continuously gassed with 95%  $O_2$  + 5%  $CO_2$ . [ $^3$ H]-ACh release was evoked by electrical stimulation of the phrenic nerve with trains of 750 supramaximal intensity pulses of 40  $\mu$ s duration delivered at a frequency of 5 Hz. Two stimulation periods were used, starting respectively at 12<sup>th</sup> ( $S_1$ ) and 39<sup>th</sup> ( $S_2$ ) min after the end of the washout period. Test drugs were added 15 min before  $S_2$ . Their effects on transmitter release were expressed by the ratios  $S_2/S_1$  as compared to  $S_2/S_1$  ratio in control experiments.

Exogenously applied ADO (3-500  $\mu$ M) had a biphasic effect on evoked [ $^3$ H]-ACh release. ADO (30  $\mu$ M) significantly ( $P < 0.05$ ) reduced transmitter release by  $32 \pm 3\%$  ( $n=4$ ). At a higher concentration (500  $\mu$ M) the nucleoside increased evoked [ $^3$ H]-ACh release by  $44 \pm 3\%$  ( $n=4$ ). The inhibitory effect of ADO was mimicked by selective activation of  $A_1$  receptors, with R-PIA (e.g. 300 nM,  $61 \pm 8\%$ ,  $n=3$ ), whereas application of CGS21680C (3 nM), a selective  $A_{2A}$  receptor agonist, increased [ $^3$ H]-ACh release by  $63 \pm 2\%$  (3 nM,  $n=4$ ). Pretreatment of motor nerve terminals with the nucleoside transport inhibitor, NBTI (30  $\mu$ M), did not significantly ( $P > 0.05$ ) modified the inhibitory effect of ADO (3  $\mu$ M;  $-22 \pm 11\%$ ,  $n=4$ ); NBTI (30  $\mu$ M) was also unable to change the facilitatory effect obtained with ADO (500  $\mu$ M,  $51 \pm 17\%$ ,  $n=4$ ). In contrast, blockade of ADA activity with EHNA (50  $\mu$ M) transformed the inhibitory effect of ADO (3  $\mu$ M) into a significant ( $P < 0.05$ ) facilitatory action of  $57 \pm 26\%$ ,  $n=3$ ), without affecting ADO (500  $\mu$ M)-induced facilitation ( $56 \pm 20\%$ ,  $n=3$ ) of evoked [ $^3$ H]-ACh release.

Data indicate that inactivation of exogenously added ADO by ADA might be more relevant than the nucleoside cellular uptake regarding the co-ordinate shift from inhibitory  $A_1$  to facilitatory  $A_{2A}$  receptors regulating transmitter release from stimulated motor nerve terminals.

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# Adolescents eating out habits in a semi-urban city of Portugal

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**Introduction:** Modern lifestyles are characterized by a lack of time for everyday activities such as cooking, which lead to an increase of eating out of home. With raising importance in the overall food intake, eating out is often associated with less healthy eating habits and the risk of developing chronic degenerative diseases such as obesity. In adolescence, it is common to adopt unhealthy eating habits that can remain in adulthood. Assessing such dietary habits might help to better shape the development of strategies for conducting an early change by improving healthier eating out choices.

**Aims:** To characterize adolescents' eating out habits.

**Methods:** 100 adolescents, aged 13 to 17 years old, from five public schools placed at Ílhavo (a semi-urban Portuguese city) were evaluated by a self-administered questionnaire. Based on the Hector Eating Out Questionnaire, habits such as frequency by meals, weekend importance, places of consumption and time changes were assessed.

**Results:** The 51 girls and 49 boys involved had a mean age of 14.3 (sd=0.8) years old.

Either every day or at least 2-4 times a week, a large part of the students had lunch, midmorning and afternoon breaks out of home, respectively 64%, 61% and 47%. Some students also referred the same high frequency of eating out (either every day or at least 2-4 times a week) for breakfast, dinner and supper, respectively 9.5%, 13% and 7%.

Breakfast and lunch has been referred to be consumed out mainly during the week (57% and 70%), while an eating out dinner was mostly made during weekends (67%).

In comparison with the previous year, the eating out frequency has reduced for 32% of respondents, and has increased for 26% of them. The reasons stated for eating out less were mainly "more time available" and "health reasons". In opposition, eating out more often was justified by "less available time/convenience".

The places more referred for eating out occasions were: for breakfast – cafe/bar (37%), school/canteen (30%), bakery shops (22%); for lunches - school/canteen (43%), friends/family house (16%), take-away/home deliver (13%), fast-food restaurants (10%); for dinner – restaurants (28%), friends/family house (26%), fast-food restaurants (21%), take-away/home deliver (18%).

No significant statistical differences between girls and boys or among schools were found.

**Conclusions:** The inquired adolescents eat out quite often, mainly at lunch, midmorning and afternoon snacks but also in some extent at breakfast and dinner. The time available was the main reason stated for change the eating out frequency. In addition, schools were an important place of out of home food consumption. Although more research is needed to access the nutritional quality of eating out meals, the investment in school meals provides a great opportunity to widen the quality and variety of foods in adolescents eating out occasions.

# Antifungal activity of the essential oil of *Thymus villosus*

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## OBJECTIVES

The genus *Thymus* (Lamiaceae) is a complex group of aromatic plants widely distributed across the Iberian Peninsula. Several species have traditionally been used as medicinal plants, namely for their antiseptic properties [1]. In this context, the objective of the present work was to investigate the antifungal activity of the essential oil (EO) of *Thymus villosus* on a range of representative human pathogenic *Candida* and *Aspergillus* species.

## Methods

The EO of *T. villosus* was obtained from the flowering parts of the plants by hydrodistillation and analysed by gas-chromatography and gas-chromatography/mass spectroscopy. The antifungal activity was evaluated against several strains from five *Candida* spp. (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. dubliniensis*) and three *Aspergillus* spp. (*A. fumigatus*, *A. flavus*, and *A. niger*) using the reference CLSI (formerly NCCLS) broth macrodilution protocol M27-A3 [2]. Minimum fungicidal concentrations (MFCs) were the lowest concentrations showing no growth after incubation of 20  $\mu$ L samples from clear tubes in the macrodilution test in Sabouraud dextrose agar at 35°C.

## Results

Minimum inhibitory concentrations (MICs) of the EO were quite low, ranging from 0.16 to 1.25  $\mu$ L.mL<sup>-1</sup>, for *Candida* species and from 0.64 to 2.5 for *Aspergillus* spp.. Furthermore, the EO displayed a clear fungicidal activity, with MFCs equal to or just one dilution above the respective MICs, including against isolates with decreased susceptibility to commercial antimycotic drugs.

## Conclusions

This work revealed a powerful antifungal activity of the EO of *T. villosus* against *Candida* spp., in line with previously reported results for related *Thymus* oils [3], thereby supporting further more in depth *in vitro* studies, as well as the potential of the EO of *T. villosus* for the clinical management of mucocutaneous candidiasis.

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# Antifungal and Antibacterial Activity of the Crude Extract and the Secondary Metabolites from the Marine Fungus *Eurotium cristatum*

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Compounds with unique scaffolds have been discovered in almost all classes of marine organisms, including fungi [1]. Marine ecological niches have been described as a particular and promising search for new antimicrobials to combat antibiotic-resistant strains of pathogenic bacteria [2]. The emergence of multidrug resistance among the latest generation of pathogens suggests that the discovery of new scaffolds should be a priority [3]. The aims of this study were: i) to verify if the extract of *Eurotium cristatum*, a fungus isolated from the marine sponge *Mycale* sp., behaves as antibacterial and/or antifungal; ii) to isolate and identify the secondary metabolites from this fungal extract and verify their antibacterial and/or antifungal potential. The ethyl acetate extract of the fungus *E. cristatum* (ECE) furnished 2-(2',3'-epoxy-1',3'-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl) benzaldehyde (**1**), 1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthracenedione (physcion, **2**) and the dioxopiperazine alkaloid echinulin (**3**). The structures of these compounds were established by NMR spectral analysis (<sup>1</sup>H, <sup>13</sup>C, DEPT, COSY, HSQC and HMBC) [4]. The broth micro-dilution method was used to assay the ECE and compound **2** to evaluate their antimicrobial activities against three bacterial species (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and three human pathogenic fungi (*Candida albicans*, *Trichophyton rubrum* and *Aspergillus fumigatus*). ECE was found to be more active against *T. rubrum* and *S. aureus* (MIC's = 128 µg/ml), and less active against *C. albicans*, *A. fumigatus*, *E. coli*, *P. aeruginosa* (MIC's > 512 µg/ml). Compound **2** exhibited MIC's > 128 µg/ml for all microbial species, which is in agreement with the previous reports [5, 6]. Further purification and biological assays will proceed to identify new chemical entity with antibacterial and/or antifungal activities.

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# Anti-inflammatory Activities of Chiral Xanthenes - Activity and inhibition of bee venom phospholipase A<sub>2</sub> and ovine COX-1 and human recombinant COX-2

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The Arachidonic acid cascade is a major pathway of inflammation. It is involved in the release of prostaglandines, leukotrienes and thromboxanes [1]. Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) is a water-soluble enzyme that hydrolyses the sn-2 ester bonds of enantiomeric L-phospholipids which acts on the beginning of the inflammatory cascade [1]. Cyclooxygenases (COXs) are also key enzymes in the biosynthesis of prostanoids and exist in two isoforms (COX-1 and COX-2). COX-1 is a constitutive enzyme, expressed in almost every cell type. On the other hand, COX-2 is highly expressed by cells that are involved in the inflammatory process. The stimulation of these cells results in the production of large amounts of PGs which are pro-inflammatory mediators. The inhibition of COXs results in a suppression of the inflammatory process. Due to their role in the production of pro-inflammatory mediators, PLA<sub>2</sub> and COXs became possible targets when considering the design of new anti-inflammatory drugs due to their action on the early stages of inflammation. One group of promising compounds, that have been described to exhibit a broad spectrum of biological activities, belong to the chemical family of xanthone derivatives described and were tested within the current work for their inhibitory activity of PLA<sub>2</sub> and COX-1 and 2.

Although it is a water-soluble enzyme, PLA<sub>2</sub> acts at the lipid/water interface and its activity can be regulated by membrane composition, so biomembrane model systems composed of EPC + 20% DPPG were used for mimicking the cellular membranes. The effect these compounds on the hydrolytic efficiency of PLA<sub>2</sub> was investigated by fluorimetry using the AcryloDated Intestinal fatty acid binding protein (ADIFAB) as a fluorescent indicator for the measurement of free fatty acid released from phospholipids. The probe fluorescence was followed at 432 nm and the decrease in the fatty acid concentration produced over time after contact between the enzyme and the tested drugs reflected the PLA<sub>2</sub> inhibition.

The evaluation of the inhibition of COX-1 and COX-2 activities was performed by using the COX Inhibitor Screening Assay Kit. This kit directly measures PGF<sub>2α</sub> by SnCl<sub>2</sub> reduction of COX-derived PGH<sub>2</sub> produced in the COX reaction. The prostanoid product is quantified via enzyme immunoassay (EIA) using a broadly specific antiserum that binds to all the major PG compounds. This assay includes both ovine COX-1 and human recombinant COX-2 and allows screening isoenzyme-specific inhibitors.

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## Are ready-to-eat salads an important vehicle of pathogenic and comensal bacteria resistant to antibiotics?

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The increase demand for fresh fruits and vegetables is causing an expansion of the market share for minimally processed vegetables along with recognized food safety problems. We analyzed the microbiological quality of Portuguese ready-to-eat salads (RTS) and their role in the spread of bacteria carrying antibiotic resistance (AB<sup>R</sup>) genes.

RTS (n=50; 7 brands; split or mixed leaves, carrot, cornmeal) were collected in 5 of the main supermarkets (2010). The evaluation of microbiological load and quality followed the international standard methods for counting aerobic mesophilic, coliforms, *Enterococcus* sp and detection of *Salmonella* sp or *Listeria monocytogenes*. Samples were also plated in different culture media with/without AB before and after a pre-enrichment step. AB<sup>R</sup> was studied by agar diffusion method (CLSI) and ESBL expression by double disk synergy test (DDST). Species were identified by PCR (Gram positive), API ID32GN or 16rRNA (Gram negative). AB<sup>R</sup> genes, integron types and *E. coli* phylogenetic groups were searched by PCR and clonality by MLST in specific isolates.

A high number of RTS presented poor microbiological quality (86% for aerobic mesophilic, 74%-coliforms, 4%-*E. coli*), but no pathogens. Different AB<sup>R</sup> phenotypes and genotypes were seen to both Gram positive and Gram negative bacteria. *E. coli* detected in 13 samples (n=26; phylogenetic groups A-7, B1-10, B2-1, D-8) presented resistance (%) to tetracycline (73; *tetA* and/or *tetB*), streptomycin (50; *aadA*), sulfametoazole (46; *sul1* and/or *sul2*), trimethoprim (46; *dfrA1* or *dfrA12*), ampicillin (46; *blaTEM*), nalidixic acid (27), ciprofloxacin (8) or chloramphenicol (4). Two integron types (*dfrA1* + *aadA*, *dfrA12* + *aadA*) were detected in 11 isolates. Multidrug resistant *E. coli* (n=2; D) belonged to the widespread ST69; the *fumC* alleles of other *E. coli* were highly diverse and identified as 8, 48, 65 and 100. DDST gave a positive test for 2 *Raoutella* sp (2 samples) carrying an ESBL identified as SHV<sub>2</sub>. Among enterococci (n=108; *E. faecalis*-20, *E. faecium*-18, *E. gallinarum*-5, *E. hirae*-9, *E. casseliflavus*-40 and *Enterococcus spp*-16) AB<sup>R</sup> (%) was seen for tetracyclines (6; *tetM* and/or *tetL*), erythromycin (3; *ermB*), nitrofurantoin (1) or ciprofloxacin (1).

The present study positions RTS within the spectrum of ecological niches that may be reservoirs/vehicles for AB<sup>R</sup> bacteria/genes with clinical interest (e.g. *E. coli*-B2 or ST69; ESBL) being these findings worthy of attention as their spread to humans by ingestion cannot be dismissed.

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# ***Aspergillus* spp. in air, soil and plants from vineyards treated with azole antifungals**

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The emergence of antifungal drug cross-resistance among clinical pathogens, like *Aspergillus* spp., represents a considerable human health risk. In agriculture, fungi are responsible for important losses of crop yields and for production of mycotoxins. The widespread use of fungicides is essential to protect a wide variety of crops from fungi, like in the vineyards (1). Azoles have been extensively used in agriculture and medicine, this led to the development of resistances which are becoming a problem nowadays. Azoles differing in structure but exhibiting the same mode of action are used to treat both fungal diseases of plants and humans (2). Considering that the azoles represent an important class of antifungals used in human therapeutics and in vineyard treatments, and the possible cross-resistance within these azoles, the final aim of this research is to contribute towards a better understanding on whether the massive use of farm fungicides is linked to resistance to drugs used in the treatment of people with life-threatening infections.

In this research samples of air, soil and plants were collected in four vineyards from “Quinta da Lage”, Pinhão at Douro region. The vineyard “BIO” was managed in organic farming (without application of non-natural fungicides). The other tree vineyards “TF0, TF1 and TN5” were managed in integrated pest management and fungicides were applied whenever needed according to pest risk assessment: in “TF0” no azoles have been sprayed; in “TF1” penconazol (azole compound) was sprayed once (May) and in “TN5” penconazol was sprayed tree times (6<sup>th</sup> May, 25<sup>th</sup> May and 16 June). In each vineyard, air, soil and plant samples were collected in three moments: before any fungicide application, just after all fungicides applications, and before grape harvest. Grapes samples were collected after all fungicides applications, and before grape harvest.

The total number of fungi was evaluated in MEA and DG18 medium and the strains of *Aspergillus* species were isolated and identified by morphological methods. Forty strains of *Aspergillus* were isolated and identified: 26 belonging to *niger* group, 1 *A. fumigatus*, 1 *A. flavus*, 2 *A. terreus* e 10 *Aspergillus* spp. All the strains were isolated from air and soil samples. In this study we did not find *Aspergillus* spp. on leaves or grapes. *Aspergillus* strains will be evaluated for their susceptibility to azoles used in human therapeutic (Posaconazole, Itraconazole and Voriconazole).

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# Assessment of the bone regeneration process in the ovariectomized rat model of osteoporosis

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Osteoporosis is characterized by a reduction in bone mass and disruption of bone architecture, resulting in increased bone fragility and fracture risk (1). These fractures are widely recognized as a major health problem in the elderly population. Osteoporosis is of additional relevance in women entering the menopause due to the lack of estrogen production - associated with an anti-resorptive stimulus to the bone tissue. Hormonal disequilibrium is known to favour bone resorption, which in turn leads to skeletal fragility and increased risk of fracture (2).

It is believed that one of the early and transient effects of estrogen deficiency is to increase the activity of osteoclasts. Increased osteoclastic activity causes augmented depth of erosion of bone by these cells, contributing to the trabecular penetration and disruption of bone architecture that characterizes postmenopausal osteoporosis (3).

The aim of this study is to evaluate bone regeneration process in a rat model of osteoporosis. This evaluation was made in a valid animal model of primary osteoporosis - ovariectomized Wistar rats, which mimics the systemic human condition. This model relies on the usual changes that bone tissue undergoes after ovariectomy. The animals were randomly divided into two groups: sham operation and ovariectomy group (Ovx). The animals of each group were assigned to 2 sub-groups (n=6): critical size and subcritical size defects. Critical size defects of 5mm  $\varnothing$  were created on the skull of Ovx and sham animals, previously to the placement of a commercial ceramic-based biomaterial (Bio-Oss®), known to report adequate biocompatibility. Non critical size defects of 3mm  $\varnothing$  were created on both groups and left untreated to access the intramembranous ossification process. Routine histological, radiographic and microtomographic evaluations were conducted at adequate time points. Furthermore, the plasmatic levels of calcium, phosphor and alkaline phosphatase were also assessed. Result analysis allowed to verify that the regeneration process was impaired in Ovx animals, compared to Sham animals, in which regards the biomaterial-mediated bone regeneration model. Also, the assessment of the intramembranous ossification process, with the subcritical model, reported an impaired biological response in the Ovx group.

Osteoporosis seems to greatly affect the biological response to biomaterial's implantation and the structured events of the intramembranous ossification process.

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# Assessing the adequacy of the DREC's food capitations for students of a primary school

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Children food habits may assure complete development and growth and may contribute to a healthy life. Schools have an important role on adoption of healthy eating habits working together with health professionals and families.

One of the main concerns of Education Ministry is food at school. Recently the Division of Innovation and Curricular Development created a Circular 14/DGIDC/2007, legislating the organization of school canteens, including general rules about meals to be served at schools as well as the individual food quantities necessary for each age group.

The aim of this work was to evaluate the nutritional adequacy of meals served at elementary schools at Guarda municipality, taking into consideration the individual quantities defined by Central Region Division of Education (DREC) and to evaluate the amount of food wastage.

This work was developed at a school canteen located at an elementary school of S. Miguel Schools, Guarda. There were included on these study 41 individuals that were served with the food amounts indicated by DREC. A descriptive correlation study was developed and the coorte was considered prospective. A convenience sample was used by non probabilistic sampling.

The amount of wastage of each meal was account individually and registered on an Excel Spreadsheet. Nutritional evaluation of two weeks schools menus was developed using the System of Planning and Evaluation of Scholl Meals (SPARE).

There was no wastage of soup since the totality of children under evaluation consumed the amount of soup served. Concerning the main dish the main meal components waste were fish and vegetables. No significant amounts of fruit were wasted.

Besides the need of school policies to improve children consumption of fish and vegetables associated to initiatives involving families, teachers and school assistants it will probably be also necessary to adjust individual food amounts indicated by the DREC in order to reduce food wastage and help controlling meals cost.

# Benzo- $\gamma$ -pyrone as a privileged structure for the rational design of adenosine receptor ligands potentially useful in anticancer therapy

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During the last decade different approaches to treating cancer have been developed based mainly on specific targets that are mostly expressed in tumor but not in normal cells. Interestingly, adenosine receptors (AR) levels in various tumor cells are up regulated, a finding which may suggest that a specific AR may serve as a biological marker and as a target for specific ligands leading to cell growth inhibition. These facts prompted to the development of novel, selective and potent AR receptor ligands suitable for chemotherapeutic purposes.

Lead discovery and optimization, guided by structure-activity-relationships (SAR) of new AR ligands based on chromone scaffold ((4*H*)-1-benzopyran-4-one) is the aim of the present work. Accordingly, a library of novel chromone derivatives was obtained (PCT/IB2008/050674). The compounds have been screened for their affinity towards different ARs subtypes (A1, A2A, A2B and A3) by radioligand binding assays. The results obtained so far will be presented in this communication.

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# Biocompatibility of antipsychotic loaded solid lipid nanoparticles (SLN) for oral delivery

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**Introduction:** Regarding its advantages, the oral route is the favorite of patients. Nonetheless, due to the harsh conditions along the gastrointestinal tract (GIT), the transport of poorly water-soluble substances, like some antipsychotic drugs [1], is difficult. The main problem is their resistance to be wetted in GIT fluids. One strategy to improve oral bioavailability of such drugs would be formulating them by means of solid lipid nanoparticles (SLN) [2], which are derived from o/w emulsions by replacing the liquid lipid by one solid at both room and body temperatures. SLN are made from physiological compounds, which predicts absence of toxicity. However, the use of high concentrations of these carriers and the production methods itself can lead to toxicological concerns [3]. Therefore, the assessment of the cytotoxicity of SLN formulations is a demand. The aim of this work was to prepare an antipsychotic-loaded SLN formulation and to assess their biocompatibility as drug delivery systems for the GIT.

**Experimental:** SLN placebo and drug-loaded formulations were prepared by ultrasound technique. The mean particle size and polydispersity index (PI) were measured by photon correlation spectroscopy. The zeta potential was accessed by laser doppler electrophoresis. The cytotoxicity of placebo and drug-loaded SLN formulations was evaluated by the MTT assay by means of Caco-2 cell cultures.

**Results:** Aqueous milky-like SLN placebo and drug loaded dispersions with colloidal sizes (~96nm) and low PI values (~0.2) were obtained. The absolute ZP values were high (~|-35|mV), predicting good long-term stability. The MTT assays show over 90% of cell viability after exposure to SLN formulations, suggesting biocompatibility with Caco-2 cells.

**Conclusions:** These results show that SLN are suitable carrier systems for the incorporation of the poorly water-soluble drugs, intended for oral administration. The long-term stability studies are going on, in order to determine particles sizes and electrochemical stability of formulations for longer periods. Additional studies are also being run to assess the encapsulation parameters (i.e. encapsulation efficiency and loading capacity), drug release profile from SLN, for further *in vitro/in vivo* correlations studies.

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## **CDKN1B (p27) alterations in primary hyperparathyroidism**

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Primary hyperparathyroidism (PHPT) is a common endocrine disorder which is due to an excessive autonomous production and release of PTH by the parathyroid glands. The majority of PHPT cases are sporadic and related with parathyroid adenoma (80-85%), hyperplasia (15-20%) or carcinoma (1%). About 10% of the PHPT cases are hereditary forms that include MEN 1 gene mutations (Multiple Endocrine Neoplasia type 1) and RET gene mutations (Multiple Endocrine Neoplasia type 2). Germline mutations in *MEN1* gene predispose to MEN1 syndrome, but in about 20 – 25% of clinical MEN1 cases no mutations can be found [1]. Recently, it has been identified a germline mutation in *CDKN1B* gene, in one suspected MEN1 patient with parathyroid and pituitary tumors [2,3]. At variance with the familial forms, the molecular mechanisms underlying the pathogenesis of sporadic PHPT are incompletely understood.

In the present work, we aim to evaluate the prevalence of *CDKN1B* mutations in PHPT patient's tumors.

Constitutional DNA samples were obtained from thirty apparently sporadic PHPT patients. Germ-line and somatic mutations in *CDKN1B* gene was searched by PCR/SSCP and direct DNA sequencing. Evaluation of CDKN1B (p27 protein) expression was performed by immunohistochemistry.

No mutations in *CDKN1B* gene were detected in any case. The polymorphic alteration, V109G, was detected in 6 (20%) cases. These results show that none of the cases in our series corresponds to hereditary forms of MEN4. The p27 immunohistochemistry study revealed that all the cases analyzed had nuclear staining, however 9 (36%) showed cytoplasmic immunostaining, raising the possibility of p27 misregulation in sporadic PHPT.

In conclusion, these results showed that in our series, *CDKN1B* mutations seem not to have a major role in parathyroid tumorigenesis. Moreover, it was observed an abnormal p27 expression. Further studies are in course in order to fully characterize the molecular alterations underlying PHPT, and evaluate the possible correlation between the allelic variants of V109G polymorphism and the risk to develop this pathology.

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# Characterization of the expression of SIRT1 and SIRT7 in the human corpus cavernosum during aging

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**Introduction:** Vascular aging is associated with silent modifications in many normal homeostatic processes, resulting in endothelial dysfunctions. Erectile dysfunction (ED) which is highly prevalent in the aged men is considered one earlier signal of endothelial dysfunction. This could be provoked by changes in molecular factors, as eNOS and ET-1, antagonistic in what concerns to vascular motility [1]. However, recent evidences suggest that upregulation of SIRT-1 and SIRT-7 [2,3] could counteract physiological aging particularly of vascular tissue.

The aim of this project was to study the changes in the expression of SIRT-1 and SIRT-7 and related molecules in the corpus cavernosum of healthy male humans during chronological aging.

**Methods:** Samples of human corpus cavernosum (CC) were collected from healthy organ donors without ED or known ED risk factors at the Hospital of S. João Porto and divided in two groups according to the donor age (Young - 16 to 35 years and Aged – 59 to 74 years). Immunofluorescence and immunohistochemistry methods were performed to evaluate SIRT1, SIRT7, eNOS, ET-1 and Hif-1alpha expression in CC from both groups employing specific primary antibodies (Santa Cruz Biotech.). All slides were observed, and images were captured in an optical microscope connected to an Axiocam MRm camera (Carl Zeiss MicroImaging GmbH). These proteins were semi-quantified by Western blotting.

**Results:** ET-1 expression was evident in endothelial and smooth muscle cells and eNOS restricted to the endothelium, while SIRT1/SIRT7 were predominantly expressed in fusiform smooth muscle cells. Hif-1 alpha was observed in the periphery of the vessels. Western blotting results do not demonstrate significant variation in expression of the studied proteins in the CC during aging.

**Discussion:** Our results demonstrate for the first time the expression of SIRT1 and SIRT7 in the human corpus cavernosum tissue. However, further molecular studies are required to clarify their role in the mechanisms of endothelial dysfunction progression. Real-time PCR of the studied molecules are under course. Supported by UP/Santander Totta

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# Chemoresistance in Pancreatic Cancer Stem Cells

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Pancreatic cancer is currently the sixth leading cause of cancer-related mortality in Europe, being the most lethal of solid tumours and having the worse prognosis of any major malignancy (5-year-survival of 3%). This disease is characterized by late diagnosis due to lack of early symptoms, high propensity to metastatic development and high resistance to chemo- and radiotherapy [1]. It is currently believed that a small subset of tumour cells is, in part, responsible for chemoresistance. These cells, known as cancer stem cells (CSCs), have the ability to self-renew and to differentiate into several lineages [2, 3]. The aim of the present work was to identify possible therapeutic targets to circumvent chemoresistance in pancreatic CSCs.

The pancreatic cancer cell line S2-013 was subjected to a cisplatin treatment with the purpose of achieving an enrichment of the putative CSC subpopulation (known to express CD133 [4]). Sensitivity to cisplatin was tested in the enriched population versus the parental cell line by the SRB assay. Expression levels of proteins involved in chemoresistance, namely apoptosis-related proteins, were assessed by Western Blot.

The obtained results showed that cells from the S2-013 cell line previously treated with cisplatin expressed higher levels of CD133 cell surface marker and presented higher chemoresistance. The putative CSC enriched population presented overexpression of Bcl-2 when compared to the parental cell population, which could be related to the differences found in sensitivity to cisplatin. To understand if the membrane protein CD133 was directly involved in chemoresistance, RNAi technology was used to knock-down CD133 expression. No differential chemoresistance was observed following CD133 silencing, indicating that this protein is not directly responsible for chemoresistance.

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## Chromone as a scaffold for the development of potent and selective MAO-B inhibitors

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The Monoamine oxidase (MAO) is an enzyme present in mammals in two isoforms - MAO A and MAO B that play a crucial role in neurotransmitters metabolism, representing an attractive drug target in neurodegenerative diseases and depression.

During our project on drug discovery for the treatment of neurodegenerative diseases, we developed versatile libraries incorporating privileged structures with benzo- $\gamma$ -pyrone substructure, sustained on chromone scaffold ((4*H*)-1-benzopyran-4-one). The SAR study performed allow concluding that chromones that have substituents in position-3 of  $\gamma$ -pyrone nucleus act preferably as MAO-B inhibitors with IC<sub>50</sub> values in the micromolar to nanomolar range. Our findings, supported by theoretical and docking studies, pointed out a crucial and undisclosed role of the presence of a carboxylate/amide group in C3 of the pyrone ring in order to obtain highly potent and selective MAO-B inhibitors.

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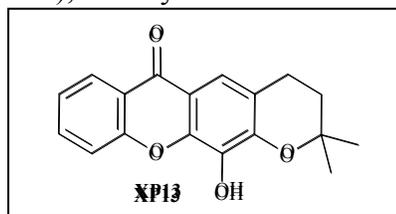
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# Development of Biodegradable Nanoparticles containing a Cytotoxic Pyranoxanthone

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Interest on xanthenes has been growing considerably due to the broad spectrum of biological activities exhibited by this class of compounds, namely antitumor [1]. Among them 4-dihydro-12-hydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-b]xanthen-6-one (**XP13**), a synthetic derivative, exhibited interesting antiproliferative and apoptotic effects in leukemia cell lines [2]. However, its poor aqueous solubility is a major obstacle for the assessment of *in vivo* studies. Previous work have demonstrated that the incorporation of xanthenes in polymeric nanoparticles was an efficient advance to overcome problems associated with their poor aqueous solubility and improved their biological activities [3]. To investigate the usefulness of this nanotechnology-based approach on **XP13** antitumor activity, **XP13**-loaded polymeric nanoparticle formulations were prepared by solvent displacement technique and emulsification solvent diffusion, and characterized regarding to their particle size, zeta potential and drug incorporation efficiency. Different nanospheres and nanocapsules formulations displayed mean diameter of about 150 nm and 250 nm, respectively and both systems exhibited negative surface charge. An HPLC method was developed and validated for the content determination of **XP13** in nanospheres and nanocapsules formulations. Incorporation efficiency values for nanocapsules (>70%) were higher than those corresponding to nanospheres (≈25%). Future work will involve the study of different formulations parameters in order to enhance the incorporation of **XP13** in nanoparticles.



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## DOPA-TRANSPORT IN AN IN VITRO CELLULAR MODEL OF DOPAMINERGIC NEURONS THE SH-SY5Y CELLS

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**Background and Aim** -3,4-dihydroxyphenylalanine (DOPA) is the immediate product of the rate-limiting step in catecholamine biosynthesis and the precursor of all the endogenous catecholamines. Treatment with DOPA remains to date the most effective treatment of the slowness of movement, increased muscle tone, and tremor that are typical of PD. The human neuroblastoma cell line SH-SY5Y has been widely used as a cellular model of dopaminergic neurons for Parkinson's disease research. In this study we investigated the transporter involved in DOPA uptake in SH-SY5Y cells.

**Methods and Statistics** DOPA levels in cells were evaluated by high performance liquid chromatography with electrochemical detection. Results are presented as arithmetic mean  $\pm$  standard error mean.

**Results** SH-SY5Y cells take up DOPA in a time dependent (linear until 6 minutes) and concentration dependent (2.5-2500  $\mu$ M) manner. Non-linear analysis of the saturation curves revealed for DOPA a  $K_M$  (in  $\mu$ M) of  $570 \pm 97$  and a  $V_{max}$  (in nmol/mg protein/6 min) of  $611 \pm 34$ . The uptake of DOPA (2.5  $\mu$ M) was reduced by the inhibitor of the L-type amino acid transporters 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH, 0.1-1000  $\mu$ M) ( $IC_{50} = 47 \pm 2$  nM;  $E_{max} = 24 \pm 10$  % control uptake) and neutral amino acids (1mM), but not by the inhibitor of the A-type amino acid transporters *N*-(methylamino)-isobutyric acid (MeAIB, 0.1-1000  $\mu$ M) nor by the acidic and basic amino acids (1mM). DOPA uptake (2.5  $\mu$ M) was unaltered by lowering the pH from 7.4 to 6.2. In the absence of sodium there was a 20% reduction in the  $V_{max}$  values for DOPA uptake. Accumulation of DOPA in SH-SY5Y cells was largely inhibited by the L-isomers of the small and large neutral amino acids (alanine, serine, threonine, cysteine, leucine, isoleucine, phenylalanine, methionine, and tyrosine), histidine, tryptophan, valine, asparagine and glutamine. Whereas the amino acids glycine, proline and the basic amino acid arginine also produced an inhibition of DOPA uptake, albeit minor, the basic amino acids lysine and cystine, and acidic amino acids aspartate and glutamate did not inhibit the uptake of L-DOPA.

**Conclusions** DOPA uptake in SH-SY5Y cells was sensitive to inhibition by BHC, but not to MeAIB, and was more sensitive to inhibition by neutral than to basic and acidic amino acids. Although most of DOPA was entering the cells in a  $Na^+$ -independent manner, a minor component of DOPA uptake ( $\sim 25\%$ ) was found to require extracellular  $Na^+$ . In general, these findings support the view that DOPA may be transported by systems  $B^0$  ( $Na^+$ -dependent) and L ( $Na^+$ -independent). The fraction of DOPA that is handled through system L is through a high-affinity ( $K_M$  values in the micromolar range) and pH-insensitive transport, which are characteristic of the LAT1.

## Effect of beta-blockers on redox status in patients with chronic heart failure

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**PURPOSE:** Reactive oxygen species contribute to the pathogenesis of chronic heart failure (CHF). Beta-blockers (BB) are a cornerstone in the treatment of CHF. Their antioxidant effect is being explored and constitutes a commercial strategy to differentiate between them. Therefore, we aimed to evaluate antioxidant and oxidant status in CHF patients treated with different BB (bisoprolol or carvedilol) and in those not taking BB.

**METHODS:** 30 CHF patients (NYHA functional class I to IV) were selected from the Heart Failure Clinic of Hospital S. João. On the day of the visit, patients were examined and blood and urine samples collected. Plasma total antioxidant status (TAS) and 24 h urinary excretion of H<sub>2</sub>O<sub>2</sub> and isoprostanes was evaluated using commercial kits. Furthermore, we also measured serum uric acid (UA), brain natriuretic peptide (BNP), urinary creatinine and cystatin C.

**RESULTS:** Patients were stratified into mild (NYHA classes I and II) and severe (NYHA classes III and IV) CHF. Plasma TAS (mM Trolox), urinary isoprostanes (ng/mg creatinine/day) and H<sub>2</sub>O<sub>2</sub> (nmol/mg creatinine/day) were markedly increased ( $p < 0.05$ ) in severe CHF patients (TAS:  $3.9 \pm 0.1$  vs  $3.1 \pm 0.1$ ; isoprostanes:  $2.1 \pm 0.1$  vs  $1.6 \pm 0.1$ ; H<sub>2</sub>O<sub>2</sub>:  $0.03 \pm 0.009$  vs  $0.01 \pm 0.002$ ). Serum UA, cystatin C and BNP were also elevated ( $p < 0.05$ ) in the same group of patients. Furthermore, plasma TAS was significantly correlated with serum UA ( $r^2 = 0.21$ ,  $r = 0.46$ ,  $p < 0.05$ ). Severe CHF patients not treated with BB had increased UA ( $115.6 \pm 21.2$  vs  $63.5 \pm 4.0$  mg/l,  $p < 0.05$ ), urinary H<sub>2</sub>O<sub>2</sub> ( $0.05 \pm 0.02$  vs  $0.01 \pm 0.001$ ,  $p < 0.05$ ) and BNP ( $2414 \pm 274$  vs  $639 \pm 274$  pg/ml,  $p < 0.05$ ). Although no significant statistical difference was found in plasma TAS between severe CHF patients treated and untreated with BB, the ratio TAS/UA was significantly lower ( $p < 0.05$ ) in untreated patients ( $0.036 \pm 0.006$  vs  $0.064 \pm 0.005$ ). BB treatment had no effect on urinary isoprostanes. Furthermore, no differences were observed between bisoprolol and carvedilol effects on redox status, serum UA levels, cystatin-C and BNP.

**CONCLUSIONS:** Severe CHF patients have increased plasma TAS, urinary isoprostanes and H<sub>2</sub>O<sub>2</sub>, as well as elevated BNP and cystatin C. Since serum UA seems to contribute to the higher TAS in these patients, the ratio TAS/UA may be a better marker of plasma antioxidant status. Treatment with BB increases the ratio TAS/UA and reduces urinary H<sub>2</sub>O<sub>2</sub> and plasma BNP. In addition, no differences were observed in the redox status, BNP or cystatin C levels in patients treated with carvedilol or bisoprolol.

# Effects of Chronic Ethanol Treatment and Withdrawal upon the Neuropeptide Y Content of Nucleus Accumbens of Rats: a Stereological Study

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The nucleus accumbens (NAc) is known for its role in mediating the reinforcing effects of drugs of abuse. Neurons producing neuropeptide Y (NPY) are abundant in the CNS and the NAc is one of the brain regions with highest concentrations of NPY. This neuropeptide displays a pivotal role in anxiety, stress, reward, and drug abuse. It may influence ethanol intake by regulating basal levels of anxiety, and by modulating the sedative effects of ethanol and/or its rewarding properties. In rodents, chronic ethanol treatment (CET) and withdrawal (W) alter NPY levels in several brain areas. It has also been demonstrated in our Institute that the number of NPY-immunoreactive (NPY-ir) neurons of the NAc is reduced in aged rats and that the subsequent treatment with nerve growth factor (NGF) restores these age-induced changes. NGF is essential for neuronal survival under normal conditions. In addition, this neurotrophin, whose levels are decreased during CET and W, plays a key role in NPY expression. We examined the effects of CET and W in the total number and somatic size of NPY-ir neurons in the NAc of rats. It was further investigated if the administration of NGF would interfere with the content of NPY in the NAc.

A total 20 Wistar male rats were used. Rats were assigned to control (n=4), young CET (n=4), old CET (n=4), young W (n=4) and young W NGF-treated (n=4) groups. At the end of the experiments, rats were perfused transcardially. From each brain, four adjacent series of sections were obtained. One of these was immunostained for NPY. The optical fractionator and the optical rotator methods were used to estimate the total number and the somatic volume of NPY-ir neurons, respectively.

We found no significant effect of CET and W on the total number of NAc NPY-ir neurons of young rats. On contrary, in alcohol-fed aged rats the expression of NPY was reduced to about 33% of the levels in controls. Our data also show that NGF significantly increases the expression of NPY in the NAc of W rats treated with NGF relative to control and W rats. Furthermore, it was observed that the somatic size of NPY neurons is similar in all analyzed groups.

**These results** demonstrate that age interfere with the effects of CET in the expression of NPY in the rat NAc. They also **corroborate earlier findings** showing **that** NGF regulates the phenotype of NPY-ir neurons. This study might help to explain the still cryptic role of NPY in alcohol intake, dependence and withdrawal. In addition, it provides hints to develop new therapies for neuropsychiatric disorders in which the NPY-ergic neurotransmitter system and/or NGF levels might be disturbed.

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# Evaluating cell proliferation and production of extracellular matrix by rat mesangial cells in primary culture: setup of the protocol and effect of glucose

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Diabetic nephropathy (DN) is a microvascular complication of diabetes [1]. Hyperglycemia is the main factor that determines the development and progression of this pathology [2]. It is known that in DN there is excessive cell proliferation of mesangial cells [3] as well as an increase in the production of extracellular matrix [4], namely collagen. These changes contribute to the development of glomerulosclerosis. This work intended to setup protocols to evaluate the proliferation and the production of extracellular matrix (collagen) of rat mesangial cells in primary culture and to verify the effect of glucose on this parameters.

The cell proliferation was quantified by a colorimetric immunoassay, based on the incorporation of 5-bromo-2-deoxyuridine (BrdU) into DNA. Collagen production was quantified by a colorimetric assay, after incorporation of *Sirius Red* dye in the triple helice of collagen.

Although fetal bovine serum (FBS) increased mesangial cell proliferation, that was not the case after 32h-exposure to 30 mM or 50 mM of glucose, when compared to the control (10 mM), either with or without insulin in the culture medium. After 42 hours of exposure to glucose, proliferation was inhibited, and this effect was lessened by the insulin. FBS also increased astrocyte proliferation in a concentration-dependent way. As for the collagen production, 30 mM glucose increased extracellular production.

The proposed protocols were established and it was possible to reproduce the effect of glucose on collagen production described in the literature. Regarding the proliferation assay, the protocol was also established, although future experiments need to clarify the time-course of the proliferative effect of glucose described in the literature.

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# Evaluation and control of food wastage at an University food unit

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Food service units at schools, universities, industry and hospitals need a complex management system involving several areas such as human resources, equipment and material, acquisition processes as well as financial and administrative management.

In order to allow the easier organization usually food units standardize the quantity of food served on meals, frequently not adequate for a significant part of users.

Food wastage, corresponding to the rest between foods prepared and not served.

The aim of this study was to evaluate the index between waste / consumption (IRI), by quantification of the number of served meals and food wastage an university food service unit.

The food unit serves 800 meals daily at lunch, including general menu, vegetarian and option. The work was developed during four weeks in order to allow the evaluation of parameters of an entire menu cycle. There were evaluated 22 days, 50% including meat and 50% fish in the general menu.

A convenience non probabilistic sample was used.

An Excel spreadsheet was created including food quantities prepared and wasted and the (IRI) was calculated.

The IRI was approximately 27%, average of 22 days, higher in the days with a fish menu (34%) and lower on meat menus days (22%) as expected.

According to Aragão (2003), these values are considered unacceptable since acceptable limit lies on 10%.

It was observed that each canteen user is served with approximately 860g of foods, from which only 620g are effectively consumed, corresponding to 70 g of food waste and 170g leftovers.

It seems that it is possible and desirable to reduce food wastage in order to optimize food unit productivity.

Potential strategies to achieve this goal may include the obligation booking meals previously, avoiding the disparity between estimated number of meals and the number of cooked meals, aiming to contribute for meals cost controlling.

## Evaluation of different QuEChERS for determination organochlorine pesticides in carrots

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Pesticides were and are developed to combat pests of agricultural crops and thereby improve food production<sup>[1,2]</sup>. Therefore they are widely used in agricultural practices, both in production and/or during the post-harvest treatment for transport purposes<sup>[2]</sup>.

However, the use of pesticides may cause potential health risks to human life if harmful residues appear in food and this can constitute a threat to food safety and quality<sup>[1,2]</sup>. Various pesticides, such as organochlorine pesticides (OCPs), are able to persist in the environment and of biomagnification in food chain. Therefore most of OCPs are considered persistent organic pollutants (POPs)<sup>[3]</sup>. To OCPs has been associated a range of both acute and chronic health effects, such as cancer, birth defects, neurological damage and several OCPs are also suspected to act as endocrine disruptors<sup>[4]</sup>.

Nowadays the presence of pesticides in food is receiving worldwide attention. The government authorities have the responsibility for setting the maximum residue limits and to regulate the concentration of pesticides in food<sup>[1]</sup>. The aim of this study was to develop a method to evaluate OCPs in carrots.

The analyzed compounds include: HCH ( $\alpha, \beta, \delta$ ), HCB, lindane, aldrin, 4,4'-DCBP,  $\alpha$ -endosulfan, dieldrin, *p-p'*-DDE, endrin,  $\beta$ -endosulfan *p-p'*-DDD, *o-p'*-DDT and methoxychlor. The extraction was performed with QuEChERS (the Quick Easy Cheap Effective Rugged and Safe). Five types of QuEChERS with different composition were studied. The selected one, EN 15662, had the following composition: 4g MgSO<sub>4</sub>, 1g NaCl, 1g Na<sub>3</sub>Citrate 2H<sub>2</sub>O, and 0,5g Na<sub>2</sub>HCitrate 1/2 H<sub>2</sub>O. Several parameters were optimized in order to achieve the best recovery and the lowest limit of detection. Gas chromatography with electron-capture detection was employed for the determination of pesticides. Recoveries varied from 11% (HCB) to 150% (HCH) with RSDs of 0.2-11% for GC amenable agrochemicals. Linear calibration curves were obtained to the 15 pesticides. Concentrations ranging from 5 to 70  $\mu\text{g}/\text{kg}$  in carrots were studied.

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## Evaluation of the growth inhibitory effect on human tumor cell lines of the secondary metabolites isolated from the sponge-associated fungi *Eurotium cristatum*

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Marine natural products have captivated many researches over the years due to a need for sources of diverse and pharmacologically active leads, especially in the area of anticancer drugs [1,2]. The aims of the present study were to: i) verify if the extract of the culture of *Eurotium cristatum*, a fungus isolated from the marine sponge *Mycale* sp., could inhibit the growth of human tumour cell lines; ii) isolate and identify the secondary metabolites from this fungal extract, iii) verify the potential of the metabolites as growth inhibitors in the same human tumour cell lines and iv) examine the cell cycle profiles of NCI-H460 cells following treatment with this fungal extract. So far, the ethyl acetate extract of the culture of *E. cristatum* (ECE) has furnished three compounds (**1-3**): 2-(2', 3-epoxy-1',3'-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl) benzaldehyde (**1**), 1,8-dihydroxy-6-methoxy-3-methyl-9,10- anthracenedione (physcion, **2**) and the dioxopiperazine alkaloid echinulin (**3**). However, we have recently isolated three more compounds from this extract and we are in the process of determining their structure. The ECE and the isolated compounds (**1-3**) were evaluated, by the SRB assay, for their growth inhibitory activity on three human tumour cell lines: breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and melanoma (A375-C5). Results showed that ECE was active in all three cell lines, providing GI<sub>50</sub> values = 44.3 ± 1.2 µg/mL, 45.5 ± 7.5 µg/mL and 71.3 ± 2.1 µg/mL for MCF-7, NCI-H460 and A375-C5, respectively. Compound **1** was found to exhibit moderate growth inhibitory activity against all three cell lines (GI<sub>50</sub> = 58.3 ± 1.2 µM, 46.0 ± 5.5 µM and 116.7 ± 7.2 µM for MCF-7, NCI-H460 and A375-C5, respectively), whereas compound **3** showed only weak inhibitory activity against MCF-7 (GI<sub>50</sub> = 109.7 ± 0.3 µM) and NCI-H460 (GI<sub>50</sub> = 96.7 ± 1.5 µM) but was inactive at the highest concentration tested (150 µM) against A375-C5. Compound **2** was found to be inactive in all three cell lines at the highest concentration tested (150 µM). Finally, the cell cycle profile of NCI-H460 cells treated with the GI<sub>50</sub> of ECE was studied. ECE was able to cause a slight cell cycle arrest in the G1 phase, with a corresponding decrease of cells in the S and G2/M phases. In conclusion, compound **1** was the most potent compound from the *E. cristatum* extract regarding tumour cell growth inhibition [3].

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## Exemestane-induced cell death in MCF-7<sup>aro</sup> breast cancer cells

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Breast cancer is one of the most common diseases that affect women worldwide. Estrogens are pivotal in the growth and development of hormone-dependent cancers, such as breast cancer. The biosynthesis of estrogens from androgens is catalyzed by aromatase, a cytochrome P-450 enzyme, so this enzyme is an attractive target for selective inhibition and for decreasing estrogen levels. In fact, aromatase inhibitors (AIs) are an effective alternative to the classical tamoxifen, for the endocrine therapy of estrogen-dependent breast cancer in postmenopausal women [1]. Exemestane is a third-generation AI that inhibits aromatase irreversibly. However, its mechanism of action in cells is not totally understood neither is the eventual development of resistances in some patients. We had previously used an ER-positive aromatase-overexpressing breast cancer cell line (MCF-7<sup>aro</sup>) for the study of anti-aromatase activity of newly synthesized AIs [2,3]. In this work MCF-7<sup>aro</sup> cells were used in order to study the biological effects of exemestane in breast cancer cells. Cells were treated with exemestane (5-15  $\mu$ M) during 3, 6, 9 and 13 days. Cell viability was evaluated by MTT and LDH assays. Morphological alterations were analyzed by phase contrast microscopy, Hoechst and Giemsa staining. The results showed a decrease in cell viability in a time and dose-dependent manner. This effect was accompanied by morphological alterations such as chromatine condensation and fragmentation and cytosolic vacuolization. During progression of exemestane-induced cell death it was observed an increase in acidic vesicular organelles (AVOs), as assessed by acridine orange staining. Pretreatment with autophagy inhibitor 3-methyladenine (3MA) decreased the appearance of AVOs, though exemestane still induced cell death. These results suggest the occurrence of autophagic cell death processes although other types of death are also involved.

These results may be important for the elucidation of the cancer cell death mechanisms induced by aromatase inhibitors and contribute to better understand the appearance of resistances.

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# Food handlers' perception of minimally processed products

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Consumers increasing concern about food and health associated with reduced time to prepare meals has been determining the search for convenient products, with fresh like quality, namely fruits and vegetables [1]. The use of minimally processed products (MPP) allows foodservice units to offer a greater variety of menus, reducing costs and minimizing cross contamination. At the same time their inclusion in menus reduces the problem of seasonality; and enables standardization of menus, saving time and labour force [2,3]. Nowadays there are still few food units adopting MPP, because these products are viewed as being more expensive than conventional ones and are frequently regarded by employees, as are less natural and easily perishable, but above all, they realize that the use of these products entails a renovation of the entire production of meals, including a reduction in the number of employees in the activities of pre-preparation [3,4].

The main goal of this study was to identify and analyze the level of awareness of foodservice operators, in respect of MPP. It is a cross-sectional study with an analytical component. The study was conducted by applying a multiple choice questionnaire to food handlers.

The majority of the institutions included in this study were hospitals, located in the North of Portugal, which cooked and served between 100 and 400 meals per day. Approximately 90% of the institutions had less than 10 employees working at the food service. 182 food handlers (180 women and 2 men), were interviewed, aged 20-58 years. The educational level was on average low, more than half of handlers had an education level less than or equal to the 2nd cycle of basic education. Regarding to employment status, the food handlers were officials of the Institute and most performs functions of kitchen helper. All food handlers responded that their food service unit used the MPP, pointing the less time spent on preparation, the less waste produce and the variety offered as the main advantages for using them. In spite of recognizing these advantages most of them prefer the traditional ones, highlighting the reasons for this choice freshness, sensory quality and the fact that more operators are needed to prepare them.

A promotion and a demystification of this category of products is necessary to food service managers and employees, since they present an excellent alternative to fresh equivalents.

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# Formulation of polymeric nanoparticles containing a biologically active xanthonic derivative

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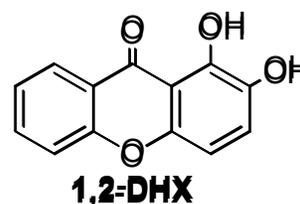
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The xanthone structure is recognized as a very interesting scaffold for the development of compounds with a wide range of different biological activities, particularly as growth inhibitors of tumor cell lines and as PKC modulators [1]. However, it has been estimated that roughly 40% of all investigational compounds fail development because of poor bioavailability that is often associated with aqueous insolubility. Regarding this, it is possible that one nanotechnology-based strategy can address solubility problems and improve biological activity of these compounds [2].

The aim of this work was therefore to formulate polymeric nanoparticles incorporating one of the xanthonic derivatives, 1,2-dihydroxyxanthone (**1,2-DHX**), for *in vitro* studies. Three different methods were used, two for the preparation of nanospheres and one for the preparation of nanocapsules. Furthermore, the nanoparticles obtained through these methods were analyzed in order to assess their granulometric characteristics and zeta potential.



Different nanospheres formulations displayed mean diameter of about 150 nm and exhibited negative surface charge. We concluded that two of the methods applied, one for each type of nanoparticles, were successful since they allowed us to prepare nanoparticles with the expected characteristics. However, further studies still need to be performed in order to evaluate the incorporation capacity of these nanoparticles and to investigate the usefulness of this nanotechnology-based approach on the improvement of 1,2-DHX biological activities.

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## Genotyping *Pseudomonas aeruginosa* collected from Portuguese cystic fibrosis patients

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*Pseudomonas aeruginosa* can easily grow in the airways of persons with cystic fibrosis. Highly discriminating, reproducible and efficient typing methods are required to answer important questions concerning *P. aeruginosa* nosocomial transmission between patients, its pathogenicity and treatment efficacy.

The aim of this study was to apply the multilocus sequence typing (MLST) scheme developed by Curran *et al.* [1] to Portuguese populations of *P. aeruginosa* in order to evaluate its genetic diversity.

Isolation of *P. aeruginosa*, yeasts and *Aspergillus fumigatus* was performed from sputum samples of cystic fibrosis patients, obtained from two healthcare centres. Five from 7 loci suggested by Curran *et al.* [1] in the development of a MLST for *P. aeruginosa* were used - *acsA*, *guaA*, *nuoD*, *ppsA* and *trpE*.

The associations between these microorganisms were described, as well as the improvement of MLST for *P. aeruginosa* by new primer selection and optimization, and the selection of the most discriminating polymorphisms. *P. aeruginosa* were found associated to the isolated fungi or alone. The optimized MLST was successfully executed for 5 of 7 loci. Only 59 single nucleotide polymorphisms were needed to discriminate the complete group of strains collected from our samples and the ones present in on-line databases. This accounts for 3% of the genome previously required for sequencing employing MLST scheme.

The wide spread of the sequenced loci through the genome allow us to make very trustworthy assumptions about the identity of a given strain by screening only a small number of nucleotide positions. The role of *P. aeruginosa* in patients with allergic bronchopulmonary aspergillosis remains unclear but its presence should not be neglected.

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# Improving Solubility of a BCS Class II Drug Using Solid Dispersions

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The objective of this study was to improve the solubility of a Biopharmaceutics Classification System (BCS) Class II drug using solid dispersions. BCS is a guide for the prediction of the intestinal drug absorption and classifies a drug according to its solubility and permeability. A BCS Class II drug has low solubility and high permeability [1]. In this study, the drug used was phenytoin (diphenylhydantoin, DPH), an antiepileptic agent extensively used for the treatment of generalized tonic-clonic epileptic seizures. Solid dispersions (SD) are a group of solid products consisting of a least two different components, generally a hydrophilic matrix and a hydrophobic drug. This technique allows a particle size reduction of drug to nearly a molecular level and when the system is exposed to aqueous media, the carrier is dissolved and the drug is released as very fine particles for quick dissolution and absorption. Solid dispersions were obtained by two methods: fusion method and solvent evaporation method [2, 3].

Fusion method consisted in heating the matrix and the drug at 85 °C followed by a cooling process to form the SD. In the solvent evaporation method, both drug and matrix was solubilized in a common solvent, ethanol, which was evaporated under vacuum. The SD's were analyzed using Differential Scanning Calorimetry (DSC) technique and compared with the respective physical mixture (PM). DSC is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature.

Because of the characteristics of the DPH, which has a melting point around 300 °C and did not melt with the fusion method, it was not possible to obtain SD using this method. The next step was to obtain SD using the solvent evaporation method which is a technique used when it is not possible to melt one of the components, drug or matrix. Three matrixes were used with this method: Methocel K4M (HPMC), Explotab<sup>®</sup> (Sodium Starch Glycolate) and Povidone (PVP K30) but it was the latter which yielded better results on DSC. SD with DPH and PVP K30 were prepared at ratios 50:50, 40:60 and 30:70. Compared, by DSC, with their respective PM and with others SD with different ratios, it was the formulation 30:70 (DPH:PVP K30) which had better results.

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## ***In vivo* Modulation of MicroRNA-155 Expression in a Murine Model of Diet-Induced Obesity. Preliminary results.**

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**Introduction:** Proinflammatory activation mediates the metabolic and cardiovascular complications of obesity such as diabetes mellitus, dyslipidemia and hypertension. MicroRNAs (miR) are a novel class of inhibitory molecules that modulate gene expression at a post-transcriptional level. Recently, miR-155 was shown to be overexpressed in several proinflammatory states such as sepsis and autoimmune diseases. In the present study we evaluated the modulation of miR-155 in a murine model of diet-induced obesity.

**Methods:** Eight-week-old male C57BL/6 mice (n=26) were used. Baseline miR-155 expression (n=3) was evaluated in heart, lung, liver, spleen, adipose tissue and gastrocnemius samples. Obesity was induced by *ad libitum* exposure to a high-calorie diet (HCD; 5.4 kcal/g; 35% fat; 35% sucrose; n=15) and compared to regular diet exposure (ND; n=5). In order to assess the feasibility of *in-vivo* modulation of miR-155 a subset of animals was treated with chemically modified miR-155 (AgoMir-155; 20mg/kg, ip; n=3) and evaluated for its effects on angiotensin receptor 1 (AT1R) target gene expression. MiR and mRNA were extracted using a guanidium-thiocyanate selective silica-gel membrane-binding method (QIAGEN, No. 217004). Two-step real-time RT-PCR (LightCycler II®, Roche) was used to perform relative quantification of microRNA and mRNA. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U1 snRNA (RNUA1) were used as mRNA and miR internal control genes, respectively. Results are expressed as mir-155/RNUA1 or AT1R/GAPDH, in arbitrary units (1AU = Mean of control/basal group). Statistical significance was set as P<0.05.

**Results:** MiR-155 expression was higher in adipose tissue (28.5 AU), followed by spleen (20.2 AU), skeletal muscle (15.8 AU), heart (13.5 AU), lung (5.0 AU) and liver (1.0 AU). Exposure to the HCD was accompanied by progressive obesity compared with normal diet fed littermates (45.6 vs. 35.7g). Mir-155 was significantly upregulated in HCD group, compared with ND (7.6 vs. 1.0 AU). Injection of AgoMir-155 effectively increased mir-155 levels (7.5 vs. 1.0 AU) with a trend towards lower AT1R mRNA levels.

**Conclusion:** MiR-155 is highly expressed in adipose tissue, skeletal muscle and the heart, being upregulated in our murine model of diet-induced obesity. Moreover we were able to modulate miR-155 expression *in vivo* using a chemically modified analog. These results suggest miR-155 as a potential therapeutic target in obesity.

# Incorporation of colloidal lipid carriers in semi-solid formulations for topical drug delivery

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**Introduction:** Concerning their advantages, lipid-based drug delivery-systems, the so-called solid lipid nanoparticles (SLN), gained attention during the last years [1]. SLN can be incorporated into hydrogels acquiring a semi-solid consistency that permits an increase of topical drug bioavailability and long-term stability of nanoparticles [2, 3]. The aim of this work was the preparation of SLN dispersion and its further incorporation into perfluorocarbon (PFC) based hydrogel. The rheological behaviour of prepared hydrogels and the influence on the particle size was evaluated, before and after incorporation of the nanoparticles.

**Methods:** Placebo SLN formulations were prepared by ultrasound technique. Particle size before and after hydrogel incorporation was measured by photon correlation spectroscopy (PSC) and laser diffractometry (LD), respectively. Zeta-potential (ZP) was accessed by laser Doppler electrophoresis. Fresh SLN formulations were incorporated into PFC hydrogels in a concentration of 40% (w/w) of the dispersion in the gel. Comparative evaluations of shear rate versus shear stress of the hydrogels were done by continuous shear investigations. In addition, the morphology of semi-solid formulations was observed under cryo-scanning electron microscopy (cryoSEM), before and after SLN incorporation.

**Results:** The mean particle size of SLN on day 0 was 98nm with a ZP of -36 mV. The particle size was maintained in the nanometer range after hydrogel incorporation. cryoSEM images showed that the hydrogel net remains after nanoparticle incorporation. Rheological analysis showed typical plastic systems, which are suitable for topical drug administration. Tixotrophy was also observed after incorporate nanoparticles in the hydrogel.

**Conclusions:** The prepared SLN-based hydrogel formulations seem to be suitable carriers for the preparation of topical adhesive systems for drug delivery. The long-term stability studies are going on, in order to determine particle sizes, electrochemical stability and rheological behaviour of the hydrogel-based formulations for longer periods.

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## Induction of oxidative stress by *tert*-butylhydroperoxide in Caco-2 cells

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Excessive reactive oxygen species (ROS) in the cells, either because they are formed in excess or because they are not adequately removed by the antioxidant defense machinery, cause oxidative stress, modifying and damaging DNA, carbohydrates, proteins, and polyunsaturated fatty acids [1]. In agreement with this, excessive production of ROS has been implicated in the onset and progression of a number of pathologies, from atherosclerosis to neural degenerative diseases, inflammation and cancer [2]. At the intestinal level, ROS have been implicated in the intestinal injury caused by inflammatory bowel diseases, ischemia-reperfusion, hemorrhagic shock, enterocolitis, xenobiotics and ingested oxidized fatty acids [3].

The aim of our work was to investigate the effect of increasing levels of oxidative stress in the intestinal epithelium homeostasis. For this, we investigated the effect of *tert*-butylhydroperoxide (TBH) upon oxidative stress markers and upon viability and proliferation of human intestinal epithelial cells (Caco-2 cells). Induction of oxidative stress by TBH was evaluated by measuring lipid peroxidation (TBARS assay) and glutathione (total (GSx), oxidized (GSSG) and reduced (GSH)) levels.

Increased levels of lipid peroxidation were observed with increasing concentrations of TBH (135±14%, 155±42%, 199±46% and 207±56% of control for TBH 30, 100, 1000 and 3000 µM, respectively (n=4-6)). TBH (30-1000 µM) showed no effect upon GSx, GSSG and GSH levels. However, TBH (3000 µM) caused a significant decrease in GSx levels (from 3.38±0.51 to 2.00±0.22 pmol/mg protein; n=8) and in GSH/GSSG ratio (from 6.96±2.70 to 0.98±0.27).

TBH (1-1000 µM) was devoid of effect upon Caco-2 cell viability (evaluated with the lactate dehydrogenase (LDH) assay). TBH (1-100 µM) was also devoid of effect upon cell proliferation, but 1000 µM TBH caused a 20% decrease in this parameter.

In conclusion, TBH seems to induce oxidative stress in Caco-2 cells. At least for the lower concentrations, TBH does not seem to influence Caco-2 cell proliferation and viability. So, TBH appears as a useful model to investigate the effect of oxidative stress in this cell line.

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# Cross-infection in dental materials: a clinical study among FMDUP students

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**Introduction/Aims:** The control of cross-infection is an imperative issue when dealing with dental impression materials in Dentistry.[1-4] The goals of this research were defined with the purpose of surpassing failures in the existing literature about the ideal disinfecting method for alginate impression: 1. Quantitative analysis of the aerobic bacteria transferred to the alginate after the dental impression - cross-infection. 2. Evaluate the disinfecting efficiency of sodium hypochlorite and tap water as procedures of avoiding cross-infection.

**Material and Methods:** This study was carried out in the Faculty of Dental Medicine, University of Porto, Portugal. The selection criteria was age, DMF index (decay/missing/filled), frequency of tooth brushing per day, manual toothbrush, powered toothbrush and mouthwash use. For each participant, one impression was taken in alginate from the mandibular arch. These samples were submitted to disinfection methods and microbiology analyses.

Student's t test was realized and the analysis of variance (ANOVA) for multiple comparisons was also done.

**Results:** After mouth contact, alginate microbial count increased to  $2,68 \times 10^3 \pm 6,19 \times 10^2$  CFU/mm<sup>2</sup>. It was verified that after water wash, microbial count decreased 50% while sodium hypochlorite disinfection decreased microbial count to near zero values.

**Conclusion:** Dental impression materials can act as vectors transmitting several microorganisms. Sodium hypochlorite disinfection is an efficient disinfection method. Tap water rinsing can reduce microbial count but does not disinfect totally dental impression materials.

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# Influence of Sex Steroids on the Morphology of the Parvicellular Neurons of the Hypothalamic Paraventricular Nucleus

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This study aims to investigate the potential contribution of the dendritic trees for the sex-related differences found in the volume of the medial parvocellular subdivision of the paraventricular nucleus of the hypothalamus (PVNmp), as well as the influence that estrogens might have in determining these neuroanatomical sexual dimorphisms.

Gonadal hormones, namely estrogens, affect a variety of reproductive and non-reproductive physiological functions neurally regulated by numerous structures such as the PVN. The PVNmp is one of the subdivisions involved, among other functions, in the regulation of the hypothalamic-pituitary-adrenal axis (HPA axis). Previous studies have found that the volume of the PVNmp is larger in males than in females, although no differences were found in the total number of its neurons. These differences in the volume may be due to variations in the length of the dendritic trees or in the number of afferents that reach this nucleus. This last point could be explained by differences in the concentration of estrogen receptors in PVN neurons.

Adult male and female Wistar rats were maintained throughout the experiment under standard laboratory conditions: 12-h light/dark cycle and temperature of 22 °C. Solid diet and water were available *ad libitum* until the day of sacrifice. Fifteen days before the end of the experiment, two groups of female rats were gonadectomized under deep anesthesia and the remaining animals were assigned to another two groups: intact males and intact females. Two days before the sacrifice one group of castrated female rats were subcutaneously injected with estrogen-benzoate (EB) and the other group with sesame-oil (vehicle). After perfusion, the hypothalami were processed for Golgi-impregnation. Approximately nine PVNmp neurons were sampled per animal and their dendritic arborizations were traced by hand using a camera lucida attachment. The drawings were used to estimate the number and to measure the length of the dendritic segments, as well as to evaluate the length of their terminal segments and the spine density.

The total length of the dendritic arborizations and the mean length of the terminal segments were significantly higher in intact females than in intact males. No significant differences were found between gonadectomized groups. We also found that the spine density was consistently higher in intact females than in males, as well as in the EB group relative to the vehicle group.

These results prove that there is a marked sexual dimorphism in the parvicellular neurons of the PVNmp, with striking differences in the length of their dendritic arborizations and in spine density. They also reveal that the sex differences in dendritic length seem to depend only on the organizational effects of sex steroids, whereas those in spine density also result from the activational effects of estrogens.

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# Ingestão de Cerveja e níveis plasmáticos de Homocisteína – Estudo Quase-Experimental

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**Background:** The elevation of serum homocysteine is an independent and significant risk factor for vascular diseases. The prevalence of hyperhomocysteinemia in the general population is between 5-10%, and these values increase to 30 to 40% in the elderly. Some studies have documented a decrease of homocysteine levels with moderate consumption of beer. However, this inverse association lacks experimental evidence. **Objective:** To evaluate, in older adults, the effectiveness of non-alcoholic beer and non-alcoholic beer with a high content of folic acid in reducing hyperhomocysteinemia.

**Participants and Methods:** A quasi-experimental study, involving 40 older adults with advanced age with high serum homocysteine levels ( $>11\mu\text{mol/L}$  in women and  $>12\mu\text{mol/L}$  in men) was conducted. The older adults were divided into three groups. During a period of three weeks, the control group ( $n=23$ ) consumed 500mL of water per day, the second group ( $n=8$ ) 500ml non-alcoholic beer per day and the third group had 500 ml non-alcoholic beer with a high content of folic acid ( $40\mu\text{g}/100\text{mL}$ ) daily. Levels of homocysteine, folic acid and cobalamin were evaluated at baseline and after this intervention.

**Results:** No statistical significance differences between groups were found at baseline and at the end of the intervention for the levels of folic acid and cobalamin. A decrease of homocysteine levels was observed in the group supplemented with non-alcoholic beer with high folic acid content (mean= $3.55\mu\text{mol/L}$ , interquartile range= $2.1\mu\text{mol/L}$ ) compared to the control group ( $0.9\mu\text{mol/L}$ , interquartile range= $1.5\mu\text{mol/L}$ ),  $p<0.05$ . A non statistically significant reduction was also observed between the control group and the alcohol-free beer group ( $2.45\text{ mmol/L}$ , interquartile range =  $6.3\text{ mmol/L}$ ).

**Conclusion:** Non-alcoholic beer with a high content of folic acid intake during three weeks significantly decreased homocysteine levels in older adults with advanced age.

**Key-words:** folic acid, cobalamin, homocysteine, non-alcoholic beer, older adults with advanced age.

# Interaction of anthocyanins with new antimalarial drugs: anticancer properties

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Anticancer properties have been described for antimalarial compounds, particularly quinoline derivatives and imidazoquinones, as well as promising results have emerged in the treatment of malaria for compounds with anticancer properties, such as polyphenols.

Thus, initially, this study aimed to evaluate the growth inhibitory effect of dipeptidyl-primaquine derivatives and imidazoquinones (antimalarial imidazolidin-4-ones derived from primaquine) on human cancer cell lines BxPC-3, Caco-2, HT-29 and MCF-7 (Fig 1.). In a second phase, the most promising antimalarials were co-administrated with natural anthocyanins on the estrogen responsive human breast cancer cell line (MCF-7) to assay any synergic or antagonistic effects.

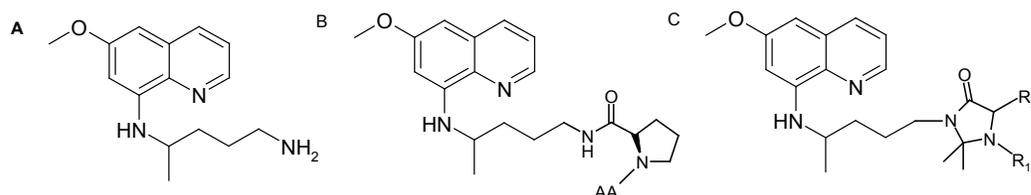


Fig 1. Primaquine (A), Dipeptidyl-primaquine derivatives (B) and Imidazoquinones (C) tested.

Overall, the tested compounds showed the highest growth inhibitory effects on the breast cancer cell line. Imidazoquinones, in general, have higher growth inhibitory effects than *N*-dipeptidylprimaquine derivatives on human tumoral cell lines tested. These results suggest that a combinatory therapy with anthocyanins and antimalarial drugs, in a proper concentration, can be useful in the treatment against cancer (Fig 2.).

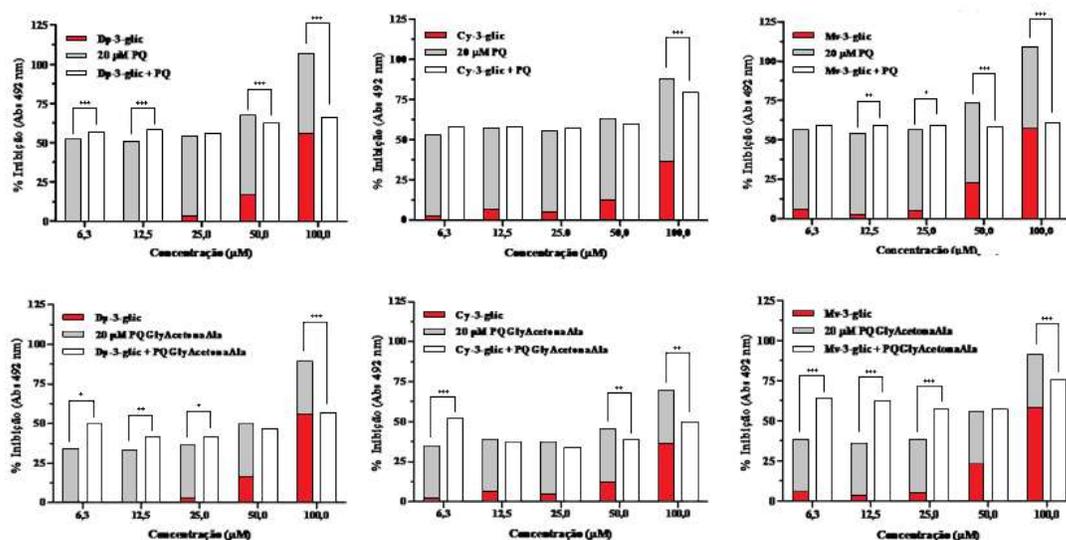


Fig 2. Effect of anthocyanins on the growth of the MCF-7 cell line, in the presence of a constant dose of antimalarial drugs. Cells, seeded in 96-well plates, were treated with a broad concentration range (6.3 – 100.0 µM) of each compound, in the presence of 20.0µM of each antimalarial drug, for 48 h. Each value represents the mean ± SEM (n = 3-12). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

## The interrelationships of informational image: aesthetics versus practical function, form vs. function

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An artifact of design is able to provide three functions: practical, aesthetic and symbolic. Often users do not limit to establish physical relations with graphical artifacts. On the subconscious level, users establish psychological relations with these artifacts. This process takes place through the aesthetic function of design, which often causes a feeling of greater or lesser acceptance of the user regarding the object of attention.

The work proposed, studies the representation of graphical interfaces to databases and the role that aesthetics play in the same function, namely in order to facilitate the user to: navigation through the data presented, the readability of information and immediate understanding of it.

In the current school year, the degree in Communication Sciences celebrated ten years of existence. This decade of operation seemed to us a good topic for this project.

Thus, it was considered appropriate to use the data from the students as the basis dataset for the work. Therefore, with different level of aesthetic, a set of graphical objects was created based on tabular data with respect to: Year of entry; Number of students entering the course, per year; Sex; Strand chosen (Public Relations, Journalism, Multimedia); Year of graduation.

Subsequent experiments were prepared in the form of an inquiry about user preferences regarding the developed set of graphical objects. This inquiry was filled by a group of 70 students, currently attending a degree in Communication Sciences, University of Porto. The study results show a clear preference for receiving images with a certain level of aesthetic presence. We can therefore say that the aesthetic dimension can itself also be considered a cognitive tool.

# Via Mascot

## A contribution to the unveil of nowadays mascot's mechanisms of communication and significance, as an artifact of graphic design.

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The present study has as main target the artifact which is nowadays meant as a “mascot”. After an approach to the consolidation of the concept itself, the mascot is analyzed through the spectrum of graphic design, with the objectives of understanding the power of its skills, as an extension of communication at the service of this discipline, and simultaneously, with the purpose of contributing to a more scientific perspective on this subject, apparently not pretty much studied.

To achieve this effect, the subject was exposed to models of communication of both processual and semiotics schools, focusing on studies emphasized by Per Mollerup [1] supported on John Fiske's [2] interpretations, from unavoidable authors such as Saussure, Peirce, Guiraud, Jakobson and Shannon & Weaver. This analysis is accompanied by examples that serve not only as brief case studies but which also constitute a sample of the mascot's "state of art".



Fig. 1 – Ebah, the metalinguistic mascot.

By these means, it was possible to assess that the mascot can be understood as a graphic phenomenon which feeds itself through a metaphorical power, serving a bigger entity by fulfilling its program guidelines.

It were also determined the necessary items to the projectual and creative process that underlies the legitimate act of creating a mascot.

Following these topics, and serving also as a way of self-knowledge of the object itself, based on Jakobson's metalinguistic communication function, it was created a mascot named Ebah (Fig. 1). It's program main aim is to somehow embody a metaphor of the “genetic potential” of all mascots.

**Keywords:** mascot, graphic design, communication, identity, program.

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# Dyslexia Assessment with the Phonological Battery of the University of Porto

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Recent studies show that 10% of children under 18 years old in the EUA have learning disabilities [1]. The majority of those children, 70 to 80%, have specific difficulties in reading and writing skills [1]. Dyslexia is the most prevalent learning disability, and fortunately one of the most studied and well understood [2]. Overcoming these difficulties is a hard task, it requires specialized literacy intervention and support, which by itself presupposes effective and early assessment. In Portugal, the assessment and evaluation of dyslexia is still somewhat problematic. Despite the worthy practices of some professionals, there are few empirically valid and organized evaluation procedures using Portuguese data samples. Today there is wide agreement that, at the cognitive level, dyslexia is characterized by a phonological deficit [3], thus its assessment is an essential component of dyslexia evaluation. For this reason we built the Bateria Fonológica da Universidade do Porto (BFUP), and are now testing it in its final form.

In this poster we will feature the collection of phonological assessment tasks included in BFUP. The tasks are organized into five domains that are relevant for assessment of dyslexia, these are: initial screening, reading, writing, phonological awareness, and short-term memory. The tasks in these domains were retrieved through an extensive literature revision, have good support from the literature, and were carefully adapted to the Portuguese language. Specifically, we have used age appropriate tasks to develop two different versions of the battery: one adapted and validated for children evaluation (1-6<sup>th</sup> grade) [1] and the other focused on adolescent and adult phonological assessment.

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# "Work Commitment and motivation: The role of family-friendly practices."

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The work-family dilemma is currently a prominent issue to workers, employers and society in general. It is, therefore essential to take measures which facilitate the articulation between work and family roles: first, so that workers can experience less interference of work demands in family responsibilities and, secondly, to enable companies to retain their best employees by offering them favorable conditions for reconciling work and family. Considering that workers tend to accommodate their schedules to their professional activities more than the opposite , it is of great importance to understand the extent in which the dedication of workers to their profession is related to the degree of support that their employers provide to them with regard to their family life. Therefore, the aim is to determine the extent to which family-friendly measures positively influences commitment and motivation to work, as well as the experience of work-family experience. In the study to be presented - developed with both a quantitative and qualitative methodology -, participated five Portuguese companies and has involved the managers of Human Resources departments, as well as 400 employees belonging to the company, with children between 6 and 12 years old. The most impressive results indicate that there is a concern by employers in providing working conditions that can satisfy the personal needs of their workforce and retain their employees. However, the practical implementation of these measures is not very expressive when considering employees' commitment and incentive to work. Regarding work-family relationship, it appears that men tend to experience great parenting-work positive spillover. There were not found significant gender differences in the experience of work-parenting and parenting-work negative spillover.

# Organizational Culture and Human Resources' Training and Development – a study about the McDonald's Hamburger University

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McDonald's is known worldwide as a leader in the fast food industry. There exists over 30 000 restaurants in more than 120 countries and it is known that this enormous expansion has an important franchising system supporting it. It became an interesting subject for the authors to understand how the McDonald's standardization could be so successfully extended to the most different parts of the world. For this reason, a conceptual paper was developed with the goal of understanding how the McDonald's Organizational Culture was passed to the franchisees through Human Resource's Training and Development.

Organizational culture is the set of shared, taken-for-granted implicit assumptions that a group holds and that determines how it perceives, thinks about and reacts to its various environments<sup>1</sup>. Human Resources' Training and Development refers to the systematic processes initiated by the organization (or by its direction) resulting in relatively permanent changes in knowledge, skills, or attitudes of organizational members<sup>2</sup>. Organizational culture constrains the human resources' practices, and these should take the first one into consideration, as a way of not being ineffective. On the other hand, the human resources' practices can create, shape or make the organizational culture stronger<sup>3</sup>. Based on information found about the McDonald's in articles, web sites and other media content, and having in consideration the conceptions of some experts in Organizational Psychology, we tried to know this organization's culture focusing on its shared values, taking also into consideration the influence of different countries' national culture. We also explored the importance that McDonald's gives to training and development, so that we could understand the dynamic between these two main themes.

We could perceive a mutual influence between Organizational Culture and Human Resource's Training and Development in this organization. The creation of the Hamburger University is a prime example of the power that the organizational culture has in McDonald's training, and the last one is also recognized as a major factor for the full ownership and identification of their human resources with McDonald's Culture. We could understand that besides the Training given at Hamburger University can express a concern about the adaptation to the different cultures where the business is installed, it is also a way of developing this organization's culture.

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# The Use of Learning Strategies in the Classroom of Portuguese as a foreign Language

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Learning a foreign language involves much more than memorizing contents. Therefore, the study herewith presented entitled “The use of learning strategies in the classroom of

Portuguese as a Foreign Language” is intended for identifying the learning strategies that foreign students of PFL use as they learn the target language best. In the first chapter, this research provides a general view over the several authors that support the underlying theory of learning strategies, which sustains the research performed and whose main purpose is to improve teaching procedure by reflecting upon the teaching and learning process. Within the scope of strategic competence, the learners need to get to know themselves by being aware of their specific needs and learning how to use learning strategies conscious and intelligently in order to overcome less positive features.

Thus, the learners must change their attitude facing the learning process, becoming more active, more participant and reflective upon their own process of learning. In the second chapter, from the gathering of data through questionnaires filled by learners, the present study is meant to search the way students learn PFL better and eventually establish a connection between this use of learning strategies and certain variables such as age, level, gender, mother tongue, literacy level, among others. So, the main purposes of this study are to recognize learning strategies used more frequently from the answers given on the questionnaires; to identify the strategies with greater potential to promote linguistic competence in foreign language; to analyze the (in)existence of the relationship between learning strategies and variables related to language learning; and to promote a strategic instruction in foreign language class through a whole of class activities that develop metacognitive reflection of the teaching and learning process, as the third chapter describes.

Keywords: Strategies; Learning; Competence; Autonomy; Metacognition

# Globalization: A Transformation in the World System and the paradigm of Social Sciences.

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Present being significantly in academic discussions since the twentieth century, the concept of globalization is constantly debated within the social sciences, so it is important to know and understand the theories that bear on the debate of the globalization. Thus, this paper seeks to expose the thought of two authors working the issue and have relevance in this context, Boaventura de Sousa Santos and Octavio Ianni. From his theoretical contributions, we can understand, briefly, the conceptualization of the overall process and how it came to influence the social sciences, with regard to its paradigm.

Since the late twentieth century, the concept of globalization permeates many discussions inside and outside the social sciences, in articulating their different perspectives: economic, political, social and cultural development, bringing with it a number of dilemmas for society today. Several prominent theorists of sociology, for example, have addressed this issue, and if not cockiness of such assertion, it can be said that globalization was one of the most important issues in academic debate for decades. Discussed by authors such as Giddens and Castells <sup>1 2</sup> and, linked to post-modern and multinational capitalism, globalization has turned out to be a part of sociological theory and is now of paramount importance to understand how they can provide social relations.

Therefore, this article aims to reflect on this theme through the literature review of two essays about globalization as the centerpiece: "The processes of globalization" and "Globalization: a new paradigm of social sciences", respectively authors Boaventura de Sousa Santos and Octavio <sup>3</sup> Ianni<sup>4</sup>. A from the first can reflect on the concept of globalization, so that can understand, how was the phenomenon and how it comes to influence social relationships. Later, with Ianni, you can see how globalization has changed the thinking in the social sciences, changing the paradigm of science.

This quick theoretical overview about the concept of globalization is capable of making us realize how complex it is his understanding in the world system and the social sciences.

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# The contribution of GIS to understand the relationship between episodes of extreme temperature and thermal variability in the transition period Spring - Summer and the occurrence of myocardial infarction in the municipality of Porto

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This study looks forward to present a methodology developed in GIS and aims to relate both the extreme temperature episodes and the thermal variability during the transition period Spring-Summer, as well as the occurrence of heart attacks within the municipality of Oporto. Having in mind the different areas of analysis– climate, biogeophysical and socio-economical variables and health – we tried to develop a spatial support for the variables that could contribute to increase the morbidity or the mortality related to this pathology and, subsequently, we aimed at finding out temporal and spatial connections between temperature and heart attacks. The outcome makes it clear that it is possible to establish a connection between both, namely in days where the maximum and/or the minimum temperatures were unusually high or low, or when the variation in the thermal pattern had suddenly changed overnight. Strangely, there wasn't any coincidence between the periods officially defined as part of the heat wave. We ended by building up heart attack risk charts for Oporto, during uncommon thermal periods.

# Assessing the Quality of Public Service - An Application to the Office of Urban Service - Santa Maria da Feira - Portugal

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The public sector in Portugal, as in major modern economies, controls a significant part of the economic resources. In a period of strong budget restrictions organizations in the public sector have been under the spotlight of the political and academic debates. Furthermore, public organizations are nowadays more concerned in finding new forms of management, leading to effectiveness with more value to the citizens. Indeed, public organizations recognized the potential of internet and technologies of information (TIC) in the provision of services and thus in the relationship with stakeholders. In this context, *e-government* emerged combining TIC and classic techniques of public administration. This study was established to develop and to validate two instruments of service quality in public services, one for the traditional and other for the virtual relationship.

A questionnaire was developed comprising 23 items for the traditional service attendance, called PUBLICPERF - *Public Performance* and 48 items for the virtual service, called E-PUBLICPERF - *E-Public Performance*. This study was conducted based on a convenience sample (n = 107) of citizens/users of the Office of Urban Service of Santa Maria da Feira.

The dimensions of service quality identified for the traditional service provision were: tangibility, reliability, responsiveness, assurance and empathy. For the virtual service, the quality dimensions identified were: trust, tangibles, empathy, safety, convenience and responsiveness. The psychometric qualities of both instruments were demonstrated in terms of unidimensionality, through confirmatory factor analysis, internal reliability and validity of content, criterion and construct.

This study also revealed that the best performance in terms of quality perceived by citizens/users of the Office of Urban Service match the scale of empathy (traditional service attendance) and convenience (virtual service). Additionally, this study showed that the dimensions that require improvements are reliable (traditional service attendance) and responsiveness (virtual service). Limitations and implications for future studies were also discussed.

# Measuring Internet Coupons Usage based on a Logistic Model

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Coupons are electronic discount vouchers distributed on the Internet. Currently the market for coupons has changed and the Internet has become a preferential channel as a means of distribution. In Portugal, the use of this new type of coupons is of little significance compared to markets like the U.S., where they already are preferred to traditional coupons.

This study aims to characterize the profile of users of electronic coupons in Portugal and the product categories in which discount coupons can influence the purchase decisions. Moreover, this study also intends to identify the most relevant factors that influence the use of electronic coupons, based on the theory of planned behavior.

The results of this work are based on a survey answered by 208 subjects.

This study showed that consumers are open to the new form of distributing coupons. Differences between the gender of the consumer and the influence that a discount coupon can have on the acquisition of categories of goods or services were also identified.

We concluded, based on a logistic regression model, that the probability of using electronic coupons is determined by the attitudes and subjective norms, and is higher for male individuals.

## Segmentation of the culture market: a study applied to Oporto

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A basic marketing principle considers that the main purpose of companies is to meet consumer's desires and needs. However, those can only be satisfied if companies know the people/organizations that will consume the products or services offered. With a greater competition and a consumer better informed, cultural organizations face, today, more than ever, important challenges in order to increase their audiences in both number and diversity.

These changes make it relevant to analyze the profile of the cultural events' consumer in Oporto city area from a marketing perspective. To this purpose an exploratory study was developed, based on a questionnaire administration to a sample of 164 individuals, though which were tested different factors influencing culture events consumption: demographic, social and economic profiles; frequency of participation on cultural events; Oporto cultural organizations knowledge; information sources used by individuals when searching for a cultural event; preferences towards different cultural events; attitude toward cultural events; factors influencing cultural events demand (such as price, proximity, artists awareness); benefits derived from attendance; perception in relation to present cultural events supply in Oporto city.

Some of the conclusions achieved were the following: (i) the internet is the main source of information used by cultural events consumers; (ii) there is a very low affinity with the cultural activities as well a very low involvement with this kind of activities; (iii) the cultural events provide fun, contribute to the cultural enrichment and are emotionally rewarding; (iv) cinema is the cultural activity for which the individuals had shown greater interest; (v) most of the cultural sites available in Oporto city are unknown for the questioned sample; (vi) the majority of the individuals questioned are unsatisfied with the cultural events supply available in Oporto; (vii) the price, the experience and the cultural enrichment are the most valuable items in the decision process as far as cultural events are concerned.

On the basis of the results achieved it was proposed a segmentation approach, based on 10 variables related to the importance given to the following items in the decision process of attending a cultural event: price; proximity to the residence area; friend advice; advertisement; experience achieved; artist awareness; discounts; cultural enrichment achieved; specialist advice presented on media, and the possibility of being with friends/family.

The results of the present study allowed a better understanding of the cultural consumers as it includes aspects as cultural activities frequency of attendance, affinity, most valuable items consider in the decision process, most popular cultural places, and perception regarding the available cultural supply in Oporto city. Thus, it permits to suggest specific marketing objectives to cultural organizations operating in Oporto, so that more efficient strategies can be designed and implemented in order to find the best way to increase their audiences.

# ADVANCED APPLICATIONS OF PMMA

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Poly(methacril-methacrylate) (PMMA) is used in a wide range of applications, the most common of which include toys and packaging. Recently, medical applications and the replacement of glass in demanding structural conditions are gaining increasing attention. PMMA is a highly conformable thermoplastic material that can be accurately shaped, even for very complex geometries. The material is inert and biocompatible. Thus, it does not react bioactively with the organism [1]. Medical interventions will have permanent results since the organism does not absorb it and the absence of proteins in PMMA avoids any reaction with the organism [1].

The first medical application was a hip prosthesis [1]. Hitherto, the material is applied in increasingly demanding fields of medicine such as plastic surgery and ophthalmology. In plastic surgeries, the use of PMMA is justified not only by its physical properties, but also by its capacity to be injected in the organism [1] in conditions that the later can withstand and accept. The injection is possible mixing microspheres of PMMA, obtained by suspension [2] in an organic fluid. This fluid is absorbed by the organism after the injection and the tissues gradually grow around PMMA, giving the implant a perfect and more realistic result [1]. The main objective of these applications is to correct wrinkles and scars, and reshape or reconstruct some parts of the body [1].

Due to its high conformability and transparency, PMMA is a material that can be used to produce contact lenses, both intra e extra corneal, used in the correction of myopia, astigmatism, cataract and keratoconus [3].

PMMA provides some relevant advantages in the replacement of glass. Its density is half the density of glass [4], but is more transparent than the later even for larger thicknesses [5]. Transparency is very important in the construction of large aquariums because the windows must be thick enough to withstand the water pressure. An interesting example is the Kuroshio Sea Tank, at the Okinawa Churaumi Aquarium, in Japan [6]. The thermal conductivity of PMMA [4] is very low, relative to that of glass, which is very important if it is necessary to combine transparency with thermal isolation. Nevertheless, it does not filter ultraviolet radiation. Also, it exhibits low wear resistance, a problem that can be tackled with abrasion resistant coatings.

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## Bioremediation of soil contaminated with ethylbenzene

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This paper reports the study of the efficiency of bioremediation of soils contaminated with ethylbenzene using the native microorganisms of the soil. Bioremediation uses microorganisms to degrade contaminants in groundwater, soils or process-waste streams [1]. This technology is considered a promising and clean technology, particularly because of its simplicity, low cost and efficacy [2]. However, bioremediation depends on several factors such as, concentration and bioavailability of contaminants, nutrients and oxygen [3].

This paper presents the bioremediation of soils contaminated with ethylbenzene using different bacteria of the soil. The experiments were performed with a soil with 14% of natural organic matter, contaminated with 100 mg/kg, inoculated with different bacteria and supplemented with nutrients to provide better degrading conditions. The tests were maintained in an oven at 25 ° C. The work performed in soils contaminated with ethylbenzene allowed concluding that the bioremediation was efficient for all tests involving inoculated bacteria and also the native microorganisms of the soil.

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# Characterization of graphite particles in spheroidal cast irons

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The mechanical properties of ductile irons are strongly influenced by the phase composition of the metallic matrix and the morphological characteristics of the graphite particles [1].

The aim of this work is to propose a consistent procedure for the characterization of the morphology of the graphite particles using an image acquisition and analysis software. The procedure was validated on ductile iron samples produced and provided by national foundry industries.

Initially, the metallographic samples preparation and etching procedure has been established, including the number of polishing-etching cycles capable of properly revealing the real form of the graphite particles. Afterwards, it was established the morphological features for the evaluation of the graphite particles. The procedures for image acquisition and processing have also been developed.

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# Chemical modification of algae binding groups for optimization of Ni biosorption

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Algae are one of the widely used biosorbents for the removal of various metal ions from water and wastewater [1]. In the present work, *Pelvetia canaliculata* (Fig. 1), commonly found brown algae in the northern coast of Portugal [2], has been modified by different chemical agents. The sun-dried algae was washed with distilled water to remove sand and excess of sodium, potassium and others ions and dried overnight at 45 °C in an oven. Then, the biomass was protonated by stirring it with 0.2 M HNO<sub>3</sub>. Different protonated algae, (1) one cycle, 3 h, (2) two cycles, 3 h each and (3) two cycles of 24 h each were prepared in order to see the effect of protonation. The subsequent conversion to Na-biomass was then accomplished by stirring the protonated biomass with 0.5 M NaCl solution. Different dosages varying from 1 to 5 g/L were tested using one to four batch cycles at different contact times (1 h to 24 h). Na-biomass was also prepared with virgin algae. Digestion results revealed that the Na content in the algae prepared directly from virgin algae was 1.3 mmol/g, while it was very low (0.16-0.28 mmol/g) in the algae prepared after protonation.

The kinetics of the process was studied in 1 L capacity perfectly mixed sorber, operating in batch mode. 0.5 g of algae prepared by different methods was added to 1 L of the nickel solution (50 mg/L, pH ~ 4). 10 mL aliquots were collected at different time intervals. It was found that Na-algae prepared directly from virgin algae (without protonation) showed the highest uptake of nickel (~ 44 mg/g), while low capacity (13 mg/g) was observed for Na-loaded algae (after protonation). Further research is under progress at LSRE, FEUP.



Figure-1: Image of *Pelvetia canaliculata*

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# Chemical Sustainable Laboratories – Equipment Management

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The design of Chemical Sustainable Laboratories is an emergent research area of REQUIMTE / GRAQ - ISEP research group. The key aspects of sustainable laboratory design include: increased energy and water conservation and efficiency; improvements to the interior and exterior environments, leading to increased productivity; reduction or elimination of harmful substances and waste; efficient use of materials and resources; recycling and increased use of products with recycled content [1].

Software systems are one of the requirements in determining a sustainable development namely making more efficient the resources management. The purpose of this project is the design and development of a web based software system [2,3] to support a more efficient equipment management.

The system will be available, anywhere and in anytime, to the registered users providing a set of functionalities according to their role in the community. The members of laboratory's community will have access to the data and features of the existing equipments as well as to make applications for equipment requisition. Any equipment breakdown could be easily reported to the system allowing a fast knowledge of the situation and as soon as possible fix the problem. The responsible team for the equipments will have the necessary information and a relevant set of alerts to make an appropriate resource allocation.

All the information will be saved and can be aggregated according to several criteria using several strategies to support the management team of the laboratory in their decision making, in the future, using a specific technology will be design a decision support system.

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<http://martinfowler.com/eaaCatalog/>

## Autonomous Driving Demonstrator - CondeDois

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This project concerns the mobile robotics and has the objective of participating in the national festival of robotics which will be take place on IST (Superior Technical Institute) in Lisbon. The competition is about making an autonomous robot drive a 8 shaped course with semaphores, road signs, parking, etc., We propose a robot with differential traction and mainly vision based. It should be able to travel through a trajectory and make its own decisions when facing obstacles, road signs etc.

The robot that will use relative location based on odometry taken from the Hall Effect sensors of the brushless motors used. For absolute localization the robot identifies world markers by using Real Time Vision System. For additional performance, an infrared radar will be used to remotely sense obstacles.

The control software uses *Lazarus*, a free-cross platform IDE (Integrated Development Environment). This software includes all the control processes of the robot including decision, path planning, control and localization. Manual control is also possible using a wiimote device. Several trajectory controllers are available like linear, circular and *Spline* trajectories. Path choices are made with the A\* algorithm.

A virtual model of the robot was tested using SimTwo, a 3D simulator with ODE (Open Dynamics Engine). ODE is a physics engine and concerns two main components, the rigid body dynamics simulation and a collision detection engine. This simulator is a very important tool that allows verifying and testing of the algorithms before use them in the real world.

Camera control software uses the Open – CV framework and communicates via sockets with the main application.

In conclusion, the presented robot will hopefully be able to perform well in the competition and latter will be adapted for less structured environment for use in demonstrations.



Figure 1 – Path Planning with obstacles and 3D Simulator

## Damages during the installation of geotextiles

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Geosynthetics are polymeric materials used in the construction of many infrastructures, such as: waste landfills, roads, railways, tunnels, dikes or reservoirs. In the past years, geosynthetics are being used in substitution of traditional construction materials, due to technical, economical and environmental advantages. These relatively-new construction materials can be divided into three main groups: geotextiles, geomembranes and related products (geogrids, geonets and geocomposites and *geo-others*). The geotextiles are the most used geosynthetics, since they can perform many different functions (separation, protection, reinforcement, drainage and filtration).

The mechanical actions that geosynthetics are subjected during the installation process can cause adverse changes in their physical, mechanical and hydraulic properties; these changes must be known and taken into consideration when designing an infrastructure with geosynthetics. The damages occurred in the geosynthetics (during the installation) can be evaluated by laboratorial tests or by field tests (installation under real conditions and immediate recovery of the materials for analysis).

In this work, several non-woven geotextiles (with different structures) were damaged in laboratory (according to ENV ISO 10722-1 [1]) using three different granular materials (corundum, limestone and granite). The changes occurred in the short-term behaviour of the geotextiles were evaluated by tensile tests (following the procedures described in EN 29073-3 [2]) and by water-permeability tests (according to EN ISO 11058 [3]). The results obtained for the damaged specimens were compared with the results obtained for reference specimens (undamaged).

*Acknowledgements:* FCT (Fundação para a Ciência e a Tecnologia – Portugal) (Research project PTDC/ECM/67547/2006).

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# Data Paths in Urban Sensing Vehicular Networks

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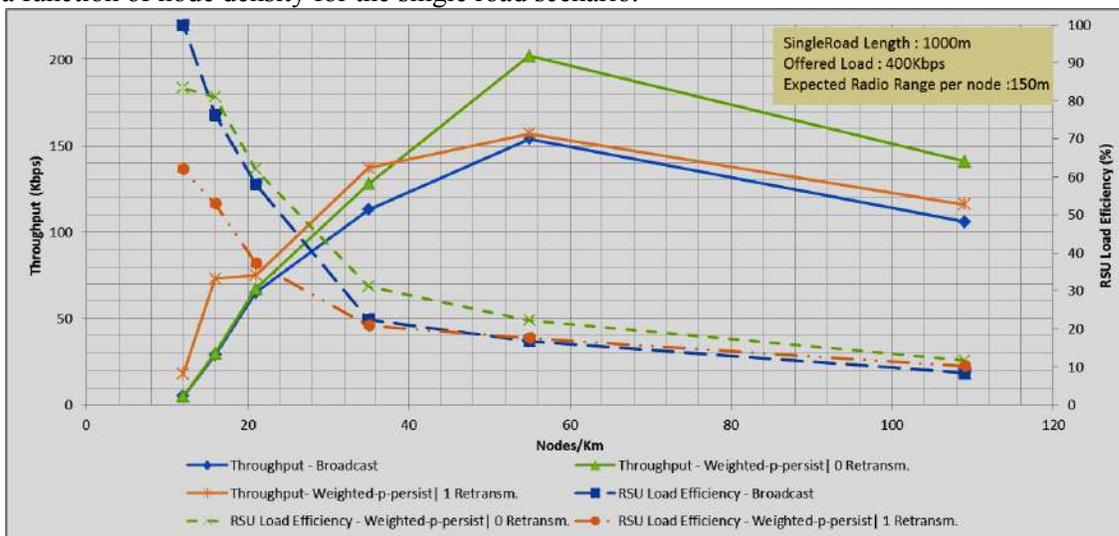
Vehicular Ad-Hoc Networks (VANETs) are a new type of network aiming to wirelessly connect road vehicles–Vehicle-to-Vehicle communication (V2V), and vehicles with Road Side Units (RSUs) – Vehicle-to-Infrastructure (V2I). VANETs enable the deployment of novel applications for safety, traffic efficiency and infotainment.

With new applications come new communication requirements that can't be addressed by existing protocols. One such example is routing, which is traditionally topology-based: a route from a vehicle to an RSU at several hops away doesn't last long enough to be useful in a VANET, due to the unstable network topology.

In this work we analyze the possibility of replacing traditional routing with a simple directed broadcast scheme to achieve multi-hop communication. Using ns-3 simulations, and two road topologies (single road and Manhattan grid) we analyzed the behavior of two different protocols:

- Simple Broadcast: every node forwards the message immediately;
- Weighted-p-persistence (W-p-P)[1]: initially, only a subset of nodes forwards the message. However, all nodes end up transmitting if they don't hear the message being sent by someone else (persistence). Optionally, a further retransmission can be allowed to increase reliability.

Below is a plot showing the tradeoff between goodput and RSU load efficiency (goodput/throughput) as a function of node density for the single road scenario.



In this scenario W-p-P provides higher goodput than simple broadcast. In high-density scenarios, retransmissions actually increase the number of collisions and hurt performance. On the flipside, in low-density scenarios, retransmissions improve packet delivery, increasing goodput. However, this increase is accompanied by an even larger surge in overhead traffic, meaning overall efficiency is actually lower: when retransmissions are disabled each node forwards each message at most once; when enabled each node can forward up to two times. In simple broadcast each node forwards exactly once, and that's why its efficiency falls between the other two.

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# Durability of geotextiles in coastal and marine environments

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Geotextiles are polymeric materials used in the construction of many Civil Engineering infrastructures. One particular field of application of geotextiles is in the construction of coastal and marine protection structures, such as: dykes, groynes, jetties, breakwaters or revetments (these structures can be used to retard and control erosion phenomenon). In marine environments, the geotextiles can be in permanent contact with many damaging agents, like solar radiation and other weathering agents, biological agents, seawater or the action of waves, tides and currents. An extended exposure to these agents can cause undesirable changes on the properties of the geotextiles, affecting their performance and shortening their lifetime.

In this work, four non-woven needle-punched polypropylene geotextiles (with different chemical stabilisations) were exposed to some degradation agents present in coastal and marine environments. The geotextiles were: (1) immersed in the ocean (depth of about 4 m), (2) exposed to sunlight and other weathering agents (exposition carried out near the sea) and (3) exposed to the action of tides (materials immersed, or not, depending of the tide). These expositions are being performed in Azores Islands (Faial and Pico) and will last for 36 months (samples taken for characterisation after 6, 12, 24 and 36 months).

The durability of the geotextiles was also studied by accelerated laboratorial tests; some EN, ISO and ASTM standards are available for that purpose. Laboratorial tests included exposition to artificial weathering (in a laboratory weatherometer) and high-temperature immersion tests.

The damages suffered by the geotextiles were evaluated by tensile tests (according to EN 29073-3 [1]). The results obtained for the exposed specimens were compared with the results obtained for reference specimens (without any damages).

*Acknowledgements:* Carvalhos Lda. (Lousã), Apto-Administração dos Portos do Triângulo e Grupo Ocidental S.A. (Horta) and FCT (Fundação para a Ciência e a Tecnologia – Portugal) (Research Project PTDC/ECM/67547/2006).

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# Eco-Efficient Biodiesel Production through Process Design and Simulation

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The analysis of the process economic potential (EP) and the total potential environmental impact (PEI) is increasingly important in the design of chemical processes, thereby allowing one to improve their eco-efficiency.

In this work the biodiesel production process is designed and simulated using the process simulator ASPEN Plus®. A detailed kinetic model of the transesterification reactions taking place in the reactor is considered in the simulations.

Objective methodologies are used at an early stage of the process conceptual development, allowing one to evaluate and select the best option from a large number of process design alternatives. To assess the PEI of biodiesel production this study applies the "Waste Reduction (WAR) Algorithm" developed by Young and co-workers [1] and using the freely available software [2]. For evaluating the process EP, an economic analysis of the transesterification process is carried out following the guidelines presented by Douglas [3].

Considering the several variables influencing the process, results show how the residence time in the reactor affects the triglycerides conversion to fatty acid methyl esters, and how it influences the overall process EP and the total PEI.

Results also show the trade-offs between the studied alternatives, allowing one to conclude that the optimum operating residence time in the reactor is 120 min, which corresponds to the lower PEI and the higher EP.

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# Effect of surface wettability on osteoblast adhesion and cytoskeleton organization using SAMs of alkanethiols on gold

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Surface chemistry and wettability are well known parameters affecting cell behavior of anchorage-dependent cells such as osteoblasts. These are important issues to consider while developing biomaterials for bone regeneration applications.

In the study we evaluated the effect of surface wettability on the adhesion and cytoskeleton organization of osteoblast cells MC3T3-E1. For this purpose, mixed self-assembled monolayers (SAMs) of alkanethiols on gold with four different levels of hydrophilicity were prepared, using SH-(CH<sub>2</sub>)<sub>11</sub>OH and SH-(CH<sub>2</sub>)<sub>15</sub>CH<sub>3</sub>, at different concentrations in solution (0% 80% 90% and 100% of OH-terminated thiol). Surface characterization was carried out by X-ray photoelectron spectroscopy (XPS) and water contact angle (CA) measurements. XPS results revealed the real percentages of each element on the surface[1]. As expected, the oxygen atom percentage increased with increasing concentrations of OH-terminated thiol. CA measurements revealed a range of wettabilities from hydrophobic (0% OH SAMs) to hydrophilic (100%OH SAMs).

MC3T3-E1 cells were seeded on SAMs previously equilibrated in culture medium, and cell adhesion and cytoskeleton evaluated after three hours of cell culture. Cell adhesion and cytoskeleton organization were evaluated using the MTT assay and F-actin/DNA staining, respectively. MTT results revealed statistical significant higher cell numbers on surfaces exposing OH-groups, as compared to those exposing only CH<sub>3</sub> groups (0% OH SAMs), regardless of OH concentration. However, a significant positive correlation was found between cell numbers and the percentage of OH groups. Fluorescence microscopy imaging showed a higher level of cytoskeleton organization on 100% OH SAMs, as compared to the hydrophobic surface (0% OH SAMs) (Fig. 1).

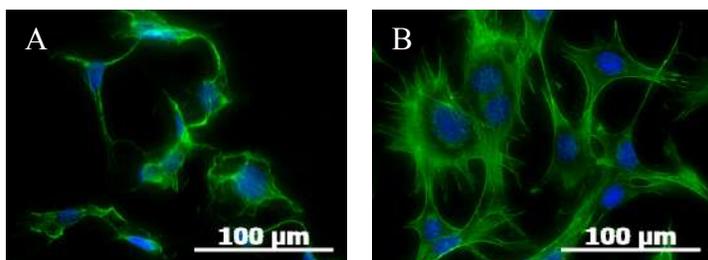


Figure 1 - F-actin (green) / DNA (blue) of MC3T3-E1 cells cultured on: (A) 0% OH SAMs (100% CH<sub>3</sub> SAMs); (B) 100% OH SAMs. Images obtained by fluorescence microscopy.

The lower cell numbers and cell spreading observed on the 0% OH SAMs may be associated to conformational changes of serum proteins upon adsorption to this hydrophobic surface [1].

These results highlight the relevance of model surfaces such as SAMs to investigate the effect of surface chemistry on cell behavior.

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# Electromiographically Controlled Artificial Muscles

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The skeletal muscle contraction process, which governs every voluntary motion in the human body, is the result of chemical activation of muscle fibers which translates into a chain sequence of ion transport mechanisms and, consequently, the generation of a time-varying electric potential, known as the electromyographic signal (EMG). In the presented work the EMG signals from the biceps and triceps muscle were proposed as a control mechanism for the bending response of artificial muscles based on ionic-electroactive polymer-metal composites (IPMCs). These materials present an attractive possibility due to their large strain rates (~20%) when surface electroded and exposed to relatively low voltages (<10V).

An EMG acquisition and conditioning analogic circuit was designed based on a double-differential X gain instrumentation amplifier cascade followed by a 4<sup>th</sup> order Butterworth topology bandpass filter with bandwidth 10-500Hz. Digital processing using MATLAB r2010b normalized, rectified and extracted the sliding mean value of the signal. Further processing, using Fourier and Wavelet Transforms, allowed the extraction of 24 characteristics from the biceps and triceps EMG signals which served as inputs for an automatic neural network classifier by back propagation with two outputs: lift and low the arm. These conditions will be used, in further work, as a control mechanism to define a specific voltage on the electrode surfaces of the IPMC artificial muscle.

The circuit was successfully implemented using three-surface Ag/AgCl electrodes. The classifier was trained, using signals acquired with the Biopac system in a process that still has to be perfected for the signals acquired with the developed circuit, with 80% of the 100 acquired signals (50 of each classified state). To perfect the process a rank of the attributes was created to use only the most significant in an attempt of lowering errors and processing time. With this process errors obtained never exceeded 5%. The artificial muscle material was not put into practice yet but the research showed that most applications involved expensive materials like platinum or gold to act as electroding surfaces. In this work it is suggested that palladium is used, for a better conductivity/price relation, as an electroding surface for a NAFION membrane.

This project still has a lot to be perfected but, in the end, it might offer an innovative application of both electromyography and the use of artificial muscles for rehabilitation, such as the use of artificial muscles to control prosthetic devices.

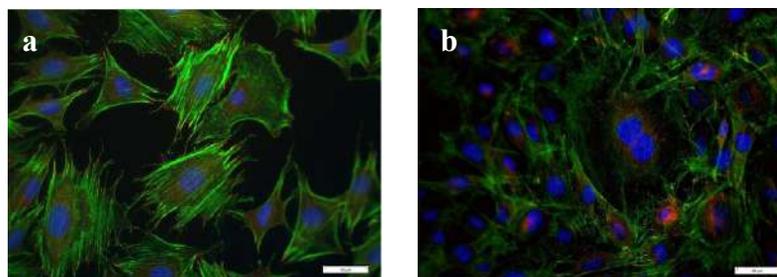
# Extracellular matrix protein adsorption as a tool to promote cell adhesion: Effect of surface chemistry

**A. Costa<sup>1</sup>, A. Ferreira<sup>1</sup>, F. Ferreira<sup>1</sup>, N. Silva<sup>1</sup>, I. C. Gonçalves<sup>1,2</sup>, I. F. Amaral<sup>1,2</sup>**

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Fibronectin (FN) is an extracellular matrix protein with multiple domains that mediates cell adhesion [1]. The objective of this study was to evaluate the effect of FN in mediating cell adhesion to molecularly-designed surfaces exposing different chemistries. For this purpose, self-assembled monolayers (SAMs) of alkanethiols on gold exposing two different terminal chemical functionalities (OH and CH<sub>3</sub> groups) were prepared. The successful preparation of -OH and -CH<sub>3</sub> terminated SAMs was checked by contact angle (CA) measurements and FTIR-IRAS. Water CA measurements revealed CA values in the range of those referred in literature, CA of  $\cong 36,5^\circ$  and  $101.6^\circ$  being found for -OH and -CH<sub>3</sub> terminated SAMs, respectively [2]. Fourier transform infrared reflection absorption spectroscopy (FTIR-IRAS) spectra was used to provide information on the packing of the alkane chains. The presence of asymmetric CH<sub>2</sub> stretching modes at 2918 and 2919 cm<sup>-1</sup> on -OH and -CH<sub>3</sub> terminated SAMs, respectively, confirmed a crystalline-like packing of the alkyl chains on both surfaces, suggesting perfect monolayer formation [3]. SAMs were pre-adsorbed with hFN (20  $\mu$ g/mL FN solution in PBS) during 1 hr, and subsequently seeded with a cell suspension of mouse MC3T3 osteoblastic cells at  $2 \times 10^4$  cells/cm<sup>2</sup>. Cell adhesion was assessed after 3 hrs of cell culture, using the MTT assay. Results revealed statistical significant higher cell numbers ( $\cong 10$  fold) on FN-coated OH SAMs as compared to FN-coated CH<sub>3</sub> SAMs. Cytoskeleton organization and focal adhesion formation were investigated by F-actin and vinculin staining, respectively. Results revealed a higher cell density and higher level of cytoskeleton organization on FN-coated OH SAMs as compared to FN-coated CH<sub>3</sub> SAMs, in line with cell adhesion results (Fig. 1).



**Figure 1.** Cell cytoskeleton organization of MC3T3 cells seeded on (a) FN-coated CH<sub>3</sub> SAMs and (b) FN-coated OH SAMs. F-actin (green); Vinculin (red); DNA (blue).

The present results highlight the effect of surface chemistry on cell behavior of osteoblastic cells by influencing the adsorbed layer of fibronectin [4].

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## Fibronectin-mediated endothelial cell adhesion to Chitosan thin films

Moço, A. A.<sup>1</sup>, Pimentel, A. S.<sup>1</sup>, Santos, T. L.<sup>1</sup>, Silva, A. S.<sup>1</sup>, Amaral I. F.<sup>1,2</sup>,  
and Gonçalves I. C.<sup>1,2</sup>

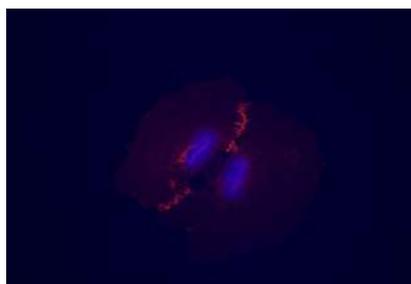
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In the present study, thin films of Chitosan (Ch), the partially deacetylated derivative of chitin [1], were prepared and examined regarding their ability to promote fibronectin (FN) adsorption and its effect on endothelial cell (EC) adhesion.

For this purpose, Ch thin films were prepared by spin-coating. Surface characterization was performed by ellipsometry and ATR-FTIR. Ellipsometry revealed films with average thickness in the range of (12,4188±3,5442)nm. ATR-FTIR spectra showed the presence of characteristic peaks assigned to Ch functional groups. Protein adsorption was performed incubating Ch thin films with a 20 µg/mL human FN solution in PBS (37°C; 1 hr). In order to explain the differences found in terms of cell behavior upon incubation with FN, immunofluorescent studies were carried out to detect the presence of FN cell binding domains at the surface.

The cell culture assays were performed using a cell line of human microvasculature endothelial cells and human umbilical vein endothelial cells (HUVECs). The EC behavior on Fn-coated Ch thin films was followed by assessing cell adhesion, cytoskeleton organization, and the expression of EC characteristic phenotypic markers. Cell adhesion was determined 3 hrs after cell culture, using a quantitative colorimetric assay (MTT assay). Results showed statistical significant lower numbers of adhered cells on Ch thin films as compared to TCPS ( $p < 0,05$ ), and no significant differences between the two Ch surfaces, regardless of the FN coating ( $p > 0,05$ ). However, ECs on Ch thin films previously adsorbed with FN revealed a well organized cytoskeleton with polymerized F-actin which was not observed on uncoated films, as shown by fluorescent microscopy of samples processed for F-actin/DNA staining. Further immunofluorescent staining of the endothelial phenotypic cell marker PECAM-1 (CD31) revealed the expression of this EC marker at intercellular junctions of confluent ECs cultured on Ch (Fig.1), a cell adhesion molecule required for capillary-like tube formation [2].



Taken together, these results show that ECs were able to adhere to Ch thin films while retaining the EC phenotype. Though pre-adsorption with FN did not affect significantly cell adhesion, it promoted clear morphological changes, namely by contributing to the establishment of a well organized cytoskeleton.

**Figure 5** – Immunofluorescent staining of the endothelial phenotypic cell marker PECAM-1 on FN-coated Ch thin films

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# Geosynthetics durability in railways – abrasion and damage during installation (DDI)

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The application of geosynthetics in railway structures has shown to be a technically and economically attractive solution, especially for rehabilitation works, due to the increase of train speeds and carried axle loads, and to the higher levels of security required. At the works in general, the installation procedures effects are very important and usually imply significant and immediate reductions of geosynthetics properties [1]. In railways the effects associated with DDI can be considered more serious given the nature of the material used in contact with geosynthetics (more aggressive). Another important question is the friction suffered by geosynthetics caused by the ballast particles (abrasion), which tend to have small but continuous displacements by the cyclic load action [2]. Therefore, it is important to know DDI and abrasion effects to ensure proper design (usually in terms of reduction coefficients) and efficient performance of geosynthetics materials during established lifetime.

In order to define the DDI and abrasion effects on geosynthetics used in railway structures, three materials with different structures were studied. After simulation of these phenomena (together and separately), the short-term mechanical behaviour (tensile strength) was evaluated. The chosen materials include: a nonwoven polypropylene geotextile, with a mass per unit area of 800g/m<sup>2</sup> (nominal strength of 50kN/m); an extruded biaxial polypropylene geogrid (nominal strength of 40kN/m); and a unidirectional geocomposite, consisting in a nonwoven polypropylene geotextile reinforced by polyester filaments of high strength (nominal strength of 75kN/m).

After inducing DDI and abrasion (together and separately) the geocomposite, with higher nominal strength (75kN/m), was the one that showed greater reductions in its tensile strength and elongation at break. The geogrid, with the lower nominal strength (40kN/m) was the geosynthetic that presented more optimistic results in terms of tensile strength, proving to be little sensitive to the induced DDI and abrasion effects.

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# Hospitals Sustainable Project Practices

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The concept of sustainable development acts through diverse meanings and common activities to humanity, which has an implicit mutual goal: a society that might persist throughout many generations with a flexible and whole vision which will allow it to maintain the social and physical system that sustain it. Cities can and should be an open field to sustainable guidelines since its scale complexity becomes an impact (positive or negative) over the environment as deep as its dimension.

On this scenario, construction industry wants to achieve a product that fulfills functionality requirements being at the same time profitable, safe and durable throughout its life cycle. The product must integrate itself on its environment with the smallest negative impact.

These principles are leading to a sustainable construction concept, integrating many different scientific areas and research fields, in which architecture plays a main role. Sustainable architecture is the concept that describes project oriented to a social, environmental and economical equity.

In the construction industry, the concept of sustainable development covers all building life cycle, since the extraction and transformation of raw materials, planning, building, usage, maintenance and demolition or deconstruction. It is noticeable the negative impact of this industry over the natural environment, so the sustainable construction must be a reality and present in all new projects.

Unifying this architecture issue and the sustainable construction to the hospitals humanization brings up the question of what is a sustainable hospital and which are the best practices to create this type of buildings.

Admitting this research should rely in a common basis of evaluation allowing the sustainable building to be classified, when it comes to matters of project and construction, its guidelines to each parameter of evaluation are achieved through comparison with other similar buildings, and when possible also define the Benchmarks to each one of the categories analysed.

# Microalgae emerging role: energy, environment and resources

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The growth of the world's population is highly associated with an increasing expense of energy. Fossil fuel, due to its relatively low cost and abundance, has been the main support of that growth for hundreds of years [1]. It has recently become clear that the rising in the consumption of fossil energy, at the rhythm verified in the last few decades is unsustainable [2]. In one hand, the substantial decrease of the world's reservations has become clear in the high price fossil fuels have reached [3, 4]. On the other hand, its use leads to the release of greenhouse gases, contributing to the severe global warming phenomena [5, 6].

An intense research has been performed to find alternative energy sources, including biofuels [1]. Nowadays, they have been produced using several sources, such as soybean, corn and agricultural/forestall waste [5, 7]. However, the use of food resources in energy production increased their prices, which was not accepted by the international public opinion. Studies in progress indicate microalgae as a technically viable alternative for biofuel production, with the advantage of not having a major impact in agricultural holdings [5]. The biomass achieved with the culture of microalgae can also be used as source of food additives, animal feed, drugs, cosmetics and fertilizers. Furthermore, microalgae can be applied as a CO<sub>2</sub> fixing agent and in biological processes of wastewater treatment [8]. However, the culture of microalgae still has relative high costs for all presented applications. Thus, this study aims to show the research directions needed to improve the economical viability of microalgae culture.

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# Tetrodotoxin occurrence in Portuguese coastal waters: First report on Atlantic temperate waters

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Tetrodotoxin (TTX) is a low molecular weight marine neurotoxin, studied mainly associated with contamination of puffer fish in Asia, although its already detected in several taxa not closely related (bacteria, marine invertebrates, terrestrial vertebrates)[1]. Nevertheless, recent reports show that the toxin is spreading to more temperate waters (Mediterranean Sea and Atlantic Ocean) and global warming and anthropogenic action are pointed as the main causes [2-4]. A poisoning case occurred in 2007 in Malaga, Spain, by ingestion of indigenous specie of a gastropod, *Charonia lampas*, captured in the southern Portuguese coast, raised the urgent need for screening coastal areas in order to prevent new poisoning cases and for a better understanding of this phenomenon [3]. This work was performed under the project ATLANTOX and the main objectives were the review of existing information concerning TTX occurrence in temperate waters and of their effects on invertebrates and the detection of TTX along the Portuguese Coast. Due to the fact of TTX travelling along the food chain, our object of study focused on the benthic organisms, especially gastropods and echinoderms, collected at eight sampling points distributed along the Portuguese coast. Once caught the animals were analyzed by the techniques of TLC and LC-MS/MS, *Artemia salina* neurotoxicity assays were also performed. TLC was used as a preliminary test, LC-MS/MS was used as the main detection and quantification technique. The *Artemia salina* bioassays are standard tests for detection of toxicity and the model it's perfect because of its rapid response and low maintenance. Results confirmed TTX presence on the Portuguese coast. Although in small quantities not enough to have a clear extract concentration/response relation in the *Artemia salina* bioassays, TTX and its analogues were detected by LC-MS/MS in some native species not yet reported as TTX-bearers such as *Paracentrotus lividus*, *Monodonta turbinata* and *Gibbula umbilicalis*. TLC was proved for not being a good preliminary test showing 13 false positives results due to the probable interference with B vitamin, present in large quantities in this type of animals. Beyond the scientific interest of this study, there is also a very important issue that is its impact in terms of public health.

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## Characterization of biomaterials for film production

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Often, resources are used to process the waste, while money and energy were dispending. However waste can be a resource. Sustainable waste planning and management reduces the use of natural resources allowing the recover value from waste materials.

In this project we propose the valorisation of chicken feathers, goat and bovine hair and human hair to produce films, focusing on methodologies that are being developed, to implement its application at industrial level in Portugal. Although these materials are similar in composition since all are keratin-based materials, they are quite distinct from each other.

The preparation of materials was carried out according to their type and source. The chicken feathers were washed with hot water containing detergent, then thoroughly rinsed with water and dried at room temperature. The human, goat and bovine hair were washed with water at room temperature and dried at 60 °C.

The samples were characterized concerning humidity, fat content, Kjeldahl nitrogen, mineral matter amount and total organic matter. The fat content was determined by a Soxhlet extraction method and the protein content by a Kjeldahl method. Organic matter was defined as the amount of sample material that vaporized during incineration at 600 °C for 3 h (i.e. amount of sample weight loss during ignition), and, conversely, inorganic matter was defined as the amount of residual solids following ignition (i.e. fixed solids or mineral ash). The analytical determinations followed standard procedures [1].

These parameters were determined to evaluate its influence in the quality of films produced considering mechanical properties (stress and elongation at break, Young's modulus), colour, solubility and moisture sorption isotherm.

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# Determinations of endocrine disruptors in surface water using SPE and gas chromatography–tandem mass spectrometry.

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Endocrine disrupting compounds (EDCs) include a wide variety of pollutants, namely substances of different sources of contamination: domestic (estrogens), industrial (plasticizers and heavy metals) and agricultural (some pesticides). Pesticides, even those which are not EDCs, occupy as well a unique position among the chemicals detected in the environment, since they are deliberately used [1]. Endocrine disruptors studied in this work are pyrethroids, triazines, organophosphate and organochlorine. These compounds found in the environment and most of these compounds are registered as potential endocrine disruptors. With the adoption of the new directive 2006/118/CE December 12th of 2006, it was included the search for pesticides in water with agricultural origin to assess their quality beyond the already contemplated search of nitrates on directive 91/676/CEE. Because of the low concentrations of pesticides in water, analytical methods should include extraction and pre-concentration to reach the required limits of detection (LODs) [2]. This study will cover the preparation of extracts and the optimization of the analytical parameters of the separation, and detection. Gas chromatography coupled to an ion trap mass spectrometry was showed excellent accuracy at low concentrations for a large number of pesticide residues analyzed in water. The programmed temperature vaporizer injector mode allows even greater input sample volume, resulting in a lower LOD. Therefore type of injection is used in this work [3]. In order to reduce the overall analysis time, substantial efforts are made to develop suitable sample preparation procedures based Solid Phase Extraction (SPE). Pesticides extracted from liquid phase into the solid phase are eluted later with a small amount of an organic solvent [4]. The studied pesticides are: lindane, methoxychlor, iprodione, bifenthrin, fenpropathrin, fenvalerate, deltamethrin, cyhalothrin, cypermethrin, HCB, cyfluthrin, atrazin de isopropyl, simazin, atrazin, permethrin, endosulfan, DDD, pp'DDE, aldrin, vinclozolin, 2,4-D, Malathion and alachlor.

In a single step of sample preparation it is possible to achieve good recoveries for ECDs with a large range of solubilities. Taking account the Portuguese legal value for individual pesticide (0.1 µg/L), this method allowed a lower quantification of them and so it is suitable to do water analysis.

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## Determination of Ibuprofen in River Samples Applying Solid Phase Extraction Followed by Liquid Chromatography

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Pharmaceuticals are useful tools to prevent and treat human and animal diseases. Following administration, a significant fraction of pharmaceuticals is excreted unaltered into faeces and urine and may enter the aquatic ecosystem and agricultural soil through irrigation with recycled water, constituting a significant source of emerging contaminants into the environment [1]. Understanding major factors influencing their environmental fate is consequently needed to value the risk, reduce contamination, and set up bioremediation technologies [2]. Several investigations have shown that substances of pharmaceutical origin are often not eliminated during wastewater treatment, and also not biodegraded in the environment. Consequently, the occurrence of pharmaceutical compounds in the aquatic environment has been recognized as one of the emerging issues in nowadays [3].

Ibuprofen (IBU) was introduced in the late 1960s as a safe nonsteroidal anti-inflammatory drug (NSAID) for the treatment of a wide range of complaints, including pain, inflammation, arthritis, fever and dysmenorrhea and it is the third most popular drug in the world. Therefore, IBU has been detected in some environmental samples, especially in water and sediment. The high mobility of IBU in aquatic environments is closely related with its physiochemical properties [4].

In this work, a solid phase extraction method followed by liquid chromatography technique with fluorescence and diode array detections (LC-FLD-PAD) has been developed for the quantification of IBU in Portuguese river waters from the North of Portugal. Six samples were analysed and no presence of IBU were detected.

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# Caffeine and chlorogenic acid in spent coffee grounds

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Coffee is one of the most popular beverages in the world. Spent coffee grounds however, remaining after beverage extraction, represent an important residue, due to the huge amounts disposed daily as well as to the potential contamination that it can represent to the environment, if disposed inappropriately.

The aim of this ongoing project is to contribute to the chemical characterization of spent coffee, aiming to define average values and variability, in order to support planning of strategies for its reuse. Two compounds are reported in this work: chlorogenic acid, an important antioxidant compound, and caffeine, here regarded as a potential environmental pollutant by soil contamination (leaching). A total of 57 spent coffee samples were collected in several coffee shops (43), from 16 brands. An HPLC/DAD methodology was validated for the purpose, with high reproducibility and low quantification limits.

Caffeine was present in all the samples analyzed, with 285 mg/100 g (dw) on average, and varying from 71 to 772 mg/100g. Chlorogenic acid, more specifically 5-CQA, presented a mean content of 89 mg/100g (dw), and values up to 235mg/100g, but below the quantification limits in 4 samples, probably due to its lower stability under storage.

These values indicate that, although the majority of caffeine and 5-CQA is extracted into the beverage, particularly espresso coffee, spent coffee grounds retain significant amounts of bioactive compounds. With caffeine amounts varying usually from 1 to 2% in roasted coffee, and those of chlorogenic acid from 0.2 to 0.8%, these figures indicate that spent grounds retain at least 20 % of the original caffeine and 5-CQA amounts. The values are highly dependable on the brand, probably associated with the amount of robusta coffee in the blend, richer in both caffeine and chlorogenic acids. Also, the extraction efficiency of the machine is an issue to address, as well as the volume of the beverage extracted.

The residual amounts of caffeine and chlorogenic acid in spent coffee after espresso extraction deserve attention regarding potential re-use alternatives.

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# Natural and anthropogenic influences on metal content in surface waters of River Douro estuary

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Among the inorganic contaminants found in river waters, metals and metalloids, particularly those classified as heavy metals, are micropollutants of special concern [1]. Heavy metals are toxic (some are potential endocrine disruptors [2]) and exert a cumulative effect [3], thus leading to various types of diseases in humans even when ingested at small doses for considerable periods. They pose a threat to biota and, finally, to the humans (at the top of the food chain) [1]. Metals in river waters mostly arise from industrial effluents, highly toxic to the aquatic life.

In order to evaluate both the natural and anthropogenic influences on the metal content of the waters of the Douro River estuary, we studied the concentrations of 20 trace elements (Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sb, Ba, Tl, Pb, Th, U) in 88 estuarine samples collected during the period of one year (11 sampling sites, both at high and low tide, in the four seasons of the year). An Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)-based analytical methodology was used.

Chemometric data analysis enabled to observe different types of behavior, e.g., some metals showed significantly higher concentration in the area close to the river mouth (Cd, Cr), while other (Pb, Zn, Cu, Ni) showed higher levels in the samples from the middle of the estuary (anthropogenic origin?). The remaining metals, excluding some particular values attributed to occasional discharges, did not varied significantly either over time or along the 11 sampling sites defined in this study (i.e., they did not show significant spatio-temporal changes in its levels in the estuarine water).

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# Microbial diversity and environmental constrains in extreme Antarctica environments

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The functionality of the extreme Antarctic terrestrial ecosystems is maintained by the simplest of any natural biological community, composed mainly by microbial organisms. In this study, we investigated the linkage between the diversity and abundance of total bacterial and ammonia oxidizers (archaea and bacteria) and the soil geochemistry in the remote Transantarctic Mountains (Darwin Mountains, 80°S).

Ours results demonstrated that general bacteria were found to be widely distributed in our sampling area. However, more complex communities, in terms of diversity, were related to the weakly developed younger drifts with higher soil C/N ratio and lower total soluble salts content (fostering low soil conductivity). From a total of 210 clones of archaea (AOA) and bacteria (AOB) amoA, only 6 archaea and 9 bacteria OTUs were recovered, showing low AOB and AOA amoA genes diversity in these extreme Antarctica environments. Sequences from different locations were found to be distributed throughout the AOA and AOB phylogenetic tree, without any distinct phylogenetic clusters allocated to different sites. Our results revealed that Antarctic terrestrial environments offer a unique natural environment, with extreme gradients in organic matter, moisture, salinity, nitrogen and carbon availability to investigate the environmental factors that dictate the distribution and diversity of microbial communities specifically the ammonia oxidizers.

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# Morphological and molecular characterization of estuarine cyanobacteria.

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Due to the increasing anthropogenic pollution, cyanobacterial excessive growth is becoming a major concern. Cyanobacteria blooms require major attention due to the ability of some species to produce cyanotoxins potentially hazardous [1].

The presented works had as primary goal to identify and evaluate the capacity for cyanotoxin production of 13 cyanobacterial strains isolated from estuaries of several Portuguese rivers (Douro, Minho and Vouga).

The proposed objectives were achieved through microscopical observation for morphological identification of the strains according to Komarek and Agnostidis [2]. The molecular identification (gene amplification through PCR procedures with posterior sequencing and identification via NCBI database) was also done as also the assessment of cyanotoxin producing potential of the strains.

The results indicate that most of the strains collected and isolated were filamentous (*Leptolyngbya* sp., *Phormidium* sp., *Microcoleus* sp. and *Nostoc* sp.) and that some strains may produce cyanotoxins.

The performed molecular screening showed potential for the production of microcystins in 7 of 13 analyzed strains.

As for the screening for Na<sup>+</sup> dependent cyanotoxins, the cyclic peptide microginin and for the cyanotoxins nodularin and saxitoxin, no amplification was encountered, suggesting the absence of these compounds.

This work reports that there is potential for the occurrence of potentially hazardous cyanotoxins on the estuaries of the surveyed rivers and therefore a monitoring program is advised.

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# Toxicity assessment of marine cyanobacteria from the filamentous genera *Leptolyngbya*

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Cyanobacteria have been long recognized as harmful organisms due to production of potent toxins with negative effects on the ecosystems and human health. Most of the toxic episodes concerning cyanobacteria have occurred on freshwater ecosystems. However, in the last years, an increase in cyanobacteria bloom formation has been also registered in coastal areas and marine cyanobacteria have been also described as toxic organisms at the marine ecosystems [1-4]. In this work, marine cyanobacterial strains of the genera *Leptolyngbya* isolated from Portuguese rocky shores were adapted to large scale laboratory culture and were tested for toxicity. Cyanobacteria freeze-dried biomass (100 mg/mL) was extracted with a dichlorometane:methanol solution, an 80% methanol solution and deionised water, in order to extract compounds with different polarities. In order to infer about the potential risks to the marine ecosystem equilibrium, extracts were tested for toxicity against the marine microalgae *Nanocloropsis* sp. and the brine shrimp *Artemia salina*. The bioassay with *Nanocloropsis* sp. was performed in 24 wells polystyrene plates with 2 mL of each cyanobacteria extract or cyanobacteria culture medium and an initial cell density of  $1 \times 10^5$  cell/mL. After a period of 72 h incubation cell density was measured by counting cells in a Neubauer chamber. *A. salina* bioassay was performed in 96 wells polystyrene plates. *A. salina* cysts were incubated in natural filtered seawater for 24 h. Ten to fifteen individuals were placed into 200  $\mu$ L of cyanobacteria extracts and were incubated in the dark at 25°C. After 24 and 48 h, dead nauplii were counted. Results were expressed as percentage of mortality. In what concerns to *Nanocloropsis* sp. assay the results showed that cyanobacteria extracts induce an increase in cell density when compared to the control. Also, when *Nanocloropsis* sp. was incubated in the cyanobacteria culture medium a stimulation of growth or no significant inhibition was registered, which can be attributed to the release of cell growth factors to the cyanobacteria culture medium. Considering the *A. salina* assay, the results showed that some extracts induce mortality. The results indicate that *Leptolyngbya* marine strains contain compounds that are toxic to marine invertebrates but induce a positive allelopathic effect on other phytoplankton groups. The nature of the compounds responsible for the effects observed was not assessed but the conclusions that we can draw is that more than one compound was produced.

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# Veterinary antibiotics in freshwater: fate and toxicity to cyanobacteria

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Several veterinary antibiotics have been found in different ecosystems, due to their discharge in the aquatic media. Inputs from manure application to soils (which can be transported to surface waters), direct discharges of livestock industries effluents into the aquatic environment (directly drain out to rivers and lakes) and livestock industries waste water treatment plants effluents, are among the sources of these compounds in the aquatic environment. Therefore, a significant amount of pharmaceuticals can reach streams and rivers. However, to date little attention has been paid to their interactions with freshwater phytoplankton.

Cyanobacteria, being prokaryotes, are generally more sensitive than eukaryotic phytoplankton to antibacterial agents. Therefore, in this work we evaluated the toxicity of five veterinary antibiotics to the freshwater cyanobacterium *Microcystis aeruginosa*.

A strain of *Microcystis aeruginosa*, (LEGE 05195) isolated from Torrão reservoir (Tâmega river) was grown in Z8 culture media doped with concentrations of tetracycline, minocycline, oxytetracycline, enrofloxacin and ceftiofur in a range from 10 µg/L to 10 mg/L, during 96h. The *M. aeruginosa* response to the antibiotics was assessed through monitoring the cells growth. The toxicity of the antibiotics was ascertained by calculating the concentration bringing about a specified inhibition of growth. These results will be presented at the conference.

## Acknowledgements

This work was partially funded by Fundação para a Ciência e a Tecnologia, Portugal, through a fellowship awarded to M. Baptista (SFRH/BD/44373/2008) and the CONC-REEQ/304/2001 re-equipment project.

# Treatment of groundwater contaminated by arsenic using iron oxides/hydroxides

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In natural waters inorganic arsenic appears commonly in the oxidation states III and V, respectively as arsenides (As III) or as arsenates (As V). Both forms are toxic and carcinogenic. In contaminated regions, the range of As concentrations found in natural waters is wide, ranging from a few  $\mu\text{g/L}$  to more than  $5000 \mu\text{g/L}$ . European Union has recently lowered the permissible maximum concentration for drinking water from  $0.05 \text{ mg/L}$  to  $0.01 \text{ mg/L}$ , in accordance with the Council Directive 98/83/CE [1].

Available technologies for arsenic removal are co-precipitation, adsorption to solid surfaces, ion exchange, membrane processes and electro-coagulation. Jiang [2] reported that the commonly used technologies in developing countries are oxidation, coagulation-precipitation, and adsorption by activated carbon, activated alumina, and iron oxide coated materials. Membrane technologies have shown to be effective but the cost is not always affordable.

The main goal of this research is the study of the operation conditions for the arsenic removal, both as As (III) and As (V), using a granular iron oxide as sorbent; so far we have been using ARM 300 from BASF.

Column experiments were performed in order to study the effect of the specific flow rate and of the oxidation state of arsenic. When it increases it leads to a decrease in the breakthrough time corresponding to a column saturation of 50%.

A loading sorbent capacity around  $9500 \mu\text{g/g}$  was obtained for an inlet concentration around  $1050 \mu\text{g/L}$ ; tests also suggest that As (III) is easier adsorbed than As (V).

The results obtained demonstrate the feasibility of applying this material to treat natural underground waters either at surface, in adsorption columns, or in-situ, as a reactive medium for permeable reactive barriers.

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## Voltammetric studies on the photodegradation of pesticide MCPA

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In the past decades, many studies have been focused on the potential hazards of a diverse number of persistent organic pollutants (POPs), especially pesticides. These chemical substances are in general very stable compounds that resist to photolytic, biological and chemical degradation, thus persisting in the environment with long half-lives. Recently, there has been a high concern about the hormone-like effects on the endocrine and reproductive systems of wildlife and humans of many POPs because of their lipophilicity and tendency to accumulate in food chains and bodies.

Chlorophenoxyacetic acids form an important group of systemic herbicides that includes MCPA [(4-chloro-2-methylphenoxy)acetic acid] (Fig 1), a herbicide widely used for the control of annual and perennial weeds in cereals. This pesticide is currently classified of restricted use since it affects surface and groundwater. In the absence of light MCPA is found to be a stable compound, but it can undergo photodegradation induced by sunlight.

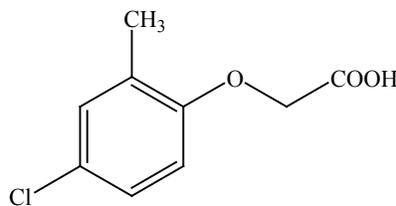


Figure 1 – Structural formulae of MCPA

It was already described that the excitation of the anionic form of MCPA, at wavelengths shorter than 300 nm, lead mainly to the formation of a hydroxylated derivative (major compound) and *o*-cresol (minor compound). In acidic solution, irradiated at 254 nm, the photochemical behavior of MCPA is more complex: the main pathway is a photochemical rearrangement leading to 5-chloro-2-hydroxy-3-methylphenylacetic acid. However when a MCPA solution is irradiated in near-UV light or in sunlight 4-chloro-2-methylphenol is the main photoproduct.

The aim of the present project is related with the study of the photocatalytic transformation of MCPA throughout the identification and characterization of photooxidative products and intermediates..

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# Can microorganisms collected in beaches affected by the Prestige oil spill biodegrade petroleum?

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Oil spills in large scale are great threats which can cause a wide destruction of coastal marine environments. Sandy beaches can be contaminated deeply into the intertidal zone. So it is necessary to develop efficient ways to clean up the buried oil. Concerning the low cost, compared with chemical and physical treatments, and because it is a non-destructive technique, bioremediation is being widely employed. With the present work we intended to investigate the potential of natural sediment microbial communities, collected at two beaches affected by an oil spill, to degrade hydrocarbons. For that controlled laboratory experiments were carried out. The nutritional conditions needed to optimise this biodegradation potential were also tested.

For the experiments sediments were collected in two beaches (O Rostro and Neminã, N.W. Spain) directly affected by the Prestige oil spill. Sediment was mixed with Bushnell Haas (BH) medium supplemented with 2% NaCl and was kept under constant agitation in 50 ml bottles during 15 days. The experiment included 6 treatments for each sediment: (i) BH medium; (ii) BH medium + petroleum; (iii) BH medium + petroleum + extra nitrogen (20 mM NO<sub>3</sub><sup>-</sup>); (iv) BH medium + petroleum + extra nitrogen (40 mM NO<sub>3</sub><sup>-</sup>); (v) BH medium + extra nitrogen (20 mM NO<sub>3</sub><sup>-</sup>); (vi) BH medium + extra nitrogen (40 mM NO<sub>3</sub><sup>-</sup>). At the beginning and at the end of the experiments, sediment samples were analyzed for hydrocarbons total content (TPHs, determined by Fourier Transform Infrared Spectroscopy (FT/IR) after their extraction by sonication) and microbiological abundance (TCC, total cell counts obtained by DAPI direct count method).

Obtained results indicate that TCC increased in all treatments that had petroleum and also in all treatments that had addition of extra nitrogen (at both concentrations). For instance, for O Rostro sediments, at the end of the experiment, the treatment (ii) (BH medium + petroleum) had sediment TCC values 100 times higher than treatment (i) (only BH medium). Hydrocarbon degradation was observed in all treatments with petroleum + extra nitrogen for both sediments, being the maximum of degradation, 85%, obtained in the presence of 20 mM NO<sub>3</sub><sup>-</sup> for the O Rostro sediment.

In conclusion, microorganisms collected in beaches affected by the Prestige oil spill presented potential for the degradation of petroleum, when the appropriate amount of nutrients is present in the medium.

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## Response of hydrocarbon degrading microorganisms in a beach affected by the Prestige oil spill (NW Spain)

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The Prestige oil tanker sank off the Galician coast, on Cape Finisterre (NW, Spain) in November 2002 releasing 53,000 tons of heavy-fuel in the open ocean, causing a major oil spill. Seven years after the accident, microorganisms collected from one of the most affected beaches (O Rostro, N.W. Spain, 43.0 °N 9.3°W) showed the ability to degrade, from bioremediation laboratory experiments, up to 85% of the hydrocarbons initially added to the sediment (Guedes et al. 2010). Samples from these experiments were used in the present study in order to ascertain the microbial response in terms of abundance of hydrocarbon degraders, bacterial diversity and presence of specific genes responsible for hydrocarbon (naphthalene) degradation.

Sediment was mixed with Bushnell Haas medium supplemented with 2% NaCl and incubated for 15 days, using 50 ml serum bottles under constant shake. The experiment included 4 treatments: (i) medium; (ii) medium + fuel oil; (iii) medium + fuel oil + 20 mM NO<sub>3</sub><sup>-</sup>; (iv) medium + 20 mM NO<sub>3</sub><sup>-</sup>. Hydrocarbon degrading bacteria were assayed using a modified most probable number (MPN) procedure. Diversity of general Bacteria, Actinomycetales, β- and α-Proteobacteria was characterized by denaturing gradient gel electrophoresis (DGGE) analysis. The presence of the gene that encodes naphthalene dioxygenase (*nahAc*), responsible for the degradation of naphthalene, was also studied by PCR amplification using specific primer sets.

Hydrocarbon degrading bacteria results showed a significant ( $p < 0.05$ ) increase in their abundance in the presence of fuel oil. In terms of community structure, a clear stimulating response was observed for some of the studied bacterial groups, namely Actinomycetales. Agarose gel electrophoresis highlighted the presence of 2 bands: 136 pb and 377 pb that corresponds to the amplification of the naphthalene dioxygenase genes that is possible to used as potential biomarkers for PAH degradations activity.

In conclusion, microorganisms collected at a beach affected by non-recent oil spill were, nevertheless able to respond to a new oil contamination, by increasing abundance of hydrocarbon degraders and changing community structure.

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## Marine cyanobacteria ability to grow at varying levels of metallic nanoparticles

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Nanotechnology gained a relevant place in almost every field of production in the recent years. In different environmental compartments, namely air, water and soil, the impact of the nanomaterials on the health and the environment is now being questioned. When the metals and materials take the form of nanoparticles (NPs) its shape and size changes and, consequentially, the hazards are yet to be explored. The NPs discharged into the environment find their way through waste disposal routes and eventually end up in wastewater treatment plants (WWTPs). But in many instances the NPs will not be removed at WWTPs and will contaminate directly the aquatic and soil environments, the seashore eventually being the final repository of these particles. Hence, an understanding of the behaviour of the NPs in costal waters, when interacting with phytoplanktonic (nano- and picoplankton) and microphytobenthic communities, is in order. Cyanobacteria are important members of these primary producers either in open waters or in the near shore ocean.

In this work the marine benthic cyanobacterium *Synechocystis* sp. (LEGE 06099), collected at the Portuguese shore (Praia de Moledo), was exposed to different concentrations of Co, Fe, Mn and Ni. Metals supplied as NPs or as salt were tested in parallel, in Z8 culture medium (with a salinity of 10%), for a 72h-period. The growth yield was recorded and the data was used to calculate the toxicity of the different metal species to the cyanobacterium. These results will be presented at the conference.

### Acknowledgements

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# Viability of Constructed Wetlands for saline wastewater treatment and plant biomass energetic valorization

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Wastewater derived from marine fish farming requires treatment before discharge to comply with environmental and legislative issues. However, conventional treatment methodologies are not fully developed for high salinity effluents and represent an added-cost for the farmer. More performing and cost-effective techniques are needed.

Constructed Wetlands (CW) are effective and low cost systems for effluent treatment, offering some valorization possibilities via biomass production. Some experiments with freshwater fish farm effluents are reported, but very few data exist with saline wastewater [1]. Hence, this study aims to test the feasibility of treating the final effluent from a marine Recirculating Aquaculture System (RAS) with salt tolerant *Typha latifolia* in a VSSF (vertical subsurface flow) constructed wetland configuration. Furthermore, through a novel and untested new methodology, this study seeks to test the feasibility of converting the produced vegetative biomass into biofuel [2].

The experimental methodology comprehends laboratory scale CWs with VSSF configuration using *Typha latifolia* planted in a substrate of expanded clay. Tests in triplicate with either RAS saline wastewater or a low concentration of a commercial nutritional solution without salt (3% N, 1,5% P<sub>2</sub>O<sub>5</sub>, 3% K<sub>2</sub>O, 0,015% Fe and trace metals) are being applied. Temperature and humidity are monitored daily and treatment efficiency is evaluated for nitrate, nitrite, ammonia, phosphate, TN and TP in water samples collected weekly. Plant growth rate is also weekly evaluated.

A methodology for a methanogenic inhibited anaerobic fermentation of the produced vegetative biomass is currently under development.

Preliminary tests with *T. latifolia* grown under laboratory conditions irrigated with a commercial nutritional solution show an average growth of 18,00 ± 15,86 cm (43,20 cm maximum height) over a period of one and half months. These findings reveal a good adaptation of the plants to the laboratory conditions, the artificial substrate, as well as to the low dosage of the tested nutritional solution.

Based on these promising preliminary findings it is expected a good biomass production with aquaculture effluents to be used in fermentation tests currently under development.

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# Ecological and socioeconomic viability of sustainable harvest of wild mushrooms in the forestland of *Baixo Tâmega*

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In Portugal, lists of mushroom biodiversity in the main ecosystems are scarce and dispersed. Surveys on mushrooms' habitat requirements per species are even scarcer.

On the other hand, mushroom harvest in Portugal is not yet regulated and every Autumn we witness the invasion of public and private forestlands by non-authorized harvesters, many of them foreigners, taking with them not only an important national natural resource but practically all the income from its sale.

In this work, since mushrooms play a very important role in the equilibrium of ecosystems and represent an important source of income for the rural populations [1], we tried to stress the importance of a sustainable mushroom harvest for both the ecology of the agro-forest ecosystems and the social and economic welfare in the rural areas in Portugal and to provide decision makers with useful sustainable forest management tools.

Thus, we started by acknowledging the habitat requirements of three well known ectomycorrhizal fungus (*Boletus edulis*, *Lactarius deliciosus* and *Cantharellus cibarius*) and built three ecological potential maps in order to create a quick and cheap model to define the areas of intervention for these three fungi. In this case, due to lack of data, we focused on the type of forest habitat requirements of these three fungus species.

Finally, we present an array of suggestions aimed at policy makers based on proven ideas implemented by other institutions and researchers that put together sustainable mushroom harvest projects in other regions of Europe. We envisage assisting those who have the will and the power to promote the well-being of human population and ecosystems services.

The obtained maps show a very fragmented, yet comprehensive, area of potential distribution of the three mushrooms in the region of *Baixo Tâmega*. Still, the proposed model to assess the areas of distribution of the three mushrooms lacks confirmation from field work surveys on the existence and productivity of these mushrooms in order to define main potential areas for a sustainable harvest.

We further conclude, based on social, demographic and economic analyses, that *Baixo Tâmega* meets all conditions to be a successful mushroom sustainable producer region, if all the right stakeholders are involved in the process.

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# Laboratorial characterization of meteorites

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Asteroids represent the sole surviving population of early inner solar-system planetesimals [1-2]. They have not formed a planet due to Jupiter's gravity influence and fragmentation produced by several collisions with other asteroids. Nowadays, asteroids provide our only *in situ* record of the conditions and processes that the inner portions (~1.8-3.5 AU) of the late solar nebulae and the infant solar system have experienced [1]. Several advances in our knowledge of the diversity of asteroid mineralogies have been made since the beginning of the use of charge-coupled device (CCD) detectors to obtain the visible and nIF reflectance spectra of small objects [3]. The bulk composition, mineralogy and petrology of a meteorite are functions of the original bulk composition of its parent body and the conditions of heating and melting that it has experienced during its formation.

Our aim in this work is to study several meteorites of different types for investigating meteorite processes and the associated thermophysical history. In a first step, thin sections of the meteorites will be prepared for optical mineralogy studies and electron microprobe analysis. These thin sections will be taken from a layer below the exposed fusion crust. In a second step, powder samples are to be prepared by milling slices of the original meteorites for X-ray diffractometry. In a third step, thin sections previously prepared will be analysed by optical mineralogy using a petrographic microscope, allowing the identification of the component minerals and their microtexture. Electron microprobe analysis in the same samples will help to complement this information by providing chemical composition profiles along selected paths such as grains boundaries, shearing planes and chondrules internal structures. Finally, X-ray diffractometry on powder samples will be performed in order to determine the crystal structure of the constituent phases and to discriminate eventual polymorphic phases.

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# Optical Fibre Pressure Sensors for Small Scale Studies of Groundwater Flow

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The underground movement of water through soil and rock is an important phenomenon in Civil Engineering. Its study is simplified if small scale prototypes are built where several combinations of soil and water in static/dynamic conditions can be studied in detail. These prototypes demand multi-point measurement of pressure and therefore an array of pressure sensors is required. Optical fibre sensors are an attractive technology for this application in view of its intrinsic multiplexing capability, associated with the characteristic of the optical fibre being simultaneously sensing element and communication channel.

For simplicity, the sensing head design was based on pressure induced optical power modulation and two configurations were researched (Figure 1), one based on the in-series connection of fibres of different core diameters (hetero-core sensing head) and the other relying in a reflective configuration associated with a pressure deformable membrane.

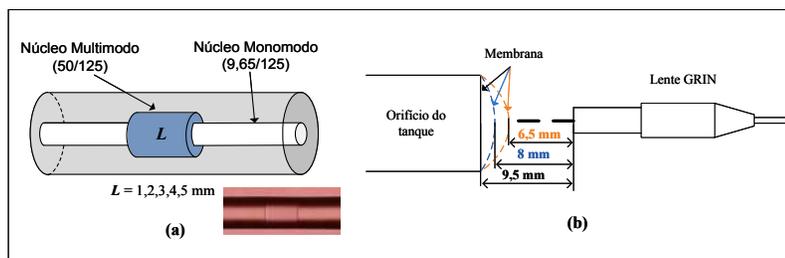


Figure 1. Optical fibre configurations studied for pressure sensing:

- (a) based on the use of hetero-core fibers
- (b) based on a reflective geometry.

The tests performed indicated the second configuration shows better characteristics, therefore it was selected. A water tank prototype was built with a reflective optical fibre pressure sensor (inset of Figure 2-a). The results shown in Figure 2 indicate the sensor has a linear response for pressure changes above 1.5 kPa, but is observed some hysteresis that can eventually be reduced by choosing another material for the membrane.

Figure 2 also shows a multiplexing layout that was studied in combination with reflective sensing heads and an OTDR (*Optical Time Domain Reflectometer*). The results are shown in Figure 3 for sensors 1 and 2, indicating a fairly linear behaviour of the sensor outputs with the mirrors displacement; it is also observed residual crosstalk.

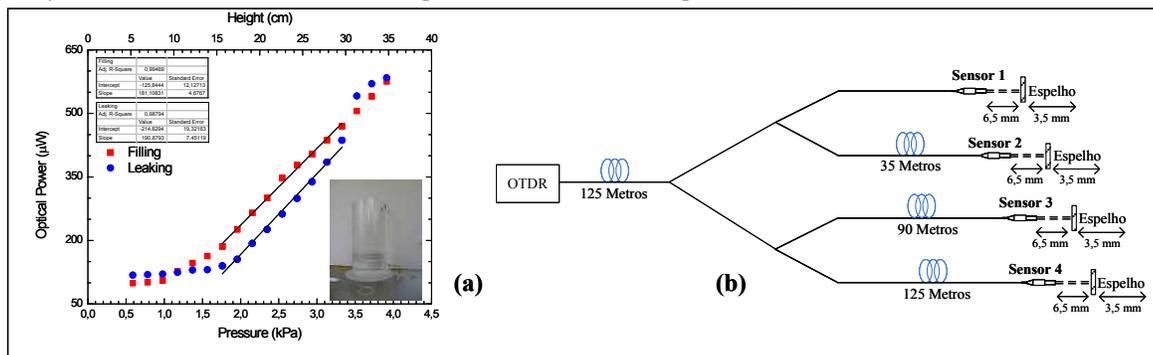


Figure 2 (above). Response of the optical fibre sensor to hydrostatic pressure in a water tank (a), and layout for multiplexing four sensors (b).

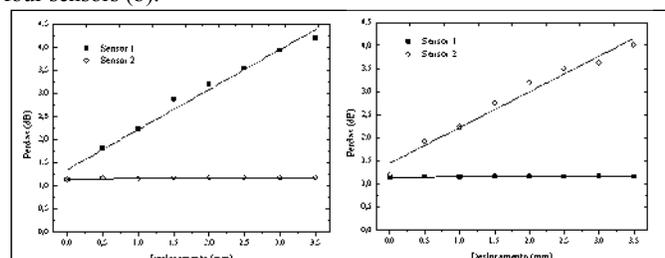


Figure 3. Response of sensors 1 and 2 and test of sensor crosstalk.

## PLD processing and characterization of MTaO<sub>3</sub> (M=K,Na,Li) thin films

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ABO<sub>3</sub> perovskite oxides constitute an important family of functional materials. Due to their wide variety of physical properties, including non-linear dielectric behaviour, piezoelectric, piroelectric, ferroelectric and relaxor response, perovskites are particularly relevant for electronic and microelectronic applications [1].

Some of the most interesting perovskites are MTaO<sub>3</sub>, with M=Li, Na, and K, and the corresponding solid solutions. They exhibit a reach variety of states, like paraelectric, ferroelectric, dipolar glass and relaxor phases [2]. Moreover, these systems are easily polarizable, being strongly sensitive to dilute impurities, and external parameters, like stress and electric field, thus enabling tailoring their properties. These features make them very attractive to be used as elements for integrating advanced technological devices. For this kind of applications, thin films are highly requested. Moreover, they can also be integrating elements of heterostructures of periodic alternating thin films, forming superlattices, which may exhibit novel interesting physical properties.

Several techniques have been used to process thin films of these systems. Pulsed laser deposition (PLD) has been widely used, since it carries very attractive processing advantages: (i) simple experimental configuration; (ii) Fast film deposition; (iii) Highly adequate for deposition of oxides thin films; and (iv) Processing of epitaxial and homogeneous thin films. Conversely though, PLD is not adequate to process large area thin films [3].

In this work, the processing and characterization of thin films based on MTaO<sub>3</sub>, with M=Li, Na, and K is reported. Thin films were processed by pulsed laser deposition technique. In order to obtain monophasic, homogeneous thin films, the deposition parameters, like substrate temperature, oxygen pressure, substrate-target distance, laser power and pulse frequency were adjusted to obtain high quality thin films. The quality of the as-processed thin films was ascertained by analysing their structure, microstructure, and stoichiometry by x-ray diffraction, and SEM-EDS techniques.

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# Reconstructing Dark Energy equation of state for several instruments

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The origin and evolution of Dark Energy are two of the most interesting topics of Cosmology and, due to the recent instruments improvement, we are able to say that we know much more than we used to about Dark Energy and consequent expansion of the Universe.

When Edwin Hubble, with his telescope, noticed that all galaxies were moving away from us faster than ever, he certainly changed deeply the way that physicists imagined our Universe. One of those physicists was Albert Einstein, who had always believed in a static Universe and who used his own equations to show an static Universe. By the time he did so, the famous cosmological constant was introduced as a factor of a static Universe, but, after Hubble's observations and Friedmann's modifications in Einstein's equations, the cosmological constant has appeared as a factor of an accelerating Universe, which has not the effect that Einstein initially predict. Currently, there are three distinct forms to interpret the dark energy: a modified theory of gravitation, a cosmological constant and a scalar field generally called quintessence. There also are other models, but it is clear that these three are the most studied.

In this project, we will consider that the reason for an accelerated Universe is the quintessence, which we consider to be coupling to electromagnetism. Also, we consider that this coupling is done by considering a cosmological variation of the fine structure constant. Also, with the CODEX precision, we will reconstruct the Dark Energy equation of state and compare it with other instruments, such as ESPRESSO and UVES-LP.

For this reconstruction, we have been using a principal components analysis in order to reduce the number of components, of a prediction based in Bayesian analysis, and the Fisher Matrix of the likelihood function of our model.

# Study of the ionospheric anomalies in the Chilean region using GPS-TEC technique

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Earthquakes are one of the most feared natural disasters due to their potential destructive power, both in terms of material and human casualties, and also due to their unpredictability. We know that some regions in the world are more prone to seismic activity but we lack the accuracy to pinpoint a more restricted location in space and time where the earthquake will happen and its magnitude. In the past, the most successful short-term prediction of impending earthquakes relied on the observation of abnormal animal behaviour. In the last couple of decades, a few new techniques have been developed in order to enable us to identify some evidences of significant undergoing seismic activity which can lead to strong earthquakes in a more specific location and time. Moreover, it may also be done with some days in advance allowing preparations and, if necessary, evacuations to be made. One of these newer techniques is the monitoring of the ionospheric total electron content (TEC) using GPS-TEC technique for the detection of ionospheric TEC anomalies associated with seismically induced electromagnetic emission phenomena [1,2].

The GPS-TEC technique takes advantage of the worldwide coverage of the GPS satellite constellation and several permanent ground station networks. In this work, GPS data was post-processed on GAMIT-GLOBK software to evaluate the feasibility of this technique. In particular, we analysed the TEC time series associated with the M8.8 Chile earthquake of 27<sup>th</sup> February of 2010 [3]. Our preliminary results show that unusual and significant variability of TEC values can only be ascertained for a period of few hours before and after the earthquake.

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# Study of the ionospheric seismo-electromagnetic anomalies using GPS-TEC technique

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Numerous evidences accumulated over the last decades in seismogenic regions indicate that, in most cases, large earthquakes are preceded by seismo-electromagnetic (SEM) phenomena (among other) with effects both at the surface and ionosphere [1,2]. The monitoring of these earthquake precursory signals is presently a research area of growing interest and activity that aims to provide an important contribution to earthquake short-term prediction.

Understanding the lithosphere-ionosphere coupling mechanisms requires a continuously operating global monitoring system, such as the Global Positioning System (GPS) [3]. The GPS network consists of 24 satellites, evenly distributed in 6 orbital planes around the globe at an altitude  $\approx$  20200 km. Each satellite transmits signals on two frequencies ( $f_1 = 1575.42$  MHz and  $f_2 = 1227.60$  MHz) with two different codes, P1 (or C/A) and P2 and two different carrier phases, L1 and L2. Since the ionosphere is a dispersive medium, the speed of propagation of the electromagnetic waves transmitted by the GPS satellites depends on the frequency of radio waves. The carrier phase advance and group delay of GPS transmitted radio waves in the ionosphere is proportional to the total electron content integrated along the propagation path, which is known as TEC. According to the GPS-TEC technique [4], one can derive TEC by comparing the phase delays of the L1 and L2 signals. TEC measurements provide two-dimensional cross-section maps of the ionosphere's electron density on a regional or global scale.

In this project, we will apply the GPS-TEC technique to the detection of ionospheric TEC anomalies resulting from large earthquakes, using freely available GPS historic record data of international permanent GPS ground stations networks. We will also assess the use of the portuguese GPS ground station network for such regional ionospheric studies of recent low magnitude earthquakes.

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# A novel approach in the study of the male copulatory system of *Bulla striata* (Cephalaspidea)

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The vast majority of opisthobranch gastropods are simultaneous hermaphrodites. As in other cephalaspideans, in *Bulla striata* the male copulatory organ is situated on the right side of the head and connected to the hermaphroditic opening by a ciliated seminal groove running along the right side of the body. However, there are still some controversial aspects about the male copulatory system of cephalaspideans, and a lack of nomenclatural consensus for its anatomical parts. To improve current knowledge about the genital organs in cephalaspideans, this study aims to obtain a detailed description of the male copulatory system of *B. striata* using histochemical techniques and transmission electron microscopy (TEM). Semi-thin sections (2cm thick) for light microscopy and ultrathin sections for TEM were obtained from male genitalia fixed with glutaraldehyde, post-fixed with osmium tetroxide and embedded in Epon.

The first part of the male copulatory system of *B. striata* is a tubular vestibular area that opens in male genital aperture. A muscular structure that has been called penial papilla is located at the internal end of the vestibular area and it is connected to a long coiled tube with a blind end. The penial papilla and the coiled tube are encircled by a muscular sheath. The vestibular area consists of a muscular wall with a lumen lined by an epithelium containing ciliated cells and a large number of mucus-secreting cells. The last ones presenting a purple metachromatic coloration in semi-thin sections stained by methylene blue and azure II. Moreover, two types of sub-epithelial secretory cells were discovered. The main body of these cells, which includes the nucleus, is embedded in the muscular wall, but they possess a long apical cytoplasmic extension that crosses the epithelium to release secretion into the lumen. In semi-thin sections, one of these sub-epithelial cell types is characterized by a red metachromatic coloration while the other contains small blue stained granules. In the penial papilla the lumen is lined by a non-secretory cubic epithelium. The initial segment of the coiled tube does not contain secretory cells, but in the longer glandular prostatic region the epithelium contains a large number of tall secretory cells interspersed with ciliated cells. These secretory cells contain vesicles which are stained by PAS and tetrazonium coupling reaction employed for polysaccharides and protein detection in semi-thin sections, respectively. Observation by TEM revealed many secretory vesicles with an electron-dense content. The epithelium of the coiled tube is surrounded by a muscular wall and many spermatozoa were found within the lumen of the prostatic region. At the posterior end, the muscular wall of the coiled tube merges with the muscular sheath. In this terminal segment, the epithelium contains only a few secretory

cells, but many spermatozoa are also found in the lumen. Probably, the mucus-secreting cells of the vestibular area are involved in lubrication, whereas the secretion produced in the glandular part of the coiled tube is necessary to maintain the stored spermatozoa.

# Ability of prostate cancer cell lines to modulate osteoclastogenesis by paracrine mechanisms

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Paracrine crosstalks between prostate cancer cells and bone cells have an important role in the development of bone metastasis. With this study, the osteoclastogenic effects, mediated by paracrine mechanisms, of one normal prostate cell line (PNT2) and two prostate cancer cell lines with different bone metastatic characteristics (PC3 – osteolytic metastasis; LNCaP – osteoblastic/mixed metastasis) was assessed on cultures of human osteoclast precursor cells.

Osteoclastic precursors were isolated from peripheral blood and were cultured in the presence of conditioned media (CM) collected from the prostate cancer cell lines after 48 hours, 7 and 14 days of culture. When indicated, cell cultures were treated with inhibitors of MEK, NFκB, PKC, MAPK, JNK and p38 signaling pathways, and a PGE2 production blocker. Cell cultures were analysed (days 7, 14 and 21) for tartarate-resistant acid phosphatase activity and histochemical staining and for the presence of multinucleated cells with actin rings and expressing vitronectin and calcitonin receptors.

It was observed that CM from PC3 cell line exhibited the highest osteoclastogenic effect, with the maximum response being observed with the CM collected after 7 days of culture. CM from LNCaP cell line also elicited a high degree of osteoclast differentiation, especially when CM was collected after 48 hours of culture. Finally, CM from PNT2 cell line displayed the lowest osteoclastogenic-inducing ability, particularly when CM was collected after 48 hours or 14 days of culture. The characterization of the intracellular mechanisms involved in the observed behaviors revealed important differences among the different cell lines tested.

In conclusion, it was demonstrated that prostate cancer cell lines have the ability to induce osteoclastogenesis by paracrine-related mechanisms. The characterization of the intracellular processes involved in the different cellular behaviors can open new insights on the study of bone metastasis induced by prostate cancer cells.

# Acute corticosteroid treatment increases chromosomal instability? A preliminary study.

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Corticosteroids are widely used in medicine, and its side effects are the subject of constant investigation. Its potential genotoxicity apparently doesn't have a harmful effect when used in normal physiological conditions and in current therapies. However, once the genotoxicity results in chromosomal instability (CI), which in turn is associated with higher incidence of cancer, it is of great importance to study the dose/effect relationship for establishing limits on the possible harmful effect of CI with carcinogenic potential [1].

In this work we present a preliminary study to evaluate a relationship between the acute use of corticosteroids as a clinical treatment and the spontaneous CI.

Blood samples were collected from two different donors treated with an acute therapeutic dose of corticosteroid injection and healthy donors; 0,5 ml of whole blood was added to culture medium, composed of 6 ml RPMI 1640, 1 ml FBS, 0,1 ml antibiotics (penicillin and streptomycin), 0,1 ml phytohemagglutinin (PHA) and 0,1 ml L-glutamine, in appropriated cell culture tubes (final volume of 7,3 ml). Cells were cultured for 72h, at 37°C, under an atmosphere of 5% CO<sub>2</sub>. At the end of the culture period, 1 hour before cell fixation, colcemid was added. Cells were collected by centrifugation, exposed 15 min to 0,075 M KCl at 37°C and fixed 3 times in iced methanol/acetic acid (3:1). The resulting suspensions were dropped onto microscope slides and stained for 4 min in 4% Giemsa solution made in PBS buffer.

Chromosome instability was determined in very well spread metaphases with intact morphology of chromosomes. The frequency of breaks (which include chromatid and chromosome breaks and tri- and tetra-radial figures) and the number of breaks per cell were scored in 100 metaphases per treatment by one independent scorer. The results showed an increase of the CI in the lymphocytes from patients, compared with controls.

To our knowledge, this is the first study that shows an influence of corticosteroid treatment in the increment of CI. Further studies are necessary to validate these results. As a future perspective, we would like to evaluate the corticosteroid effect in other clinical situations, for instance, patients with a chronic exposure to this compound.

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## Ageing fox with a smile

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Age determination is an important tool for population dynamic studies and so several methods have been developed and used to obtain an estimate for Red Fox (*Vulpes vulpes*) populations. There is a great diversity of methodologies [1] but only one is commonly accepted as being the most accurate, which is based on the count of the dentine layers [2].

Foxes killed during battues in Portuguese hunting zones were brought to the laboratory for analysis. The age of each fox will be estimated by several methods: (1) Upper incisor teeth were observed for tooth wear marks [3]; upper canine teeth were extracted from the skull after maceration and (2) will be radiographed (the print will be used to measure external diameter of the tooth and the size of the pulpar cavity); some will be further (3) decalcified with nitric acid (36-48 h), rinsed with water and a sagittal cut (20 µm) stained (hematoxylin) and used to count the incremental lines under an optical microscope; in others (4) the tooth root will be ground by hand down to nearly half its thickness using sandpaper with decrement coarseness and the sagittal section obtained examined under a stereoscopic without any staining.

So far 14 foxes were obtained. All are adult animals, as they had permanent teeth. Some foxes (n=2) presented teeth problems (broken or missing). At the present, age could only be evaluated by the incisive tooth wear analysis, and the two oldest foxes (both vixens) had probably almost five years, this in agreement with the available scientific literature that reports that Red Fox longevity in the wild is short. The results should however be considered with caution not only due to the small sample size, but also to the low accuracy of the method used. Teeth are being prepared for the X-ray analysis and subsequent incremental line counts, so precise age data is not available yet.

The knowledge of the Red Fox age structure will hopefully allow to understand other aspects of their biological cycle, namely if age influences the species feeding behaviour or its reproductive cycle, both important for the definition of management strategies for the Red Fox in Portugal.

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## Antimicrobial activity of phlorotannins from brown macro algae

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In brown algae (Phaeophyta), phenolic compounds are referred to as phlorotannins, since most of them are polymers of phloroglucinol (1,3,5-trihydrobenzene). In spite of some structural differences, phlorotannins exhibit chemical properties and physiological roles similar to those of the tannins synthesised by vascular plants [1]. They protect brown algae against UV irradiation, and are also discussed as a chemical defence to deter herbivores. Nowadays, diverse effects on biological systems are reported, namely anti-inflammatory, anti-allergic, anti-viral, anticancer, bactericide, antioxidant and anti-diabetic activities, as well as radioprotective effects [2, 3].

In the present study interest is focused in the determination of antibacterial activity of phlorotannin purified extracts from ten species of Phaeophyta collected along the Portuguese west coast for the first time.

The results obtained show a high antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Enterococcus faecalis* and *Micrococcus luteus*) and Gram-negative bacteria (*Salmonella typhimurium*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*).

These results confirm the antimicrobial potential of phlorotannins and contribute to the valorisation of macro algae species from the Portuguese west coast.

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## Chelae dimorphism in the green crab *Carcinus maenas* (Crustacea: Portunidae): Fine-scale differences

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Chelae dimorphism or heterochely is a common feature in crustaceans, mainly in crabs. These animals often present several morphological differences between claws, such as in the chelae width, propus and dactyl length and dentition. However, most of the studies conducted so far, did not have analyzed these characteristics in detail. In this context, the present study aimed to compare in detail the right and left claws of the green crab *Carcinus maenas* of both sexes. In order to accomplish this, the carapace dorsum and claws of each individual crab were photographed using a digital camera coupled to a magnifying glass with a scale. Afterwards, images were analyzed using the cell<sup>^</sup>B<sup>®</sup> software. A total of 25 morphologic features were measured in each individual crab, being 3 related to the carapace (width, height and area), while the remaining 22 being related to the claw, namely the propus and dactyl length, area and maximum angle of the gap between them, as well as the number of tooth and its angles of both claw parts. A total of 28 crabs were analyzed (13 males and 15 females). Considering the ratios of carapace parameters as variables, and hence correcting for size, males and females were significantly different (ANOSIM, R=0.094, p<0.05). The angles of propus tooth are more acute than dactyl's in both claws of both sexes (ANOVA; p<0.001). There were no significant differences between all variables measured of the right and left claws for in animals of the same gender. Our results are somewhat in accordance with previous studies, since we also recorded a sexual dimorphism in *C. maenas* claw's, but not between right and left claws of both genders. These results are relevant since the use of more accurate measurements can either confirm or refute previous studies, which used less accurate techniques. So, in this context it is important to keep investigating morphological characteristics, especially using new digital techniques, which can highlight differences, which may have significant relevance in the behavior and the ecology of the species.

# Chemical and biological characterization of *Glycine max*, *Vigna radiata* and *Medicago sativa* sprouts

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Consumption of sprouts, common in Asia, has been growing in occidental countries, because they are one valuable dietary supplement and considered a natural healthy food. Sprouts are believed to be rich in health-promoting phytochemicals compared with their mature counterparts. The germination improves nutritional quality of seeds, because the levels of some anti-nutritional factors decrease or even disappear during germination process, while some compounds with antioxidant activity increase. However, despite the popularity of sprouts as a healthy food, very little is known about their health-promoting qualities or about factors that may affect their phytochemical composition [1,2].

The aim of this work is to establish the metabolic profile and to evaluate the antioxidant capacity of distinct sprouts of species used in human diet: *Glycine max*, *Vigna radiata* and *Medicago sativa*. The phenolic compounds and sterols were analyzed by HPLC-DAD, organic acids by HPLC-UV, fatty acids and volatile compounds by GC-MS. Antioxidant activity was assessed by DPPH, nitric oxide and superoxide radical scavenging assays.

The results obtained reveal some qualitative and quantitative differences among the metabolites composition of the three analyzed sprouts. Also the antioxidant potential of the three species was distinct, although they were all found to have good activities.

Thus, this study indicates these matrices as interesting sources of bioactive compounds, contributing to improve their inclusion in human diet.

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# Computational studies of the interaction between phospholipid bilayers and potential new drugs

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Iron is an essential micronutrient for microbial pathogens and its accumulation as been suggested to potentiate the susceptibility to mycobacterial infections, so one possible way of fighting this infections will be through the use of iron chelating agents [1]. Recently one of this agents was developed with promising results [1,2], and in order to improve the knowledge of its properties, we aim for the study of its interaction with computational phospholipidic bilayer models, initially composed of a single type of phospholipids, such as DLPS, DMPC, DMPG, DLPE, etc. The development of these bilayers models will also provide us with different molecular mechanics parameters that may be used for other studies by our laboratory and others, since they will be included in a publically available database.

For our membrane simulation we have used classical molecular dynamics (MD), which is the most commonly used method for biomolecular simulations, and it has shown great progress in membrane simulations [3,4]. Quantum mechanics was also used for the parameterization of phospholipids in order to calculate partial atomic charges.

At this stage of our project we are validating our simulations through the comparison with experimental data, and in terms of the volume per lipid parameter, we are in good agreement with the experimental values available [5]. We will then be prepared to study the interaction between our models and the potential new drugs.

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# Coreopsis tinctoria: Chemistry and biological potential

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*Coreopsis tinctoria* flowering tops infusions have been traditionally used in Portugal to control hyperglycemia. However, this is a poorly studied matrix. In this work, fatty acids profile was characterized by Gas Chromatography-Ion Trap-Mass Spectrometry (GC-IT-MS) analysis. Sixteen compounds were determined, being saturated fatty acids, namely myristic, heptadecanoic and behenic acids, the ones present in highest contents.

Volatile compounds were also determined by Head Space-Solid Phase Microextraction followed by GC-IT-MS (HS-SPME/GC-IT-MS), being identified 19 compounds, distributed by distinct chemical classes: aldehydes, monoterpenes and sesquiterpenes.

The antioxidant potential of this matrix was checked by several *in vitro* chemical assays. A concentration-dependent activity was noticed against DPPH (IC<sub>50</sub>=0.043 mg/mL), nitric oxide (IC<sub>50</sub>=0.107 mg/mL) and superoxide (IC<sub>50</sub>=0.237 mg/mL) radicals (Fig.1).

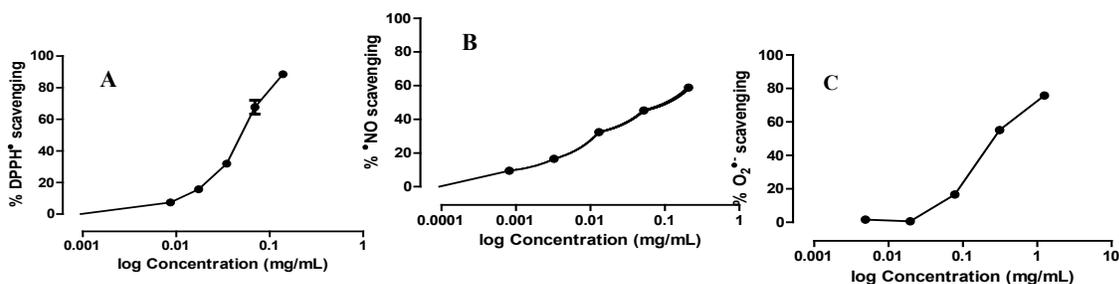


Fig. 1 Effects of *C. tinctoria* aqueous lyophilized extract against (A) DPPH, (B) nitric oxide and (C) superoxide radicals. Values show mean  $\pm$  SE from three experiments performed in triplicate.

Additionally, antimicrobial potential was also assessed against Gram-positive and Gram-negative bacteria, being more active against Gram-positive bacteria. In a general way, the chloroform/methanol extract was more effective than aqueous lyophilized extract.

The results obtained are very promising, constituting a base for the possible application of this matrix in food, cosmetic and pharmaceutical industries, due to its biological potential.

## Acknowledgments:

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## Crystallization and refinement of human Transthyretin (TTR) in complex with Nordihydroguaiaretic acid (NDGA)

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The search for specific and effective drugs to treat familial amyloidotic polyneuropathy, a disease characterized by the formation of amyloid structures of Transthyretin (TTR), has been focused in finding different substances with stabilization potency for this protein.

The main objectives of this work were to crystallize and resolve the structure of TTR in the presence of a putative tetramer stabilizer, the Nordihydroguaiaretic Acid (NDGA). We could observe that NDGA binds deeply in the hormone-binding channel. The drug, with a symmetry related di-substituted phenyl rings, is tightly anchored into a pocket of the binding site by its innermost ring and establishes extra hydrogen bounds between the outermost phenyl ring and the protein. The hydroxyls substituents in NDGA outer ring are involved in electrostatic interactions with the N<sup>ε</sup> of a lysine and the O<sup>ε2</sup> of a glutamate residue. Also the alteration in the spatial orientation in some residues side chains result in the formation of new intersubunit hydrogen bonds. These interactions induced by NDGA are believed to increase the stability of the tetramer possibly avoiding the formation of amyloid fibrils.

## Design and construction of integrative vectors for the generation of $\Delta hoxW$ and $\Delta hupW$ mutants of *Nostoc* sp. strain PCC 7120

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Cyanobacteria can produce hydrogen (H<sub>2</sub>) in a clean and renewable way, utilizing solar energy and water as sources. In these organisms two types of enzymes are directly involved in H<sub>2</sub> metabolism, namely nitrogenases and hydrogenases. *Nostoc* sp. strain PCC 7120 harbors two different hydrogenases (i) an uptake hydrogenase, responsible for the recycling of the H<sub>2</sub> produced by nitrogenase and (ii) a bidirectional hydrogenase that may consume or produce H<sub>2</sub>. The maturation of these NiFe hydrogenases is a complex process in which several accessory proteins are required for the assembly of a functional enzyme. The last step in the maturation of the large subunits of both hydrogenases is the cleavage of a C-terminal peptide, which allows the subsequent assembly of the large and small subunits. This cleavage is putatively achieved by specific proteases HoxW and HupW, for the large subunits of bidirectional and uptake hydrogenase, respectively. The main goal of this work was the construction of integrative vectors to generate  $\Delta hoxW$  and  $\Delta hupW$  mutants of *Nostoc* sp. strain PCC 7120 in order to clarify the role of each endopeptidase in the maturation of the cyanobacterial hydrogenases.

For this purpose the flanking regions of the genes *hoxW* and *hupW* were amplified from *Nostoc* sp. strain PCC 7120 genomic DNA by PCR, in which internal primers (5' Rev and 3' Fwd) have 3' complementary sequences containing *Xma*I recognition site. The fragments obtained were joined together by overlap PCR and the product cloned into pGem T-easy vector (*hoxW* construct) or directly joined by successive cloning steps (*hupW* construct). A selective cassette from pK18mobsacB, providing resistance to kanamycin or resistance to kanamycin and sensitivity to sucrose was inserted into *Xma*I recognition site.

The constructions obtained were confirmed both by restriction analyses and sequencing. The vectors generated will be inserted into *Nostoc* sp. strain PCC 7120 by triparental mating, and the genetic structure of the excojugants will be analyzed. In the mutants the arrest of the maturation of the hydrogenases large subunits is expected and will be confirmed by Western blotting analyses using antibodies against the unprocessed and processed (C-terminal cleaved) forms, unveiling the specificity of HoxW and HupW endopeptidases for each hydrogenase. This work will provide new insights on the cyanobacterial hydrogenases maturation process that may be applied to the generation of genetic engineered strains for photobiological H<sub>2</sub> production.

# Detection of partridge meat for the authentication of “Alheiras de caça” using polymerase chain reaction

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The manufacture of traditional meat products is a long-established tradition in Northeastern region of Portugal, in particular the case of “Alheiras”. Besides the traditional “Alheiras” mainly produced with pork and poultry meat, others are currently available in the market, which are produced with different game meats, such as “Alheiras de caça”. Since this kind of meat products are prepared using more expensive meats, they are prone to adulterations due to the economic profit that might result from the replacement or decrease of those high valued meats.

Consumers require clear and accurate information about the products they purchase and, therefore, the assessment of food composition and authenticity is becoming a very important issue to allow accurate information and to avoid unfair competition among producers. Thus, it is important to develop analytical tools to access the authenticity of this kind of foodstuffs, contributing to their valorisation.

Due to the higher stability of DNA molecules compared with proteins, and for its fastness, accuracy and sensitivity, polymerase chain reaction (PCR) techniques is the most suited for the species-specific detection in food products [1].

The aim of this project was to develop species-specific PCR techniques, able to specifically identify meat species in “Alheiras de caça”, namely partridge meat. For this, reference meat mixtures containing known amounts of partridge meat were prepared and “Alheiras” were acquired in the market. DNA was extracted using the Wizard method. Purity and DNA yield were assessed by spectrophotometry. To specifically detect partridge species, specific primer targeting the mitochondrial 12S rRNA gene were used to obtain 141 bp DNA fragments [2]. Pork and poultry species were also detected by PCR to evaluate the amplification capacity of extracts [3]. The results showed the detection of partridge until the level of 0.01% addition in pork meat. The technique was successfully applied to commercial samples of “Alheiras de caça” indicating possible misleading labelling in some of them. Other game species, such as quail, pheasant, duck and hare are under study.

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# Discrimination of *Trisopterus luscus* (Linnaeus 1758) stock(s) in the northern of Portugal using otolith elemental fingerprints

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The pouting, *Trisopterus luscus* (Linnaeus, 1758), is one of the most important fish species captured by northern Portugal traditional fisheries. In spite of a substantial decrease in fish catches, the existent works either on the population structure or on the management of the species are scarce. In this study chemical analysis performed by inductively coupled mass spectrometry in whole otolith samples of 90 pouting of age group 2+ provided location-specific elemental signatures. Sampling took place in shallow waters along the coastline in three fishery grounds of the Portuguese north coast (Viana do Castelo, Matosinhos and Aveiro) between February and March 2010. Otolith fingerprint analysis detected the presence of several informative trace elements. Molar concentrations for each site were analyzed through uni and multivariate statistical tests. Sr, Ba, Mg and Li differed significantly among locations. Canonical analysis allowed us to discriminate juvenile stocks and determine, with a good percentage of classification (69%), the original sampling areas pouting. The observed elemental site-specific differences in pout otoliths suggest a high level of site-fidelity in relation to their growing/feeding areas. Pouts from these locations can be regarded as a single, although not necessary homogenous, stock. Furthermore this study also suggests that the stocks of juvenile fish studied are partially mixing and cannot be assumed to be separated units for fisheries management purposes.

# Effect of temperature and dietary protein: energy ratio on the performance of juvenile Senegalese sole (*Solea senegalensis*)

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*Solea senegalensis* has been considered a promising species for Mediterranean aquaculture, because of its high price and market demand [1]. The Senegalese sole can be found in the North African coast and in the Mediterranean Sea, both temperate and subtropical regions. This species presents difficulties in the transition from larvae to juvenile state, experiencing a relatively high mortality rate of larvae [2]. The information about the nutritional needs of juvenile sole is still scarce but it is known that the requirement of dietary protein is higher for flatfish than for finfish [1]. The aim of this study is to improve knowledge about nutritional needs of this species under intensive culture conditions and optimize the protein: energy ration in order to reduce its high cost.

The trial lasted 63 days and it evaluated the effects of temperature and diets on growth of juvenile sole. The experience included four diets with different percentages of protein and fat: 55% of protein and 16% of Lipids (55P16L), 55% of protein and 8% of Lipids (55P8L), 45% of protein and 16% of Lipids (45P16L) and 45% of protein and 8 % of Lipids (45P8L).

The values of growth rate obtained for the specimens kept to 18°C are close in all cases to the double of the values obtained for fish that remained at 12°C. The diet 45P16L, at 18°C, was the one that obtained the highest growth rate, despite being the least consumed. The 45P16L also had the largest feed efficiency and protein efficiency ratio in both temperatures, having higher value at 18°C.

The response of the specimens varied with temperature and diet. The data suggests that the best temperature for efficient growth of Senegalese sole juveniles is around 18°C. The diet with the best results was 45P16L and the data suggests that occurred a better use of lipids for energy purposes not being used a high proportion of protein to that.

## Acknowledgments:

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# Effects of metalaxyl on cell growth and viability of *Solanum nigrum* L. cell suspension cultures

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The use of pesticides provides many benefits and makes a significant contribution to increase yield and to reduce crop losses from pests and diseases [1]. Excessive use of these chemicals poses a threat to the environment because of their persistence and potential to bioaccumulate in soils and groundwater. Phytoremediation is the use of plants and their associated microbes to degrade, stabilize, and/or remove contaminants as metals and organic compounds from soils, groundwater and sediments. This technology is a low cost, effective and noninvasive alternative to conventional methods for environmental remediation [2]. Previous studies reported *Solanum nigrum* L., as a promising candidate to phytoremediate hazardous compounds from soils [3]. The systemic fungicide metalaxyl is widely used in agriculture and has a tendency to accumulate in soils and groundwater. As plant tissue cultures are a powerful tool frequently applied in phytoremediation research [4] and *S. nigrum*, is a model in plant tissue culture, the present study addressed the effect of metalaxyl on *S. nigrum* cell suspensions in order to characterize stress responses at the cellular level, and to investigate the potential use of cell suspensions as a model research system. Pale green and friable calli were used to establish cell suspensions in Murashige & Skoog medium (1962) supplemented with 2 mg/dm<sup>3</sup> 2,4-D and 0.5 mg/dm<sup>3</sup> BA, at pH 5.7. Cells were propagated on a rotary shaker (120 rpm), in the dark; at 25 °C. Subcultures were performed every 14 days, for approximately 1 month after induction, thus providing, biological material for further studies. To evaluate the effect of metalaxyl on cell growth, cells were transferred to identical MS medium supplemented with 0 µM, 71.60 µM and 143.20 µM of metalaxyl. Cells were harvested at 0, 48, 96, 168, 216, 264 and 312 h during the growth cycle, and cell growth was monitored by measuring cell fresh and dry weight. Significant decreases on fresh weight could be observed at 96 h for both metalaxyl treatments, the higher concentration being responsible for a bigger decrease throughout the experience. No significant variations were found for the control treatment throughout time, but significant decreases on dry weight were found both treatments only at 264 h. Cell viability was evaluated by plasma membrane permeability to trypan blue. Metalaxyl resulted in a reduction of cell viability on a concentration-dependent manner. These results shown that growth of *S. nigrum* cell cultures was inhibited mostly when exposed to the highest metalaxyl concentration. As well the significant inhibitory effect on cell viability indicated the establishment of stressful conditions by the fungicide. These preliminary results suggest that *S. nigrum* cell suspensions can be a good research model for studying the metalaxyl effects on plant stress metabolism.

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# Effects of photoperiod and induced anoxia during over-watering on *Arabidopsis thaliana*.

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Plants often experience challenging hypoxic conditions imposed by soil water logging or complete flooding. We call the state induced by this condition as soil anoxia [1].

It has been shown that anoxia induces oxidative stress in plants. In this study we propose to explore the response of *Arabidopsis thaliana* growing in different photoperiods to watering conditions in order to optimize the growth conditions of wild type *A. thaliana*.

For this study, we used trays where seeds were sown directly, two of these seed trays were placed in a long-day growth chamber (16h light) and two other seed trays were placed on a short-day growth chamber (8h light). For each of these two growth conditions, two different situations regarding the watering; 1 – over-watering, 2 – control normal watering, were applied. *A. thaliana* is a facultative long-day plant.

During the experiment the rosette's diameter and the number of leaves were weekly controlled, as well as the final biomass. After the first siliques mature we will measure the plant biomass dried and without the roots.

Our preliminary results show that plants on trays subjected to anoxia conditions present smaller and weaker plants. We also expect to obtain differences regarding the photoperiod conditions.

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# Effects of proton pump inhibitors on osteoclastogenesis: in vitro studies with human osteoclasts and osteoblasts

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Proton pump inhibitors (PPI) are widely used in many pathological conditions, like, for example, gastroesophageal reflux, dyspepsia and peptic ulcers. Their mechanism of action relies in the inhibition of H<sup>+</sup>/K<sup>+</sup> ATPases, responsible for gastric acid secretion. Osteoclasts are specialized cells that solubilize extracellular bone matrix through the production of acid, by proton pumps that belong to the same family as the gastric ones. Thus, PPI appear as good candidates to modulate osteoclast resorption activity. This work intended to characterize the effects of three PPI on human osteoclastogenesis in co-cultures of human osteoclasts and osteoblasts.

Osteoclastic precursors were isolated from human peripheral blood and were co-cultured with MG63 osteoblast-like cells. Cell cultures were treated with different concentrations (10<sup>-7</sup>-10<sup>-3</sup> M) of omeprazole, esomeprazole and lansoprazole. In order to characterize the intracellular mechanisms affected by PPI, cell cultures were treated with MEK, NFκB, PKC, MAPK, JNK and p38 signaling pathways inhibitors, and a PGE2 production blocker. Cell cultures were characterized throughout a 21 day period for tartarate-resistant acid phosphatase activity and histochemical staining and the presence of multinucleated cells with actin rings and expressing vitronectin and calcitonin receptors.

The PPI tested revealed a dose-dependent inhibition of osteoclast development. The effect was more pronounced for lansoprazole. Nevertheless, the osteoclastogenic inhibition was verified at levels higher than 10<sup>-6</sup> M for the three PPI. The characterization of the intracellular mechanisms revealed that different PPI can act by different manners on PBMC.

Taken together, PPI have the ability to decrease human osteoclastogenesis, when osteoclastic precursors were co-cultured with osteoblastic cells. Understanding the subjacent mechanisms can open new perspectives in the utilization of such compounds in pathological conditions characterized by a hyperactivation of osteoclastic cells.

# Effects of the temperature in the agonistic behaviour of a widely distributed key-species of temperate estuarine systems

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Nowadays climate change is considered one of the most severe threats to life in Earth. This phenomenon is affecting life in almost all terrestrial and aquatic ecosystems worldwide. In this scenario of rapidly environmental changes, species needs to quickly adapt, finding new places to live or even modifying their physiology and behaviours. In this context, the present study aimed to report how water temperature increasing may affect the agonistic behaviour of a widely distributed and easily found keystone species in temperate estuaries: the shore crab *Carcinus maenas*. In order to test how crabs will react to temperature increasing, an ethologic experiment was performed in the lab. The experiment consisted in observe the agonistic behaviour of crabs under different levels of temperature (10, 16 and 22°C) during a 5min dispute for food in a tank. Two crabs *per* fight were used, but only one had its behaviour recorded. Crabs were kept isolated from each other and in starvation during one week prior to the experiment. Three types of standard behaviours (approach, attack and retreat) were previously established based on pilot study and literature. At 10°C crabs displayed a significantly lower number of agonistic behaviours than other temperature levels (ANOVA;  $p < 0.01$ ). At this temperature crabs were almost inactive, rarely displaying agonistic behaviours. While when submitted to elevated water temperatures (22° C), crabs behave differently, with some becoming extremely agitated, while others showed an indolent behaviour, barely moving. Fights where food possession by crabs changed, presented a significantly higher number of agonistic events than fights in which changes did not occurred (ANOVA;  $p < 0.01$ ). Our results highlight that the species may become more aggressive in the habitats where it inhabits, due to upcoming water temperature increasing. The consequences of this behavioural change are somewhat unclear at the moment, and due to the ecological importance of *C. maenas* in temperate estuarine systems, it is essential to perform field and laboratory studies in order to assess effects of this behavioural change in the ecosystem.

# Enzymatic hydrolysis by commercial proteases of Brewers' spent grain proteins

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Valorisation of by-products is an innovative concept in the field of residues management contributing to a sustainable development. Brewer's spent grain (BSG) is an abundant, protein-rich coproduct from the beer industry. Its use is still limited, being basically used as animal feed. It has received little attention as a marketable commodity, and its disposal is often an environmental problem. Nevertheless, it can be of value as a raw material. The industrial production of protein hydrolysates by commercial proteases is a field to explore.

In the present study protein hydrolysates were enzymatically produced from BSG. To that end, BSG protein concentrate (BPC), prepared by alkaline extraction of BSG and subsequent acid precipitation, was enzymatically hydrolyzed with commercial Alcalase and Protamex® over a broad pH range (pH 5-10).

Activity profiling of commercial Alcalase and Protamex® (a mix of Alcalase and Neutrase) as a function of pH and protease concentration were determined at 37°C using BSG proteins as substrate (Fig.1 a, b, respectively).

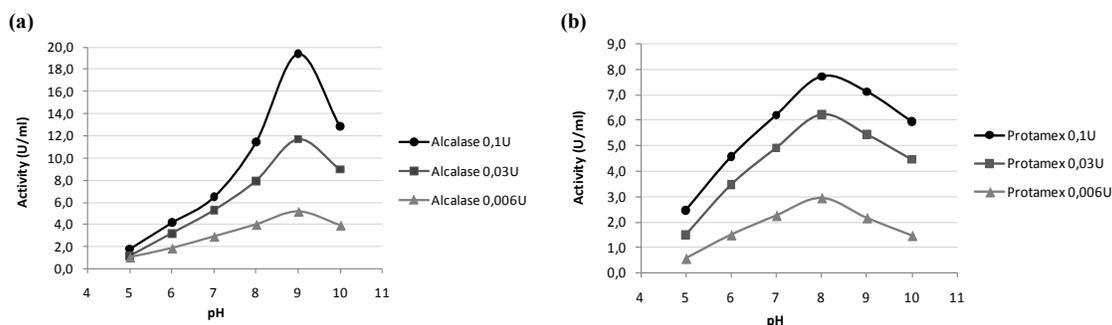


Figure 1 – Activity profile of Alcalase (a) and Protamex® (b) as a function of pH and protease concentration.

Concerning to hydrolysis of BSG proteins the optimum pH for Alcalase is 9 and for Protamex® is 8. At optimum pH, higher activity is observed for Alcalase than for Protamex®. As expected, higher proteolytic activity is obtained for higher protease concentrations. Alcalase activity between pH 5 and 7 was similar, when protease concentration increased, however, a significant increase of activity was observed at pH 9. These results suggest that the use of alkaline pH (8 – 9) increases the extent of hydrolysis of BSG proteins.

# Evaluation of the conditions for photoconversion and imaging of Kaede and EosFP by laser scanning confocal microscopy in transformed plant cells

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The possibility to locally change the emission wavelength by focused UV light makes Eos fluorescent protein (FP) a superb marker for experiments aimed at tracking the movements of biomolecules within the living cell [1]. Monomeric EosFP-based probes retain all the qualities of single-colored fluorescent proteins while providing the additional capability of photoconversion. Both green and red fluorescent forms of mEosFP are stable and thus provide the highly desirable intracellular controls during prolonged live imaging [2]. Kaede, another photoconvertible fluorescent protein that changes from green to red upon exposure to violet light, has been used as an intracellular optical marker to monitor cellular and intracellular movements. Here we propose to study the conditions of conversion and imaging of Kaede and mEosFP in *Nicotiana tabacum* leaf epidermis transient expression. mEos and Kaede fused with a Golgi marker - sialyl-transferase (ST-Eos/ST-Kaede) were engineered and tested simultaneously with a mEos version with a signal peptide for endoplasmic reticulum (SP-Eos). *Nicotiana tabacum* leaves were *Agrobacterium*-infiltrated with the three constructs and imaged in a confocal laser-scanning microscope (SP2 CLSM, Leica), with a 488 excitation laser for green fluorescent detection and a 568 excitation laser for red fluorescent detection. mEosFP is easily photoconverted to a red color following an approximate 10s exposure to UV illumination, while the images of ST-Kaede taken 3 days after infiltration were inconclusive. More testes are in progress using plant aspartic proteinases cardosin A and cardosin B which have been extensively studied in our laboratory either in native plant *Cynara cardunculus* or in genetic transformed models such as *Nicotiana tabacum*. Therefore, fusions of mEos and Kaede will be performed to investigate the use of photoconvertible fluorescent proteins as an important tool to explore the trafficking pathways of these proteins in living cells.

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# Evaluation of the susceptibility of Portuguese autochthon flora to the pine wilt nematode (*Bursaphelenchus xylophilus*)

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Pine wilt disease, caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, was first detected in Portugal in 1999<sup>[1]</sup>. It was the first report of this nematode in a native conifer within the EU. Its insect vector is *Monochamus galloprovincialis* and, once infected, most plants show symptoms of needle chlorosis and usually die in just a few months<sup>[2]</sup>. Although it is well known that plants of the *Pinus* genus are the preferred host of the PWN, it isn't known what other Portuguese flora species may be susceptible to the PWN. In this work we aimed at determining if *Picea abies* and *Cupressus lusitanica* are susceptible species to *B. xylophilus*.

One year old seedlings were maintained on a 16h, 25°C light, 8h, 18°C darkness, photoperiod at 80% of humidity. *B. xylophilus*, HF strain, was grown on barley seeds with *Botrytis cinerea* at 26°C, in the dark, and extracted using Baermann funnel technique. Eighty seedlings of each plant species were inoculated with 1000 nematodes in a sterile water suspension and monitored for 28 days; another set of 80 seedlings were inoculated with sterile water and used as control. Chlorophyll extraction and quantification was done according to Abadía *et al.*<sup>[3]</sup>. Nematode extraction from seedlings for posterior quantification was done using Baermann funnel technique. Total soluble phenolic content and was determined according to Azevedo<sup>[4]</sup>. Also, the morphology of both species was visualized with electron microscopy and compared with the morphology of the known PWN susceptible host (*Pinus pinaster*).

It was found that neither species presented visible symptoms of disease, either because more time was needed for symptoms appearance, or because these species are resistant to the nematode. *P. abies* presented a larger amount of nematodes than *C. lusitânica*, probably because it's a conifer, and so, has a more identical morphology and physiology to pine species. This similarity of morphological characteristics between *P. abies* and *P. pinaster* was confirmed by visualizing stems of these species under scanning electron microscopy. Both in control and infected plants of *P. abies* and *C. lusitânica*, the chlorophyll leaf concentration decreased during the experiment. In control seedlings this was probably due to the mechanic injury occurred during the inoculation whereas in infected seedlings this might have been caused by mechanic injury or the action of the nematode population itself. Quantification of total phenolics showed that inoculated plants increased phenolic compound production.

More studies are underway to determine the susceptibility of *P. abies* and *C. lusitânica* to the pine wilt nematode *B. xylophilus*. This work includes the quantification of lignin, optical microscopy and investigation on activation of defense-related genes.

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## Evidence of an introduction of *Podarcis sicula* from Italy to Spain associated with importation of olive trees (*Olea europaea*)

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The Italian lizard *Podarcis sicula* (Rafinesque, 1810) is widespread mainly in the Italian Peninsula but we can also find these lacertids in the East coast of the Adriatic Sea and in many islands like Corsica and Sicily. [1] This species appears to be a good colonizer because has been reported the introduction of some populations of this lizard in places like France, islands of Turkey, Istanbul, North Africa and even populations in North America. In the Iberian Peninsula there are a few isolated stable populations in Cantabria and Almeria. They may be the result of the connection between these places with Italy during the Spanish Civil War. Recently a new introduction was discovered in Lisbon, Portugal [2].

This species occupies a great variety of habitats, especially in Southern Italy, like dry fields and fluvial places, natural or urbanized. In this study we analyse the first finding of a population of *P. sicula* in a plant vivarium in La Rioja, Spain, where had been imported centenary olive trees from Italy [3]. Lizards were seen for the first time in the Spring of 2009, and seem to have been using the olive trees for thermoregulation and refuge. In the same area, there is another lizard, *Podarcis hispanica* that lives on the same trees but never at the same time. *P. sicula* has high competitive skills and is able to displace other lizards, representing a risk for endemic populations of species with similar characteristics [4]. As the introduction of *P. sicula* in other areas can thus be harmful for endemic lizards, introduced individuals were eradicated and genetic analyses were employed to assess their origin. Two mitochondrial DNA fragments, 12S and 16Ss rRNA, were analysed, and a simple neighbor joining analysis demonstrates that these individuals were very close to the samples from Meridional Italy, Sicily, Sardinia and Croatian sequences.

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## Fatty acids in brown macroalgae of the Portuguese west coast

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Marine organisms are rich in a variety of compounds used in nutritional and/or pharmaceutical areas [1]. The chemical diversity of marine lipids, especially unsaturated fatty acids, makes them an interesting option for a healthy diet. Macroalgae are known to have a low total amount of lipids (usually less than 5%), with a high proportion of polyunsaturated fatty acids (PUFA), which can represent more than 50% of fatty acids composition [2]. Although PUFA's profile depends on the species and environmental factors, in general, polyenoic C<sub>18</sub> and C<sub>20</sub> fatty acids of the omega-3 and omega-6 series predominate in most brown algae species. Particularly, brown macroalgae usually contain high proportions of eicosapentaenoic (20:5 ω-3) and arachidonic acids (20:4 ω-6) [3]. Consumption of omega-3 and omega-6 fatty acids has been associated with reduced mortality from cardiovascular disease, suppressed arthritis-associated inflammation, and decreased risk of cancer [1]. So, macroalgae can be regarded as a healthy dietary alternative to fulfill fatty acid needs.

In this work, ten brown macroalgae species (*Cladophorus spongiosus*, *Cystoseira nodicaulis*, *Cystoseira tamariscifolia*, *Cystoseira usneoides*, *Fucus spiralis*, *Halopteris filicina*, *Padina pavonica*, *Saccorhiza polyshides*, *Sargassum vulgare* and *Stypocaulum scoparium*) collected along the Portuguese west coast were studied for their fatty acids composition. The profile was determined by GC-MS after alkaline hydrolysis and derivatization of fatty acids to their methyl ester derivatives [4].

As far as we know, this is the first survey on fatty acids in Phaeophyta species from the Portuguese coast. The obtained results can be used to value this underexploited natural resource, either for direct human nutrition or to be used as a poultry supplement for the production of eggs rich in omega-3 PUFA or in aquaculture to enhance the omega-3 PUFAs content of fish. Furthermore, they may have interest for biotechnology and cosmetics.

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# Feeding behaviour of the brown shrimp *Crangon crangon* from Minho estuary (NW Portugal)

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The brown shrimp *Crangon crangon* is a long known key-species in estuarine epibenthic communities: in one hand it is an important prey for fish and crustaceans, and in the other hand it is a relevant predator on early life stages of flatfish and on several meiofaunal invertebrates. However, brown shrimp feeding behavior has not been studied yet.

In the present work, laboratory experiments were designed to investigate [1] how *C. crangon* responds in the presence of food and [2] which senses are mainly used to locate the food source. After 3 days of starving, the behavior of ten individuals was registered in two separate trials: one using an acrylic board acting as a physical barrier between the animal and the food, and the second without the board. The initial positions of the study objects (predator and prey), the location time and the search time taken to find the food were registered during 10 minutes of observation. Ten different types of food, some of them available in the natural habitat, were tested independently. In some cases, separate tests were performed with the same prey type frozen/dead and fresh/alive.

In the presence of a food item, the first appendages to move were the antennules and then the antennas suggesting that they are the appendages used primarily to detect the food. Following the other appendages such as the maxillipeds and pereopods were also active. In addition, other observations led to unexpected results that could mean that shrimps can use the optical sense to locate the food, as some of them tried for several times to reach food by digging up the sand under the board using the pereopods. Additionally, different food had different response intensities from the animals, depending on food characteristics like alive/fresh or dead/frozen.

# First records of marine Tardigrades in the Portuguese coast

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Tardigrades are micrometazoans that can be found in marine, freshwater and terrestrial habitats. Their body is composed by a cephalic segment, three trunk segments and a terminal segment. Each trunk segment and the terminal segment bear a pair of legs terminated in either claws or digits. They have a complex pharyngeal apparatus used for feeding on the fluid of bacteria, algae and other small invertebrates. Sense organs (e.g. eye spots, cephalic cirri) are seldom present. Marine tardigrades are present in all oceans, ranging from the intertidal zone to abyssal depths, inhabiting a great diversity of sediments from fine mud to coarse sediments, rocks and algae. Their small size, the low number of specimens frequently found, the need of expertise to accurate taxonomic identifications and specialized methods of study, such as the sampling procedures, are difficulties certainly responsible for the paucity of knowledge about marine tardigrades namely about their diversity, ecology and geographical distribution.

Taking in mind the importance of the knowledge of the marine zoological resources, particularly enhanced by the recent Portuguese proposal, submitted to the United Nations, to extend the Exclusive Economical Zone (EEZ), we decided to study the patterns of distribution of the tardigrade species in relation to some abiotic factors along the northern coast of Portugal where tardigrades are completely unknown. Actually, up till now, only two (eventually three) marine tardigrade species have been recorded in the Azorean Sea [1, 2].

Tardigrades were extracted from samples of sand, sediments scrapped on rocks and algae collected in the intertidal zone in two beaches of the region of Porto: “Homem do Leme”, in May 2009, and “Memória” in November 2010. To release tardigrades from the sediment, samples were fresh-water shocked, decanted through a 64 µm mesh net and fixed in 10% buffered formaldehyde. Tardigrades were mounted in Hoyer’s medium and photomicrographs were made under oil immersion using phase contrast microscope.

In this work the first three Portuguese marine tardigrades species, *Batillipes similis*, *Echiniscoides sigismundi* and *Echiniscoides* sp., are recorded and figured. Remarks on their geographical distribution and on the morphological variability in *Echiniscoides* are also provided.

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## Foxes at our doorstep? Urban foxes in Vila Nova de Gaia

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Urban foxes first appeared in residential areas of British cities in the 1930s [1]. Although thought to be a British phenomenon, they are common in English, Welsh and Scottish cities, fox populations have been latter reported in many other European, Northern American, Australian and Asian cities as well [2]. This is however the first report of one urban fox population for the Iberian Peninsula.

Data on the occurrence of the Red Fox in the area of the municipality of Vila Nova de Gaia is being gathered since October 2010. Local authorities (City and Parish Councils) and several institutions/associations related to animal care were contacted; online questionnaires were made available on the project web page to collect information on sightings and hunting of foxes within the study area; records of injured foxes delivered at the Parque Biológico de Gaia recovery centre dating back to 1996 were also analysed. A cartographic survey of the available habitat for the fox is being performed.

All the information gathered confirms the presence of the Red Fox in Vila Nova de Gaia and gives a preliminarily assessment of its current geographic distribution. The Parque Biológico de Gaia and its neighbouring area are one of the Red Fox sanctuaries; however according to most of the other records the distribution of the species largely corresponds to the predominantly agricultural and forested areas characteristic landscape of the southern region of the municipality.

Data collected by the Parque Biológico de Gaia on the Red Fox is scarce, so its analysis must take that into consideration. The sex ratio is almost balanced and foxes continue to breed in the municipality area as confirmed by the age of the collected animals, most of them being juveniles; also, most records also point out to an increase in mortality during the first months of the juvenile phase period.

Taking into account the urban fox's needs and their home range we can consider that the available habitat for the species is still considerable, even though its reduction and fragmentation (urbanization and associated linear structures) is also unfortunately predictable [3]. Further information about the urban carnivore populations and their habitat is urgently needed.

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## Hunting zones- foxes in the chicken coop?

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The Red Fox, *Vulpes vulpes*, is known to be an omnivore, but also opportunistic carnivore species [1]. Frequently considered to have an impact on game birds, especially reared and released game species, and on livestock, a number of contradictory articles can be found in the scientific literature. Previous studies of fox's diet in Portugal reported that the fox is not a small-game predator [2].

Fox carcasses obtained in hunting areas in the Portuguese territory were dissected and their stomach removed and stored at low temperatures (-20°C). Processing the stomach contents included determining its weight (to the nearest g) and measuring its volume (to the nearest ml), followed by the separation of the components through 1.2 mm sieve for further identification [3]. The identified items are included in the following categories: mammals, birds (including eggs), other vertebrates (like reptiles, amphibians and fishes), insects, earthworms, other invertebrates, plants and fruits, and refuse.

A small number of stomach contents were examined until now, but some data can be already presented. The percentage of empty stomachs was 35.7%, the stomach contents weighted 108.8 g ( $\pm$  80.6 g) and showed a mean volume of 120.5 ml ( $\pm$  77.8 ml). The diversity of items found in each stomach contents analyzed is not big, as is not yet globally; fox food include so far mammal's remains (hairs and skin), legs and wings of insects and a few plants and fruits.

Our results are still preliminary but they already seem to confirm the opportunistic feeding behaviour described for the Red Fox. However data are still insufficient to withdraw any conclusions about the impact of fox predation on game species. Only with the expected increase of the sample size it will be possible to obtain a more precise image of the red foxes diet diversity, and to verify if there are any seasonal or geographical differences.

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# Identification and analysis of cadmium transporting HMA genes in *Solanum lycopersicum*

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Tomato (*Solanum lycopersicum*) is one of the model plants from the Solanaceae family and the first to have its genome sequenced and publicly available (the ongoing International Tomato Genome Sequencing Project (ITGSP) is available at <http://solgenomics.net>).

Metal transport and uptake of metals are essential for plant growth, development, nutrition and signal transduction. In addition, plant metal transport systems are a very important part of mechanisms for decreasing the toxicity of certain metal ions.

Therefore, many metal transporters are known to transport toxic cations (such as heavy metals) along with cationic nutrients.

The HMA family (P1B-type Heavy Metal ATPases) is a type of heavy metal transporters responsible for the metals loading to the xylem. The HMA1 to HMA4 genes in *Arabidopsis thaliana* are known to possess Cd transport ability.

Although this family of transporters has been already described for *Thlaspi* and *Arabidopsis* species, is still largely unknown on Solanaceae plants.

Using sequence information from the ITGSP, with data mining and gene prediction techniques, 2 distinct HMAs (HMA1 and HMA2) were identified and characterized in tomato. HMA1 was identified in the tomato genome and the cDNA prediction program wise2 deduced a coding ORF of 2457 bp. This coding sequence has 71% identity with HMA1 of *A. thaliana*.

Another gene, HMA2, was also identified in tomato with a wise2 predicted coding ORF of 2983 bp. This coding sequence has 69% identity with HMA2, 3 and 4 of *A. thaliana*, but only up to the first 2100 bp of the ORF. The remaining [2100, 2983] bp has a very low identity to the *A. thaliana* HMAs. In order to confirm if this result was due to an erroneous wise2 ORF prediction, an EST BLAST search was conducted with the referred interval, revealing at least one EST of tomato with 100% identity (Accession number AW737974). This shows that this portion of the gene is expressed and points to a correct ORF prediction of wise2.

A more broad EST BLAST search also shows that this identity difference between *A. thaliana* HMA2/3/4 and HMA2 of tomato is consistent in the Brassicaceae family (which includes *Thlaspi* and *Arabidopsis* species) and is characteristic of the Solanaceae family.

We expect that these metal transporters identified in Tomato will be a stepping stone in the understanding of how each of the transporters identified specifically contributes to the metabolism of heavy metals in Solanaceae. This knowledge will have multiple applications in biofortification and agronomy, in particular for studying the impact that exposure to certain heavy metals have on edible Solanaceae.

# Identification of a novel carbonic anhydrase isoform in larval lamprey (*Petromyzon marinus*)

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Carbonic anhydrase (CA) plays a key role in CO<sub>2</sub> excretion and acid-base regulation in vertebrates [2] catalyzing the reversible hydration/dehydration reaction of carbon dioxide and bicarbonate [1]. Since CA's catalyse many physiological processes it was likely one of the first enzymes to appear [2]. The lamprey is a basal vertebrate and to date only a single CA isoform has been identified; however, at least 15 isoforms have been characterized in other vertebrate groups [2]. Lampreys are characterized by a complex life cycle in which the larvae (initial stage) or ammocoetes are benthic filter feeders which undergoes a dramatic morphological and physiological transformation into parasitic feeding adults [3]. In the case of *Petromyzon marinus*, the post-metamorphic juveniles migrate to the sea to commence feeding.

The aim of this study was to determine if additional CA isoforms are present in *P. marinus* and if expression patterns are life history stage and tissue dependent. In order to answer this question a Polymerase Chain Reaction (PCR) approach was taken using degenerate and consensus sequence primers with samples from different tissues of adult lamprey (gill, anterior, middle and posterior gut, muscle kidney, heart and liver), ammocoete gill and transformer gill. High expression was found in ammocoete and transformer gill. Sequencing of the PCR product from ammocoete revealed a new CA isoform which has 76% sequence identity with the published CA isoform from lamprey and 64% and 61% for teleost and mammalian CA isoforms, respectively (Fig. 1).

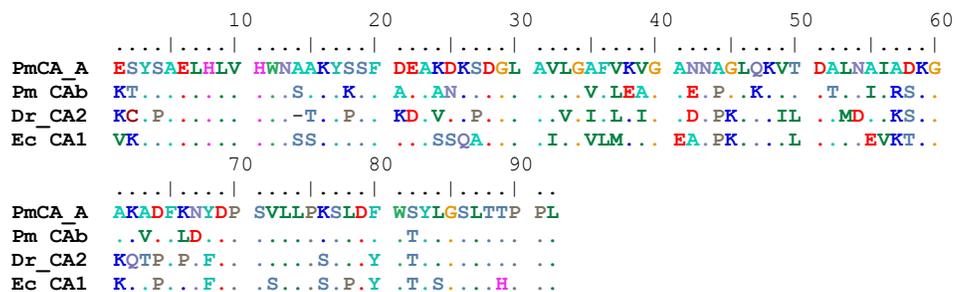


Fig. 1. Alignment of *P. marinus* partial sequence obtained from ammocoete gill (PmCA\_A) with published *P. marinus* CAB (AAZ83742.1), *Danio rerio* (NP954685.1) and *Equus caballus* (P00917.3) using ClustalW Multiple alignment within BioEdit 7.0.9.0.

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## Identification of *Quercus spp.* pollen profilin-like

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Profilin is a ubiquitous protein that has been implicated in a variety of physiological and pathological processes. Profilins are small, 14 to 17 KDa proteins expressed in eukaryotes and certain viruses and they are highly conserved proteins. These proteins are involved in cell development, cytokinesis, membrane trafficking and cell motility. At subcellular level, the profilin is involved in actin polymerization dynamic and also interacts with polyphosphoinositides (PPI) and proline-rich domains containing proteins. Depending on the organism, profilin is present in a various number of isoforms, it comprises sequences between 100-131 amino acids. However, the significance and the functions of the different isoforms are not yet fully understood. Allergenic profilins were identified in tree, grass and weed pollen, in plant-derived foods, as well as in latex. [1-2].

*Quercus* pollen is present in the atmosphere since Mars to June which has respiratory allergenic proteins. The aims of this work were to identify and characterize profilin of *Quercus spp* pollen. Samples of pollen from different *Quercus* species (*Q. suber*, *Q. robur*, *Q. rubra*, *Q. faginea*, *Q. ilex*, *Q.imbricaria*) were collected during the Spring of 2010. Soluble proteins extracts were quantified, assayed by sodium dodecyl sulphate-polyacrilamide gel electrophoresis (SDS-PAGE). Immunobiochemical techniques were applied to these extracts using specific antibodies to *Zea mays* profilin. The results revealed the presence of profilin-like protein in *Quercus* pollen extracts.

Total RNA from *Q. suber*, *Q. rubra*, *Q. robur*, *Q. imbricaria* and *Q. faginea* pollen were analysed in agarose gel. By RT-PCR using degenerate *primers* for profilin was obtained a *cDNA* amplification of ~ 400 bp. Further studies, will be conducted in order to clone amplified *cDNA* into a plasmid vector and the cloned profilin fragments will be sequenced. Profilin sequences will be analysed by bioinformatic methods.

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# Immunolocalization of arabinogalactan proteins (AGPs) in *Trithuria* reproductive structures, the closest living relative of water-lilies

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*Trithuria*, the sole genus in the family Hydatellaceae, is an important group for understanding early angiosperm evolution because of its sister relationship to the ancient lineage, Nymphaeales (water lilies). Although also aquatic, *Trithuria* is a very small plant, less than 1 cm, mostly annual and with small reproductive structures that have both characteristics of flowers and inflorescences and they may represent a transitional, pre-floral stage in the evolution of the flower [1]. Also remarkable, is the presence of a strategy that is unique among flowering plants, but common to all gymnosperms; the pre-fertilization allocation of nutrients to the embryo-nourishing tissue [2].

AGPs are a kind of hydroxyproline-rich glycoproteins that are massively glycosylated, ubiquitous in plants, and particularly abundant in cell walls, plasma membranes and extracellular secretions. AGPs can be localized in tissues and cells through the use of specific monoclonal antibodies that bind to the structurally complex carbohydrate epitopes. The selective labeling obtained by using these monoclonal antibodies in *Arabidopsis* and in other plant species, made us conclude that some AGPs can be present as molecular markers during development and that they are probably related to important steps of the plant sexual reproductive cycle [3].

In this work we propose to evaluate the presence of different AGP epitopes in the reproductive units of *Trithuria*, assess differences present during development and address some fundamental questions related to evolution and genetics.

Our preliminary results are showing that in the ovules, there are some specific antibodies labeling extensively the long style filaments, the outer integument and the perisperm cell reserves. In the anthers curiously, all the pollen grains are collapsed and there are also some specific labeling related to the anther wall and most probably, to the pollen intine wall.

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## Impact of axenic culture conditions in *Leishmania infantum* virulence

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Protozoan of the genus *Leishmania* undergo several developmental transitions during its life cycle. *Leishmania* life cycle alternates between two morphologically distinct forms, the promastigotes (insect stage) and the amastigotes (vertebrate stage). The use of sandflies to grow the promastigote form, although possible and ideal, has a number of limitations due to issues related to biosecurity and handability preventing their use. As an alternative it is possible to achieve indefinite promastigote growth outside the sandfly using a several established media. These media enable the growth of the promastigote form in a controlled environment. Although commonly used, these media have been shown to induce loss of virulence over time. This can be due to either loss of virulence factors or due to disadvantageous adaptations to the media. These alterations in the physiology of the parasite that are induced by the growth in the different media, can lead to misleading and often contradictory experimental results. It is then of great interest to access the influence of the different media in the parasite virulence.

The aim of this work was to compare the biochemical and virulence modifications of a cloned line of virulent *L. infantum* (MHOM/MA/67/ITMAP-263) promastigotes maintained in four different culture mediums: Schneider, RPMI 1640, SDM and RPMI-SDM without serum. *L. infantum* parasite cultures were used for the realization of growth curves and the preparation of logarithmic and stationary-phase parasite total protein extracts. These were compared by SYPRO staining after protein separation by SDS-PAGE electrophoresis. The analysis of the parasite viability was performed using Annexin V and propidium iodide staining. Moreover, the percentage of metacyclic promastigotes (the most virulent form of the parasite) in each culture was quantified after Ficoll density gradient purification and by quantitative PCR to stage-specific genes. To evaluate their *in vitro* virulence, primary bone marrow-derived macrophages were used as infection target using fluorescent CFSE-labelled promastigotes. The percentage of infection was measured by the quantification of CFSE-positive cells in a flow cytometer.

This approach will shed some light on the association between the culture media and the parasite virulence.

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# Influence of Sex and Gonadectomy in the Anatomy and Neurochemical Organization of the Dorsal Parvicellular Division of the Hypothalamic Paraventricular Nucleus

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The production of glucocorticoids in adrenal cortex is dependent on parvicellular neurons of the hypothalamic paraventricular nucleus (PVN), by means of the central component of the hypothalamo-pituitary-adrenal (HPA) axis, and through its autonomic-related descending division, strongly related to neurons mainly located in the dorsal parvicellular (PVNdp) division of the nucleus. Several studies have shown that females produce more epinephrine and corticosterone than males after exposure to a stressful situation. However, discrepant sex-related differences in ACTH levels have been encountered. In order to understand this distinctness, we have speculated that it could be connected to PVNdp dimorphism in what concerns to nuclear volume, total number and volume of its neurons and also vasopressin (VP)- and corticotrophin-releasing hormone (CRH)-positive neurons. To analyze if gender differences may be contingent on gonadal steroids, we studied the repercussion of gonadectomy in males and in females. Our results show that the volume and total number of neurons of the PVNdp were not significantly influenced by sex or gonadectomy. They also reveal that intact females had larger neurons than intact males and that difference disappeared after gonadectomy. Our results further show that the total number of CRH-immunoreactive neurons was not significantly influenced by sex or gonadectomy but the total number of VP-immunoreactive neurons varied as a function of sex and gonadectomy since intact females had significantly more VP neurons than intact males and gonadectomy led to a significant decrease in the number of VP-immunoreactive neurons in both sexes and also abolished the sex differences of intact groups. Therefore, our study shows that the anatomy and neurochemistry of the PVNdp present gender differences in rats, and that these differences are related to circulating levels of sex steroid hormones. This suggests that the PVNdp might be important in the establishment of the sexually dimorphic production of corticosterone.

# Influence of silicon on the osteoclastogenic behavior of co-cultures of human osteoclasts and osteoblasts

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Bone tissue is characterized by the presence of an extracellular mineralized matrix, mainly composed by crystals of hydroxyapatite. In addition, low amounts of sodium, magnesium and silicon are also found in the matrix. Although it is acknowledged the importance of silicon for the proper development of bone, its effects on bone cells, particularly on osteoclasts is still scarce. Furthermore, this issue assumes a growing relevance in the context of bone regeneration, where silicon appears as an element with a high potential of utilization. The aim of this work was to analyze the osteoclastogenic cellular and molecular effects of serum concentrations of silicon on co-cultures of human osteoclasts and osteoblasts.

Osteoclast precursor cells (PBMC) were collected from human peripheral blood and were co-cultured with MG63 osteoblast-like cells. Co-cultures were maintained in the absence or presence of different concentrations of silicon (0.04 – 1µg/mL). When indicated, cell cultures were also performed in the presence of MEK, NFκB, PKC, MAPK, JNK and p38 signaling pathways inhibitors, and in the presence of a PGE2 production blocker. Cells were analyzed throughout 21 days (7, 14 and 21) for tartarate-resistant acid phosphatase activity and histochemical staining and the presence of multinucleated cells with actin rings and expressing vitronectin and calcitonin receptors.

It was observed that silicon had the ability to modulate the osteoclastogenic response of co-cultures. Specifically, it inhibited osteoclast differentiation in a dose-dependent way. This effect was caused by significant changes in the intracellular signaling pathways assessed.

Taken together, silicon appears to have a negative effect on osteoclastogenesis on co-cultures of human osteoclasts and osteoblasts. Furthermore, this effect is observed when silicon concentration is in the range of that found in human serum (0.2-4.0µg/mL).

## Inoculation of *Capsicum annuum* L. with Rhizobia: bioactive compounds and antioxidant activity

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Pepper (*Capsicum annuum* L.) is one popular vegetable because of the combination of attractive colors, characteristic taste and aroma, but also for its nutritional value. Peppers contain a wide array phytochemicals, including phytosterols, fatty acid, volatile compounds, organic acid, phenolic compounds, vitamins and carotenoids. Epidemiological data have been reported possible positive roles of these compounds in human health, as in the prevention of certain cancers, cardiovascular diseases, neurodegenerative disorders and stimulation of the immune system [1,2]. Inoculation of some vegetables with soil bacteria, called rhizobia, is a technique to promote nitrogen fixation and improve their nutritional status. This symbiosis may possibly improve biomass production and induce chemical changes in pepper fruit.

The aim of this work was to assess the modifications in chemical composition and antioxidant activity of pepper fruits inoculated with rhizobia belonging to *Rhizobium* and *Mesorhizobium* genus. Fatty acids and volatile compounds in pepper extracts were analyzed by GC-MS, phenolic compounds and sterols by HPLC-DAD and organic acids by HPLC-UV. Antioxidant activity was evaluated by DPPH, nitric oxide and superoxide radical scavenging assays.

Inoculation with *Rhizobium sp.* (TVP08 and PETP01) and *Mesorhizobium mediterraneum* (PECA20) strains lead to different results. Overall, fruit inoculated with *M. mediterraneum* showed more sterols and organic acids comparatively with control. Regarding volatiles, samples inoculated with both *Rhizobium sp* strains have ketones, esters, sesquiterpenes and alcohols in higher amounts; inoculation with PECA20 resulted in major levels of aldehydes. *Rhizobium sp* TVP08 lead to higher diversity and amounts of fatty acids. Phenolic compounds were not significantly affected by inoculation. Samples inoculated with PETP01 revealed to be the most active against DPPH and nitric oxide.

Thus, it can be highlighted that important modifications in the chemical profile of pepper fruit inoculated with rhizobia and consequently, changes in antioxidant potential, were produced.

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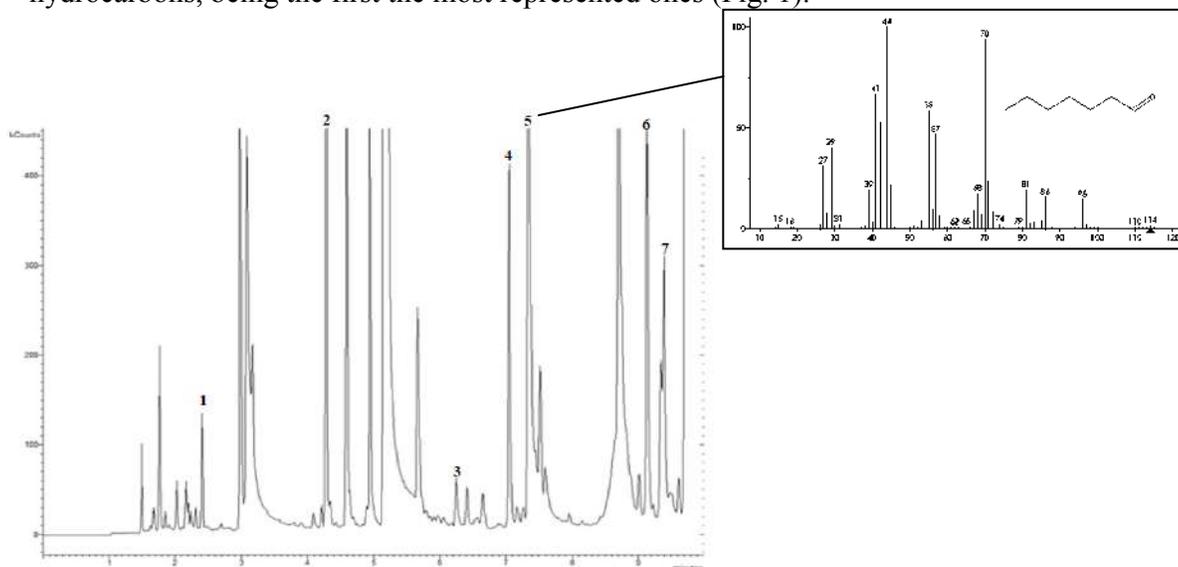
# Insight into chemistry and biological potential of *Osmundea pinnatifida* (Rhodophyta)

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In the last decades there has been a growing interest in the search for bioactive compounds in marine organisms. Red algae (Rhodophyta) have revealed to be an interesting source of bioactive molecules, namely of halogenated compounds. For the present work *Osmundea pinnatifida* was selected, a species with a ubiquitous distribution, from Europe to America and Australia, found in rocks of relatively sheltered areas and used in human diet. An aqueous extract was prepared, which was further subjected to phytochemical and bioactivities studies.

No phenolic compound was identified by HPLC/DAD. On the other hand, twenty six volatile compounds were determined by GC/MS, which included aldehydes, norisoprenoids, alcohols, ketones, terpenes, aromatic hydrocarbons, halogenated volatiles hydrocarbons and other hydrocarbons, being the first the most represented ones (Fig. 1).



**Fig. 1.** GC/MS chromatographic profile of *O. pinnatifida* aqueous extract (0-10 min scan) and MS spectrum of heptanal. (1) trichloromethane; (2) toluene; (3) (*E*)-2-hexenal; (4) 3-heptanone; (5) heptanal; (6) (*Z*)-2-heptenal; (7) benzaldehyde.

Some antiradical activity was observed against DPPH radical (14% for the highest tested concentration, 1.11 mg/mL) and a concentration-dependent effect was noticed against nitric oxide ( $IC_{15}=159 \mu\text{g/mL}$ ). However, no antimicrobial capacity was found against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* between 0.78 and 25 mg/mL of lyophilized extract.

This work was developed within the optional curricular unit “Bioactivity of Natural Matrices” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

## Maiden, barren or pregnant vixens?

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The knowledge of the reproductive biology is fundamental to understand the dynamics of any given populations. The Red Fox, *Vulpes vulpes*, is not an exception, reproduction being regulated by food availability and social constraints [1]. Red fox fertility may be appraised by its litter size, but it might be reduced by intra-uterine mortality and not all cubs survive within the den. The analysis of the vixen's uterus is an important tool since it allows the detection of barren foxes and the determination of the number of embryos, or of the placental scars after the reproduction period.

Two methodologies are being used to evaluate the average litter size. One is based on the actual number of visible embryos and the other is the placental scar count (PSC). This method is based on the analysis of the aspect of the uterine tissues (frame structure and color). The first step is searching for visible scars (crater breaking the lengthwise folds of the tissues with blood residues) in fresh uterus, carefully recording the position and its description. For the second step, the uteri are stained [2] and re-examined under a dissection microscope; stained scars are again recorded and described [3].

Only 14 adult Red Foxes (hunted in the Portuguese territory) were obtained so far. The Red Fox is a monoestrous species and the females are in heat for a short period (1 to 6 days); as mating occurs between the end of December and the beginning of February, pregnant foxes were not yet observed, as expected. Eight of the foxes analyzed were females (1.3♀:1♂). The mean length of each horn of the uterus was 11.2 cm ( $\pm$  1.99) and mean weight 3.4 g ( $\pm$  1.02). Although placental scars were not found in 4 vixens (all with 2 years or older) we cannot conclude if they were maiden or barren, because the analysis of the fresh tissues can underestimate previous pregnancies. The mean number of placental scars was 4 ( $\pm$  1), in agreement with the reported for other European fox populations [1]. The number of scars seems to be related with the fox's size, both length and weight.

Our results constitute a first step toward demographic analysis aiming at modeling the population of the Red Fox. The expected increase of the sample size will also allow a more precise estimation of litter size; the estimation of the reliability of the placental scar counts for the Red Fox is also important due to the difficulty to determine the litter size in the wild.

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# Modification of the arabinogalactan proteins AGP6 and AGP11 of *Arabidopsis thaliana* for their localization and purification

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Pollen grains and pollen tubes present a very specific transcriptome during their development, with several structural proteins being up-regulated and others being exclusively expressed. Two of such proteins are AGP6 and AGP11, members of the arabinogalactan protein family. AGPs are assumed to have an important role in angiosperm sexual reproduction, and AGP6 and AGP11 have been shown to be fundamental for the correct pollen development in *Arabidopsis*. However, due to their functional redundancy, AGPs have been difficult to study, and the individual sub-cellular localization has never been done due to the lack of specific antibodies.

Using the Multisite Gateway technology, we have inserted a c-myc tag between the signal peptide and the protein core of each of these AGPs, and used their native promoters and the CaMV 35S to drive their expression. With the native promoters, using anti-myc antibodies we will be able to specific localize AGP6 and in the pollen grain and pollen tube wall; with the 35S-driven overexpression we will be able to purify, by affinity chromatography with anti-myc antibodies, each of the proteins for further biochemical studies.

## Mutant cardosin A constructs and their expression in *Nicotiana tabacum* for the study of the Plant Specific Insert

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Cardosin A is an aspartic proteinase (AP) commonly found in cardoon pistil tissue (*Cynara cardunculus*, L.). Sub-cellularly, this AP is found to accumulate in vacuoles and, like other plant APs, it possesses a specific internal segment called the “Plant Specific Insert” (PSI). This domain has been implicated in vacuolar sorting, both due to its action as a sorting signal and to its ability to interact with lipid membranes. It has also been associated with other cellular processes, namely in membrane reorganization and solute leakage during seed germination and in defense mechanisms. In an attempt to unveil its true function in both the trafficking and maturation process of cardosin A, we have applied several mutagenic PCR techniques for the construction of mutated forms of cardosin A. Results were analyzed through Western blotting after protein isolation and vacuoles preparations from whole leaves and, in some constructs a fluorescent protein (mCherry) was incorporated. This approach, allows us to visualize its sub-cellular localization and *in vivo* movements, through confocal microscopy. Preliminary results show that point mutations inserted into the amino-acid sequence to prevent the cleavage of the PSI, result in untimely cell death and plant senescence. However, we were able to observe that the protein processing was not affected by this mutation. Furthermore, the swapping of the PSI from cardosin A with the PSI from another AP – cardosin B – results in a mixed western blotting profile, indicating that the original protein may have gained some of the characteristics of cardosin B processing, such as a slower pattern of accumulation and its absence in the vacuole preparations. However, to better assess the physiological role this domain may play in APs, further studies are necessary and are being undertaken in our lab.

# Osteoclastogenic behavior of human osteoclast precursor cells cultured in the presence of hydroxyapatite nanoparticles

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Nano-Hydroxyapatite (nanoHA) has a variety of proposed applications in bone repair/regeneration strategies, but information regarding the response of bone cells to particulate nanoHA is scarce. The aim of this work was to evaluate the behavior of human osteoclast precursor cells in the presence of different concentrations of Hydroxyapatite nanoparticles.

NanoHA was prepared by a hydrothermal synthesis and characterized by XRD analysis, FTIR and TEM. Osteoclast precursors (PBMC) were isolated from peripheral blood. At days 1, 7 and 14, different concentrations (0.1-100 µg/mL) of nanoHA were added. When indicated, cultures were treated with inhibitors of MEK, NFκB, PKC, MAPK, JNK and p38 signalling pathways and a blocker of PGE2 synthesis. Cell cultures were assessed at days 7, 14 and 21 for tartarate-resistant acid phosphatase activity and histochemical staining and the presence of multinucleated cells with actin rings and expressing vitronectin and calcitonin receptors.

NanoHA presented a rod-like shape, an average length of 55nm and 26nm in width, and a specific surface area of 64m<sup>2</sup>/g. Results showed that the presence of nanoHA influences osteoclast development, depending on the experimental conditions. Addition of the nanoparticles at day 1 did not cause a significant effect on osteoclastogenesis. However, if the material was added at day 7, cell cultures displayed an increase on the osteoclastogenic response. On the other hand, the addition of nanoHA at day 14 significantly decreased osteoclast-related parameters, in a dose-dependent manner. Also, it was observed some significant differences among the intracellular pathways involved in the co-culture behavior.

In conclusion, nanoHA particles have the ability to modulate osteoclastogenesis, influencing directly osteoclast precursor cells, and this ability is dependent on the differentiation stage of the precursors.

# Overexpression of Target Class III Peroxidases in *Arabidopsis*

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Class III peroxidases (Prxs) form a large multigene family typical of plants and they are heme-containing enzymes that catalyze the oxidation of small molecules at the expense of H<sub>2</sub>O<sub>2</sub>. Prxs have been implicated in numerous physiological processes, particularly in key processes determining the architecture and defense properties of the plant cell wall, but also in many types of stress, senescence, and the metabolism of natural compounds. However, their precise role is often controversial, and their *in vivo* mechanisms of action and substrates are still poorly characterized, especially in what concerns vacuolar Prxs. In fact, the high redundancy of Prxs (73 genes in *Arabidopsis*, 138 in rice) and their overlapping substrate profiles turn the ascription of precise functions a daunting task.

To approach the functions of specific Prx isoenzymes using all the advantages of the model plant *Arabidopsis thaliana*, we set *Arabidopsis* leaf expressed Prxs as a target, due to the usually simple Prx isoenzyme profile expressed in this organ. Transcriptomic analysis using the microarray data base GENEVESTIGATOR identified respectively Prx 42 and Prx 33/34 as the main cell wall and vacuolar Prxs expressed in *Arabidopsis* leaves. On the other hand, analysis of leaf Prx activities confirmed a simple isoenzyme profile, and the purification of the main leaf Prx activity enabled the identification of Prx 34 by PMF. qRT-PCR confirmed the high expression of these genes in leaves.

In order to investigate the physiological roles of Prxs 33, 34 and 42 through functional genetics, strategies for the underexpression and overexpression of these genes were designed. Here, we report the work performed for the generation of *Arabidopsis* lines overexpressing Prxs 33, 34 and 42. We performed PCR amplification of the cDNAs with inclusion of new restriction sites used afterwards for insertion of the PCR product in the binary vector pGreenII 235, under the control of the strong 35S promoter. The vector was then used for transformation of *Agrobacterium* GV3101 already harbouring pSOUP, the other component of the binary plant transformation system. Finally, we performed transformation of *Arabidopsis* Col0 plants by floral dipping, and putative transformed seeds were collected, sown, and overexpressing lines were selected.

In parallel, we characterized the phenotype of root growth in seedlings of previously generated overexpressing lines, which actually had shown to suffer cosuppression of the Prx genes. Both 35S lines for Prx 34 and 42 showed root hairs highly reduced or even absent, while the line 35S:Prx 33 showed normal root hairs with a slight tendency to be longer than the wild type.

## Paracrine-mediated osteoclastogenic effects of a Ewing sarcoma cell line

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Ewing sarcoma is the second most frequent bone tumor, usually affecting children and young adults. This tumor is characterized by its low differentiation state and a very aggressive biological behaviour. The main targets of Ewing sarcoma are the pelvis, thorax and long bones, where it often leads to a weakening of bone tissue. In this context, the aim of this work was to evaluate the cellular and molecular osteoclastogenic response of osteoclast precursor cells in the presence of conditioned media (CM) collected from a Ewing sarcoma cell line (91304).

Osteoclastic precursor cells (PBMC) were isolated from human peripheral blood. PBMC cultures were supplemented with CM from the 91304 cell line, collected after 48 hours, 7 and 14 days of culture. The involvement of the signaling pathways MEK, NFkB, PKC, MAPK, JNK and p38 and the synthesis of PGE2 was addressed. Cell cultures were analysed at days 7, 14 and 21 for tartarate-resistant acid phosphatase activity and histochemical staining and the presence of multinucleated cells with actin rings and expressing vitronectin and calcitonin receptors.

It was observed that the presence of CM from the 91304 cell line elicited just a slight increase on the osteoclast differentiation on PBMC cultures, when compared to cultures performed in the absence of any exogenous osteoclastogenic stimuli. The different osteoclastogenic-related signaling pathways analysed revealed important differences in their relative contribution for the observed responses.

In conclusion, this work demonstrated that Ewing sarcoma cells display a small ability to act directly on osteoclast precursors, directing them towards osteoclast development. Data regarding the intracellular mechanisms involved can reveal new insights about the bone metabolic processes affected in patients suffering from Ewing sarcoma.

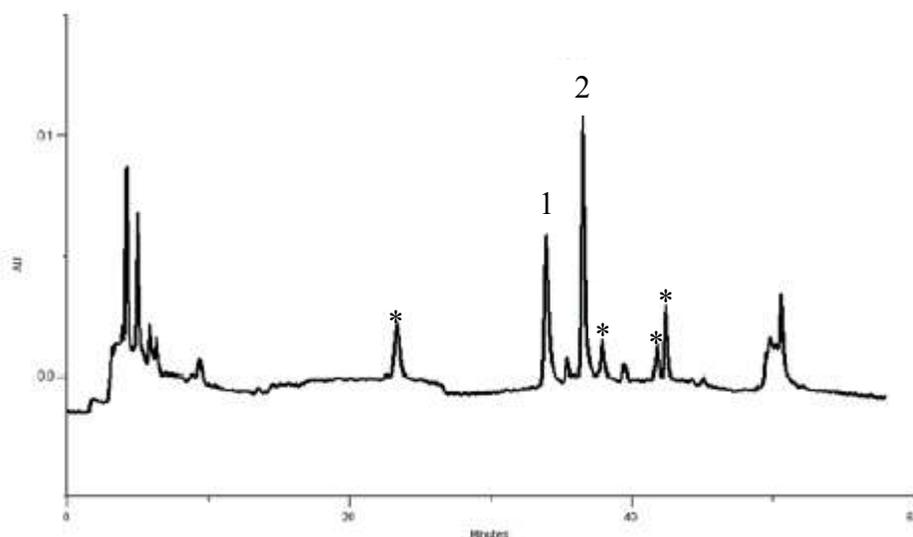
## Perspectives on *Pieris brassicae* larvae: chemistry and bioactivity

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*Pieris brassicae* is an oligophagous insect found in several areas of the world. It feeds mostly on the leaves of Brassicacea plants. In this work aqueous extracts of larvae reared on *Brassica rapa* var. *rapa* were used. A phytochemical study was performed concerning phenolic compounds (HPLC/DAD) and volatiles (GC/MS). Biological potential was assessed by DPPH and nitric oxide scavenging assays. Antibacterial activity was also checked against Gram-positive and Gram-negative species.

HPLC/DAD analysis allowed the detection of both hydroxycinnamic acids and flavonoids in the extract (Fig. 1). Several volatiles were also found, including norisoprenoids, monoterpenes, aromatic hydrocarbons, aldehydes, ketones, alcohols and esters.



**Fig. 1.** HPLC/DAD of *P. brassicae* larvae aqueous extract. Detection at 350 nm. (1) ferulic acid; (2) sinapic acid; (+) flavonoids.

The extract displayed a concentration-dependent effect against both DPPH ( $IC_{25} = 0.122$  mg/ml) and the reactive nitrogen species ( $IC_{10} = 0.020$  mg/ml), but no antimicrobial activity was observed under the tested concentrations.

According to the results obtained, *P. brassicae* larvae seems to be an interesting source of bioactive compounds, to be used in pharmaceutical, cosmetics or food industry.

This work was developed within the optional curricular unit “Bioactivity of Natural Matrices” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

## ***Pieris brassicae* butterfly: an insect with bioactive compounds**

**A. P. S. Oliveira, A. M. Coelho, A. M. Martins, C. I. Cândido, F. Martins, J. G. Louçano, J. P. Mendonça, P. Cecílio and S. Fontoura**

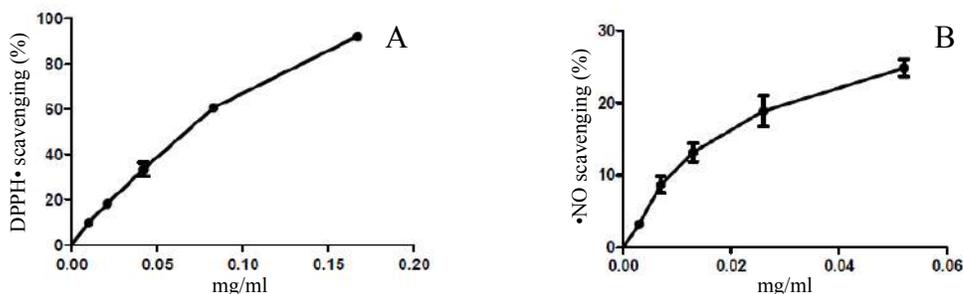
REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

The strict relationship between *Pieris brassicae* (Lepidoptera: Pieridae) and its host plants from Brassicaceae family has long attracted researchers attention. Its life cycle lasts 45 days, from egg to adult, with larvae constituting the cause of great economical losses among *Brassica* producers.

Here we evaluated the chemical composition and biological activity of butterfly *P. brassicae*, which was exclusively reared on *Brassica oleracea* var. *costata* at larval stage. We used GC/MS and HPLC/DAD to assess its metabolic profile, including volatile compounds, phenolics and carotenoids. Antioxidant capacity against DPPH• and nitric oxide (•NO) radicals was also checked, as well as antimicrobial activity against Gram + and Gram - bacteria.

The lyophilized aqueous extract showed no phenolic compound. The volatile compounds identified mainly included aldehydes and ketones, but also alcohols, a monoterpene, a quinone and a norisoprenoid. The acetonic extract revealed the presence of four carotenoids.

The extract exhibited ability to sequester DPPH• and •NO radicals, in a concentration-dependent way (Fig. 1), and showed no antimicrobial capacity up to 2 mg/ml.



**Fig. 1.** Effect of *P. brassicae* butterfly aqueous lyophilized extract against (A) DPPH• and (B) •NO. Values show mean  $\pm$  SE of 3 determinations, performed in triplicate.

Attending to results obtained it may be anticipated that *P. brassicae* butterfly aqueous extract is a source of bioactive compounds, with antiradical capacity, for which it can be exploited by industries requiring this kind of components for their products.

This work was developed within the optional curricular unit “Bioactivity of Natural Matrices” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

## Profiling the kinetics of Matrix Metalloproteinases activity following experimentally-induced myocardial infarction.

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The present study aims to elucidate the functional alterations in the cardiac stem/progenitor cell (CSC/CPC) niche composition under injury conditions, i.e. induced myocardial infarction (MI), including those that occur at the extracellular matrix (ECM) and, in particular, at the matrix metalloproteinase (MMP) activity regulation level.

MI was induced by permanent ligation of the left anterior descending (LAD) coronary artery on C57BL/6 female mice and cardiac-tissue (peri-infarction region) lysates were obtained at 2 and 7 days post-surgery. Sham-operated mice were used as control. Gelatin zymograms were performed under non-denaturing conditions. Briefly, following electrophoresis, gels were incubated at 37°C in a MMP substrate buffer and metalloprotease activity was visualized as white proteolytic bands against a blue background of Coomassie Blue-stained gelatin. MMP activity was quantified by densitometric analysis using the Quantity One software.

Increased MMP activity was detected as early as 48h post-MI although differences were more pronounced at seven days (7d) post- surgery (Fig. 1).

These results demonstrate that following MI induction, MMP2 and MMP9 activity is enhanced, suggesting that these matrix metalloproteases are involved in cardiac remodeling and ECM degradation prior to mature scar formation.

This work constitutes a first draft of the activity profile of MMPs in an experimental infarction setting and the results herein described will be further explored by in situ zymography.

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# Reproduction of *Pomatochistus microps* under controlled conditions

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The common goby *Pomatochistus microps* is a benthic, coastal fish with a short life period [1] that lives in shallow waters from Norway to Portugal and also in some Mediterranean regions [2]. It plays an important role in food chain, being predator of plankton and meio and macrobenthos and a prey of several larger fishes and birds [3]. Their reproduction tends to occur repeatedly during one season from May to August [1]. Usually, males build and defend nests under mussel shells and even rocks, by covering the structures with fine sand [4]. In this particular species male takes all the parental work by not only defending the nest from eggs' predators but also fanning and grooming the eggs. Male care ceases when the eggs hatch [4].

Temperature plays an important role in *P. microps* life cycle, especially on its reproductive period [2]. The main goal of the present work is to determine the temperature effect on reproduction, namely to estimate the temperature range in which gobies have their reproduction increased or at least in which they show more reproductive behaviors, both by female-male interaction and by clutched nests. This way we can determine the temperatures range for the species' reproductive behavior. Furthermore, this is an opportunity to preview at which temperatures males would defend their nests more actively, where eggs and larvae might grow faster and where size broods might be bigger.

Results show that without the appropriate shelters, gobies tend to compete hard for space, occasionally resulting in mass deaths, as much in lower temperatures as in higher ones. This suggests that more places of refuge would be a better choice than free water space, so they can interact without fighting and cannibalizing each other. Territorial behavior was also seen on ostreid shells and clay flower pot for higher temperatures.

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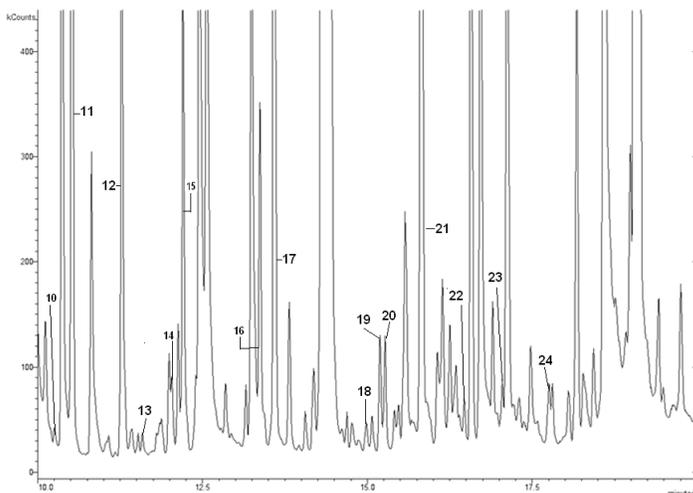
## Secondary metabolites, antioxidant and antibacterial activity of *Codium adhaerens* (Chlorophyta)

M. Taveira, A. A. Q. P. Cardoso, A. P. Machado, A. S. Araújo, D. P. Oliveira, L. Boaventura, M. F. Palha, M. J. Silva and T. M. Constantino

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The potential of marine products has attracted the attention of researchers for some time. Inspired in the expanse of the oceans and on the tremendous biodiversity they present, the pharmacological potential of secondary metabolites in marine organisms has been enthusiastically explored. Our study aimed to determine the chemical composition (phenolic and volatile compounds), as well as to evaluate the antioxidant and antimicrobial potential of an aqueous extract of *Codium adhaerens*, a Chlorophyta macro algae species usually found in the intertidal zone, covering rocks, under reduced light exposition and intense water flow.

The volatile compounds profile was determined by GC/MS. The twenty-nine identified volatiles (Fig. 1) arise from several chemical classes: alcohols, aldehydes, ketones, aromatic hydrocarbons, mono and sesquiterpenes, norisoprenoids, among others. Saponosides were also detected, but no phenolics was identified by HPLC/DAD.



**Fig. 1.** GC/MS chromatographic profile of *C. adhaerens* aqueous extract (10-20 min scan). (11) octanal; (12) 2-ethyl-1-hexanol; (13) 3,5-octadien-2-ol; (14) 2,4,4-trimethyl-cyclohexen-1-ol; (15) (*E*)-2-octenal; (16) linalyl anthranilate; (17) (*E*)-2-nonen-1-ol; (18) (+/-) camphor; (19) menthone; (20) (*E*)-2-nonenal; (21) (+/-) menthol; (22) safranal; (23)  $\beta$ -ciclocitral; (24) 1,3-bis-(1,1-dimethyl)-benzene.

The antioxidant activity of this species was checked against DPPH<sup>\*</sup> radical and nitric oxide (NO, a reactive nitrogen species), but only a reduced effect was noticed against NO. No antibacterial capacity was observed against the tested Gram-positive and Gram-negative bacteria.

This work was developed within the optional curricular unit “Bioactivity of Natural Matrices” of the 5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Paula Andrade and Patrícia Valentão.

# Seroprevalence of a novel Canine Norovirus in pet dogs

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Noroviruses (NoV) are today recognized as the leading cause of gastroenteritis worldwide among persons of all ages [1]. They have a wide degree of genetic variability and are classified in 5 genogroups (GI-GV) three of which (GI, GII and GIV) contain human viruses [2]. The most important modes of transmission are person-to-person contact and consumption of contaminated food, however, a zoonotic transmission has been suggested as a possibility due to the close genetic relatedness between human and some animal NoV [3]. Recently, our group identified in Portugal a novel canine NoV (Viseu strain) that was genetically unrelated to any other animal or human norovirus known [2].

In order to evaluate the prevalence of antibodies to the novel canine NoV in dogs we developed and validated an enzyme immune assay (EIA) based on recombinant virus-like particles (VLPs). The VLPs were produced in Sf9 insect cells infected with a recombinant baculovirus containing the capsid protein gene (VP1) of the Viseu strain.

Serum samples were obtained from 309 dogs from Portugal and 61 dogs from the USA. Sera from 8 specific pathogen free (SPF) beagle dogs were used as negative control. Each serum sample was tested for the presence of IgG antibodies against canine NoV (Viseu strain) using a direct VLP based EIA. A serum was considered positive when the optical density (OD) value was higher than the cutoff (mean of negative control serum OD + 3 standard deviations).

Overall, 226 (73%) of dogs from Portugal and 51 (83%) of dogs from the USA tested positive for IgG against the Viseu canine NoV. The age of the animal was identified as a risk factor, with young dogs (< 6 months) having a 16 fold increased odd of seropositivity to canine NoV (aOR=15.82, P<0,001).

The high canine seroprevalence found in the present study indicates that infection with this novel canine NoV strain is common among dogs of Portugal and of the USA. Given the intimate contact of dogs with humans, this virus may represent a potential zoonotic risk.

**Acknowledgments:** To Fundação para a Ciência e a Tecnologia for the grant SFRH/BD/45407/2008 to J.R.M. To Dr. Jan Vinjé, CDC, Atlanta, USA for the generous provision of the VLPs.

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## ***Solanum nigrum* L. as a tool to remediate Cr(VI)-polluted sites?**

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Phytoremediation consists in the use of plants to remove, inactivate and/or mitigate organic and inorganic polluting compounds present in soil or water. Chromium(VI) is one of the most harmful ions to living organisms and it is recognized as an human carcinogenic [1-3]. Therefore, its introduction into the environment should be avoided and its presence should be removed as far as possible. The objective of this study was to evaluate the toxicity of Cr(VI) on *Solanum nigrum* L. plants and their potential for the phytoremediation of Cr(VI)-contaminated areas. Plants were grown in a vermiculite:perlite (2:1) substrate, supplemented with a commercial liquid fertilizer for one month. Then they were divided into two groups where the control's nutrition was not altered and a shock treatment was performed by supplementing with the same fertilizer added with potassium dichromate to a 250 ppm final concentration. This treatment lasted for a week. Shoots from the Cr(VI) treated plants suffered significant reductions in fresh weight (54%) and in height (28%). Other measurable parameters like shoot dry weight, root fresh and dry weight and root length were not affected by exposure to Cr(VI). This study demonstrates that 250 ppm of Cr(VI) can be toxic and that the effects of such toxicity can be specific to different organs of *S. nigrum* plants. These data also suggest that *S. nigrum* may not be a good candidate for the phytoremediation of Cr(VI)-contaminated areas, but further studies, such as Cr quantification in plant tissues, will be performed in order to better understand the effects of chromium on plant physiology.

Acknowledgements: The authors gratefully acknowledge the Universidade do Porto for financial support (Project MetalloChromium), with the contribution of Santander Totta.

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# Study of cardosin B Plant Specific Insert in protein processing and transport to the vacuole

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Cardosin B is an aspartic proteinase (AP) first isolated from cardoon (*Cynara cardunculus*, L.) pistils. This AP is known to be secreted in the native plant flowers, accumulating, however, in vacuoles when expressed in *Nicotiana tabacum* leaves. The PSI (Plant Specific Insert) domain is quite common among plant APs and it is thought to possess other functions than vacuolar sorting within the cell, such as interactions with cellular membranes or defense mechanisms. However, regarding the maturation and trafficking of cardosin B, the function of PSI is still uncertain. Thus, as an attempt to uncover the real implications of PSI in this matter, several mutagenic PCR techniques were performed in order to obtain mutant cardosin B constructs: cardosin B with mutations in PSI's cleavage sites and cardosin B with cardosin A's PSI. Constructs were expressed in transiently transformed *Nicotiana tabacum* leaves and protein extracts from leaves and vacuoles were analyzed by Western Blotting. Furthermore, a fluorescent protein (mCherry) was incorporated in one of the constructs, allowing its sub-cellular localization and tracking by confocal laser scanning microscopy (CLSM). At this point, results show that cardosin B sorting was not affected by the mutation in the cleavage sites of the PSI. The transport to the vacuole of this truncated form was, however, slower than in the non-mutated form. When the PSI was replaced by cardosin A's PSI, the sorting of the AP was not affected, though the processing showed to be belated. Nevertheless, further studies are still necessary in this matter so as to unveil the role of PSI in the sorting and processing of APs.

# Study of the effect of copper in planctomycetes

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The Earth is a complex system that integrates different ecosystems and reactions between them, as well as several and varied organisms. Most part of these ecosystems is marine (1), so it is important to better understand the marine organisms, their metabolisms and interactions with the various elements. Copper is one of those elements essential to life by integrating many metalloenzymes, proteins, and intervening in other important reactions (2). However, because of its condition of heavy metal, copper can be responsible for many cells damage (3). It is known that heavy metals pollution in coastal zones is a worrying problem nowadays. Metals are accumulated preferentially in sediments, and since much of the benthic community is bacterial, the latter have a key role in the fate of these compounds. In our work we are aiming to study the effect of copper in a species of planctomycetes, strain LF2, isolated from the biofilm community of *Laminaria* sp. collected in a polluted environment, Foz, Porto. Several assays have been done to optimize the medium to perform the experiments and be able to assess the levels of available copper to the cells. The behavior of the cultures is evaluated in exponential growing phase after addition of increasing levels of copper. Strain LF2 seems to present a great resistance to copper.

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# Study of the involvement of arabinogalactan protein 1 (AGP1) on pollen tube guidance in *Arabidopsis thaliana*

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During sexual plant reproduction, the general pattern of development that leads to gamete formation is mostly clarified, but little is known about the molecular mechanisms that regulate the transition of the sporophytic to the gametophytic type of development. All steps of the complex process which is the pollen grain development into the pollen tube and the efficient way it reaches the target cells in the embryo sac are dependent on a complex network of signalization events still undefined and most likely involving molecules of different types. Arabinogalactan proteins (AGPs) belong to a family of hydroxyproline-rich glycoproteins, which structure indicates important functions on both vegetative and reproductive growth and development as well as in programmed cell death. Studies with selective marking obtained with monoclonal antibodies directed to the AGP's glycosidic parts have pointed out the involvement of some AGPs or of their sugar residues during the gametophyte development and during the pollen tube guidance into the embryo sac, at least on the final part of its pathway, the arrival at the embryo sac [1]. Knowing that the AGP1 has been proved to be present in the pollen tube [2] as well as in the female gametophyte [3], the main objective of this study is to learn more about the AGP1 function, in one of the most fundamental stages of sexual plant reproduction, the pollen tube guidance into the embryo sac which precedes double fecundation. In order to do so we propose to start by studying the phenotype of two mutant lines for AGP1, SALK\_149861C and SAIL\_247\_H01. The homozygous will be checked by RT-PCR and sequenced. We also propose to study the role of AGP1 in attracting or guiding pollen tubes and for that the AGP1 gene will be amplified through PCR using properly designed primers, and constructions will be made using the Gateway technology vectors with the 35S promoter to transform cells over expressing AGP1 and evaluate the attraction capacity of these cells *in vitro*.

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# Use of ectoparasites of Chub mackerel, *Scomber colias* and Atlantic mackerel, *Scomber scombrus* as biological markers

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Chub mackerel, *Scomber colias* Gmelin, 1789 and Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758 (Scombridae) are two fish species inhabiting the northern coast of Portugal, with economical importance for fisheries. The knowledge of the parasitological fauna of fishes from the northern coast of Portugal is still scarce, in particular that of these two species of mackerels, which bear great morphological similarity. The present study aims to use ectoparasites as biological markers to easily distinguish *S. colias* from *S. scombrus*. To accomplish this objective, 40 *S. colias* and 42 *S. scombrus* were analyzed, and their ectoparasites collected and identified. In *S. colias*, 4 species of ectoparasites were observed: *Pseudokuhnia minor*, *Grubea cochlear* and *Kuhnia scombri* (Monogenea) as well as individuals of the family Gnathiidae (Isopoda), the latter described for the first time in this host. *P. minor* had the highest prevalence (97.5%) and mean abundance ( $23.95 \pm 22.34$ ). In *S. scombrus*, 6 ectoparasite species were recovered: *K. scombri*, *K. sprostonae* and *G. cochlear* (Monogenea), *Caligus pelamydis* and *Clavellisa scombri* (Copepoda) and an individual of an Aegidae (Isopoda). *C. pelamydis* occurred with the highest prevalence (69.0%) and mean abundance ( $3.12 \pm 4.33$ ). Comparing the parasitological parameters (prevalence, P, and abundance, A) among the two host species, several significant statistical differences were found. This allowed us to consider 4 ectoparasite species as good biological markers to distinguish *S. colias* from *S. scombrus*: *C. pelamydis* (recorded only in *S. scombrus*, with high prevalence values), *P. minor* (present only in *S. colias* with very high prevalence and abundance values), *G. cochlear* and *K. scombri* (recorded in both fish species, but being more prevalent and abundant in *S. scombrus* – P= 28.6% and 66.7%, A= 0.38 and 2.00, respectively - than in *S. colias* – P=2.5% and 17.5%, A= 0.03 and 0.53, respectively).

## Acknowledgments

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# Vacuolar function regulation by Isc1p in *Saccharomyces cerevisiae* – consequences in cell death induced by oxidative stress and aging

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Sphingolipids are important structural components of cell membranes and their metabolites play important roles in cell signaling, being involved in cellular processes such as endocytosis, exocytosis, mitochondrial function, oxidative stress and aging [1].

An increase in the cellular levels of the sphingolipid metabolite ceramide has been associated with apoptosis, cell cycle arrest and cellular senescence. Ceramide can be produced via *de novo* biosynthesis pathway or through the hydrolysis of complex sphingolipids. In *Saccharomyces cerevisiae*, the inositolphosphosphingolipid phospholipase C (Isc1p), the yeast orthologue of mammalian neutral sphingomyelinase 2, is the only enzyme known to be responsible for the hydrolysis of complex sphingolipids. Cells lacking Isc1p display mitochondrial deficiencies, hypersensitivity to hydrogen peroxide and a shortened chronological lifespan. These phenotypes were correlated with iron homeostasis since *isc1Δ* cells exhibit an iron overload[2]. Moreover, the deletion of *SIT4*, which encodes the catalytic subunit of the ceramide activated protein phosphatase 2A, suppresses H<sub>2</sub>O<sub>2</sub> hypersensitivity and the shortened chronological lifespan of *isc1Δ* cells.

The vacuole, yeast organelle analogous of the mammalian lysosome, plays a key role in iron homeostasis and protein turnover. In this work we have been studying the role of the vacuole in oxidative stress sensitivity and shortened chronological lifespan of *isc1Δ* cells. The proteolytic function of the vacuole was assessed measuring the activity of the vacuolar Pep4p protease [3]. Our results show a reduction in the activity of Pep4p enzyme in *isc1Δ* cells that is suppressed by *SIT4* disruption. The decrease in Pep4p activity led us to study the Pep4p localization and vacuole morphology. For this, cells were transformed with a plasmid expressing a GFP-tagged Pep4p protein and incubated with a fluorescent probe FM4-64 that labels the vacuole membrane, and cells were observed by fluorescence microscopy. Pep4-GFP signal co-localized within the vacuole in the parental and *isc1Δ* cells indicating that Pep4 activity decrease is not due to Pep4p mislocalization. However, we observe an aberrant morphology of the vacuole in *isc1* cells, which is characterized by over 20 vacuole-like compartments per cell randomly distributed, in opposition to the big central round vacuole in *sit4Δ* and *sit4Δisc1Δ* cells and the cluster of 3-10 subcompartments observed in the parental strain.

The overall results suggest that Isc1p has a key role in vacuolar function and biogenesis and Sit4p-mediated vacuolar dysfunctions may be involved in oxidative stress sensitivity and shortened chronological lifespan of *isc1Δ* cells.

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**U. PORTO**

*Posters II*  
*Friday, February 18<sup>th</sup>*

# Artistic intervention in the city of Porto

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*Non-Places, Introduction to an Anthropology of Supermodernity*[1], by Marc Augé, raises important issues around the changes of men and cities in the last century. Regarding the problem of anthropology, the author analyses the phenomenon that changed the behaviour of modern man. In this book, the author tries to demonstrate that there are recent spaces - spaces of transit and consumption (non-places) - that have no reference to the past.

The concepts developed by the author are controversial and inquisitive, making the reader assume a reflective and critical position. From reading this book, I have developed my own ideas of fighting the acculturation shown by the anthropologist, in response to Marc Augé awakening questions.

Students of Fine Arts have image as a tool for action. A power, if well used, can be very effective. In this sense, I have conceived a form of intervening art in "anthropological places", which still exists in the city of Porto, in contrast to non-places. In support of this action I have chosen the tile, since it is the predominant element in these anthropological places (Igreja do Carmo, Igreja de Santo Ildefonso, a typical house of Foz Velha, São Bento train station, the exception being Casa da Música). The tile in this case represents a symbol of Portugal's history and an authentic reference to its people's identity. In this way, I created a *pastiche* of the ancient painting technique of narrative tile panels, introducing the theme and imagery of "non-places". The purpose of this intervening art is to cover "places" that often present themselves to the city dweller as a symbol of belonging, with paintings that represented the contrasts of "non-places", closer to reality and the new membership of the contemporary individual.

What if our places and non-places would mingle in the way that I propose? What strangeness would this cause the individual in the course of their daily activities, if confronted with another image of himself? What impact would this strangeness cause to his own image of the city and himself?

References:

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# The Role of Design in the new emerging space industries: New Opportunities for Portugal's Industrial Design

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Since the very beginning of time mankind have always been curious about the possibility of living in “the sky”. First there were detailed descriptions idyllic gods and their lives until a new age of knowledge brought science fiction.

Today industrial design in space industries is still seen as a science fiction related unnecessary luxury however, with new growing industries like space tourism this kind of thinking is starting to change. We now look at an emerging market where much is still to explore in matters of innovation.

The purpose of this presentation is to motivate designers and industrialists to engage in a new type of market that much like the beginnings of aviation promises to offer a revolution not only in economics but also in technology, psychology, physiology and most of all sustainability.

Design is not a luxury it is a reflection of the needs of a specific culture in a specific time. With this in mind, the presentation will follow a path starting with the analysis of past experiences as the basis for the reflection about the role of design in space exploration. Then some practical examples will be shown hoping to achieve a clearer view of a still very closed industry.

Some sociological conclusions can be withdrawn about the evolution of the space vision as a collective imaging and how the public interacts and understands these new concepts.

At last some data about Portugal's involvement in hi-tech engineering projects and design innovation and a brief conclusion about future opportunities in these fields.

# Learning Spaces

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Learning Spaces is a critical analysis about the contemporary learning, social and cultural spaces that are emerging in universities. This research focuses specifically on emergent learning spaces that are shared by the different departments of a university campus, and it concentrates on studying the architectonic or spatial characteristics that facilitate and promote the learning process as form and configuration, texture, acoustic and other characteristics alike.

Accordingly, a set of studies related to the learning processes were analyzed critically in order to identify in them the presence of certain activities considered significant within this process. Then the authors tried to understand how these activities would be encouraged and reinforced by certain spatial characteristics of those learning spaces: program, size, interrelation between areas and atmospheric parameters (light, sound, air quality, temperature, and smell).

The capacity to learn and attain certain knowledge is a capability inherent in every human being: everyone learns. Even though we all may have different learning intervals, we are all capable of achieving physical, emotional, social and intellectual competencies that evolve throughout our life. All these different competencies are interrelated and complemented by each other. So, for example, when we think of a Learning Space that aims to strengthen and encourage the intellectual work produced in university, all the other dimensions shouldn't be dismissed. The aim must be to include and explore activities that allow the growth of the students in the largest sense possible so that a real formative effect on the mind, character and physical ability of an individual takes place. This learning process must be a means by which knowledge, skills and values from one generation to another are effectively communicated and understood. The student centered learning theories, which are now believed to be more efficient, believe that undergraduates should be allowed greater flexibility and choice of their study curriculums, have the possibility to engage in different studying activities and to make a varied and dynamic use of university spaces. Thus, it is very important that universities offer spaces with height levels of comfort and capable of multi-purpose uses in order to guarantee their performance across student's multiple needs and learning tasks.

Important case studies for this work that allowed the analysis and monitoring of the first learning space of University of Oporto and the design of a new E-Learning Space Café where: (1) The E-Learning Café Asprela UPorto (2008) and (2) The E-Learning Café Botânico U. Porto.

The main conclusions of this work are first that Learning Spaces should support not only the specific teaching and studying exercises, but also other activities related with our physical, emotional, social and intellectual competencies. Second, that interactive and communication technologies are now part of the basic infrastructures and that these digital artifacts should be the result of a complex network of spaces offering different levels of visual and acoustic privacy and formal characteristics.

# [Cell, Crystal, Level, Leaf] The inhabiting in the “*interseccionisms*” of the social space and the approach to new tools of a projective thinking: the relational transition.

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The reality of the anonymous and common architecture, servant of the big number of people, moves over from the richness of the individual-architecture interaction, as it's approaching to the dense context with the consumerist mentality.

The present dissertation consists on a personal search of a projective thinking, that is ruled by relational precepts, looses itself from the formal content of the architectonic space in order to search, beside the seen architecture, the felt one. In this way, the space is decomposed in limit, its materiality and its consequent social component [1], the domain.

Cell, crystal, level and leaf are four symbols used to decode spatial circumstances, more precisely circumstances of the inhabit atmosphere's threshold, existing on the identified as social space's “interseccionism” [2].

In this threshold, the “reality's interseccionism” of the poet's vision crosses with the relativity's one, defining the circumstances of the architectural atmosphere. The well known dualities interior – exterior and private – public are no more than relativities on this complex system, fact that makes them valuable tools of work in this reflexive point of view. Beside that, the change of its associations shows the four selected situations of “interseccionism” corresponding, each one of them, to different attitudes of space producers, the architects.

The four symbols clarify the sequence of the different interseccionisms, pointing to the searched projective thinking, in an approach to opening inhabiting atmospheres to the exterior (improving its qualities) and, at the same time, working with transitions between the domains.

In the end, the experience of the quotidian spaces by the individual must be constructed not as a sequence of contrasting realities but on a transition of spatial circumstances through the formal and social articulation and gradation.

The notion of space transitions, connected to the continuity of the quotidian course of the habitant, must be linked to the notion of relational articulation, in behalf of a projective attitude that is coherent with the search of the richness of the architectural atmospheres. The pursuit of relational transition passes through the accentuation of the “interseccionisms” in order to reinforce the balance between different scales, circumstances and relativities.

From the precept and the project the tools presented must be seen as opportunity to have a more fresh project experience that, working with the form, isn't conditioned by formalisms and that, in the projectual visiting, doesn't neglect the importance of the singularity of each individual's experience.

[1] Concept explored by Henry Lefebvre in: Lefebvre, Henri (2000), *La production de l'espace*, Anthropos, Paris.

[2] Concept created by Fernando Pessoa, expressing the intersection of realities.

# To (in)form project arousers. Gleaning and duration. Bernard Rudofsky, Aldo van Eyck, Lina Bo Bardi: Landscape, Street, Play

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Resulting from the uneasiness about the exercise of the architect's profession, in a contemporary frame that bends to consumers' principles and to the cult of the image, it is intended with this study to find an equilibrium, the balanced reflection of the ideals to be executed that may accompany the emergency for a definition of patterns, for fear that one shall not yield to a numb progress or to the persistence of an outdated past.

The present thesis consists of a search for project arousers, the definition of strategies to be followed in the processes of architectural production that may certify integration, interaction and play. For that purpose, the scales of landscape, street and components were considered. These project arousers are seen as essential for the construction of an identity, appropriation and relationship between man and the world.

Gleaning has been identified as a way of observation, of (in)formation about what a project posture can be. That is how observations, local research, memories and the works of architects considered references have been compiled – information which has been gathered, reorganized and re-evaluated, in a pre-project task.

Resulting from the approximation made along the enunciated methods, the conclusions concern to 3 main themes, in order to embrace the totality of the projective thinking:

Landscape: the search for integration – an intrinsic relation with nature that is achieved by symbiosis and not mimicry, assuming the autonomy of architecture. Permeability to phenomena, contact with the ground, framing of the sky and scaling man are fundamental in the construction of active contemplation laboratories, regarded as workshops to reach the living duration;

Street: allows interaction – the street as element of reference and articulation in the urban fabric that must be varied and animate the walking experience. The in-between spaces acquire the value of transition, the interstices where happenings are convoked and social life flows, provided by series of alleys (stages for occasions) which gain meaning in their proximity and in the network they form together;

Components: provide play – the possibility of a playful universe where fantasy contaminates the house and the city as responding to the human needs of liberation. Marginal and banal things, by the means of subversion, acquire some sacredness and recreate daily experience, in the construction of gears-spaces that promote action-reaction, incorporating stimulus for the reinvention of the normalized environment.

A dialectic is pretended in the interaction architect-inhabitant. The intent and porous stare are exercised to alert to existing durations, at time, to reach place instead of space, occasion instead of time. Timeless quality is pretended, where time is suspended and duration represents the density of the present. To project the duration is to desire the synchronic place, the inscription in a contemporary setting, proposing the recycling of “the look” in order to the reinvention of “the making”.

# Take-away architecture

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Increasingly, concepts such as ephemeral and transportable architecture are more considered and developed in architectural research, looking for a “global house”, some kind of modular system that might be applied in any place, under any conditions: meteorological, geographical, cultural, etc.

The purpose of this research is not to be another one looking for that “ideal solution”, but to look beyond for the consequences that such concept would imply in the world as we know it.

It will not take long until the architecture’s tradition to build for the place will be lost, being replaced by a settlement of any depersonalized modules or containers’ housing, crowding the city like a virus, which, like any other, will quickly spread destroying everything around it without any order or reason, just for his own survival.

What will then happen to the cities, their spaces and their experiences? Will walk in cities’ centers, like the Porto’s world heritage historical center equal to stroll around the loading and unloading Leixões’ docks?

What will be the role of the architect if people could go to a retail outlet and buy his home with an instruction booklet “do-it-yourself” style?

Is this "Lego" city an evolution as it is indeed considered, or analysing it more deeply we come across a case of inevitable regression?

Is there a new era of nomadism on a worldwide scale approaching our times? There won’t be a home, a place with its own history that holds the Man to his roots but instead, a standardized concept of cities that may be born or die by the human hand without any kind of stability or expiration date.

Aren’t we regressing to the times when, some on foot, others with carrying animals, were loaded with the supplies they could find in a place and proceeded to the next unknown destination? The difference nowadays, is that we limit ourselves to meet the weight restrictions of a low-cost airline, on arrival at whatever destination we get to, search the architectural "IKEA", and for a negligible cost we can get our cubicle. The question that arises then is: where to put that cubicle?

The growth of the "stacked cities" hardly will be able to be ruled by an expansion pattern as the predetermined allotments we have nowadays. Won’t this transition provoke a sudden spatial anarchy that when people would notice, even the city's public spaces would be occupied like if they were a summer festival camp?

Today the architect thinks it is his role - the design of these flexible modules, transportable, and above all, sustainable! What will happen in the future to the same architect who invented them? Of course he will no longer be necessary since he standardized architecture to such a level that turned it into a franchising, and soon he will see his city abandoned because of his own invention, since their cherished self-contained modules allowed people to move and install their “houses” anywhere in the world, never coming to settle in a place since nobody will have reasons for it. We are speaking of a migratory man in a worldwide scale.

# Vista Alegre porcelain factory: an example of sustainable architecture

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The present paper gives notice of my master thesis in Architecture<sup>1</sup> that investigated the built environment of the Factory of Vista Alegre. Our work tries to inscribe this particular Portuguese urban complex in the context of theory and history of architecture by revealing the implications of architectural and productive utopia in urbanism and in society. It underlines the “intentions” that made this entrepreneurial initiative unique, in the domain of the production of ceramics and glass. In order to demonstrate the architectural relevance of this factory site, we researched the cultural context of the foundation of the factory, dating from 1824, and we identified the theoretical and practical foundations that may have affected its building up from a three-fold perspective: architecture, urbanisation and planning.

First hand and documental sources were dominant material of our research while an intensive activity of architectural survey of the urban growth, at the levels of street, building type and samples, was necessary because no accurate data was available at day, regarding previous studies of the case, and each street corresponds roughly to a specific period characterised by well identified and visible elements.

The study of the urban design and development processes showed different evolution phases of the neighbourhood of Vista Alegre. Periods of expansion correspond to specific projects that seem to point towards a recurrent transformation and modernization of the space.

The manufacturing site of the Vista Alegre arises as a sustainable and autonomous space. The urban structure promotes the equilibrium between the constructed area and its environment as well as the relation between the individual and the society.

European similar cases such as Sevres, New Lanark, Guise and Bois-du-Luc were personally visited. From the comparison with other urban complexes of reference we can observe the importance attached to the human dimension of the set, in Vista Alegre. The homogeneity and bucolic beauty of the place inspires the artists and has the ability to help fixate the craftsmen in the area, away from other population centres. Essential components of education and culture stand out, although the social stratification is most evident in Vista Alegre.

From the architectural point of view, the Vista Alegre compound, is markedly Portuguese and adopts innovative features that blend with the traditional image which still holds today.

The private and public recognition of these values should guarantee this urban compound its preservation as one of the rare “living” examples both of architectonic patrimony and of the material Portuguese culture of porcelain. Our work aspires to be a positive contribution for this purpose.

<sup>1</sup>This dissertation was supervised by PHD Arch. Maria T. Fonseca

# Recent Interests of our Architectural Community: A reflexion based on the themes chosen for Provas Finais at FAUP

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This paper presents a study [1] on the multitude of subjects and areas of interest brought into architecture by its community, both students and professionals, during the last decade (1999-2009).

This study is divided in two parts with different methodologies.

The first part is based on existing bibliography about the discipline of architecture in the academic context. It intends to give the reader a specific background on the subjects of research work in the university (focusing on the architecture schools), the interdisciplinary condition of contemporaneity and the importance that schools of architecture have on transmitting specific knowledge to future professionals.

The world we live in today, a stage for social and cultural mutations, is constantly changing and evolving. Architecture, as an intervenient agent in this reality, has to adapt itself for a perfect synchronization with the surroundings. Therefore, it suffers interferences, including and absorbing a diversity of other discipline's knowledge. In this context, professionals and students of architecture frequently feel the need to extend the borders and the *modus operandi* of the discipline.

The second part of this study focus on the research works carried out at FAUP in the last ten years, using as study-case the final work "Prova Final" submitted by the students to complete their studies. This second part intends to show the diversity of themes chosen by each student for each research, a possible demonstration of how transversal and ample may be the architecture practice today.

Limiting the field of study to the period of 1999-2009, a catalogue with selected information of every "Prova Final" was made. The information gathered includes the names of author and teacher supervisor, a miniature of the cover and the index; but most importantly, we did an effort to provide a brief description of the object and objectives of each research, as well as its methodology and structure. Using this information, we were able to classify all study-cases according to their main theme and subthemes (previously determined), in order to analyze the interests of our architectural community and the evolving tendencies of the research fields followed by the students in ten years.

In the end of this survey, many results are expected. From mere graphics showing the diversity of topics chosen and those most/less used as research field, to a reflection on the interdisciplinary interests of the architectural community, based on a systematic illustration of this multitude.

[1] This study is being carried out by Luis Lima in the context of his master dissertation, with the orientation of Prof. Gonçalo Furtado (FAUP).

# School Canteen Design

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The design of a school canteen shall be subject of detailed study regarding its functional activity, and shall consider several factors such as legal aspects, production technical requirements, architectural and working conditions as well as economic considerations.

The objective of this study was to systematize the information needed to design a school canteen, with meals served at school but also prepared for the elaboration of meals to be transported to another schools, covering all aspects of its functioning in order to achieve the optimal working conditions for the employees and the best hygienic conditions for the food elaboration.

In addition to the literature review there were analyzed some architectural projects of school canteens and have been done some technical visits to allow checking on-site compliance with the legislation, the operational suitability of the equipment as well as working areas and materials used.

From the analysis it was found that there are numerous structural nonconformities that affect compliance with the good food and hygienic requirements which could be avoided if, at the design stage of the project, these factors have been considered.

Taking into consideration all the “best practices” obtained through the research done within the present work, it has been done one “optimum” school canteen project where all the analyzed factors were considered and that can serve as reference for future works.

# Vitruvius' Models - Drafting of "Kit" with material about ancient Theaters and Temples prescribed by Vitruvius in his *De Architectura*

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The study, linked to the Laboratory of three-dimensional models of the Faculty of Architecture and Urbanism of the USP, suggests, from the reading and interpretation of the treatise *De Architectura*, written by Vitruvius in the first century BC, an elaboration of didactic material designed for beginning students in undergraduate Course of Architecture and Urbanism, in order to stimulate knowledge through years of design and construction of models.

In place of simulating famous buildings of the Roman Era on a smaller scale, the aim of this paper is to develop models that, during assembly, may enable the student to understand the accurate proportional relationships and geometric composition at work in architecture.

Coupled with the development of physical models, were also treated to the unique attention of Vitruvius and extensive research to copies of theaters and temples that still exist with a view to prepare a graphic design consistent with its requirements and that, moreover, reach a deeper level of detail, he intended to cover the minutiae concerning about composition of ancient buildings. Thus, we generated plans and elevations that culminated in an elaborate assessment of the specific theater and ancient temple. Grounded in these designs, we proposed a virtual model able to ratify in three-dimensional language, all details relating to each element of the Roman Theater. It was possible to investigate details of access, commensurability of order (Doric and Ionic), and facade composition, among others. The following process approached the design of physical models which refers to the assembly process, the main objective of the research. That is, models whose assembly forms conducive to learning as giving to do the procedures observed by the ancients in the design of their buildings.

All the work had an excellent reception and performance. From the point of view that has taken different and complementary procedure of work, the whole study led to the apprehension of new knowledge in different areas. It was possible to advance the capabilities of research and analysis of information, a fact that occurred during the examination of the treatise *De Architectura*, especially when comparing different authors and interpretations.

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# A Empirical Investigation of the Main Determinants of Private and Manufacturer Label Purchases

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The competition between store and manufacturer brands has increased in Portuguese stores during the last decade. Given the increased penetration of private brands, it is of utmost importance to study the determinants of their adoption by consumers.

This work was developed with the aim of studying and evaluating the main determinants of the consumer choices between private and manufacturer labels for 16 categories of commodities, which are part of everyday life of most consumers: milk, toilet paper, oil, wine, water, cookies, rice, yogurts, breakfast cereals, tea, juice, cheese, butter, coffee, cleaning and frozen products.

The literature review revealed the existence of a large number of determinants for the adoption of the manufacturer brands or private brands, which were organized into the following groups: perceptual factors, psychographic factors, behavioral factors, demographic and socio-economic factors.

In terms of methodology, a structured questionnaire was designed and answered by a convenience sample of 200 consumers during 2010.

The results highlight a strong penetration of the private brands in consumer purchases. It was also found that the influence of perceptual, behavioral, psychographic, demographic and socio-economic factors differ across the considered product categories. Indeed, we conclude that these determinants act on the choice of private and manufacturer labels, but according to the product purchased. This study also revealed that the main determinants of the number of product categories in which consumers acquired the private brands are the perceived quality of private brands, risk aversion and the budget constraint.

# Democracy in Times of Crisis

Igor Cardoso

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The liberal Benjamin Constant, during a discourse pronounced in *Athenée Royale de Paris* in 1819, said: “Il résulte de ce que je viens d'exposer, que nous ne pouvons plus jouir de la liberté des anciens, qui se composait de la participation active et constante au pouvoir collectif. Notre liberté à nous, doit se composer de la jouissance paisible de l'indépendance privée.” His words consecrate what would be imposed during the XIX century in Europe as the hegemony of a representative system through the liberal ideas. The struggles during the French Revolution have made clear to the French politician that a direct participation democracy, as it existed in classic Athens, was no longer possible in the modern times.

This way, little by little, at the ending of the 1800's, the workers, through syndicates and political parties, sought in the representative regime the realization of some of their claims. While in some countries, like England and France, the incorporation of some labour laws was considered as a caution measure to the sustainability of the liberal regimes, in others, like the Post-Bismarck Germany, differently, the liberals saw themselves pressured by the political advances of the socialists. After the depletion of the economical and social structures, besides the strong psychological fatigue, with the Great War, Europe found itself in real disillusion with the political alternatives presented until then.

Nevertheless, as well as the circumstances in which the social and political conditions changed differed in the various European countries, the reactions of the liberals were also different towards the new experienced catastrophes and the growing adhesion to the communist ideas. However, despite of the different reactions cited above, my research seeks to understand, in general way, how it was possible that some liberal regimes could converge, at the political ideas level, with the emerging authoritarian regimes beginning in the 1920 decade, whether they are classified as fascists or not.

To do so, I seek to analyze the mainstream ideas of the scholars of the classical culture, in the Interwar Period, regarding the Athenian democracy, keeping in mind the adopted perspective towards the participation of the masses and the social equality of the ancients. Some names, among others examined, are those of Glotz, Guiraud, Wilamowitz and Pasquali.

At last, I sense that the study about representatives regimes, the emerging and fragile democracies and the outbreaking of authoritarian regimes in a period of crises, is relevant for the current times, when we live again the discredit of the traditional political institutions, verified in the decline of popular participation and in the belief of solutions that are more individual for social problems.

# Detection of biotech maize event MIR604 in processed foodstuffs

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Despite the controversies surrounding genetic modification technology, the production of genetically modified (GM) crops has increased during the past decade, especially in developing countries. In fact, biotechnology has revolutionized agriculture whereas it is being applied to the development of GM crops with advantageous characteristics such as resistance to herbicides, pests and diseases [1]. In 2009 the global area of biotechnological crops reached 134 million hectares, from which 25% corresponded to GM maize [2]. Maize is the second most important GM crop with the highest number of authorized GM events (22) for food and feed in the EU [3]. The EU legislation demands the labeling of food products containing more than 0.9% of GM material (Regulation (EC) no. 1829/2003), while for unapproved genetically modified organisms (GMO) the tolerance threshold is 0.

Most methods for GMO detection and quantification are based on polymerase chain reaction (PCR), since protein-based assays are not suitable for highly processed food. The aim of the present project was to detect the maize event MIR604, one of the most recently approved events in the EU. DNA was extracted using the Wizard method [4]. Yield and purity of DNA were assessed by spectrophotometry, while amplifiability was evaluated by targeting two endogenous maize genes: *invertase* and *zein*. The screening of GM products was carried out by qualitative PCR targeting the 35 S promoter and the NOS terminator. For the positive results, event-specific PCR primers were used to detect MIR604 event. The screening and specific detection results evidenced some contaminated food samples (grains, flours, snacks, rusks, etc) in the Portuguese market.

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# Detection of almond as a potential allergen in chocolate matrix using the polymerase chain reaction technique

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Food ingredients are considered as causative factors in IgE mediated food allergies. These are recognised as a major health concern with 3-4% of adult population and 6% of young children being affected by food-allergic disorders [1]. Tree nuts, such as almond, are responsible for triggering severe allergic reactions in sensitised individuals. In recent years, their use in food also led to a rising number of adverse reactions to tree nuts and peanut, which has been intensified in Europe and USA [2]. The inadvertent ingestion of tree nuts might be the result of cross-contamination during production or misleading labelling of foods, representing a risk to allergic individuals, who may develop from mild urticaria reactions to potentially lethal anaphylactic shocks. As a complete absence of food allergens in the diet is the only means to protect the health of sensitised individuals, it is of major importance to be able to verify their presence at residual levels.

In principle, any molecule that is specific for an allergenic ingredient can serve as a marker of its presence in food, but mainly proteins and, more recently DNA have been targeted for this purpose. Since DNA is more stable to physical and chemical treatments than proteins, the use of the polymerase chain reaction (PCR) techniques have been proving to be useful alternatives to detect trace amounts of allergenic foods.

The aim of this work is to develop a PCR technique to detect almond as potential allergen, in a chocolate matrix. For this purpose, 15 model mixtures containing known amounts of almond in chocolate matrix were prepared as references materials for establishing sensitivity and quantitative assays. DNA was extracted using the Nucleospin<sup>®</sup> Food kit and the Wizard method. The concentration and purity were verified by UV spectrophotometry. For the detection of the gene encoding Pru du 6 (amandin) protein of almond (*Prunus dulcis*), new oligonucleotide primers were designed to produce 121 bp fragments by PCR amplification. After overcoming the difficulties to obtain amplifiable DNA from chocolate matrices due to the presence of PCR inhibitors, almond-specific fragments were obtained. The results of the PCR amplification allowed detecting almond residues (0.05%) in the chocolate matrix, using the Wizard extraction method. PCR technique can be considered a reliable tool to specifically identify trace amounts of tree nuts, such as almond. However, considering the need to detect minute amounts of allergens, further work is still necessary to increase even more sensitivity.

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## DNA barcode *loci* in the authentication of fruit juices

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Fruit-based products such as juices, jams, jellies, purées and other fruit preparations are being increasingly consumed in modern societies. These kinds of products have usually premium prices representing favoured targets for adulteration. Adulteration of fruit-based products can be performed by blending original products with cheaper and more available fruits not respecting labelled contents. Several approaches have been used in the authentication of such products including HPLC and infrared, mass and NMR spectroscopy [1]. Notwithstanding molecular DNA based methodologies are widely used in food analysis only a few methods have been reported for fruit juices [2].

The main objective of the present study was to evaluate the possibility of using the highly polymorphic regions of candidate plant DNA barcodes (*e.g. nrITS, trnH-psbA, rpoC1 and trnL*) [3] for the authentication of fruit juices. DNA extraction methods were evaluated in several products and the referred *loci* sequenced for the most commercially relevant fruit species. Sequences were retrieved from GenBank when available. Three sequence discrimination techniques were tested: (i) PCR-RFLP, (ii) direct sequencing and (iii) HRMA (High Resolution Melting Analysis) technique using the saturating dye SYTO9.

Results have permitted the definition of the more polymorphic barcode *loci* for the species under study, the *trnL* and the spacer *trnH-psbA*. Also an appropriate method for DNA extraction was selected based on several DNA quality parameters. Concerning the application of a barcode system to the samples in study the most promising technique seems to be the HRMA which permitted the discrimination of all the natural juices from the five different species (orange, mango, pineapple, peach and pear). This technique was also applied to mixtures of DNA from orange and mango at the ratio 75:25, 50:50 and 25:75. Results obtained permitted the correct correlation of the melt profiles with the content of the mixtures.

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# Isoflavones quantification in wild *Leguminosae* plants identified by a DNA barcoding approach

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*Leguminosae* (Fabaceae or bean family) constitutes the third largest flowering plant family, with 19,325 species in 727 genera. Thus, reliable and expedite identification as well as the understanding of the chemical composition variation are important for the efficient use of these plants. Plant identification at the species level can be performed by DNA barcodes which are short and standardized segments of DNA that reliably distinguishes species. Several *loci* have been proposed as a standard barcode however most of the recent work focuses four: the psbA-trnH spacer (photosystem II protein D1-tRNA Histidine), the rpoC1 (RNA polymerase beta subunit 1), the matK (maturase K) and the rbcL (Ribulose-1,6-bisphosphate carboxylase) chloroplastial genes [1]. The phenylpropanoid pathway is perhaps the best characterized of all metabolic pathways in *Leguminosae*, and many of its flavonoid products are now being used as health supplements. The isoflavones in particular have estrogenic properties and have recently gained a considerable reputation as phytoestrogen therapeutic agents in human health [2].

The main objectives of this study were the: i) collection of 12 wild plants belonging to the *Leguminosae* family and their identification by a DNA barcode approach ii) identification and quantification of isoflavones in the collected plants using a previous developed MSPD-HPLC/DAD method [3]. Four putative barcode loci were amplified and sequenced: ITS; rbcL; rpoC1 and matK. The barcode system used permitted the unequivocal identification of 10 from 12 samples. In the identified plants it was possible to quantify 11 isoflavones (puerarin, prunetin, daidzein, daidzin, glycitein, formononetin, irilone, genistein, pratensein, genistin and biochanin A). The isoflavone total content ranged from 20 µg/g to 396 µg/g.

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# Determination of tricothecenes and zearelenone mycotoxins in popcorn by QuEChERS and gas chromatography-mass spectrometry

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Mycotoxins are secondary toxic metabolites that fungi produce naturally. Due to their ubiquitous presence in foodstuffs and their potential risk for human health, prompt detection is essential [1]. Cereals and cereal-based products represent the major source of mycotoxin intake by the EU population [2]. The analysis of mycotoxins in cereal and cereal-based foods can be a real challenging task due to the minute amounts usually present in the samples, i.e. ppb and the large quantities of co-extracted compounds (e.g. fats and sugars) which can adversely affect the method and instrument performance. Chromatographic methods are the most suitable analytical approaches and require previous extraction and cleanup steps, which are often time-consuming tasks [3].

The aim of this work was to simplify the sample preparation required for the determination of several mycotoxins namely: zearelenone, deoxynivalenol, fusarenon X, 15-acetyldeoxynivalenol and nivalenol, in popcorn. For this purpose, it was used a slightly improved version of a previous developed method based on QuEChERS (QUick, Easy, CHEap, Effective, Rugged and Safe) procedure, followed by silylation of the extract with a mixture [BSA (N,O-bis(trimethylsilyl) acetamide) + TMCS (trimethylchlorosilane) + TMSI (N-trimethylsilylimidazole) (3:2:3)] and gas chromatography-mass spectrometry (GC-MS) analysis [3]. The method was validated and further it was applied for monitoring the tricothecenes and zearelenone contents in popcorn samples commercialized in Portugal.

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# Antioxidant capacity of commercial beverages, dietary supplements and teas based on plant extracts

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Tea plant (*Camellia sinensis*) is used in the preparation of several types of tea (black, green, white and red). Their aroma, taste and medicinal properties, worldwide appreciated, result from its high concentration in antioxidants, especially polyphenols, such as flavanols (catechins) and flavonols (quercetin), which might neutralize free radicals in the body[1,2]. For this reason, its moderate consumption has been associated with a lower incidence of injuries related to oxidative stress, including cardiovascular and neurodegenerative diseases, as well as some cancers[3]. These beneficial effects have led to the inclusion of this plant in several mixtures used as food supplements and commercial beverages. Additionally, other plants, like Rooibos (*Aspalathus linearis*) and Borututu (*Cochlospermum angolensis*) are also traditionally used as a source of antioxidants and can be easily found in our market.

This study aimed to evaluate total phenolics content and antioxidant activity of dietary supplements ( $n = 9$ ) and soft drinks ( $n = 3$ ), with and without green tea in its composition, sold in commercial areas, as potential antioxidants. Samples (bags, leaves and/or dried roots, extracts and soluble mixtures) were prepared, as brews, according to the manufacturer's instructions. Total phenolics content and antioxidant capacity were determined by Folin-Ciocalteu[4] method and DPPH[5] method, respectively.

Total phenolics varied between 34.6 and 337.87 mg EAG/200 ml. The commercial beverages without green tea presented the lowest levels. A dietary supplement consisting of a mixture of green tea, pineapple and hibiscus showed significantly higher ( $p < 0.05$ ) concentrations of phenolic compounds compared to other samples. The antioxidant activity of the samples was highly correlated with the phenolics content, being both influenced not only by the recommended preparation method, but also by the composition of the mixture.

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# Determination of organic acids in commercial vinegar samples by HPLC/UV

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Organic acids are important factors in physical stability and sensory perception (flavor, colour and aroma) of vinegars [1]. These constituents are formed during the two fermentation process involved: alcoholic and acetic; maturation and aging play also important roles. The total acidity of vinegars is expressed as acetic acid; the major organic acid in vinegar. The continued increasing demand for high quality vinegars requires straightforward analytical methods for the determination of the major as well as the minor organic acids. Several methods have been used to determined organic acids in vinegars, including enzymatic, electrochemical and chromatographic methods. The usual liquid chromatographic methods without derivatization require purification techniques to eliminate matrix interferences. Therefore the use derivatizing reagents such as 2-nitrophenylhydrazides or p-nitrobenzyl compounds and detection at 265 nm offers better sensitivity and selectivity [2].

In aim of our research was used a slightly improved version of a previous developed method based on the reaction of free organic acids with O-(4 nitrobenzyl)-N,N'-disopropylisourea (PNBDI) in presence of dioxane followed by HPLC/UV analysis for determination of organic acids in vinegars. The method was validated and further it was applied for determination of organic acids such as lactic, acetic, tartaric, malic, succinic and citric in twenty different vinegars samples commercialized in Portugal.

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# Effect of nanoparticles concentration on the properties of gelatin films

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A not commonly studied alternative to improve the qualities of the biodegradable films is the use of the nanoparticle (NA) as a reinforcing, creating a material called *nanocomposite*<sup>[1]</sup>. Therefore, this project purpose is the developing and characterization of biodegradable films taking as basis gelatin, reinforced by NA that has been produced by “casting”.

Materials swine skin gelatin (Gelita South America), glycerol (Synth) and montmorillonite hydrophilic NA (“Nanomer® Clay”, Sigma). Films production: 5g of gelatin/100g of FS, 30g of glycerol/100g of gelatin and 0,5 or 10g de NA/100g of gelatin. The filmogenic solution (FS) is disposed in acrylic plate using an automatic film spreader (Model Speed II, TKB Erichsen). The FS has been characterized with a rheometer (AR 2000, TA Instruments). The films have been characterized in respect to the mechanical proprieties (TA.XT2i, TA Instruments, U.S.A.) and humidity.

The FS cooling has provoked a transition sol-gel because of the formation of the adjacent polymeric chain link, with the consequently growth in the storage module (G’).

The NA incorporation has provoked a little reduction in the G’ values (storage module) and G’’ (loss module), with no effect the sol-gel transition temperature ( $T_{sol-gel}$ ) (Table 1), contrary to the behaviour observed by Daniel-da-Silva et al<sup>[2]</sup>; it has also provoked the reduction of the humidity, consequently to the reduction to the sensibility of the material to the water, and the increase in the film thickness, indicating an effect in the material density (Table 1).

Finally, the incorporation of the NA has improved the mechanical characteristics of the material, which has provoked a growth in the tensile strength and in the elastic module (EM), and the reduction of the elongation (Table 1), possibly because of the perfect dispersion of the NA in the biopolymeric matrix.

Table 1. Temperature of the sol-gel transition and the propriety of the nanocomposite films

Propriety	$C_N$ (g de nanoparticle/ 100g of gelatin)		
	0	5	10
$T_{sol-gel}$	21,9±0,5 <sup>a</sup>	22,2±0,1 <sup>a</sup>	22,2±0,1 <sup>a</sup>
Humidity (%)	20,4±1,4 <sup>a</sup>	18,3±1,1 <sup>b</sup>	17,4±0,6 <sup>b</sup>
Thickness (mm)	0,065±0,003 <sup>a</sup>	0,080±0,003 <sup>b</sup>	0,089±0,004 <sup>c</sup>
Tensile Strength (MPa)	23,5±3,1 <sup>a</sup>	31,7±1,8 <sup>b</sup>	31,1±1,9 <sup>b</sup>
Elongation (%)	48,7±5,5 <sup>a</sup>	40,4±3,4 <sup>b</sup>	38,0±3,4 <sup>b</sup>
EM (MPa)	2,1±0,6 <sup>a</sup>	5,4±1,0 <sup>b</sup>	6,6±0,1 <sup>c</sup>

$C_N$  = nanoparticle concentration; EM = elastic module

Mean ± Pattern diversion followed by same small letters, in the same line, does not present significative difference.

In conclusion, the NA incorporation has affected the gel proprieties (FS) without affect the transition sol-gel. In respect to the film, the NA has improved the mechanical proprieties, possibly as consequence of its perfect dispersion in the polymeric matrix, besides it has reduced the sensibility to the water steam.

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# Production and characterization of solid lipid microparticles encapsulating beta-carotene

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The food industry has sought innovative measures like health and welfare; in this context, have sought the development of foods with bioactive compounds, for example, carotenoids, due their nutraceutical properties[1]. There are two main problems regarding the use of carotenoids in foods that require attention: (i) the low stability of carotenoids due to their tendency to isomerization and oxidation, (ii) the relatively low bioavailability of carotenoids due to limited absorption in the gastrointestinal tract. It is therefore crucial to study how to protect these bioactives if you want their incorporation into foods, and also the use of food matrices that optimize intestinal absorption. One way to achieve both goals is using microencapsulation, which may allow, besides protection, conditions for controlled release and optimize the absorption in the gastrointestinal tract. Microencapsulation using lipid matrices, such as liposomes, emulsions and solid lipid particles, can achieve both objectives [1]. In this sense, this work aims the encapsulation of beta-carotene in solid lipid microparticles, which were produced in different compositions, and characterize them in terms of stability, taking into account some microstructural aspects.

As methodology the solid lipid microparticles were produced in different mass ratios of lipid (stearic acid and sunflower oil). The surfactants were tested polysorbate 60 (PS60) and polysorbate 80 (PS80), and to some formulations the antioxidant  $\alpha$ -tocopherol was added. Shortly, the lipids were melted, and to this melt beta-carotene was added (0.75% by weight), then hot surfactant (4% surfactant, % m / m) was added to the and brought to ultrasonic agitation at 9000 rpm for 3 minutes. Then came under magnetic stirring and then left that the system had reached 20°C. All have a 70/30 mass ratio of stearic acid/ sunflower oil. The determination of average particle diameter and size distribution was performed by laser diffraction and, X-ray diffraction to assess stability. The samples were made in triplicate.

As results, the particles with PS80 were extremely stable compared to the size distribution (and in all tests showed the particles with diameters below 3  $\mu\text{m}$ ) for periods exceeding 70 days of refrigerated storage. Preservation of beta-carotene was in the systems containing  $\alpha$ -tocopherol, but not in the absence of this antioxidant. Analyses of X-ray diffraction indicated that these systems of stearic acid and sunflower oil are also extremely stable microstructure.

In conclusion, it was possible to produce solid lipid microparticles using polysorbate 80 as surfactant, encapsulating beta-carotene, and store them under refrigeration for a period exceeding two months without it happening microstructural changes and degradation of microencapsulated compound.

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# Analysis of Teacher Education trainee on the Learning-Teaching Process

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The objective information about student performance is used by the teacher to outline the involvement of the practice and subsequently assist the modification of student behavior, so the Pedagogical Feedback (FBP) acts as a fundamental tool in the teaching process (Rosado, 2009; Hodges & Franks, 2002). The lecturer is faced with the need to constantly observe and assess your students' plays and prescribe the correction to the results detected, with reference to the pre-established objectives. Given these aspects, we analyze the performance of the teacher in the instructional process of teaching and learning during their lessons with a class of 22 students 16 females and six males of the 12th year of Humanities on Carolina Michaelis School in Porto. We adapt the protocol Gilbert et al. (1999) Systematic Analysis of Pedagogical Content Interventions (SAPCI) to define categories that were seized in the analysis of video made during the classes, which were observed in 270 minutes in 1229 distributed information units to take advantage of the observational method. The SAPCI allowed us to collect data from three of the four components that are teaching this instrument, the dimension "What," "How" and "The Who". According to the results of the study, with a frequency of 4.55 per minute FBP, the dimension "What" the trainee teacher in 52.5% of the interventions turned to the Technical Skills at the expense of 44.1% for components Tactics and 3.4% for the Rules. In the dimension "How" large parts of the interventions were aimed at positively evaluated aspects of encouragement / pressure (13.9%) and the component Without Content (29.2%) and hearing (39.8%) over others related to this dimension. The dimension "Who" that the professor surmised apprehend more FBP for Every Class (85.1%) than for the subgroup of students (5.4%) or in a single (9.5%). Thus, we find a predominance of emission of FBP which made the content broadcast seemed to be overlooked substantial criteria of the discipline that seemed to induce students to the little reflective practice and making us, in the face of frequency displayed, speculate that there was little learning.

**KEYWORDS:** PHYSICAL EDUCATION, TEACHER, EDUCATION, EDUCATION, REFLECTION.

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# **Effectiveness factors of Visual Information Processing in Action Shot at Soccer. Study on the relationship between interference color of the background and directionality of the ball in the shot in the game of football.**

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The diversity of factors contributing to the performance of players in sports with higher yields, causing a plurality of interests that seek to several studies where the decision-making, information processing and reaction time are some of these facilities. Football is constrained by contextual processes that influence the visual processing of captured images linked to the complexity of the game. This process is linked to information processing, which helps the directionality of the ball bearing in mind the influence of background such as the color of the equipment of the goalkeeper (GK). With this many responses are obtained, but there are still few studies that determine whether or not the relation of chromatic induction. And we've developed a series of tests that simulate game situations to assess the accuracy of the shot of the player, and if the same, based on the disturbance of contextual background on shot in or out of the Performance Area (PA) developed GK to capture the results. Based on data obtained in the different situations that we measure static or dynamic, with no time limit or free the the players tended to trim out of the PA of GK, however in some situations that the shoots was trim to PA of GK increased with the GK wearing a Living Color Orange clothing. However, the results of this study indicate the importance of specific training for football players optimize the shot precision are optimized reducing the environment influence on performance on this technical movement.

**Keywords:** Chromatic Analysis; Football Shoot, Information Processing, Decision Making.

# Biomechanical Analysis of 200 m freestyle

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In competitive swimming the arm stroke motion is of fundamental importance to aquatic propulsion. Thus, it becomes crucial to analyze the biomechanical parameters associated with the arm stroke motion, particularly over competitive events, in order to better understand the effect of fatigue. In this sense, the purpose of this study was to observe the effect of fatigue throughout 200 m front crawl held at maximum speed, observing the behaviour of the following biomechanical parameters: (i) swimming speed, (ii) speed (x-axis), depth (y-axis) and width of the hand (z-axis), (iii) stroke frequency, (iv) stroke length and (v) stroke index.

Ten male high level competitive swimmers ( $21.6 \pm 2.4$  years old,  $76.4 \pm 6.1$  kg,  $185.2 \pm 6.8$  cm,  $11.9 \pm 3.5$  years of experience in competitive swimming and  $109.3 \pm 2.1$  s of personal record in 200 m freestyle in short course) performed a 200 m front crawl test as in real competition, i.e., at the maximum intensity but using an individual tactic. Test was recorded by six stationary cameras (two above and four underwater).

In Table 1 it is possible to observe the effect of fatigue over each 50 m lap of the event in each of the kinematic parameters evaluated.

Table 1. Mean  $\pm$  SD values regarding the swimming velocity (v), velocity (v hand), depth (y) and width (z) of the hand, stroke frequency (SF), stroke length (SL) and stroke index (SI).

Parameters	50 m	100 m	150 m	200 m
v (m/s)	1.57 $\pm$ 0.08	1.39 $\pm$ 0.06 <sup>a</sup>	1.34 $\pm$ 0.07 <sup>a</sup>	1.35 $\pm$ 0.06 <sup>a</sup>
v hand (m/s)	2.93 $\pm$ 0.62	2.77 $\pm$ 0.38 <sup>a</sup>	2.71 $\pm$ 0.23 <sup>a</sup>	2.50 $\pm$ 0.32 <sup>a</sup>
y (m)	0.73 $\pm$ 0.02	0.71 $\pm$ 0.02	0.71 $\pm$ 0.02	0.71 $\pm$ 0.02
z (m)	0.33 $\pm$ 0.03	0.31 $\pm$ 0.02	0.32 $\pm$ 0.03	0.35 $\pm$ 0.03
SF (Hz)	0.68 $\pm$ 0.09	0.63 $\pm$ 0.06	0.61 $\pm$ 0.05 <sup>a</sup>	0.64 $\pm$ 0.05
SL (m/cycle)	2.29 $\pm$ 0.23	2.21 $\pm$ 0.17	2.19 $\pm$ 0.13	2.12 $\pm$ 0.13 <sup>a</sup>
SI (m <sup>2</sup> .c <sup>-1</sup> .s <sup>-1</sup> )	3.59 $\pm$ 0.13	3.07 $\pm$ 0.08 <sup>a</sup>	2.94 $\pm$ 0.07 <sup>a</sup>	2.85 $\pm$ 0.09 <sup>a</sup>

a: significant difference to the first 50 m lap ( $p < 0.05$ )

It is evidenced that concomitantly with the decrease of swimming velocity there is a decline of hand speed and SI. The hand speed and swimming speed are closely related, highlighting the importance of hand speed has in generating propulsive force [1]. The decrease in technical efficiency, as measured by the SI, also helps explain the reduction of speed. In addition, the behaviour of the general biomechanical parameters (swimming speed, SF and SL) obtained in this study is consistent with the literature, noting the decrease in speed as well as variations of SF and SL in response to the onset of fatigue [2].

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# Efeito do treino na Velocidade Reacção Pedal Natação Pura Desportiva e Ginástica Artística Feminina

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The main purpose of the present study was to evaluate the train effect in the Velocity Reaction (pedal) in young Gymnasts and Swimmers.

Twelve young female athletes (10 years old and, a medium of 5 years of practice experience) participated in this study. To evaluate the Speed Reaction (pedal) was used the Simple velocity Reaction Test (Nelson, 1965). The statistical procedures included the descriptive statistic (Mean and Standard deviation) and the inferential statistic (Independent Sample t Test, Paired Sample t Test and Shapiro Wilk Test). The significance level was settled in  $p \leq 0.05$ .

The present study allows concluding: (i) there are significant differences between the modalities concerning to the preferred and non-preferred foot in the first moment of evaluation (before training session); (ii) there are no significant differences between the modalities, concerning the differential, at the first moment of evaluation. (iii) there are significant differences between the two modalities, concerning non-preferred foot, at the second moment of evaluation.

The main conclusion of this study evidences that there are no significant differences between the two moments of evaluation (before and after practice) to the studied modalities, concerning to the preferred foot, non-preferred foot and the differential between those.

**Key-words:** VELOCITY REACTION (PEDAL), TRAINING SESSION, SWIMMING, WOMEN GYMNASTIC ARTISTIC.

# Front crawl technical characterization of 11-13 years oldswimmers

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Introduction: To characterize the front crawl technique, stroke rate (SR), stroke length (SL) and Stroke Index (SI) are widely used since their interaction determines swimming velocity [1]. Recently, Chollet et al. [2] proposed a tool to determine the inter-arm coordination - the index of coordination (IdC) - which is based on the measurement of the lag time between the propulsive phases of the two arms. Three different synchronization modes in front crawl are possible [1]: (i) opposition (IdC = 0), providing continuous motor action; (ii) catch-up (IdC < 0), existing a lag time between propulsive phases of the two arms and (iii) superposition (IdC > 0), which describes an overlap in the propulsive phases of both arms. In this sense, our aim was to characterize the front crawl technique of young swimmers performing at very high intensity.

Methods: One hundred and fourteen swimmers performed a 25-m front crawl at 50-m pace. Two underwater cameras was used to assess general biomechanical parameters (velocity, SR, SL and SI) and IdC, being also identified each stroke phase (entry/catch, pull, push and recovery).

Results: When dividing the sample by maturational and gender groups, it was observed that post-pubertal group showed higher SL and SI than pubertal group, and that and boys exhibited greater velocity, SR and SI values, longer recovery phase, and shorter pull phase than girls. In addition, for all groups, only the catch-up coordination mode was observed; in fact, the sum of non propulsive phases was higher than the sum of propulsive phases. Moreover, the entry/catch phase had the higher percentage compared with the other three front crawl stroke phases, and the pull phase had the lowest percentage.

Conclusion: Comparing to adults, young swimmers presented lower values in all general biomechanical parameters, having the post-pubertal group closest values to adult swimmers due to their superior anthropometric and maturational characteristics. Regarding genders, boys showed higher velocity, SR and SI as reported for elite swimmers. In addition, when considering the total sample, a higher relationship was observed between velocity and SL (than with SR), indicating that improving SL is fundamental. Furthermore, the catch-up was the coordination mode selected, being in accordance with older swimmers performing at high intensities.

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# Individual anaerobic threshold assessment in elite swimmers

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**Introduction:** One of the most used methods for anaerobic threshold assessment is based on the averaged value of  $4 \text{ mmol.l}^{-1}$  of blood lactate concentration ( $[\text{La}]$ ). However, an individualized approach was suggested before [1], rather than the use of a fixed  $[\text{La}]$ . In fact, a fixed value of  $[\text{La}]$  does not take into account considerable inter-individual differences and may frequently underestimate or overestimate real endurance capacity [2]. The purpose of this study was to assess the individual anaerobic threshold (IndAnT) in national level swimmers performing in their best swimming technique.

**Methods:** Twenty-five elite swimmers ( $20.1 \pm 2.2$  years old,  $181.2 \pm 6.9$  cm,  $73.8 \pm 8.0$  kg and  $n > 8$  training units per week) performed  $n \times 200$  m individualized intermittent incremental protocol, with increments of  $0.1 \text{ m.s}^{-1}$  for each 200 m step, and 1 min rest intervals [adapted from 1]. Initial velocity was established according to the individual level of fitness. IndAnT was determined by  $[\text{La}]$ /velocity curve modelling method and assumed to be the intersection point, at the maximal fit situation, of a combined pair of regressions (linear and exponential) [3], as observable in Figure 1.

**Results:** Velocity and  $[\text{La}]$  values corresponding to IndAnT averaged, respectively,  $1.37 \pm 0.11 \text{ m.s}^{-1}$  and  $2.31 \pm 0.67 \text{ mmol.l}^{-1}$  for front crawl ( $n=13$ ),  $1.33 \pm 0.35 \text{ m.s}^{-1}$  and  $1.93 \pm 0.33 \text{ mmol.l}^{-1}$  for backstroke ( $n=2$ ),  $1.19 \text{ m.s}^{-1}$  and  $1.36 \text{ mmol.l}^{-1}$  for breaststroke ( $n=1$ ),  $1.32 \pm 0.66 \text{ m.s}^{-1}$  and  $4.30 \pm 0.55 \text{ mmol.l}^{-1}$  for butterfly ( $n=4$ ), and  $1.35 \pm 0.08 \text{ m.s}^{-1}$  and  $3.68 \pm 1.31 \text{ mmol.l}^{-1}$  for medley ( $n=5$ ).  $[\text{La}]$  corresponding to IndAnT values were lower than the  $\text{mmol.l}^{-1}$  value in all the techniques. Concerning the swimming velocity at IndAnT, differences were only observed in medley, but its difference for  $v_4$  values is evident for training proposes.

**Conclusion:** The incremental protocol for IndAnT assessment used in the present study is specific and precise for IndAnT assessment. The results seem to confirm the fact that the  $4 \text{ mmol.l}^{-1}$  and  $v_4$  values do not represent the individualized lactate threshold in trained swimmers.

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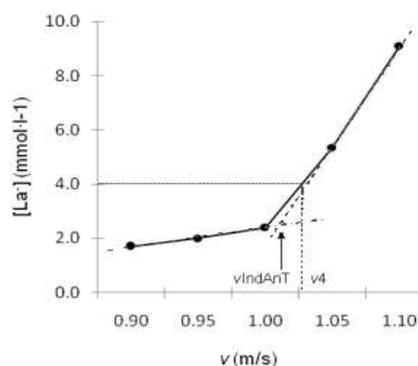


Fig.1. Example of an individual  $[\text{La}]$  / $v$  curve for IndAnT assessment represented by the interception of a linear and an exponential line ( $v_4$  is also shown).

4

# Relation between manual reaction speed and foot reaction speed, in volleyball players

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**Introduction:** Volleyball is a complex sport that requires from the athlete a combination of motor skills, cognitive and coordinative, and the greater is the demand of the game more these capabilities are required (Stanganelli et al., 2006). Therefore, the purpose of this study was to understand how were the manifest and the similarities between nelson hand reaction test and nelson foot reaction test.

**Methods:** The sample consisted of 10 athletes from the men's volleyball team of Esmoriz Gym Club, which was participating in the National Volleyball Championship in the 2010/2011 season (average age:  $23.8 \pm 4.52$ ). In order to evaluate the reaction time was used The Nelson Hand Reaction Test and The Nelson Foot Reaction Test. At both instruments were evaluated the preferred and the non preferred member. Statistical procedures included descriptive statistics (Mean and Standard Deviation) and inferential statistical test (correlation Spearman's). The significance level was set at  $p \leq 0.05$ . To process the data, was used the statistical program, Statistical Package for Social Science (SPSS) version 18.0.

**Conclusions:** After statistical analysis were not found significant differences in speed of reaction of preferred members (hand and foot) when compared with non-preferred. There was a positive correlation between reaction speed of preferred foot and reaction speed of non-preferred foot and between speed reaction of preferred hand and speed reaction of non preferred foot.

**Keywords:** Reaction Speed Manual; Reaction Speed Pedal; Volleyball; Players.

## **Relationship between a Force Velocity Training on the Manual Dexterity in Junior Swimmers.**

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The manual dexterity is a coordinative capacity extremely considered in Swimming, and most of the technical gestures of which swimming techniques are performed by the upper limbs.

The objective of this study was to observe the global manual dexterity effects in upper limbs after a force velocity dry land training in the junior swimmers. For this study we used the Test of Manual Dexterity - Plate Taping (Eurofitt Physical Fitness Test Battery,1991).

After the force velocity training, which lasted 45 minutes and was mainly performed by the upper and lower limbs, was evaluated the manual dexterity of the Preferred Member (PM), Non Preferred Member (NPM) and the functional motor asymmetry in a 10 swimmers sample (5 female and 5 male) of F.C Porto Swimming Team, aged between 14 and 17 years. The sample was balanced with respect to the member that initiates the test. We used SPSS 18.0 and analysis statistics included descriptive and inferential statistics. The level of significance was set at 5%.

We observed a statistically significant effect on muscle fatigue on speed, movement's precision of the PM, of NPM, and the consequent functional motor asymmetry.

# TO BE A SUPERVISOR: A study with Cooperating Teachers of Physical Education

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The cooperating teacher has been taking on an increasingly important role in the initial training of future teachers. The cooperating teachers, besides supervising the whole teacher practice process, act as a role model for their student training. Therefore, the supervisor is responsible for conveying a set of values and principles that promote the idea of training. The main purpose of this paper was to analyze the reasons and meanings that could impel a teacher to become a supervisor. It also aimed to detect which models, styles and supervision perspectives are embraced by cooperating teachers. Semi-structured interviews were applied to four cooperating teachers who worked as supervisors at the Faculty of Sport, University of Porto. The transcriptions of the interviews were submitted to content analysis from which the following themes had arisen: the reasons to become a cooperating teacher and the styles and perspectives they embraced in the supervision process. The results show that Knowledge upgrade turned out to be the primary motivation to start and to keep the role of supervisor, and the reflective supervision model and the supportive supervision style prevail among cooperating teachers. The relationship between supervisor and student teaching was also considered important.

**KEYWORDS:** COOPERATING TEACHER; SUPERVISOR; MODELS AND STYLES OF SUPERVISION.

# Antioxidants from broccoli and lamb lettuce: Influence of the extraction procedure on total phenolics and antioxidant capacity

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In recent years, studies on the extraction of phenolic compounds from natural products have attracted special attention. Phenols, potent antioxidants, are ubiquitous in vegetables and have demonstrated an active role in preventing the progress of certain degenerative diseases. These beneficial effects have been partially attributed to the compounds which possess antioxidant activity. The major antioxidants of vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids [1]. Phenolic compounds are secondary metabolites synthesized by plants both during normal development and in response to stress conditions [2]. Thus, the extraction of phenolic compounds from plant materials seems to be a very interesting area of research, because it will allow industries to enhance the phenolic content of commercial products obtained from vegetables.

The principal aim of this work was to investigate the influence of the extraction conditions on the yield of phenolic compounds and the corresponding antioxidant capacity. The extracts obtained in each experimental condition tested were analysed in terms of total phenolic content (TPC) by the Folin-Ciocalteu method and the antiradical power evaluated by the DPPH and the ferricyanide reducing power assays, as previously described [3]. In the case of lamb lettuce, the extraction technique (stirring) and type of solvent (methanol) were found to have a critical role in the extraction of antioxidant compounds. For broccoli extracts, yield was maximized when soxhlet extraction and water was used. Moreover, the results showed that lamb lettuce, compared to broccoli, contain high amounts of total polyphenols ( $2.78 \pm 0.27 \text{ mg}_{\text{GAE}} \cdot \text{g}^{-1}$  and  $1.46 \pm 0.02 \text{ mg}_{\text{GAE}} \cdot \text{g}^{-1}$ , respectively) and higher antioxidant capacity ( $86.3 \pm 11.8 \%$  and  $11.3 \pm 0.1 \%$ , respectively). Work is in progress in order to identify by HPLC-ESI-MS which phenolic compounds are contributing to the antioxidant capacity of the broccoli and lamb lettuce extracts.

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# Automated evaluation of imidazolium ionic liquids toxicity based on their effect on catalase

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Several works regarding the toxicity and biodegradability of ionic liquids (ILs) have shown that the “green” image of these solvents is misplaced and must be urgently reviewed. Thus, ILs toxicity must be evaluated before they enter the environment, as a part of a sustainable development of chemicals. A few enzyme inhibition assays, based not on complex animals but rather on enzymes with biological impact, have already provided very interesting information about the toxicity of ILs, in a simple and expeditious way [1]. These studies have been performed till now in batch mode with drawbacks associated with time spent, human intervention and consumption of reagents. In this context, it seems that the automation of these procedures could further increase their potential and significance. Among flow techniques, sequential injection analysis (SIA) has been profitably applied in biocatalytic procedures, including enzyme activity assays in ILs [2], proving to be a robust and accurate tool for the implementation of these studies.

This work is based on the development of a SIA procedure for the evaluation of imidazolium ILs toxicity, based on their effect on the activity of catalase, an enzyme with important biological activity. The developed methodology was based on the oxidation of the non-fluorescent probe amplex red, in the presence of H<sub>2</sub>O<sub>2</sub>, to produce resorufin, a strong fluorescent compound. Catalase activity was monitored by the decreased of the fluorescence intensity due to the consumption of H<sub>2</sub>O<sub>2</sub> by the enzyme. The activity assays were performed in strictly aqueous media and in the presence of increasing concentrations of seven commercially available ionic liquids and sodium azide, a strong inhibitor of catalase. IC<sub>50</sub> values between 0.15 and 2.77 M were obtained for the tested compounds, revealing distinct inhibitory effects. This allowed us to perform some considerations about the toxicity of the tested cations and anions.

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# Characterization of Nickel Complexes for Biomolecular Simulations

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Nickel is present in a variety of different enzymes, playing a fundamental role in many biological processes. Notable examples include the enzymes urease, Ni-Fe hydrogenase, CODH (carbon monoxide dehydrogenase), S-Methyl-Coenzyme M-reductase and JMJD2A (Jumonji Domain 2A) [1-2]. However, in spite of the high biological relevance of such systems, the almost total absence of accurate molecular mechanics parameters able to accurately describe the nickel coordination sphere in these systems, greatly limits the application of powerful computational methodologies, such as molecular dynamics (MD) simulations to these enzymes. Such methods could provide valuable atomic level information on important Ni enzymes.

Here, we introduce our studies on this important set of metalloproteins, and evaluate different computational/experimental methods for developing specific molecular mechanics parameters for the accurate description of the most common Ni coordination spheres present in metalloproteins. In addition, we summarize some of our conclusion on this subject and present some of the different molecular mechanical parameters that we have determined.

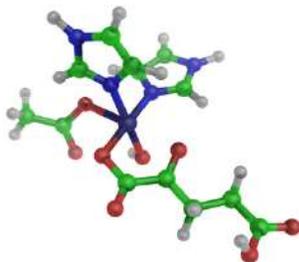


Fig1- Ni sphere from JMJD2A

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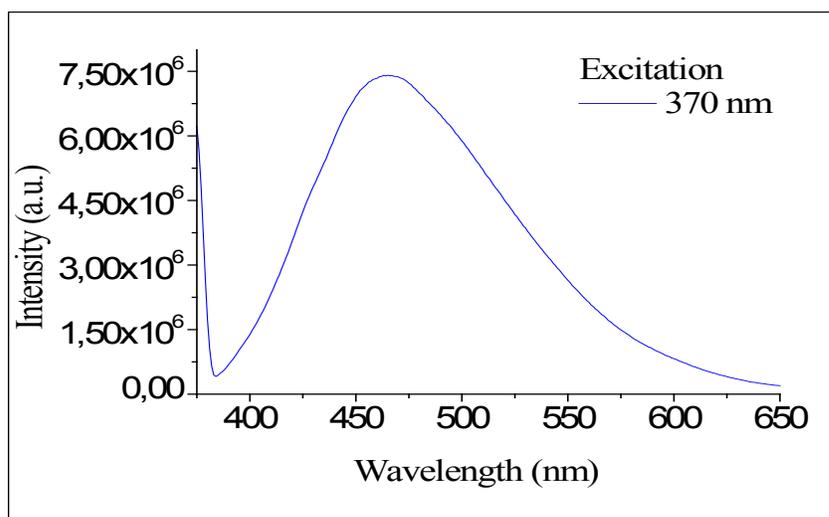
# Chemical Synthesis of Fluorescent Carbon Nanoparticles

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Carbon nanoparticles are graphitic nanomaterials that show near spherical geometry and isotropic shapes, and they fluoresce in the visible upon light excitation [1]. The light emitted by these nanoparticles depends on the wavelength of light used for excitation.

In this study, fluorescent carbon nanoparticles were synthesized directly from a carbohydrate by a one-step acid assisted ultrasonic treatment. Through this synthesis method fluorescent carbon nanoparticles were obtained. The fluorescence results showed that the nanoparticles fluoresce in the visible at different excitation wavelengths. As an example, Fig. 1 shows the emission spectrum of carbon nanoparticles.



**Fig. 1- Emission Spectrum of carbon nanoparticles.**

This communication will present a greenchemistry method using natural precursors to prepare carbon nanoparticles, by using a carbohydrate as carbon resource, and its application in the development of fiber optic sensors for the determination of reactive oxygen (ROS) and nitrogen (RNS) species in biological systems.

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# Chemiluminometric detection applied to analysis of paracetamol

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Paracetamol (acetaminophen or N-acetyl-p-aminophenol) is a popular and widely used drug for treatment of pain and fever. It occupies a privileged position among analgesic drugs, due to almost do not have side effects. It can be used in a variety of patients, including children, pregnant women and the elderly<sup>1</sup>. Due to its extensive used paracetamol or its hydrophilic metabolites may also be indicative of a contamination of water resources. In this context, a multicommutation flow system with chemiluminometric detection was employed in the determination of low levels of paracetamol in wastewaters. Chemiluminescence constitutes the basis of several high sensitive analytical procedures, even when carried out using simple instrumentation. Its association with continuous flow methods allows important improvements, showing some advantages as, for instance, reduction in the reagents and sample consumption and high sampling rate<sup>2</sup>.

In this work a multicommutation flow-system using three solenoid valves interconnected with 0.8 mm PTFE tubing downstream connected to a spiral flow cell in front of a solid state photomultiplier as detector device was employed. The developed method used a binary sampling strategy with equal volumes (around 2 mL) of sample and a 10  $\mu\text{M}$  hypochlorite solution. This mixture then converged to a third solenoid valve, which inserted around 1 mL of a 62.5  $\mu\text{M}$  luminol solution, prepared in a carbonate buffer pH 10.5, before detection. The detection of paracetamol was based in its oxidation by the hypochlorite solution, due to the presence of an amide group in the chemical structure of paracetamol. The remaining hypochlorite reacted with luminol leading to light emission. The described conditions allowed the determination of paracetamol in the range between 10 and 3100  $\mu\text{g L}^{-1}$ , with a detection limit of 2.5  $\mu\text{g L}^{-1}$  and repeatability lower than 3%.

## Acknowledgments

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# Comparative Study of Antioxidant Properties of Different Green Tea Formulations

Ana L. Morais<sup>1,2</sup>, João C.M. Barreira<sup>1,2</sup>, M. Beatriz P.P.<sup>2</sup> Oliveira and Isabel C.F.R. Ferreira<sup>1</sup>

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Tea, a product made up from leaf and bud of the plant *Camellia sinensis*, is, after water, the most consumed drink in the world. Particularly, green tea, a “non-fermented” tea (produced by drying and steaming the fresh leaves to inactivate the polyphenol oxidase and thus, non oxidation occurs) has been related to different health benefits such as reduction in the risk of cardiovascular disease and some forms of cancer, and neuroprotective power, as well as other disorders related to oxidative stress. Moreover, it was suggested the ability of green tea, when consumed within a balanced controlled diet, to improve overall the antioxidative status and to protect against oxidative damage in humans [1].

In the present work, different formulations of green tea obtained in a local supermarket were used: green tea bags (F1); green tea leaves (F2); soluble green tea (F3a and F3b); green tea liquid extract (F4). The teas were prepared according to manufacturer information, including infusions (F1- 75 °C, 5 min; F2- 75 °C, 3 min), or solubilisation (F3a- 75 °C; F3b- 21 °C; F4- 21 °C). The antioxidant properties were evaluated through several chemical and biochemical *in vitro* assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power (RP), inhibition of  $\beta$ -carotene bleaching, and inhibition of lipid peroxidation in brain tissue by formation of thiobarbituric acid reactive substances (TBARS) [2]. The DF<sub>50</sub> (dilution factor responsible for 50% of antioxidant activity, or 0.5 of absorbance, in the case of RP assay) values were calculated for all the methods in order to evaluate and compare the antioxidant efficiency of the different formulations.

Some differences were observed among the antioxidant activities of the tested green tea formulations. The best results were observed for green tea liquid extract: DF<sub>50(DPPH)</sub> = 151±10; DF<sub>50(RP)</sub> = 203±1; DF<sub>50( $\beta$ -carotene)</sub> = 18±2; DF<sub>50(TBARS)</sub> = 408±48).

This work might be useful in the definition of the best green tea formulation, considering the health benefits of this highly consumed beverage.

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# Computational study of the catalytic mechanism of *Candida albicans* N-myristoyltransferase.

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*Candida albicans* is a pathogenic fungus that causes oral and genital infections in humans. The enzyme N-myristoyltransferase (NMT) is essential for its growth and survival [1]. NMT catalyzes the formation of an amide bond between the carboxyl of myristate from the myristoyl-CoA cofactor (m-CoA) and the N-terminal glycine of protein substrates [2]. N-myristoylation of *Candida albicans* ADP-ribosylation factor produces a change in its electrophoretic mobility, the myristoylated species migrates more rapidly than the nonmyristoylated species. Therefore, NMT is an attractive target for anti-fungal therapy. The catalytic mechanism of NMT is not well known at the atomic level. It is now recognized that NMT has an ordered bi bi mechanism. The putative mechanism involves the sequential formation of a m-CoA:NMT binary complex and the formation of a ternary m-CoA:NMT:substrate complex afterwards (Fig. 1). Once this complex is formed, the catalytic transfer between m-CoA and the peptide substrate occurs. Finally Coenzyme A is released followed by a myristoylated peptide [3]. We are performing computational calculations in order to unravel the NMT catalytic mechanism with atomic detail, to be able to study the inhibition of the enzyme. We are using density functional theory (DFT) calculations, on a model with 131 atoms that represents the NMT catalytic residues, the peptide substrate and the myristoyl-CoA. This work is under development and this communication presents the first theoretical insights.

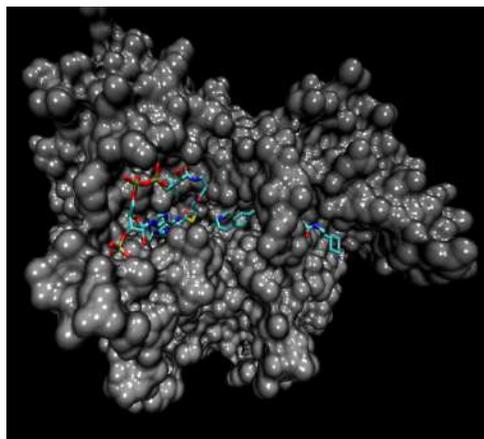


Fig.1- m-CoA:NMT:substrate complex

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# Coumarins as an Important Scaffold on Neurodegenerative Diseases Drug Discovery

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With the increase of the population life expectancy on the actual developed countries' society, the importance of drug discovery focused in the neurodegenerative diseases (ND), such as Parkinson's and Alzheimer's diseases, emerges. The few accessible treatments use monoamine oxidases inhibitors (MAOI-A and MAOI-B) and acetylcholinesterase inhibitors (AChEI) (Figure 1).

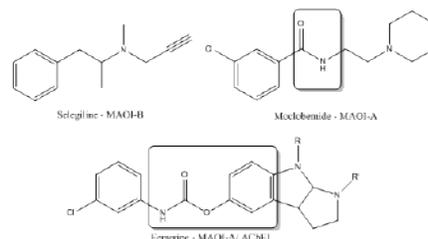


Figure 1

Coumarins are a large family of compounds of natural and/or synthetic origin that proved to have numerous pharmacological properties.<sup>1</sup> In our group, we already synthesized and evaluated new potent MAO-A and MAO-B inhibitors (Figure 2).<sup>2-4</sup>

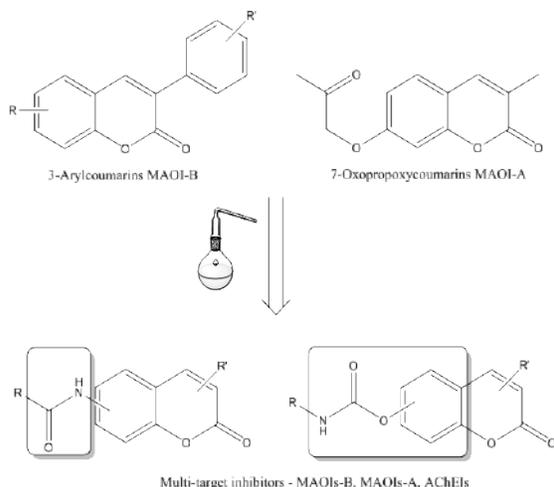


Figure 2

In this project, an innovative therapeutic solution is proposed for the application in ND, which links the development of new drugs obtained by hybridization process with their pharmacological properties: MAOI, AChEI and antioxidant activities (multi-target drugs). In this work we developed new synthetic methodologies to create novel multi-target inhibitors focusing on the coumarin scaffold (Figure 2). Biological assays targeting MAO-A, MAO-B and acetylcholinesterase are currently in progress.

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# COX/LOX Dual Activity – A novel and rational approach towards inflammation

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Inflammation is an important and extremely complex biological process. Though inflammation corresponds to a functional necessity to provide host defense and activate regenerative processes, its persistence may be deleterious to the host, resulting in cardiovascular and neurodegenerative diseases, as well as cancer. The inflammatory process often begins with the release of arachidonic acid (AA), which is then metabolized by two major enzymatic pathways: cyclooxygenase (COX) and lipoxygenase (LOX) with the production of pro-inflammatory mediators. These pathways provide a wide range of possible targets for the pharmaceutical treatment of inflammatory diseases and the dual inhibition of COX-2/LOX constitutes the most recent approach towards inflammation [1]. In line with this approach, the aim of this work was to find novel anti-inflammatory drugs with higher efficiency, less side effects and wider application on the treatment of inflammatory diseases, based on the in vitro screening study of the anti-inflammatory properties of several indole derivatives.

The evaluation of the inhibitory effects of the compounds under study against the enzymes COX-1 and COX-2 was evaluated using a specific EIA kit. The majority of the tested compounds showed inhibitory activity against COX-1 and COX-2 for the concentrations 100µM and 50µM and two of them also exhibit relative selectivity for COX-2 inhibition.

In conclusion, this preliminary study showed promising results in this approach for developing selective COX-2 inhibitors. The effect of the indole library on 5-LOX activity is presently in under study.

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## Acknowledgements:

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# DESIGN OF FLUORESCENT 3-HYDROXY-4-PYRIDINONE HEXADENTATE IRON(III) CHELATORS WITH POTENTIAL ANTIMYCOBACTERIAL ACTIVITY

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Iron is a crucial transition metal for all living cells and also an essential micronutrient in the survival of several pathogenic bacteria. The molecules developed by these microbes to obtain iron from the environment are known as siderophores, which are high affinity hexadentate iron(III) chelators.

According with these facts, the design of iron(III) chelators to use in the treatment of some infectious diseases caused by these pathogens have been considered.

Mycobacterial infections have particular interest in our group and some results obtained by us [1] have shown that a 3-hydroxy-4-pyridinone hexadentate chelator is effective at inhibiting the growth of *Mycobacterium avium* inside macrophages.

Therefore, in the present work, we report the synthesis of new hexadentate chelators (Fig.1) based on a structure of three bidentate 3-hydroxy-4-pyridinone ligands with fluorescent units of different rhodamine derivatives (X substituent in Fig. 1).

*In vitro* and *in vivo* studies of these related chelators are performed in order to understand the role of action of these antimycobacterial compounds in macrophages and study their different chelation potency for iron.

This type of studies will certainly improve our knowledge about the antimicrobial activity of these chelators and their potential for the treatment of iron-overload related diseases, as Tuberculosis.

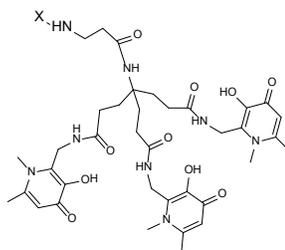


Figure 1

Financial support from FCT through project PTDC/QUI/67915/2006 is gratefully acknowledged. T. Moniz also thanks FCT for BI grant.

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# Design of novel flame retardancy cotton textiles through functionalization with nanoclays

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Textiles are probably among the most popular materials since they decorate and protect our bodies while bringing comfort into our lives. Flame retardant fabrics for industrial applications represent one of the most profitable niches in the global textile market. Since most of the textile materials are flammable, the incorporation of flame retardants onto textile fabrics becomes necessary to assure human safety under many circumstances [1]. Much attention has been devoted to the use of layered silicates (nanoclays) due to their great potential to produce materials with improved flame retardancy [2].

This work reports the fabrication of functional cotton textiles with flame retardancy properties through their derivatization with nanoclays (Fig 1). Initially, the K10-montmorillonite nanoclay was functionalized with two organosilanes with reactive bromide and fluorophenyl groups by a post-grafting methodology. Subsequently, these materials were immobilized onto cotton textile substrates.



**Fig. 1** – Schematic representation of the textiles functionalization with nanoclays.

The morphology and chemical composition of the materials were characterized by scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) and thermogravimetry (TG). The flame retardancy of the functional textiles will be examined.

**Acknowledgments:** This work was partially funded by Projecto de Investigação Científica na Pré-Graduação 2009, U.P. and Santander Totta, and by FCT and FEDER, through project PTDC/CTM/108820/2008. AM and CP thank FCT for their grants.

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# Determination of 24 pesticide residues in fruit juices using a novel dispersive liquid-liquid microextraction method (DLLME) coupled to multidimensional gas chromatography- mass spectrometry (MD-GC/MS)

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Fruit juices are low-fat and nutritious beverages whose consumption can help to fulfill the dietary recommendations to eat more fruits and vegetables. However, safety concerns about these products have increased in consumers and authorities, due to the possibility of contamination with minor amounts of toxic residues arising from agricultural practices and/or juice processing. Pesticide residues resulting from the treatment of raw fruits or from the water used in the processing are undoubtedly one of the major problems to human healthy due to its toxicity. Despite the progressive introduction of good practices in the fruit juice production, regulation and scrutiny to prove that the fruit juice are safe for consumption, the presence of pesticide residues remains a real problem [1].

The aim of this work was to develop and validate a fast analytical method for evaluation of multiple pesticide residues in juices, using a dispersive liquid-liquid microextraction method (DLLME) coupled to multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Special attention was given on the optimization of DLLME parameters, by evaluation of type and volume of extractor and dispersive solvents, to maximize yields in the extraction of 24 pesticide residues belonging at eighth different chemical classes. The potential of a dual GC column system connected by a Deans switch device was tested in combination with fast GC-MS. The developed method showed very acceptable performance characteristics, within the quality control requirements of EU guidelines [2]. The DLLME procedure in combination with the MD-GC/MS technique allowed to analyze a batch of 30 commercial juice samples within 6 h (analysis time of each sample is less than 12 min, including sample preparation and quantification).

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# Determination of lipid content of frozen sardine by microwave-assisted extraction and Bligh & Dyer methods

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Total lipid content of fish samples is an important parameter used in biochemical, physiological, and nutritional studies. Consistent methods for the quantitative extraction of lipids from tissues are of critical importance and should not only be reliable and reproducible, but also yield a minimum amount of extracted non-lipids [1].

Analytical determination of lipid content has historically been achieved with Bligh and Dyer method [2]. Modified Bligh & Dyer [2] extraction is a modification in which methanol is replaced by 2-propanol, and chloroform by cyclohexane. These methods are labor intensive, time consuming, difficult to automate and use toxic solvents.

MAE (microwave-assisted extraction) is the process by which microwave energy is used to heat solvents in contact with solid samples and partition compounds of interest from the sample into the solvent. Studies dealing with lipids MAE are scarce but seem to be a great promise to improve lipid yields.

In this work, a MAE methodology was compared with two conventional extraction methods (Bligh & Dyer and modified Bligh & Dyer) for quantification of frozen sardine (*Sardina pilchardus*) lipids. The influence of extraction method, season of capture (winter and spring), gender and frozen storage time (varying from fresh to 9 months of freezing) on total lipid content was analyzed in detail.

MAE was the method with best extraction efficiency and repeatability (CV=0.034). Bligh & Dyer method presented comparable extraction efficiency to MAE but with lower repeatability. Modified Bligh & Dyer method was very poor in terms of efficiency as well as repeatability.

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# Development of a bioluminescence assay for AMP detection and quantification

**L. Pinto da Silva, V. Monteiro and J.C.G. Esteves da Silva**

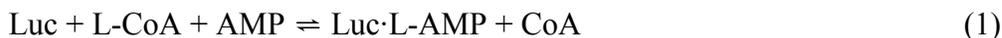
Centro de Investigação em Química (CIQ-UP), Department of Chemistry and Biochemistry, Faculty of Sciences of University of Porto, Rua Campo Alegre 687, 4169-007 Porto (Portugal).

Firefly luciferase (EC 1.13.12.7, Luc) is an enzyme that catalyzes the oxidation of firefly luciferin (LH<sub>2</sub>), giving rise to light in a two-step reaction. The most remarkable characteristics of this system are the large quantum yields of light production and its pH-dependent multicolor bioluminescence (in the range of 530-640 nm) [1].

This bioluminescence system has also several interesting analytical properties, as high selectivity and sensitivity, and is currently used in the determination of some metabolites as ATP, PP<sub>i</sub> and CoA [1, 3].

Adenosine 5'-monophosphate (AMP) is ubiquitous in living organisms and is generally regarded as a product of ATP consumption. However, it can function as a second messenger in the form of cyclic AMP, as an allosteric effector in enzymatic reactions and has been recently used as a marked for bacterial contamination.

The objective of this work is the development of a bioluminescence assay for the detection and quantification of AMP. This method is based on the monitoring by fluorescence of the Luc-catalyzed reactions depicted in equation 1 and 2. This approach exploits the fact that dehydroluciferin (L) exhibits fluorescence, to the contrary of dehydroluciferyl-coenzyme A (L-CoA) and dehydroluciferyl-adenylate (L-AMP) [4].



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# Development of polyaniline microarray screen-printed electrodes for quantification of heavy metals

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One of the main challenges facing the analytical chemist is the development of methods that respond to the growing need to perform rapid *in situ* analyses. In recent years, many of the procedures developed with this end in sight have been based on electrochemical techniques due to their high sensitivity and selectivity, portable field-based size and low cost.

Heavy metals are naturally present in the environment. The adverse human health effects associated with exposure to heavy metals, even at low concentrations, are diverse and include, but are not limited to, neurotoxic and carcinogenic actions [1].

The analysis of heavy metal ions in environmental samples remains a challenging task. Electroanalytical methods, particularly stripping analysis, are the most widely used alternative methods that compete with atomic spectroscopy or other techniques, as far as trace analytes determination is concerned.

Recently, electropolymerized polymer films have received considerable attention in the preparation of sensors, because of their good transducing properties, high stability and reproducibility [2]. Groups of monomers that displayed market analytical utility are those based upon substituted aromatic, such as aniline. Polyaniline is a conducting polymer which is easy to deposit from aqueous acid solutions, adheres strongly to the support and presents good environmental stability. The polymer has low cost production, high conductivity and high chemical durability against oxygen and moisture. Polyaniline films are known to be effective in the reduction of electroactive interferences on the response of polymer-modified sensors.

The aim of this work is to develop a new kind of sensor, based on the surface modification of screen-printed electrodes with polyaniline, to determine heavy metals in environmental samples.

Acknowledgments:

The authors thank the *Fundação para a Ciência e a Tecnologia* for the financial support through the project PTDC/AGR-AAM/102316/2008 (COMPETE and co-financed by FEDER).

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# Electrochemical biosensor for the determination of catechol

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Tyrosinase is an enzyme that contains two atoms of copper per active site with mono-oxygenase activity, which catalyzes the oxidation of the phenol group to o-quinone, thus allowing a variety of phenolic compounds to be used as substrates of this enzyme [1-2]. This work aimed to study a tyrosinase-based electrochemical biosensor for the determination of catechol. A self-assembled monolayer of cysteamine was formed on a clean gold electrode by immersing it into a  $10^{-3}$  mol/L cysteamine solution for 18 hours. To remove excess or unbound cysteamine the electrode was washed with ethanol followed by water. Next, the electrode was immersed in a 2% glutaraldehyde solution for 30 minutes for cross-linking immobilization. The electrode surface was incubated with 20  $\mu$ L phosphate buffered saline (PBS, pH 7.4) containing 10 mg/mL tyrosinase and 1 mg/mL bovine serum albumin for 18 hours. Finally the electrode was washed with PBS (pH 7.4) and stored at 4°C until use. Different concentrations of catechol in PBS (pH 6.5) were used in these studies. The results show that as the catechol concentration increases the reduction peak also increases. A linear response was observed for different concentrations of catechol ( $9 \times 10^{-7}$  -  $9 \times 10^{-4}$  mol/L) with a 0.98 correlation factor.

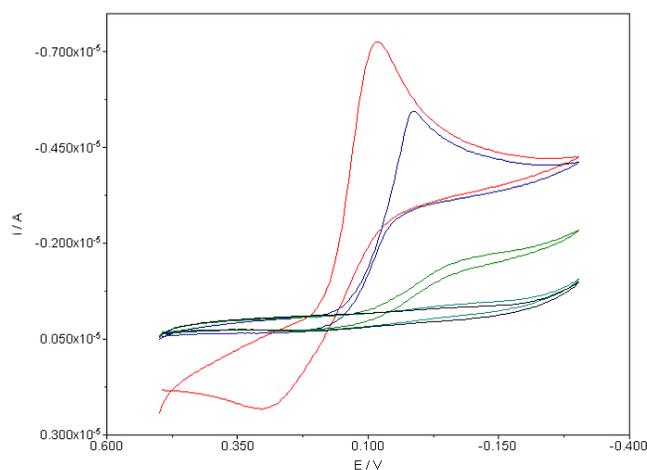


Figure 1: Voltamograms of the enzyme electrode's response for catechol in PBS (pH 6.5)

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# Electrochemical Study of Keggin-Type Polyoxometalates

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The interest in polyoxometalates (POMs) has increased in the latest years, mainly because of their enormous variety of structures and properties that make these compounds unique, being used in many fields with several applications, such as catalysts, electrochromic and photochemical materials, energy storage and optical materials<sup>[1]</sup>.

Keggin-type POMs (Figure 1.), with a general structure of  $[XM_{12}O_{40}]^{n-}$  ( $X = P, Si$ , etc and  $M = W, Mo$ ) are a early transition metal-oxide clusters consisting of  $d^0$  metal cation ( $Mo^{6+}$  and  $W^{6+}$ ) and oxygen anions arranged in  $MO_6$  octahedral units with remarkable ability to accept a large number of electrons and undergo reversible multi-electron redox processes, giving rise to mixed valence species<sup>[1-3]</sup>. The substitution of the metal cations  $Mo^{6+}$  and  $W^{6+}$  by other transition metals, such as vanadium, can be done, in order to change the properties of these clusters.<sup>[3]</sup>

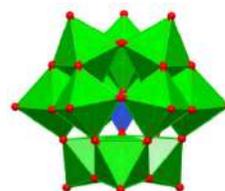


Figure 1 – Keggin-type POM

In the present work, vanadium substituted polyoxometalates were synthesized and characterised by several techniques. Their composition and structures were accessed by elemental analysis, termogravimetry, Fourier transform infrared spectroscopy and <sup>31</sup>P and <sup>51</sup>V NMR spectrometry. The electrochemical behaviour of the Keggin-type POMs in solution was studied by cyclic voltammetry in acetonitrile and acetate buffer (pH=4) using a standard three electrode cell – carbon disc and Pt foil as working and counter electrodes, respectively, and Ag/AgCl (1M NaCl) as the reference electrode. In the future, these clusters will be used on the preparation of multilayer thin films, using the LBL (Layer-by-Layer) method and their electrochromic properties studied.

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# Enantioresolution of Chiral Xanthone Derivatives by HPLC

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Xanthonones have emerged as a class of compounds with increasing interest, possessing a broad spectrum of biological and pharmacological activities<sup>[1]</sup>. In spite of this, only a handful of chiral xanthone derivatives (CXDs) have been reported to date<sup>[2,3]</sup>.

Many biologically and pharmacologically interesting compounds are chiral and pharmacodynamics, pharmacokinetics and toxicity are often different between their enantiomers, making this an important issue in drug development<sup>[4]</sup>. Since the development of chiral stationary phases (CSPs), high performance liquid chromatography (HPLC) has emerged as the preferred method for analyses and preparation of enantiomerically pure bioactive compounds<sup>[5]</sup>. Taking into consideration that CEQUIMED-UP has ongoing work in the development of pharmacologically active xanthonones, including some CXDs, it is of our interest to develop new methods of HPLC enantioresolution.

Therefore, the enantioresolution of a series of seven synthesized xanthonic racemates was investigated by HPLC on two Pirkle columns, (*S,S*)-Welch O1 (5  $\mu\text{m}$ , 100  $\text{\AA}$ , 25 x 0.46 cm i.d.) and *L*-Phenylglycine (5  $\mu\text{m}$ , 100  $\text{\AA}$ , 25 x 0.46 cm i.d.), under multimodal elution modes (normal, reversed-phase and polar organic). The analyses were performed in isocratic mode and with UV-Vis detection.

At the chromatographic conditions evaluated, the (*S,S*)-Welch O1 column demonstrated the higher power of resolution for majority of the racemates analyzed.

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# Evaluation of drug-drug or drug- metabolite interaction(s) by differential scanning calorimetry

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Present understanding that drug addiction is a chronic brain disease paves the way for pharmacotherapy. Its ideal goal is to enable a patient to maintain permanent and voluntary abstinence. However only provisional goals, such as reduced drug use, improvement of patient's functioning in the society, and minimization of addiction-related health and social hazards have been attained yet [1].

Recent research have shown that drugs not only interfere with brain normal functioning creating feelings of pleasure, but also have long term effects on metabolism and brain activity, and at one point, changes could occur in the brain that can lead to abuse and to dependence.

Two drugs with a high consumption rate and large potential for addiction are cocaine and heroin having its combined use ("speedball") increased significantly in recent years. The use of "speedball" in polydrug abusers is more rewarding than cocaine or heroin-alone [2]. The mechanisms' underlying the biological action of this kind of combination of drugs is still unknown. It is consensual that the characterization of molecular and biological processes, and the resulting effects, is essential for understanding the cause-effect and to develop an effective treatment.

Accordingly, any research efforts directed to amplify the knowledge of biological and chemical molecular mechanisms of drugs of abuse is found to be vital to decode the addiction problem. As a result, substantial work at various levels is currently required to establish the connection between the molecular mechanisms and addition activity.

With the aim of acquiring information on the relationship between structure and activity the study of the interaction of cocaine, and its main metabolites, with opiates has been carried out in the present project. The drug-drug or drug- metabolite interaction(s) were monitored by differential scanning calorimetry (DSC). The results of this study will be presented in this communication.

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# Flow systems with voltammetric detection in pharmaceutical formulations

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Isoniazid, pyridine-4-carboxylic acid hydrazide, is an antituberculosis-agent, which is used to prevent the development of clinical tuberculosis.

There are several methods for analysis of isoniazid available in literature. However, most of them do not achieve low enough detection limits, require sample pretreatment and time-consuming extraction steps prior to the analysis.

In this work, a fast, simple and precise method to analyze isoniazid based on the electrochemical oxidation of the analyte at a glassy carbon electrode is described and applied to the assay of the drug in tablets.

Determinations were performed using flow injection analysis (FIA) technique associated with voltammetric detection. This flow technique is based on the injection of an aliquot of sample into the carrier flow, not segmented, that carries the sample to the detector. Flow injection analysis system allows the reproducibility of the sampling procedures and detection and control of sample dispersion. [1]

This FIA manifold uses a Gilson Minipuls 3 peristaltic pump to propel the solutions. Measurements are performed using an *Eco Chemie Autolab PGSTAT10 potentiostat* and a “wall-jet” electrochemical cell. The cell is composed by three electrodes, a glassy carbon working electrode (3mm diameter), an Ag/AgCl (3M) reference electrode and a platinum auxiliary electrode.

Reagents of p.a. quality or similar were used without being subjected to any additional purification. In the preparation of solutions, water purified by the Millipore Milli Q system (conductivity <0.1  $\mu\text{S cm}^{-1}$ ) was used.

This method gave high sampling rates and reduced the adsorption at the electrode surface, thus requiring mechanical cleaning of the glassy carbon electrode. The electrochemical oxidation of isoniazid has been investigated by cyclic voltammetry and square-wave voltammetry. In cyclic voltammetry, an irreversible anodic peak was attained, which must be attributed to the irreversible oxidation of the amide moiety of the drug molecule. The assay of isoniazid in tablets using square-wave voltammetry showed results with high sensitivity and low limits of detection.

The proposed procedure was successfully applied to the analysis of isoniazid tablets without the need of any pretreatment.

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# Functionalization of mesoporous silica nanoparticles using microwave irradiation

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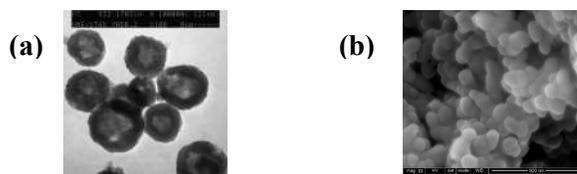
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The synthesis of mesoporous nanosilicas with controlled particle size, morphology and porosity has been a theme of intense research for a wide variety of technological applications, such as catalysis, adsorption, separation and chromatography. The functionalization of these nanomaterials with organic groups allows tuning their surface properties. [1]

This work reports the synthesis of silica nanoparticles by two different methodologies based on sol-gel, using various surfactants such as cetyltrimethylammonium chloride (CTAC), poly(vinylpyrrolidone) (PVP) and dodecylamine (DDA). [2] The synthesized nanomaterials were functionalized with different organosilanes by microwave irradiation (MW) to evaluate the potential of this method for the grafting of organic groups on the surface of mesoporous silica nanoparticles. The MW-heating represents an alternative to conventional functionalization procedures, since it is more eco-efficient and significantly reduces the reaction time and amount of solvents needed. [3]

The new silica nanomaterials were characterized by FTIR, SEM (Fig. 1), EDS, TEM (Fig.1), powder X-ray diffraction, thermogravimetry, <sup>13</sup>C and <sup>29</sup>Si solid-state NMR and N<sub>2</sub> adsorption-desorption isotherms at 77 K.



**Figure 1:** TEM and SEM images of silica particles prepared with different surfactants: a) PVP and DDA and b) CTAC.

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# Gold nanotriangles and nanocubes preparation, purification and functionalization for binding to biomolecules

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Nanotechnology, inextricably a multidisciplinary field, has an explosive growth in the past decade due to its extremely hefty applications in various fields of nanoscale electronics, optics magnetic, energy, catalysis, nanomedicines clothing, cosmetics, etc. [1]. Recently a great advancement was observed in utilizing metal nanoparticles, especially gold, for biomedical applications, owing to their unique shape/size-dependent properties, strong absorption/scattering of light, stability and nontoxic nature [2].

The main goal of this work is optimize methods of synthesis of gold nanotriangles and nanocubes. Additionally we intend to develop effective methods of purification of nanoparticles in order to obtain colloidal suspensions with physicochemical properties very well defined. Finally, the purified nanoparticles are functionalized with biological molecules using electrostatic interaction strategies and covalent bonding. The functionalized nanoparticles will be use in toxicity studies and to study its interaction with serum proteins.

The nanoparticles were synthesized using a photocatalytical process [3] and studied by UV-Vis spectroscopy. The gold nanoparticles were purified by centrifugation to remove the excess of capping agent and the effectiveness of purification process will be assessed using DLS, TEM and AFM followed by functionalization with molecules containing the thiol group, surface modification with net negatively charged compounds [2] and covalent binding of proteins (using peptide bond formation with EDC/NHS). The efficiency of functionalization will be evaluate by zeta potencial and chemical analysis using ICP and XPS.

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# Heterogeneous Catalytic Oxidation of Styrene by mono-Substituted Silicotungstates Immobilized in SBA-15

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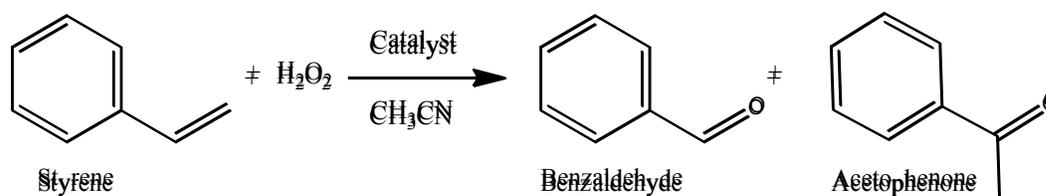
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The oxygenation of hydrocarbons has received much attention over the last few decades because their products are valuable intermediates for organic synthesis in the laboratory and in industry.<sup>[1]</sup> Polyoxometalates (POMs) have been largely used as homogeneous catalysts in the oxidation of organic substrates.<sup>[2]</sup> These compounds have a highly structural versatility and a high compatibility with eco-sustainable catalytic reaction conditions.<sup>[3]</sup>

Despite the advantages of homogeneous catalysts - low reactions times and selectivity- they have some disadvantages which include difficult catalyst separation and recycling from the reaction media and low chemical and thermal stability.<sup>[4]</sup> In this context, the immobilization of homogeneous catalysts onto solid supports is an alternative method to overcome the drawbacks of homogenous catalysis.<sup>[4]</sup>

The purpose of this work is to study the heterogeneous catalytic activity in the oxidation of styrene by hydrogen peroxide of monosubstituted silicotungstate,  $[\text{SiCo}(\text{H}_2\text{O})\text{W}_{11}\text{O}_{39}]^{6-}$  immobilized in SBA-15, previously functionalized with 3-aminopropyltriethoxysilane (APTES). The use of hydrogen peroxide in the oxidation of organic compounds is very attractive because of the simplicity of handling, the environmentally friendly nature of co-product, the high oxygen atom efficiency and the versatility. The catalyst efficiency, identification of the reaction products and selectivity will be presented and discussed.



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# Identification of photo-degradation products of geotextiles by gas and liquid chromatography with mass detection

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Geotextiles are polymeric materials used in the construction of many civil engineering structures, such as: waste landfills, roads, railways, tunnels, dikes or reservoirs. During their lifetime, the geotextiles can be exposed to many damaging agents, such as: acids, alkalis, ultraviolet (UV) radiation and other weathering agents, atmospheric O<sub>2</sub>, high temperatures and microorganisms [1]. An extended exposure to these agents can have a negative impact on the properties of the geotextiles, affecting their performance and shortening their lifetime. The degradation suffered by these materials is often retarded or inhibited by the incorporation of chemical additives (antioxidants, thermal stabilisers or light stabilisers) in their composition. Chimassorb 944 (C944) (a light stabiliser) is an example of one of those additives (in the last years, this additive is being studied by our research team [2]).

The aim of this study was to identify photo-degradation products of polypropylene (PP) geotextiles (stabilised, or not, with C944) by liquid and gas chromatography with mass detection (LC-MS and GC-MS, respectively).

First, some PP geotextiles were exposed (at 60 °C) to UV radiation (photo-degradation process) in a laboratory weatherometer (the *QUV Weathering Tester*). Then, the intact and photo-degraded geotextiles were subjected to solvent extraction (dichloromethane, 2 hours at 40 °C) in an ultrasonic bath. Finally, the organic extracts were analysed by LC-MS and GC-MS.

The LC-MS study allowed the identification of C944 in the intact geotextiles (stabilised with this additive), but not in the photo-degraded ones (C944 was lost and/or consumed during the photo-degradation process). In addition, this study allowed the identification of some photo-degradation products of the geotextiles.

The GC-MS analyses showed the presence of some compounds in the geotextiles, such as: siloxanes (present in the intact and photo-degraded samples), trialkylphosphates and phthalates (both only present in the photo-degraded samples).

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# In vitro antioxidant activity of 3-hydroxyflavones

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Flavonoids are a group of naturally occurring compounds with an assortment of structures based on a common three-ring nucleus comprised of two benzene rings (A and B) linked by a heterocyclic pyran or pyrone ring (C). The various classes of flavonoids [anthocyanidins, flavanols (catechins), flavones, flavonols, flavanones, and isoflavones] differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of A and B rings. Several compounds from this group have shown great antioxidant activity [1]. This activity is based mainly on their capacity to inactivate pro-oxidant compounds, namely free radicals, by donating electrons or hydrogen atoms, originating aroxyl radicals that are sufficiently stable to avoid the propagation of chain reactions. However, the structure activity relationship is only known for a limited number of well known flavonoids. Thus, the aim of the present study was to evaluate the putative scavenging effect of an hitherto not studied series of 3-hydroxyflavone derivatives (Fig. 1) on a array of reactive oxygen species (ROS): superoxide radical ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), and hypochlorous acid (HOCl), as well as a reactive nitrogen specie (RNS): peroxyntirite anion ( $ONOO^-$ ) using non-cellular *in vitro* systems.

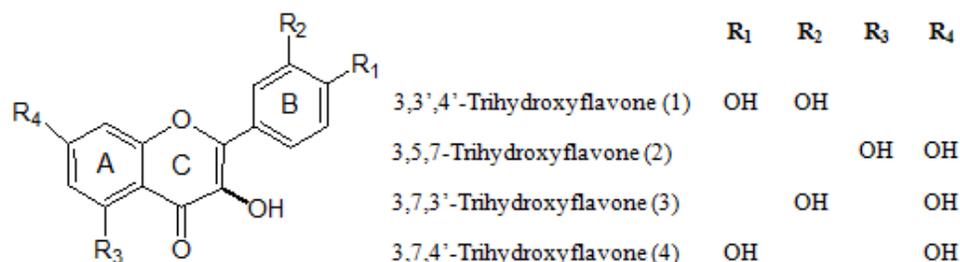


Fig. 1 Chemical structure of the studied 3-hydroxyflavones.

The results obtained in this study showed that the studied compounds effectively scavenge  $O_2^{\cdot-}$ ,  $^1O_2$ , and HOCl in a concentration dependent manner, though no activity was found for  $H_2O_2$ . From these ROS, the effect on  $^1O_2$ , and HOCl, specially those mediated by compounds (1) and (2) are very promising since they were obtained at the low  $\mu M$  range. The scavenging effect for  $ONOO^-$  was also concentration-dependent, though this time no clear structure activity relationship was found.

In conclusion, the potent scavenging activities observed in the present non-cellular *in vitro* assays for some of the studied 3-hydroxyflavones indicates that these compounds can have high interest in different fields where the antioxidant agents are required, especially if  $^1O_2$ , HOCl, and/or  $ONOO^-$  are the pro-oxidant reactive species to eliminate.

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# In vitro evaluation of the pharmacokinetics of nimesulide and meloxicam

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The Pharmaceutical Industry faces nowadays new challenges as a consequence of strong demands related with the profitability and efficiency of new drugs. Thus, it became mandatory to develop simple and fast assays for the screening of new drugs in an initial stage of their development, in order to select the most promising compounds. The implementation of these assays *in vitro* must consider the course of the drug in the human body until it reaches the therapeutic target and thus should somehow mimic some of the biological phenomena that condition its pharmacokinetics and pharmacodynamics [1]. Physiologically, the bioavailability of drugs is strongly conditioned by their absorption in the gastrointestinal tract that is influenced by the emulsifying capacity of bile salts. Several studies with drugs belonging to distinct classes revealed that bile salts affect not only the dissolution of lipophilic drugs but also their absorption from the gastrointestinal tract. So, the study of the interaction of pharmaceuticals with bile salt micelles is nowadays accepted as an important model to predict the same interaction at the biological level [2]. After absorption, the distribution and elimination of drugs are mainly controlled by their binding to plasma proteins, a process that greatly influences their pharmacokinetics. Thus, the study of drug-protein interactions became an important tool to predict the behaviour of drugs *in vivo*. In particular human serum albumin, the most abundant plasma protein, has been integrated in fluorescence assays with several drugs, and is considered a model protein for the study of drug-protein interactions [3].

In this work, two spectrophotometric methodologies for the evaluation of the pharmacokinetics of nimesulide and meloxicam were implemented. This evaluation was based in the interaction of drugs with bile salt micelles and with human serum albumin. The interaction of the drugs with bile salt micelles was quantified by derivative spectroscopy with calculation of partition coefficients ( $K_p$ ) between the aqueous and micelle phases. The study of the quenching of albumin fluorescence in association with models of drug-protein binding in the equilibrium (considering or not ligand depletion) enabled the calculation of binding constants as well as the evaluation of the binding sites and nature of forces involved in the interaction.

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# Inorganic and metallo-organic polymer based electrochromic devices

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The electrochromism phenomenon involves electroactive species whose optical properties change reversibly as response to a DC voltage/current input<sup>[1,2]</sup>. Electrochromic materials have been studied over the past years due to their scientific interest<sup>[3]</sup> and potential applications, for example, in intelligent windows<sup>[4]</sup>, in auto-dimming rear view mirrors<sup>[1]</sup> and in paper-like displays<sup>[5]</sup>. In this work, two classes of electrochromic systems were studied: one based on the reversible electrodeposition of Bi<sup>0</sup> and other based on metallo-organic polymers obtained by electropolymerisation of Ni(II) *salen* complexes<sup>[6]</sup>.

Electrochromic systems based on the reversible electrodeposition of Bi<sup>0</sup> are very complex. After the electrodeposition of a black film of metallic bismuth on the electrode surface, its complete dissolution by oxidation is not a really reversible process, most probably due to the low electroactivity of the formed bismuth films<sup>[7,8]</sup>. According to literature, the best results for reversibility and colour contrast are obtained in the presence of copper(II) ions in an acidic medium. The goal of this work was to find the ideal proportion of Bi:Cu in this system to give the highest stability and colour contrast. To achieve this goal, an experiment based in the *standard addition* method was designed: the Cu<sup>2+</sup> concentration in the medium was varied by adding small volumes of a concentrated solution of this ion, and the electrochromic behaviour of the system evaluated by cyclic voltammetry upon each Cu<sup>2+</sup> addition, using as working electrode an indium-tin oxide coated glass (ITO/glass).

The electropolymerization of [Ni(3-MeOsald)] and [Ni(3-MeOsaltMe)] was performed in flexible polyethylene terephthalate coated with indium-tin oxide (ITO/PET), by cyclic voltammetry. The respective polymeric films were characterised by cyclic voltammetry and chronoamperometry and by *in situ* UV-Vis spectroscopy (in the range  $\lambda = 300$  to 1100 nm). The corresponding electrochromic devices were fabricated using an ionic conductive polymeric gel based on a poly(ethylene glycol) (PEG)-related polymer, a lithium salt and acetonitrile.

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# Manganese(II) ferrite nanoparticles: a novel approach of synthesis conditions

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Colloidal magnetic nanoparticles are of great interest for numerous scientific and technological applications such as biology, medicine, catalysis, magnetic sensors, data storage and magneto-optical devices, magnetic refrigeration and environmental remediation [1]. In particular, spinel-type ferrite nanoparticles ( $MFe_2O_4$ , where M is a *d*-block transition metal) are preferentially used due to their stability against oxidation and their significant magnetization when compared to other metallic magnetic particles [2].

The aim of this work was to study the influence of different types of bases – NaOH - and two organic bases – base\_1 and base\_2 – in the synthesis of manganese(II) ferrite nanoparticles ( $MnFe_2O_4$ ) (Fig. 1) by the co-precipitation method [2]. The particles composition, morphology and structural properties were characterized by chemical analysis, termogravimetry, Fourier transform infrared spectroscopy and powder X-ray diffraction. The magnetic properties of the samples were studied by vibrating sample magnetometry (VSM) at 300 K.

The FTIR spectra showed the typical metal-oxygen bands in the range of  $600-400\text{ cm}^{-1}$ . The ferrite nanomaterial prepared with NaOH had a ferromagnetic behavior with a saturation magnetization of  $\sim 62\text{ emu g}^{-1}$ , while the nanoparticles synthesized with the organic bases presented a superparamagnetic behavior with a saturation magnetization of  $\sim 53\text{ emu g}^{-1}$  for base\_1 and  $\sim 3\text{ emu g}^{-1}$  for base\_2.

## Acknowledgments:

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# Microplate method for determination of cupric ion reducing antioxidant capacity of food products

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Assessment of antioxidant capacity of food matrixes such as wines, teas, vegetables and fruits has become a rather disputable issue during the past decade. In this context, analytical chemists have sought to develop or to improve antioxidant methodologies in order to attain rapid, reliable and high-throughput procedures suitable for routine analysis. Among the antioxidant capacity assays developed, the spectrophotometric methods based on electron transfer reactions, such as the cupric ion reducing antioxidant capacity (CUPRAC) assay, are widely used due to their simplicity and reduced cost.

The CUPRAC assay measures the capacity of antioxidants to reduce the Cu(II)-neocuproine complex to the highly colored Cu(I)-neocuproine, which is measured spectrophotometrically at 450 nm [1]. This method has been used to determine the antioxidant capacity of a wide range of foodstuffs; nevertheless this procedure uses a single cell spectrophotometer which limits the determination rate of the methodology.

Therefore, the aim of the present work was to implement the CUPRAC assay into a microplate format in order to attain a high-throughput method suitable to rapidly assess the antioxidant/reducing capacity of a large number of samples. Using a 96-well plate, five concentrations of standard compound (Trolox) and eighteen food samples can be analyzed in quadruplicate. The reaction between the oxidizing species (Cu(II)) and antioxidants can be easily monitored every minute until the maximum absorbance signal is reached for assessment of the total antioxidant capacity. This microplate method also reduces about 15 times the quantity of reagents used as well as the waste produced per sample analyzed.

The proposed microplate CUPRAC assay was applied to red wines ( $n = 12$ ), white wines ( $n = 6$ ), green teas ( $n = 5$ ), black teas ( $n = 5$ ) and orange juices ( $n = 3$ ). The CUPRAC values, expressed as mmol of Trolox equivalents per liter of sample, obtained for red wines ( $23.6 \pm 4.4$  mM) were about 15 times higher than those attained for white wines ( $1.7 \pm 0.5$  mM) and orange juices ( $1.5 \pm 0.1$  mM). Green teas ( $10.0 \pm 0.6$  mM) and black teas ( $4.7 \pm 0.3$  mM) provided antioxidant values 2.5 and 5 times lower than those obtained for red wines.

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# Microwave Assisted Synthesis of Coordination Polymers: MIL-47 and MIL-53

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Coordination polymers, also named Metal-Organic Frameworks (MOFs) are hybrid materials containing metal centers linked by organic ligands, extending in an infinite array. A MOF compound is typically composed of two main components: a metal ion (or metallic cluster) and an organic molecule (linker). The choice of the metal and the linker greatly affects the structure and properties of the MOF, thus obtaining networked structures that are one-, two- or three-dimensional (1D, 2D or 3D). Besides the interesting structural features MOF materials have potential applications in many scientific and technological areas, such as gas adsorption/storage, separation, catalysis, molecular sieving, drug delivery, magnetic and luminescent sensors, and others. [1]

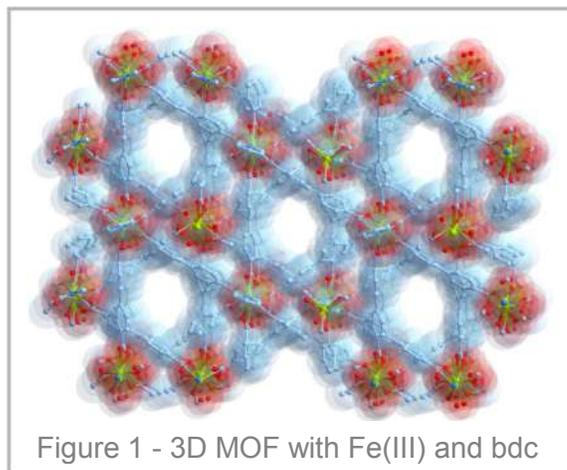


Figure 1 - 3D MOF with Fe(III) and bdc

MOFs have mainly been prepared by solvothermal methods at high temperatures using conventional electrical heating, with reaction times up to several days. Nowadays, a challenging task in the preparation of MOF materials is the reduction of reaction times and energy consumption. Consequently, Microwave Assisted Synthesis (MWAS) is being experimented for the preparation of these materials. [2]

Two distinct MOFs based in the benzene-1,4-dicarboxylic acid (bdc) as organic linker and V(III) or Fe(III), figure 1, as metal centers (respectively, MIL-47 and MIL-53) were isolated in the solid-state. Both MOF materials have been synthesized by conventional electric heating (solvothermal synthesis) and microwave heating at various conditions and characterized by vibrational spectroscopy. Further characterization will be done by RMN, and UV/VIS spectroscopies, thermogravimetric analysis, powder X-Ray Diffraction and Scanning Electron Microscopy.

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# Molecularly Imprinted Polymers Coated Carbon Nanotube Screen Printed Electrode For Dopamine Determination

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Dopamine (DA) analysis has been proven to be a very effective tool towards discerning brain functions, such as learning and memory formation. Electrochemical methods for the dopamine determination are widely used due to its simplicity, speed, low cost, high sensitivity and easy operation. Recent studies demonstrated that a CNTs modified electrode could impart strong electrocatalytic activity to some important biomolecules, including cytochrome, NADH, hydrogen peroxide, and catecholamines such as dopamine, epinephrine, and ascorbic acid [1]. The coupling of polymers with carbon nanotubes and recognition elements form a composite which importance has been increasing due to its simplicity of construction and its ability to incorporate conducting materials into porous polymers in order to form electrochemical sensors [2]. Biomacromolecules such as enzymes, antibodies and DNA have many practical difficulties in application due to their sensibility to various conditions, such as instability against high temperature, organic solvents, and pH. To overcome the above limitations, a molecular imprinting technique, the design and construction of biomimetic receptor system, with predetermined recognition for target molecule, has been proposed and developed rapidly [3]. In this report, the molecular imprinted polymer (MIP) deposited on multiwalled carbon nanotube coated electrode (MWCNTs-MIP) was tested by cyclic voltammetry (CV), by electrocopolymerization of aniline and resorcinol in the presence of the template molecular DA. The performance of the imprinted and non-imprinted (NIP) films was evaluated by difference pulse voltammetry (DPV). The calibration curve for the DPV peak current observed for DA oxidation versus dopamine concentration was ranged between  $10^{-7}$ M and  $10^{-4}$ M. This MWCNTs-MIP on the screen printed electrode surface was employed to recognize DA from ascorbic acid, glucose, urea and creatinine as interference.

## Acknowledgement:

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## Na, K, Mg, Ca and Mn determination in teas

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The growing demand of tea lies on its benefits to human health. The consumption of algae based tea is more and more recurrent due to its high levels of proteins, minerals and vitamins [1]. Although minerals are essential to normal health and development, they can become toxic in higher amounts. Risk assessments of chemical elements show high intakes that result in toxicity or nutritional problems related to low or no intakes. Depending on the amounts needed, minerals can be divided into macro (g or mg/day) and microminerals (few mg or µg/day) [2]. Na, K, Mg, and Ca are examples of important macrominerals, and Mn is considering a micromineral.

Considering the effect of bioaccumulation in algae, the purpose of this work was to develop and validate analytical methods to quantify some metals in different types of tea.

Four samples of teas were analyzed: green with cinnamon, 100 % black and two types of algae based teas. Teas were prepared from the sachets and dipping them in boiling water for 5 min. The effusions were cooled to room temperature and a microwaves digestion was performed. The method used for the quantification of Na, K, Mg, Ca and Mn was the high resolution continuous source atomic absorption spectrophotometry (HR CS AAS) with flame atomization.

Given the legislation limits (Decreto-lei nº 306/2007 from 27 of August) worrying levels for Mn in samples without algae were obtained. The teas which contain algae extracts have higher concentration of K, Na and Ca and residual quantities of Mn than teas without algae.

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## Nanosensor development for C-Reactive Protein in human serum

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C-reactive protein (CRP) is an acute-phase protein, discovered in 1930 by Tillet and Francis at the Rockefeller Institute for Medical Research. This protein is used as a marker for certain diseases such as viral or bacterial infections, inflammations or rheumatoid arthritis, and therefore currently used to detect these diseases when values are relatively high, or show a marked increase, in human serum. <sup>[1]</sup>

In this communication, the results obtained in the development of a nanosensor for CRP based on fluorescent nanoparticles, quantum dots (QDs), will be presented. QDs of CdSe and ZnSe functionalized with *O*-phosphorylethanolamine (PEA), which possesses a highest affinity for CRP, are being developed as CRP nanosensors.<sup>[2,3]</sup> QDs were capped with mercaptosuccinic acid (MSA), mercaptopropanoic acid (MPA) and mercaptoacetic acid (MAA) before functionalization with PEA.

The results of the interaction of the developed nanosensors with CRP will be presented and the figures of merit of the nanosensor discussed. Also, the potential advantages of the sensor for routine blood analysis in advanced diseases diagnostic will be analyzed.

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# Optimization of the microwave assisted synthesis of meso-tetra(pentafluorophenyl)porphyrin

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Porphyrins and derivatives are macrocyclic compounds with a key biological role and that have been object of great interest in several areas such as catalysis, photodynamic therapy and in the fabrication of molecular electronic or magnetic devices.

One important factor for a wider application of porphyrins is the improvement of their synthetic procedures. The porphyrin synthesis has been studied since 1929; however, new methodologies are still under research in order to increase the yields and lower the production costs. Alternative methods are all also searched in the context of "green chemistry".

Microwave-assisted reactions have attracted much interest because of their simplicity in operation and milder reaction conditions. The salient features of the microwave approach are enhanced reaction rates, formation of products in high yields and ease of isolation [1].

In the present study the microwave assisted synthesis of *meso*-tetra(pentafluorophenyl)porphyrin (H<sub>2</sub>TPFPP) was optimized. Different parameters were tested such as the reaction temperature and time, concentration of reagents, the mixture of solvents used and power of activation. The best results were obtained in environmentally acceptable conditions and show a dramatic reduction of reaction time and higher yields than those mentioned in literature for the synthesis under classical heating method.

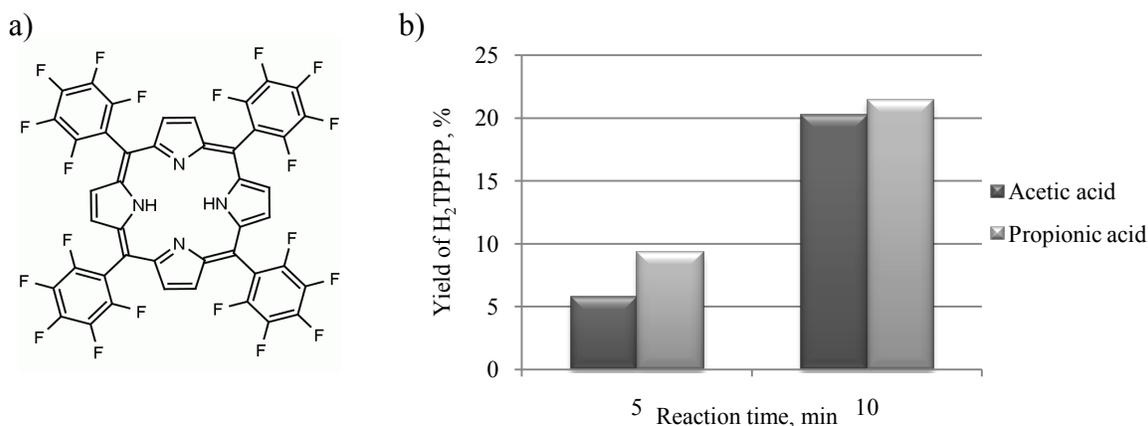


Figure 1. a) *meso*-tetra(pentafluorophenyl)porphyrin; b) Yields of H<sub>2</sub>TPFPP obtained in microwave-assisted synthesis in different conditions.

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# Oxidation of tetracene with hydrogen peroxide catalyzed by a Mn(III) porphyrin

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The use of metalloporphyrins as catalysts for oxidation reactions was initially inspired on the activity of the prosthetic group of cytochrome P450, a mono-oxygenase which mediates oxidations even of inert and apolar xenobiotic substrates, in order to facilitate its excretion from the body. [1]

Tetracene, a linear polycyclic aromatic hydrocarbon with four fused rings was efficiently oxidized in mild conditions using hydrogen peroxide as a clean oxidant in the presence of the metalloporphyrin [Mn(TDCPP)Cl] (Fig. 1). [2] The reaction proceeded at room temperature leading to quinones and epoxyquinones that have important characteristics for photo-electronic and biological applications.

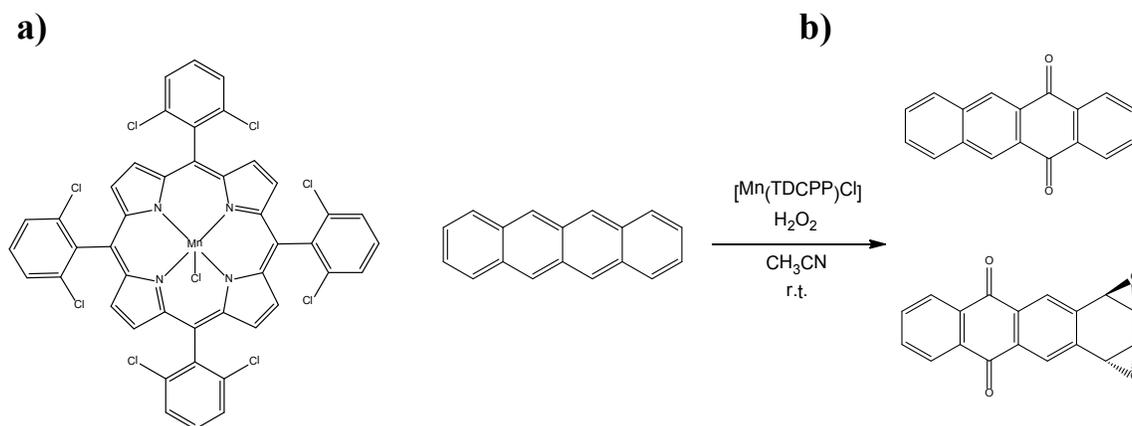


Fig. 1: a) [meso-tetrakis(2,6-dichlorophenyl)porphyrin]manganese(III) chloride; b) Oxidation reaction of tetracene.

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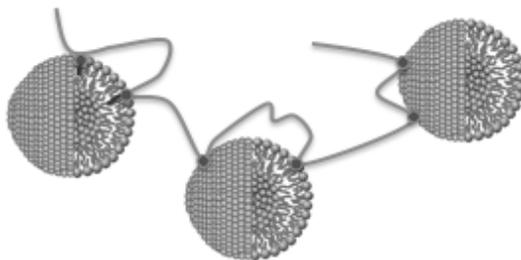
# PHASE BEHAVIOR AND MOLECULAR INTERACTIONS BETWEEN BIOPOLYMERS AND MODEL LIPOSOMES

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Polymer-surfactant systems have been intensively investigated from a physico-chemical perspective in the last two decades, with the main focus on micelles as the surfactant aggregate in interaction. Polymer-liposome systems, on the other hand, have received comparatively less attention. Yet, phase behavior features of these systems, effects of the polymer on the liposome structure and stability, and the forces/mechanisms of macromolecule-liposome interaction are all relevant scientific topics [1]. For instance, polymer-liposome hydrogels with interesting rheological properties and high potential for applications (e.g. in drug delivery) can be generated. Also in this context, model non-conventional liposomes based on lipid-mimetic molecules can be used to circumvent some disadvantages of classic lipid-based formulations [2].

In this work, we report the results obtained in the study of the phase behavior and structural effects for different biopolymer-systems, as revealed by differential scanning calorimetry and microscopy techniques. The biopolymers include polycations based on hydroxyethylcellulose, and polyanions based on modified chitosans. The liposomes are composed by either an anionic lysine-based surfactant or by a synthetic cationic surfactant. The polymer-liposome systems are highly associative due to both electrostatic and hydrophobic interactions [3].



**Figure.** Schematic drawing of a polymer-liposome gel-like network.

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# Phase solubility studies of the inclusion complex MCPA -Methyl- $\beta$ -cyclodextrin

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Cyclodextrins (CDs) are cyclic water soluble oligosaccharides that have the ability to form inclusion complexes with nonpolar molecules. Inclusion in these hosts results in many modifications to the properties of the guest compounds such as fixation of volatile materials, masking of odorous compounds, stabilisation against hydrolysis, protection against oxidation and photolysis and the modification of their physical and biological properties.

However, the application of cyclodextrins in pesticide formulations is very modest till now. At the present time, large improvements in cyclodextrin production and purification have been achieved and the price is lower than before. In this way, the application of cyclodextrins on pesticide-formulating industry could be a real possibility.

MCPA or 2-methyl-4-chlorophenoxyacetic acid (Figure 1), a typical organic compound of the phenoxyalkanoic group, is a widely used systemic and selective herbicide. It is mainly used to enhance the productivity of rice, corn, wheat and other agricultural crops by controlling perennial broadleaf weed growth. MCPA has been popularly employed for field application due to its high efficiency and lower price.

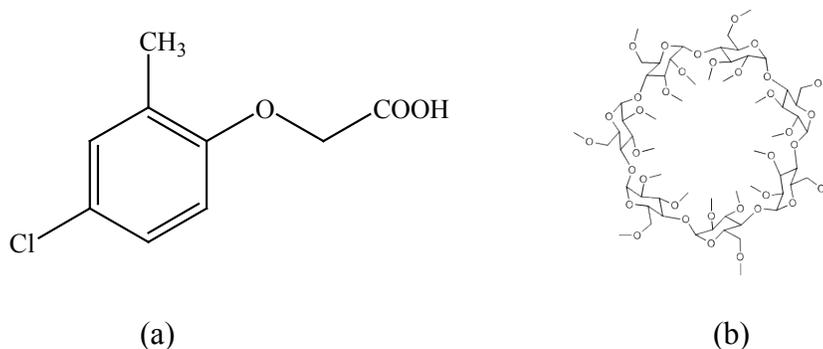


Figure 1 – (a) MCPA and (b) Me- $\beta$ -cyclodextrin.

The aim of this project is to study the formation of inclusion complexes of MCPA with methyl- $\beta$ -cyclodextrin (Figure 1) and the determination of association constants of the complex formed by using spectrophotometric methods. The results obtained so far will be presented in this communication.

## Acknowledgements

Authors acknowledge U.Porto/Santander Totta and Fundação para a Ciência e a Tecnologia (FCT), project PTDC/AGR-AAM/105044/2008, for financial support.

# Portable and automatic micro-system for chemical and toxicological control of glibenclamide

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\*The authors had equal contribution to the developed work.

This work deals with the development of a portable, fast screening and automated microsystem for the detection and quantification of glibenclamide in pharmaceutical formulations as well as in “surreptitiously” spiked alcoholic beverages. The exploitation of the multipumping flow analysis concept (MPFS) [1] allows implementing very compact and portable analytical systems, with high simplicity in automation and control, bringing together all the advantages associated to miniaturization, such as, low power requirements, reagent consumption and reduced time for analysis. The developed method was based on the fluorimetric monitoring of glibenclamide in acidic medium ( $\lambda_{\text{ex}}=301$  nm;  $\lambda_{\text{em}}=404$  nm) and the analytical signal was enhanced by performing the determinations in an organized micro-system obtained with sodium dodecyl sulfate.

Glibenclamide is a second generation antidiabetic agent widely used to lower glucose levels in patients with type II non-insulin-dependent diabetes mellitus. In addition to classic overdose due to suicide attempts, homicides with glibenclamide have been reported [2]. In fact, overdosages with glibenclamide can induce high sedation effects that can lead to conditions of lethargy. For this reason, glibenclamide can be used to incapacitate victims and commit drug-facilitated crimes. Thus, the fast screening of such drug is imperative in terms of public health.

A linear working response range for glibenclamide concentration up to  $75 \text{ mg L}^{-1}$  was obtained. The calibration curve was represented by  $\text{FI} = 0.0512 (\pm 0.0007) \times C_{\text{glibenclamide}} (\text{mg L}^{-1}) + 0.09 (\pm 0.03)$  with a correlation coefficient of 0.9992. Comparison of the results obtained in the determination of glibenclamide in five commercial pharmaceutical formulations by the proposed flow procedure, with the ones provided through a HPLC reference procedure, revealed a good agreement between both methods, with relative deviations comprised between  $-3.53$  and  $0.82\%$ . Additionally, analysis of five spiked alcoholic beverages revealed good recoveries comprised between  $98.6\%$  and  $99.6\%$  of glibenclamide from the spiked samples.

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# Preliminary results on the evaluation of a post-mortem interval (PMI) estimating approach based on the direct skin surface analysis using FT-NIR spectroscopy

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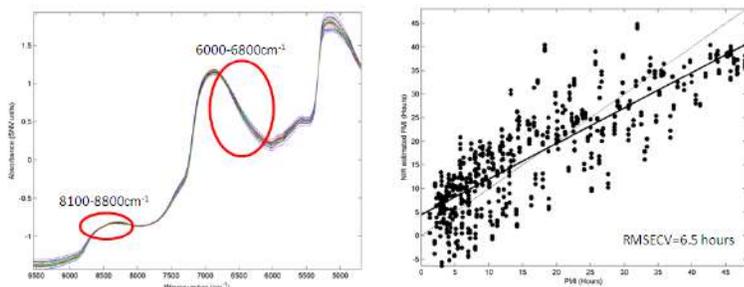
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The estimation of time since death (the so-called “postmortem interval” – PMI) is one of the most challenging problems for pathologists. Methods currently employed have considerable inaccuracy [1,2].

Fourier Transform Infrared (FTIR) Spectroscopy is a powerful tool for identifying chemical bonds in molecules by producing a spectrum that is like a molecular “fingerprint”. In its variant “attenuated total reflectance” (ATR-FTIR spectroscopy), it is widely used, including to study the human skin. Usually, these studies are done with in-compartment ATR accessories, and the sample must be brought into contact with the ATR crystal within the spectrometer sample compartment. However, in the last few years, using mid-infrared fiber optics, direct *in-situ* analysis by ATR-FTIR became possible [3].

In the scope of our Project\* we studied the utilization of FT-NIR spectroscopy to directly test the human skin for possible chemical changes occurring after dead that could correlate with PMI. Studies performed to date (n=25 corpses; submitted to autopsy at NILM) showed promising results. Figures above shows typical spectra obtained from a corpse in the 48 h post-mortem period (left) and the correlation between the predicted PMI versus the known (“real”?) time since dead (right), using 17 cases (corpses) as “calibrations cases” in a PLS model. Additional work with corpses with a more accurately known time since dead (hospital mortuary) is being performed.



**Acknowledgements:** To University of Porto and Santander Totta for financial support.

\*NA::EPMI2 - New Approaches for Estimating the Postmortem Interval - II: Direct skin surface analysis using FTIR spectroscopy (Pre-Graduate Scientific Research – Pluridisciplinary Projects, 2009 Competition).

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# Preparation of metallic nanoparticles functionalized with peptides for applications in biosensors.

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Since its technological development, nanotechnology has been an area of great interest given that involves the manipulation and fabrication of new materials and systems with unique physical and chemical properties. Nanotechnology has an important role in the development of biosensors, as nanomaterials can be bound to biological molecules to be used in bioassay applications.

In the present work the main objective is the preparation of bionanoconjugates of gold nanoparticles and laccase. Laccase from *Rhus vernicifera* is an oxidoreductase that belongs to the multinuclear copper-containing oxidases. It catalyses the monoelectronic oxidation of a variety of substrates in the presence of molecular oxygen, and contains a single set of copper ions of three distinct types [1]. Laccases find several applications in industry, including detoxification of industrial effluents from paper, textile and petrochemical industries, bioremediation agent to clean up herbicides and pesticides, etc. The nanoparticles were synthesized by the Turkevich and seeding growth methods [2,3], and functionalized with different peptides (CALNN, CALKK and KKLAC) in order to improve the adsorption of the enzyme, and the properties of the final bionanoconjugates. Bionanoconjugates (BNCs) were prepared by adsorption of the enzyme to the functionalized nanoparticles. The enzyme activity of the BNCs was compared with the enzymatic activity of laccase alone, in the same experimental conditions. Studies of laccase activity were conducted at different pH, enzyme and substrate concentrations, which were performed by UV-Vis Spectrophotometry. The adsorption of laccase to the functionalized nanoparticles will be evaluated using zeta potential measurements. The adsorption of protein can be simulated using a Langmuir isotherm, which determines the binding constant and the amount of protein required for saturation of the surface. Well-known methods of peptide bond formation chemistry using EDC/NHS for protein binding to the particles functionalized polypeptides will be adapted. This type of engagement will be done initially using laccase, which will compare the enzymatic activity of laccase using coupling via non-covalent binding (adsorption) and covalent bonding. These BNCs have the potential application on the development of sensors and in bioremediation.

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# Chemicals Reactants Exchange Network

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Sustainability is nowadays present in every activity with the goal of improving economic, environmental and social performance [1]. Chemical laboratories should also be managed in a sustainable way through the implementation of strategies and measures that will allow a better performance in the several aspects in a continuous way.

One of the main chemical laboratories problems consists in the reactants that are rarely used and that often become a waste that must be properly disposed. In order to make a better economical and resource management and also contributing to the prevention of chemical waste generation it was developed an on-line reactant exchange place.

The purpose of this project is the design and development of a web application [2, 3] “Chemicals Reactants Exchange Network” that will be used by the community like university, research, professional and educational laboratories. It intends to encourage the exchange of laboratory reactants from one laboratory to another. It will function as an information directory and exchange facilitator for reactants and it will deal with hazardous and nonhazardous reactants, overstock, and so on, bearing in mind that what is a waste to one laboratory can be a valuable resource to another.

Using the Internet to host and offer this service, any laboratory or institution mentioned above will be able to easily access and exchange the desired reactants with each other, preventing the generation of chemical waste caused by the unused reactants that represent, most of the times, a great investment made by the institution.

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# Recovery of natural antioxidants from brewing industry by-products

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The brewing industry produces substantial quantities of by-products such as spent grain, spent hops and yeast, which are rich in bioactive compounds. However, spent grain is the most abundant brewing by-product, representing approximately 20 kg per 100 L of beer produced [1]. This by-product is available at low or no cost throughout the year, and is produced in large quantities not only by large, but also small breweries. Since beer is produced from raw materials rich in phenolic compounds [2, 3], it is expected that the brewing by-products contain several antioxidants in their composition. Therefore, the transformation of these wastes into a product with high value makes it possible for these companies to reduce their cost of treatment and even to generate additional profits. Moreover, the act of by-product valorization fits in the present trend of sustainable development and environmental protection. In this perspective, the recovery of antioxidant compounds from by-products of beer production could be an interesting option to take in account.

The principal aim of this work was to investigate the possibility of using inexpensive residual products from brewing industry as sources of natural antioxidants. Brewers' spent grains was subjected to an extraction process under different experimental conditions (namely, solvent type, percentage of solvent and extraction technique) in order to study their effect on the extraction yield of phenolic compounds and the corresponding antioxidant capacity. The obtained extracts in each experimental condition tested were analysed in terms of total phenolic content (TPC) by the Folin-Ciocalteu method and the antioxidant capacity evaluated by the FRAP and ferricyanide reducing power assays as previously described [2]. The antioxidant capacity was maximized when spent grains was extracted with acetone 70% and with mechanical stirring (TPC =  $78.2 \pm 3.9$  mg<sub>GAE</sub>/100g<sub>sample</sub>). After optimization of the extraction process, by-products from beer industry, namely spent grain, spent hops and yeast, were analysed. Characterization of the phenolic composition by HPLC - UV enable us to conclude that ferulic and *p*-coumaric acids, xanthohumol and catechin were the main phenolic compounds found in the by-products analysed. Work is in progress in order to identify by HPLC-ESI-MS several other peaks that were also detected.

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# Sequential injection spectrophotometric evaluation of trypsin activity in ionic liquids: comparative study of immobilized and free enzyme

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Proteases are one of the most relevant group of enzymes and are nowadays widely used in synthetic biotransformations. Among proteases, the utilization of trypsin is well recognized as an interesting approach for the synthesis of important biological macromolecules. However, its implementation has been hindered by the poor solubility of substrates and products in strictly aqueous solvents. In this context, the particular nature of ionic liquids (ILs) makes them potential good solvents for biocatalytic processes resorting to trypsin. There are already some studies confirming the applicability of ILs in protease catalyzed processes, namely in the resolution of amino acids and quantification of peptides. However, focusing on trypsin there are only two works reporting the study of the behaviour of the enzyme in IL media [1,2]. On other perspective, other major problem associated with the utilization of trypsin as industrial catalyst is the possibility of occurring enzyme autolysis which becomes a big source of instability. This problem can be solved recurring to chemical modifications and immobilization on solid supports. However, as far as we know there is not information about the behaviour of the immobilized trypsin in IL media.

In this regard, two SIA methodologies for the evaluation of trypsin activity in ILs were developed aiming the comparative study of the behaviour of both free and immobilized enzymes. The methodologies were based on the spectrophotometric monitorization of BAPNA's hydrolysis and involved the aspiration of 100  $\mu$ L of BAPNA intercalated with small aliquots of IL. In the methodology with immobilized enzyme the aspirated zone was propelled to the enzyme reactor where it remained for 90 seconds before it was sent to the detector. In the assays with free trypsin, the enzyme was aspirated in small aliquots between BAPNA and IL. In the optimized conditions the activity of trypsin was comparatively evaluated in strictly aqueous media and in the presence of imidazolium ILs in concentrations between 15 and 85%. It was observed that enzyme activity decreased significantly in the presence of all the tested ILs in concentrations above 25%. Moreover it was noticed that the degree of inhibition was much smaller when the enzyme was immobilized. In both cases, the utilization of ILs in small concentrations (15-25%) can be an interesting approach to broaden the applicability of trypsin in biocatalytic transformations.

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## Sodium and chloride determination in Portuguese Wheat Bread (Carça)

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Over the years, our uses for salt have grown. At first, it simply provided a vital diet supplement, a means of food preservation and improves taste. It has a wide range of uses: seasoning, preservative, binding agent, colour controller, texture aid, fermentation control [1]. The reduction of the salt intake is a public health issue in many countries, because it has been identified as the main cause of some diseases such as hypertension, heart and kidney diseases, stomach cancer, osteoporosis, stroke and obesity [2]. Most of the salt in our diets is found in processed foods. Of all foodstuffs, bread has been identified as the single highest contributor to the total daily salt intake [3].

Bread has as primary ingredients yeast, flour, water, improver and salt. Sometimes flour and even the improver contribute also for the total salt content. Therefore, revenues should take this into account. Bread as part of the daily diet of the Portuguese population, and in an effort to reduce the amounts of salt intake in Portugal, the Portuguese legislation establishes a maximum level (1.4 g sodium chloride per 100 g of bread to match 0.55 g of sodium) and provides guidance on labeling with regard to salt content of prepackaged foods intended for human consumption.

For sodium and chloride determination, the AACC 40-71 [4] and Mohr methods were used and characterization was performed by analysing fifty “carça” fresh bread samples of the North of Portugal bakeries. Twenty four percent of the bread samples are above the level allowed by law.

### Acknowledgments

Thanks to the APHORT - Associação Portuguesa de Hotelaria, Restauração e Turismo and his associates for providing the samples.

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# Structural and Energetic Study of non linear Polyphenyls

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The energetic and structural study of some orto and meta polyphenyls is been a theme of research in our laboratory due to their interesting properties as organic semiconductors materials<sup>[1,2]</sup>. This work focuses the thermodynamic and structural study of some polyphenyls isomers (Figure 1), in order to explore the relationship between structure, thermal stability and their potential as semiconductors materials.

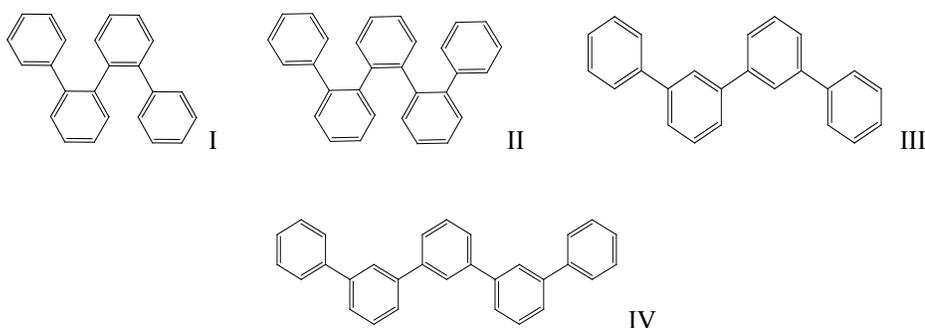


Figure 1. Schematic structural formulas of: (I) o-quarterphenyl; (II) o-quinquephenyl; (III) m-quarterphenyl; (IV) m-quinquephenyl.

Quantum chemical study (Density Functional Theory DFT) will be used in order to evaluate the most stable conformers as well to predict the vibrational frequencies IR spectra in the gaseous phase.

All the compounds were synthesized in our laboratory by Suzuki, Palladium-Catalyzed Cross-Coupling Reactions<sup>[3]</sup> and characterized by G.C. analysis and single crystal X-Ray diffraction.

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# STUDY OF UV FILTERS'S DEGRADATION AND DISINFECTION BY-PRODUCTS IN CHLORINATED WATERS

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Ultraviolet (UV) filters are fundamental constituents of sunscreen's cosmetic products formulations, since they absorb, reflect and/or scatter UV radiation, therefore protecting us from the sun's deleterious effects on human skin and health.<sup>[1]</sup>

However, these compounds experience degradation, mainly through exposure towards sunlight (photolysis and/or photo-isomerization) and also in the presence of disinfectant products, such as chlorine. Through these processes of degradation, new compounds are originated which exhibit UV radiation protecting capabilities much lower than the original compounds and are usually highly toxic and harmful towards human health and the environment.<sup>[2]</sup>

There is no official method for the identification and quantification of these compounds, their photo-degradates and disinfection by-products. Nevertheless, analytical methods are being implemented to determine and regulate their presence, both in environmental and commercial samples and/or products.<sup>[1]</sup>

This study focuses on the assertion of the stability of UV filter ethylhexyl methoxycinnamate and the identification of its photo-degradates and disinfection by-products, in chlorinated waters, using high performance liquid chromatography-diode array detection and liquid chromatography-mass spectrometry techniques.<sup>[3]</sup> This communication will present the analytical procedures to identify and quantify the photochemical and/or disinfection by-products under development and it will discuss the health risks associated with the presence of these substances in swimming pools water.

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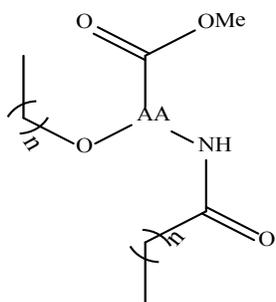
# Surfactants Synthesis Based on Amino Acids

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Surfactants are chemical compounds which present two distinct regions, a polar and an apolar one, in the same molecule. Each one of them has different affinity towards different substances. Being popularly known as soaps and detergents, surfactants are characterized by its tendency to adsorb on surfaces and interfaces allowing the decrease of surface tension. This particularity enhances the improvement of material properties to endless applications, such as, food and drug industries.

In this work, we propose to synthesize surfactants with future application on drug delivery systems, starting from a set of three amino acid derivatives on which we will condensate aliphatic chains from fatty acids or organic halides, (fig.1).



Classic synthetic methodologies will be used, in order to prepare different chemical surfactants which will enhance the chemical diversity.

In a first step the single-chained surfactant will be obtained by condensation of the amino acid derivatives (threonine, 4-hydroxyproline and histidine) with the fatty acids (lauric, myristic and palmitic) followed by condensation with organic halides (dodecyl bromide, tetradecyl bromide and hexadecyl bromide) in order to obtain the double-chained surfactant and finally saponification will take place.

Figure 1

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# SYNTHESIS AND AGGREGATION BEHAVIOR OF SERINE-DERIVED SURFACTANTS: TOWARDS BIOFRIENDLY VESICLES

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The growing use of surfactants in pharmaceutical and industrial applications requires compounds presenting high levels of biocompatibility and biodegradability, while at the same time showing the desired functional properties. The use of amino acids for the synthesis of novel surfactants with potential vesicle-forming properties provides the route to the design of biofriendly vesicles with enhanced properties with respect to classical lipid vesicles. Catanionic vesicles currently stand as a promising alternative to conventional liposomes owing to their long-time stability, spontaneous formation and, in some systems, good encapsulation properties towards biologically relevant molecules [1].

In this context, our research team has been involved in the synthesis and evaluation of the basic physicochemical properties of a great variety of ionic amino acid-based surfactants [2, 3]. A recent study on their toxicological properties has revealed the best profile for the serine derivatives [4]. Based on these results, further evaluation of the self-aggregation properties were directed towards serine-based surfactants.

In this work, we report the results obtained in the synthesis and subsequent study of the phase behavior and microstructure of some catanionic mixtures composed by monomeric single-chained cationic (C12, C16) and monomeric double-chained anionic (C8-8, C12-12) serine surfactants. The synthesis has been performed according to a methodology established in our laboratories. The characterization of the catanionic mixtures has been carried out by differential scanning calorimetry (DSC) and polarized light microscopy (PLM).

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# Synthesis and magnetic properties of ferrite nanoparticles

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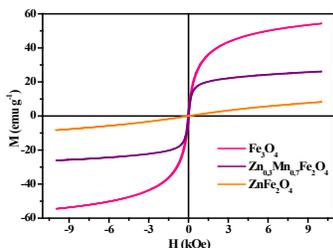
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Magnetic nanoparticles are a class of nanomaterials with tremendous interest in several areas of research, including biology, medicine, environmental remediation, data storage and catalysis. [1] In particular, spinel-type ferrite nanoparticles ( $MFe_2O_4$ , where M is a *d*-block transition metal) offer promising perspectives in these areas due to their large surface area to volume ratio and significant magnetization. [2]

This work reports the synthesis of colloidal simple and mixed ferrite nanoparticles,  $MFe_2O_4$  ( $M = Fe^{2+}$  or  $Zn^{2+}$ ) and  $M_xN_{1-x}Fe_2O_4$  ( $M = Zn^{2+}$  and  $N = Mn^{2+}$ ) respectively, by the co-precipitation method. [3] The composition, structure and magnetic properties of the nanomaterials (denoted as  $Fe_3O_4$ ,  $ZnFe_2O_4$  and  $Zn_{0.3}Mn_{0.7}Fe_2O_4$ ) were studied by chemical analysis, Fourier transform infrared spectroscopy, thermogravimetry and vibrating sample magnetometry (VSM, Fig. 1).



**Fig. 1** –Magnetization *versus* applied magnetic field of the synthesized samples.

The FTIR spectra showed the typical vibration bands of the M-O bonds and of the capping agent used. The chemical analysis confirmed the stoichiometry of the prepared materials. VSM results showed that  $Fe_3O_4$  and  $Zn_{0.3}Mn_{0.7}Fe_2O_4$  nanomaterials are superparamagnetic at 300 K with a magnetization saturation of  $\sim 54$  and  $\sim 26$   $emu\ g^{-1}$  respectively, and that the  $ZnFe_2O_4$  sample is paramagnetic at 300 K.

Acknowledgments: This work was funded by Projecto de Investigação Científica na Pré-Graduação 2009, U.P. and Santander Totta, and by FCT and FEDER, through project ref. PTDC/QUI-QUI/105304/2008. CP and AMP thank FCT for their grants.

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# Synthesis of azole of benzophenone/xanthenes by click chemistry

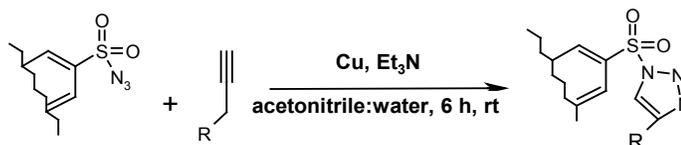
N. Machado<sup>1</sup>, D. Costa<sup>1</sup>, E. Costa<sup>1</sup>, E. Sousa<sup>1,2</sup>, M. M. Pinto<sup>1,2</sup>

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The development of enzyme inhibitors to block estrogen synthesis offers an interesting approach for the treatment of postmenopausal women with hormone-dependent breast cancers. Our goal is to obtain antitumor agents belonging to xanthone and benzophenone families, which can be used as new chemical entities towards breast cancer therapy, by inhibition of STS and/or aromatase.

The strategy applied to obtain potential aromatase inhibitors consisted in the synthesis of a diverse library of new azole derivatives by “click chemistry”. The copper(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes is a particularly powerful linking reaction, due to its high degree of dependability, complete specificity, and the bio-compatibility of the reactants without the need of purification steps. The triazole products are more than just passive linkers; they readily associate with biological targets, through hydrogen bonding and dipole interactions [1].

Herein, we present the synthesis of a new azole derivative by click chemistry (Fig. 1) as a potential aromatase inhibitor.



**Fig. 1.** Strategy for the synthesis of azoles by click chemistry

The structure was elucidated by IR, <sup>1</sup>H, <sup>13</sup>C NMR, HR-MS data. Several conditions of the Huisgen cycloaddition reaction were investigated and the better yield (62%) was obtained with the strategy represented in Fig. 1.

In the future, these derivatives will be investigated in the *in vitro* growth of cell lines, such as the MCF-7 ER(+) (breast adenocarcinoma).

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Acknowledgments: FCT I&D 4040/2007, FCT/COMPETE/FEDER PTDC/SAU-FCF/100930/2008, POCI for financial support and for the PhD grant to Elisangela Costa (SFRH/BD/30615/2006).

# Synthesis of di(hetero)arylethers in the thieno[3,2-*b*]pyridine series by Cu-catalyzed C-O coupling

P. Soares<sup>1,2</sup> and Maria João R.P. Queiroz<sup>2</sup>

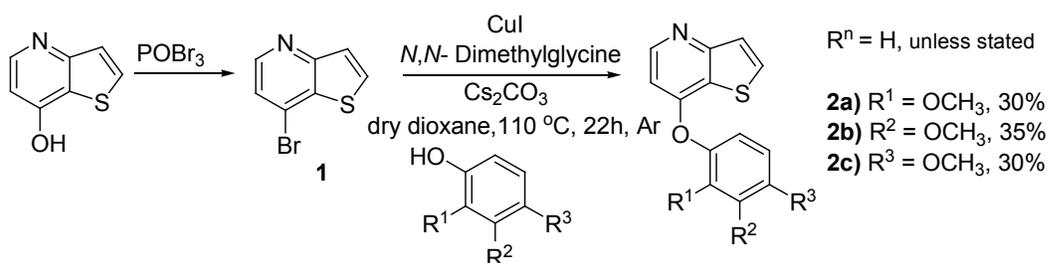
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Thienopyridines have shown interesting biological activities, namely as antitumorals and antiangiogenics [1]. Membrane growth factor receptors of tyrosine kinase, VEGFR (vascular endothelial growth factor receptor) and EGFR (epidermal growth factor receptor) are important targets in angiogenesis and cancer as they are key molecules in regulating cell functions such as growth and differentiation [2].

Di(hetero)arylether derivatives of the thieno[3,2-*b*]pyridine scaffold were shown to be tyrosine kinase inhibitors of the VEGFR [3].

Here we present the synthesis of three 7-(methoxyphenoxy)thieno[3,2-*b*]pyridines **2a-2c** by Cu-catalyzed C-O coupling of the 7-bromothieno[3,2-*b*]pyridine **1**, prepared from the commercial 7-hydroxythieno[3,2-*b*]pyridine and POBr<sub>3</sub>, with methoxyphenols, using *N,N*-dimethylglycine as the ligand and Cs<sub>2</sub>CO<sub>3</sub> as the base (Scheme).



**Scheme** – Synthesis of the 7-bromothieno[3,2-*b*]pyridine **1** and of the di(hetero)arylethers **2a-c**

The di(hetero)arylethers, were obtained in moderate yields, were fully characterized by <sup>1</sup>H and <sup>13</sup>C RMN using heteronuclear correlations (HMSC and HBMC) and HRMS, and will be submitted to antitumoral and antiangiogenic activity studies.

Thanks are due to FCT for financial support through the research centres, through the Portuguese NMR network (Bruker Avance III, 400MHz) and through the project PTDC/QUI-QUI/11060/2009 also financed by FEDER and COMPETE.

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# Synthesis of lipid nanoparticles for applications in the development of novel drug delivery systems

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Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are lipid-based drug delivery systems that gained increased attention during the last years and represent an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro and nanoparticles [1] [2]. SLN are composed of solid lipids and their matrix present perfect crystalline lattices. This type of colloidal system possesses many advantages such as: biodegradability; biocompatibility, low cost of ingredients, easy preparation and consequent capacity of being produced on large scale [1]. Despite all the mentioned advantages some problems occur with SLNs including, low drug loading and drug expulsion from the carrier during storage, due to polymorphic transformations [1]. Therefore and to overcome some of the potential limitations associated with SLN, the NLC were developed [2]. These carriers are produced with solid and liquid lipids (oils), whose blend is solid at body temperature [2]. The particle matrix contains many imperfections providing space to accommodate the active compound [2]. Hence, with NLC it is possible to increase the loading capacity for a number of active compounds and it is also possible to avoid or minimize the expulsion of the active compound during storage.

Within the current work, the preparation of SLN and NLC with the selected combination of lipids was initiated by the melting of the lipids, approximately 5-10 °C above the lipid's melting point. The lipid melt was dispersed afterwards in purified water of identical temperature by high-speed stirring in an ultra-turrax followed by sonication. Several combinations of times of stirring; times of sonication and sonication intensity were chosen to establish the best conditions for nanoparticles' production. Characterization methods of the nanoparticles produced included particle diameter and zeta potential (ZP) analysis using dynamic light scattering. In conclusion, SLN and NLC were successfully prepared and characterized and can be loaded with pharmaceutical and cosmetic active compounds.

## Acknowledgments:

Thanks are due to U.Porto and Santander Totta for financial support trough a Pluridisciplinar project. A. N. also thanks FCT for the fellowship (SFRH/BD/73379/2010).

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# Synthesis of New Proline-mimetics Tripeptides with Potential Neuroprotective Activity

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Peptides biological importance is linked to its broad range of functions in living organisms since they can take action as hormones, neurotransmitters or neuromodulators, with straight intervention on respiratory, reproductive and immunological systems<sup>1</sup>.

In recent decades, investigators deal with synthesizing structurally modified peptides in order to improve the stability and biological activity of these compounds that usually have low metabolic stability and poor absorption, with the aim of obtaining new potential pharmaceuticals<sup>2,3</sup>.

This project aims the synthesis of new potential mimetic compounds of neuropeptides PLG (*L*-prolyl-*L*-leucyl-glicinamide) and GPE (*L*-glycyl-*L*-prolyl-*L*-Glutamic acid), which perform important roles in central nervous system. Therefore, the synthesis of corresponding proline-mimetics P\*LG and/or GP\*E, might make an enormous contribute to the discovery of new potential therapeutic agents for the treatment of neurodegenerative diseases such as Alzheimer, Parkinson or Huntington.

Summary:

- Type I, II and III adducts synthesis (Fig. 1): The methodology in synthesizing this type of bicyclic, proline-mimetic precursors, is well known in our research group<sup>4,5</sup>.
- Corresponding type P\*-L and G-P\* dipeptides synthesis.
- Tripeptide synthesis (biological appraisal will be held at Faculty of Pharmacy, University of Santiago de Compostela, Spain).

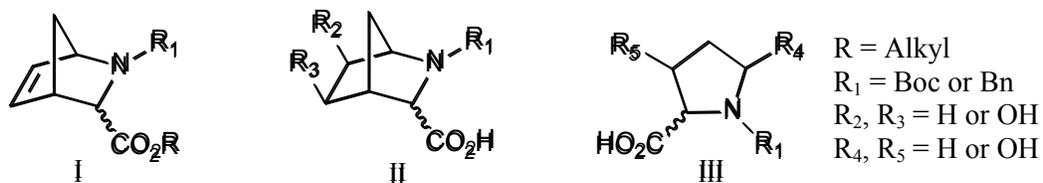


Figure 1 – Proline-mimetic precursors.

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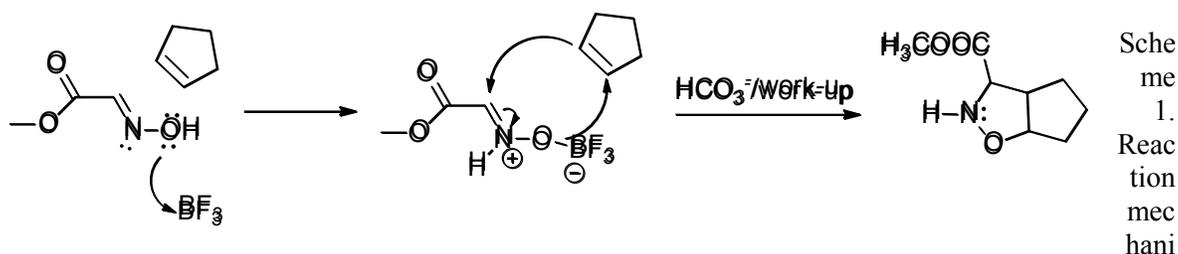
# Synthesis of non-functionalized isoxazolidines from 1,3-cycloaddition reactions

Sandra C. N. P. Costa, Carlos A. D. Sousa and José E. Rodríguez-Borges

Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal.

In the last years, our research group has been interested in the synthesis of pyrrolidine derivatives as synthetic intermediates in the preparation of aza-nucleosides<sup>[1]</sup> compound in which the oxygen atom of the sugar moiety is replaced by a nitrogen. These compounds may mimetize natural nucleosides and therefore be active against retroviruses like HIV or Hepatitis B, by inhibiting the viral reverse transcriptase enzyme. However, aza-nucleosides have shown only modest activity against tumour cells.<sup>[2]</sup> On the other hand, *N*-functionalized oxazanucleosides showed promissory antiviral activity;<sup>[3]</sup> nevertheless, only methyl or benzyl *N*-functionalized isoxazolidines may be prepared from the described methodology.<sup>[3]</sup> By this reason, the preparation of non-functionalized isoxazolidines (oxa-azasugars) at the nitrogen atom would be an important task.

In this work we present a new reaction that allows the synthesis of non-functionalized isoxazolidines from alkenes and oximes of glyoxylates. The versatility generated by the possibility of posterior *N*-functionalization with any desired groups is a decisive advantage in the preparation and design of new oxa-azanucleosides. The mechanism of this uncommon 1,3-cycloaddition reaction is illustrated (scheme 1).



sm for the synthesis of a non-functionalized isoxazolidine (oxa-azasugar) by a 1,3-cycloaddition reaction.

## References:

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# Synthesis of rhodamine fluorophores using cleaner and more efficient methodologies

**I. Cardoso<sup>1\*</sup>, A. M. G. Silva<sup>1</sup>, M. Rangel,<sup>2</sup> B. de Castro<sup>1</sup>**

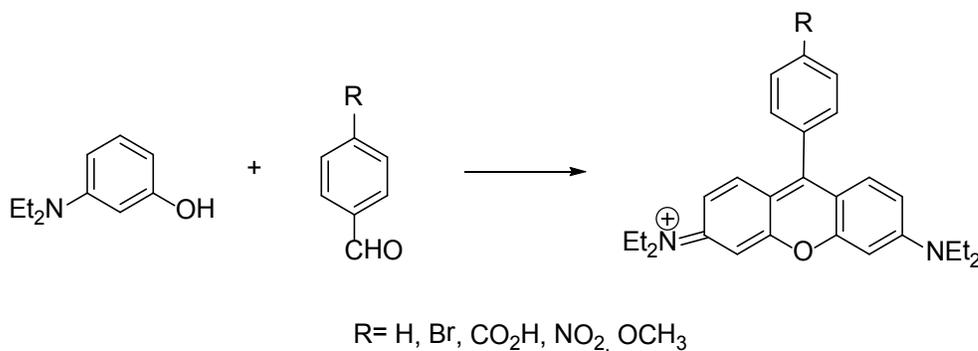
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Rhodamine fluorophores are part of the family of xanthenes. Their special interest is mainly due to its excellent photophysical properties, including high absorption coefficients, fluorescence spectrum in the visible region of the electromagnetic spectrum, high fluorescence quantum yield and photostability. Rhodamines have many applications as laser dyes, fluorescence standards, pigments and fluorescent probes to characterize the surface of polymer nanoparticles, fluidity of lipid membranes, and detection of polymer-bioconjugates [1].

The purpose of this study is to develop more efficient and less harmful protocols for the synthesis of derivatives of rhodamine. To achieve this goal we explored new routes to synthesize rhodamine without a carboxylic group in order to obtain a single regioisomer (Fig.1). Also, we developed new greener methodologies in aqueous media in combination with different types of heating, such as classic heating and microwave irradiation. Following these procedures we expect to significantly increase yields and reduce the time of the reactions [2].



**Figure 1**

Financial support from FCT through project PTDC/QUI/67915/2006 is gratefully acknowledged.

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# Thermodynamic study of fluorene-9-carboxylic acid

**Juliana A. S. A. Oliveira, M. J. S. Monte, M. D. M. C. Ribeiro da Silva**

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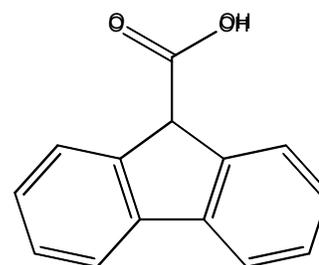
Polycyclic aromatic hydrocarbons (PAHs) are very well known as environmental pollutants, originated by several natural and anthropogenic sources, being part of a class of compounds with relevant negative impact for health.<sup>1</sup> On the other hand, PAHs are now being successfully used in organic electronics as active components in a new generation of electronic devices, like organic light-emitting diodes and organic photovoltaic cells.

One of the main goals of our Research Group has been to correlate molecular energetic data with structural characteristics of molecules, through experimental and computational studies of different classes of aromatic compounds. The lack of reliable energetic data for polycyclic aromatic hydrocarbons (PAHs) and their derivatives presented a challenge and lead us to the study of thermodynamic properties of some fluorene derivatives, in order to get key values not available in the literature.

As part of that project, we have performed the energetic study of fluorene-9-carboxylic acid (Fig. 1). This compound was studied by static bomb calorimetry, from which the energy of combustion and the standard molar enthalpy of formation in the crystalline phase were derived.

In addition, the sublimation vapour pressures of this compound have been measured as function of the temperature, using the Knudsen effusion method, from which the standard molar enthalpy of sublimation, at  $T = 298.15$  K, was derived.

The combination of these thermodynamic parameters yielded the standard ( $p^\circ = 0.1$  MPa) molar enthalpies of formation of fluorene-9-carboxylic acid, in the gaseous phase, at  $T = 298.15$  K.



**Figure 1.** Chemical structure of fluorene-9-carboxylic acid molecule

## References:

[1] Verevkin, S.P., (2004), *Vapor pressure measurements on fluorene and methyl-fluorenes*, Fluid Phase Equilibria, 225, 145-152.

## Acknowledgments:

Thanks are due to Fundação para a Ciência e Tecnologia (FCT), Lisbon, Portugal, for granting the financial support to Centro de Investigação em Química – UP and for financing the research project PTDC/QUI-QUI/102814/2008, which includes a research grant awarded to JASAO.

## Voltammetric study of pesticides MCPA and MCPP

**M. Martins**<sup>1,2</sup>, **B. Brito**<sup>1</sup>, **M. Matos**<sup>1,2</sup>, **J. Garrido**<sup>1,2</sup>, **F. Borges**<sup>1</sup>, **E.M Garrido**<sup>1,2</sup>

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Chemical pesticides have contributed greatly to the increased yields in agriculture by controlling pests and diseases and also toward checking the insect-borne diseases in the human health sector.

The discovery of the phenoxyacetic acid herbicides during the Second World War heralded the modern era of agrochemicals. The fact that they are still being used today, more than fifty years later is a testament to their efficacy, safety and low cost. When misused or as a result of accidental spillage, these herbicides have the potential to injure non-target cultivars and microorganisms, in particular herbicide degraders and others contributing to soil quality and to cause adverse side effects in mammals, including humans. Because of its widespread use and due to its persistence, polar nature, and water solubility, these herbicides are dispersed and its residues and transformation products could be present in matrices including water, soil, and cereals, and other vegetable products. Therefore, sensitive analytical methods for the routine determination of phenoxyacetic acids in water and soil samples are highly desired.

MCPA [(4-chloro-2-methylphenoxy)acetic acid] and MCPP-mecoprop [(RS) 2-(4-chloro-2-methylphenoxy)propionic acid] (Fig 1) are both phenoxyacetic acid herbicides widely used as a mixture for the control of annual and perennial weeds in cereals.

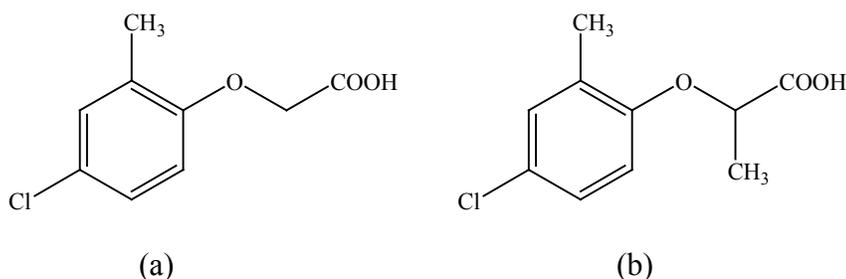


Figure 1 – Structural formulae of (a) MCPA and (b) MCPP

Electrochemical techniques are recognized methods for trace determination of metals and for determination of reducible or oxidizable organic compounds that possess surface-active properties. One of the most important advantages of electrochemical systems is the simplicity of instrumentation.

The results gathered along this project on the oxidative behavior, at different pHs, of MCPA and MCPP by using voltammetric methods (differential pulse and cyclic voltammetry) will be presented in this communication.

### Acknowledgements

Authors acknowledge U.Porto/Santander Totta and Fundação para a Ciência e a Tecnologia (FCT), project PTDC/AGR-AAM/105044/2008, for financial support.

# Analytical methods for the quantification and speciation of Selenium in dietary supplements – A review

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Consumption of selenium (Se) supplements has increased dramatically as a result of the health benefits reported, including protection of cells against oxidative stress, the normal functioning of the immune system, thyroid gland and reproductive function, as well as protection against certain types of cancers and cardiovascular diseases.

Se is an essential trace mineral but in excessive amounts can be toxic. The most common Se forms used in dietary supplements include the inorganic, sodium selenite and sodium selenate, and the organic forms, selenomethionine and selenocysteine. The recommended daily allowance (RDA) for total Se is 55 µg/day and the acceptable upper limit ranges between 300–400 µg/day, in the European Union (UE).

In this communication, we reviewed the most commonly used analytical methods for the quantification and speciation of Se in dietary supplements and the main results described in literature. A variety of analytical methods have been used, including atomic spectrometry, gas chromatography, neutron activation analysis, spectrophotometry, x-ray fluorescence analysis and voltammetry. Inductively coupled plasma mass-spectrometry (ICP-MS), hydride generation (HG) combined with atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS) and electrothermal atomic absorption spectrometry (ET AAS) are commonly used techniques for the determination of total and inorganic Se. Although important, the determination of total Se is not sufficient and must be accompanied by speciation, since Se toxicity and bioavailability depends on chemical form present. Hyphenated techniques are a fundamental tool in elemental speciation. Analytical methods based on the coupling of chromatographic and electrophoretic separation with ICP-MS detection are the most widely used for Se speciation analysis of inorganic and organoselenium compounds. Several works reported significant discrepancies regarding label declaration and analytical determinations, namely Se species, Se contents, as well as differences between batches of the dietary supplements.

Acknowledgements:

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# Development of a microwave digestion method for metal analysis in fish fingers

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Portugal is a country with great consumption of fish. Although considered a healthy food, worrying amounts of several elements can be detected depending on the origin or fish species [1].

The aim of this work was the development of a methodology for sample preparation and metals quantification (Fe, Zn, K and Mg) in fish fingers.

Usually fish samples are grind, homogenized and then reduced to ashes in a muffle furnace, followed by an acid treatment. [2] Disadvantages such as contamination and time consuming of this procedure make microwave digestion a better sample treatment [3,4]. Sample drying and digestion were performed by Microwave. Different mixtures of solvents were tested (HNO<sub>3</sub>, HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub>) being the mixture HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub> in the ratio 8:2 the one that conducted to a clear solution. Atomic absorption spectrophotometry with continuum source was the technique used for metal quantification.

Recovery studies were performed for method validation. Average values for Fe, Zn, K and Mg were 7,5, 18, 1350 and 390 mg/kg of dry fish, respectively. These values are in accordance with literature references.

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# Influence of normal stress and soil relative density on shear strength of sand-geocomposite interfaces on both sides

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Geosynthetics are polymeric materials widely used in geotechnical works such as soil reinforced walls, roads and railway embankments, soft ground improvement, slope stabilizations and foundations. The understanding of soil-geosynthetic interface shear strength is very important for the design and stability analysis of geosynthetic reinforced structures.

This study conducts a series of large scale direct shear tests under three different normal stresses (50kPa, 100kPa and 150kPa) and two values of soil relative density (50% and 90%) to investigate the interface shear strength of a granular soil (sand) against a geocomposite (RockPEC) on both sides. Tests were conducted in a direct shear apparatus developed at Faculty of Engineering. The inner dimensions of the upper shear box are 600 mm × 300 mm × 150 mm. A rigid plane as a lower shear box was used in all tests.

The test results show that the shear strength of the interfaces is dependent on the applied normal stress, soil relative density and geocomposite side (or face). As expected, for all the interfaces, the shear strength increases with applied normal stress and soil relative density (Fig. 1). The influence of the soil relative density on the interface shear strength increases with applied normal stress for both sides of the geocomposite. The shear strength for the geocomposite upper face, which was expected to be greater than the shear strength for the other face, seems to be lower for the tests performed with 50% of relative density (Fig. 1a). The values of the interface friction angle for the lowest value of relative density (50%) are similar for both sides of the geocomposite. For the greatest value of the relative density (90%), the difference between the friction angle achieved for the lower and upper face is about 3 degrees.

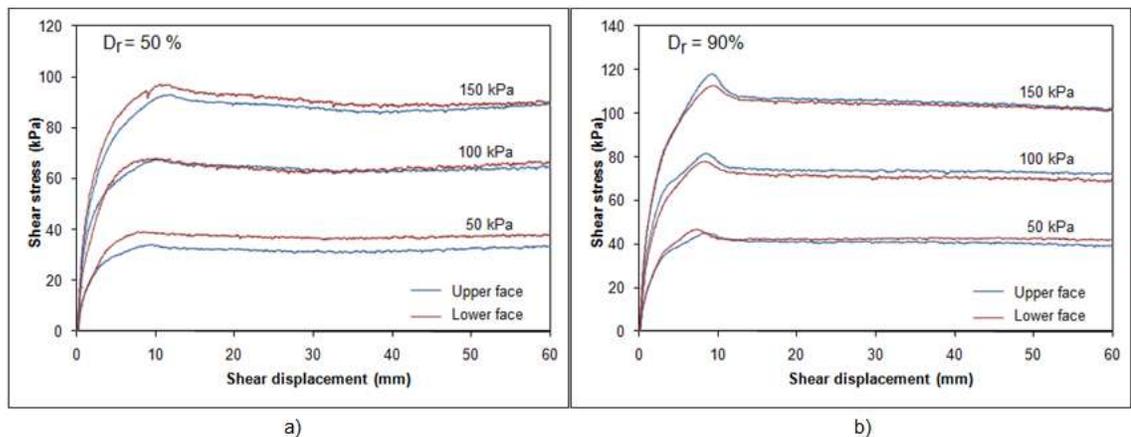


Fig. 1 – Stress-strain behavior of soil-geosynthetic interfaces: a) soil relative density = 50%; b) soil relative density = 90%.

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# Investment Casting of Microparts made of Co-Cr-Mo Alloy

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The developments in micro and nanomanufacturing technologies are a global trend due to the increasing demand for parts miniaturized with technical applications in several areas, such as, biomedical, pharmaceutical, microelectronics and mechanical engineering.

Microcasting is a metal forming process based on the lost-wax technology of investment casting which is capable of produce small structures in the micrometer range, or larger parts carrying microstructural features. This process uses a molten metal which is cast into a ceramic microstructured mold [1].

The production of microcomponents by investment casting requires a special control of the variables at each stage of the process, such as, melting and mold temperature, filling pressure and pattern and investment materials.

The purpose of this work is to perform a detailed analysis of the investment casting process, to select and to establish the most important variables for production of microparts made of Co-Cr-Mo alloy. To fulfill this objective, it is essential to characterize the influence of the process parameters on the structure, surface finish and properties of the microcomponents.

Some microparts, including specimens for tensile tests with a total length of 15 mm and a rectangular cross-section of  $0,5 \times 0,5 \text{ mm}^2$ , were fabricated using photopolymer patterns, phosphate bonded investments and centrifugal casting. The dimensional accuracy, surface roughness and microstructure characterization were performed on the microparts fabricated.

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# Laminar flow characterization in presence of a rheological agent - Tracer experiment on a tubular reactor.

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The present work started last semester as a final project of a Chemical Engineering curricular unit. It's the result of the continuous effort from our faculty to improve the laboratorial experiments available to students.

The application of a rheological agent in tracer experiments on tubular reactor assumes a particular interest in the control of the fluid's density, when facing temperature changes on room conditions.

$$(d\rho/dT)_{40^\circ} < (d\rho/dT)_{20^\circ} < (d\rho/dT)_{5^\circ} \quad \text{Eq. (1).}$$

As expressed in eq.1, the higher the temperature the less dense the fluid is.

Adding the rheological agent, viscosity is also modified. The viscosity of Newtonian liquids decreases with increase in temperature, approximately, according to the *Arrhenius* relationship:

$$\eta = A e^{B/T} \quad \text{Eq.(2).}$$

Where T is the absolute temperature, A and B are constants of the liquid. For Newtonian liquids, the greater the viscosity the stronger is the temperature dependence.

For the laboratory temperature range, density and viscosity change, directly affects the fluid flow within the reactor.

In this way when using the agent we can assure a perfect laminar flow, minoring the occurrence of eddies, as well as reactor walls adhesion, and in some cases the phenomena of convective wave flow. To verify this assumption experiments were made, kinetics and conversion for the reaction between crystal violet and sodium hydroxide were studied.

The strong temperature dependence of viscosity is such that, to produce accurate results, great care has to be taken with temperature control in viscometry.

For instance, the temperature sensitivity for water is 3% per °C at room temperature, so that ±1% accuracy requires the sample temperature to be maintained to within ± 0,3°C.

The fluid behaviour was mathematically studied through the Dankwerts'F curve for different temperature range. The reaction kinetics, were obtained in CSTR reactor in presence of the rheological agent, and the non interference of the agent was assured by the results. We can positively state that the reactor walls adhesion and eddies formation doesn't occur in presence of the rheological agent for 15 – 22 °C temperature range. But we can't ensure that there isn't formation of convective wave flow during the process.

**Our goal:** To find out the specific concentration of rheological agent that allows neglecting the formation of the phenomena.

In order to achieve our goal we have obtained a viscosity vs. concentration relation, for constant temperature, using a Brookfield viscometer.

Modeling of mixing phenomena in the presence of chemical reaction presents an even greater challenge to engineers and scientists and has been studied extensively in the last 50 years.<sup>[1]</sup>

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# Mammograms image processing

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In the western world, breast cancer is the most common form of cancer in the female population [1], [2]. Mammography is the best available tool for screening for the early detection of breast cancer reducing mortality. Even being the best tool, mammograms are difficult to interpret and the sensitivity of screening mammography is affected by image quality and the radiologist's level of expertise [1]. Enhancement algorithms have been widely used for the improvement of contrast features and the suppression of noise, which allows the expert to make a better analysis if the mammogram [3].

In this work, an algorithm based on [2], for enhancement of masses is being tested. Images from the MIAS database were used. The purpose of the enhancement algorithm is to increase contrast between the background and mammary structures. To achieve this, two different smoothing filters were experimented: a Gaussian filter and an anisotropic diffusion filter [2], [4]. After the smoothing, a top-hat operation using as structuring element a disk with radius of 80 pixels was applied in the images to eliminate the background. The following step involves the decomposition of the images into three scales using wavelets. Haar wavelet was used and images were reconstructed using only the second scale, which contains more details associated with mass boundary information [2].

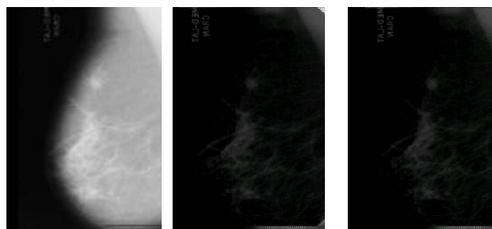


Fig. 5: a. Original image; b. Image after anisotropic diffusion filtering, top-hat operation and wavelet reconstruction; c. Image after Gaussian filtering, top-hat operation and wavelet reconstruction.

The goal of this work is to mimic the method in [2]. Until the moment, only part of the pipeline was implemented. The future work consists in implementing the remaining step and to evaluate the results.

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# Modeling of Conventional Packed-Bed and Membrane Reactors for Hydrogen Purification via Water-Gas Shift Reactors

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Hydrogen is one of the most important industrial commodities [1]. It has a great potential to be the main energy carrier of the future, supplying all energy services practically without impact on the environment, both locally and globally [2]. In recent years, the interest of innumerable companies, academic institutions and government laboratories arose in attempt to find an attractive way, both environmentally and economically, to produce and purify hydrogen [3]. In this sense, the water-gas shift (WGS) reaction ( $\text{CO} + \text{H}_2\text{O} \rightleftharpoons \text{CO}_2 + \text{H}_2$ ) provides important advantages, because produces hydrogen and simultaneously reduces the CO content (a poison for fuel cell's electrocatalysts).

Therefore, the main objective of this work was to address the WGS reaction, in particular to develop phenomenological models that can reproduce experimental results of CO conversion in a WGS reactor operating at low temperatures. The simulations were divided in two sections; in first place it was simulated the conversion obtained in a traditional packed-bed reactor, in which the maximum attainable reaction conversion is intrinsically limited by the equilibrium of the reversible reaction. Several packed-bed reactor models were then proposed and analyzed in detail, from a theoretical point of view, to analyze the effect of different parameters like the space time and Peclet number. Then, experimental work was done to validate the model's predictions, in a wide range of operating conditions: temperature between 150 and 300 °C, flow rate in the range 40 - 150 mL<sub>N</sub>min<sup>-1</sup>, etc. After comparing the simulations against experimental CO conversion data, it was concluded that the heterogeneous model considering axial dispersion and mass transfer resistances shows a better fitting. This model revealed also good adherence for other experiments employing different feed compositions (CO and H<sub>2</sub>O contents) and pressures.

In second place, a membrane reactor was simulated (using a simple ideal model), aiming to shift the reaction by employing a hydrogen-selective Pd-Ag membranes. It was found that the membrane reactor has a better performance than a packed-bed reactor, allowing in certain conditions to overcome the thermodynamic equilibrium – the limit for traditional reactors. Besides, it provides an ultra-pure H<sub>2</sub> stream, which can be sent directly to a fuel cell device.

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# Potassium catalysed degradation of wood under nitrogen and air

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Biomass pyrolysis and combustion are important processes in the production of fuel gases, chemicals and energy [1-5]. They are also relevant in the production of charcoal [6], used as a domestic fuel or as a reducing agent in metallurgy, and in the production of activated carbon [7], an adsorbent with a large range of applications. Most of the current interest in this topic is focused on sustainability, since these processes represent attractive ways of using agricultural and forestry residues as well as a major part of the components in municipal solid wastes [1-5]. Moreover, research on forest fires, requires knowledge of biomass combustion, a process in which pyrolysis plays a major role.

In this work, the effect of adding potassium inorganic compounds in the degradation of wood was studied. In particular, the catalytic effect of the addition of KOH and KCl to samples of eucalyptus and pine, as well as of cellulose, during pyrolysis and combustion, was evaluated. Materials were previously washed in order to remove inorganic compounds and analysed by thermogravimetry and differential scanning calorimetry (TG/DSC) from 30°C to 700°C, at a heating rate of 10°C/min, in N<sub>2</sub> or air atmospheres. Analysis of gases released during pyrolysis and combustion were carried out by mass spectrometry (MS). It was shown that there is a catalytic effect in the addition of potassium salts to the biomass decomposition. The anion with which the solution is impregnated has an important function, since samples loaded with the hydroxide, in general, had the highest decreases in the thermal decomposition starting temperature. That can be related with the higher basicity of OH<sup>-</sup>, compared to Cl<sup>-</sup> [8].

Acknowledgements: Fundação para a Ciência e Tecnologia (FCT) is acknowledged for the BII research grant (BII/LAB/0020/2008) for MCFS.

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# Preliminary study of the biomechanics of the glenohumeral ligament

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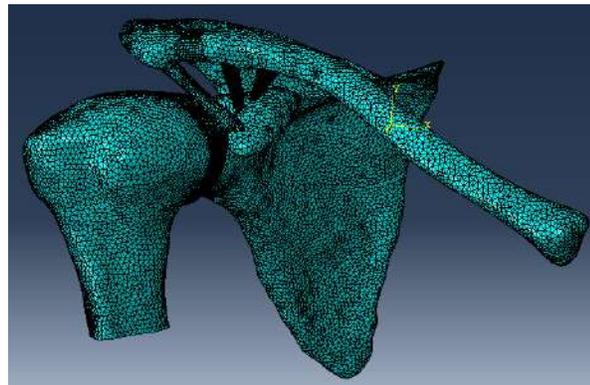
<sup>2</sup> Gaia's Hospital Center

Anatomy is an integral part not only of medical science but also of engineering knowledge in particular in the fields of biomechanics [1]. Engineering has had an very large influence in the current years on the evolution of the medical industry, from equipment development to improve the quality of services provided to patients by carrying out studies for the physical medicine and rehabilitation. Also at the biomechanics of sport has taken an active role in relation to anthropomorphic and motion analysis of athletes increasing performance and reducing injury risk.

In order to prepare such studies, has been revealing particularly important to use models that attempt to reproduce with the most rigor of the anatomical structures and how they interact. These models are obtained through various biomodeling techniques, and this area of biomechanics is crucial for the development of this project.

It was proposed for this work reproduce the shoulder joint by creating 3D models using commercial software *Solidworks* and its discretization, using the software *Abaqus* CAE, in order to study the biomechanics of the glenohumeral ligament for future application in the development of prostheses (Figure 1).

In this paper, the 3D reconstruction was designed by manual segmentation of the boundaries of anatomical structures to considered, obtained by imagiologic exams. Created the models we proceeded to its finite element discretization in order to be able to simulate the behavior of the ligament.



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Figure 1 Solid Works 3D draw of shoulder.

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# Properties of Biodegradable Films from Bovine Hair Plasticized with Glycerol

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This project involves the valorisation of waste-products from the leather industry. The aim is to produce and characterize environmentally friendly biodegradable films to be used either in packaging, food coating or agriculture as an alternative to synthetic, non-biodegradable films. In Portugal, bovine hairs are considered waste-products which are disposed into incinerators or landfills; in this context a clean alternative is highly desirable.

In this project, bovine hair was tested for the production of biodegradable films directly by thermo-compression, allowing waste valorisation and reduction of environmental pollution. The specific aims are to establish the best conditions for film formation and to characterise the resulting films.

Bovine hair with 30 % of glycerol was pressed into films at 88 kN or 157 kN and 120 °C or 150 °C for 3 minutes.

The mechanical properties (stress and strain at break, Young's modulus), colour, solubility and moisture sorption isotherms of the obtained films were tested.

Stress and elongation at break increased with the increase of processing temperature, reaching the maximum value (4.1±0.3 MPa and 0.94±0.12 %, respectively) for films pressed at 88 kN. On the other hand, Young's modulus was not significantly affected by the thermo-compression conditions.

The experimental results of the moisture sorption isotherms showed a good fit to the Guggenheim-Anderson-de-Boer (GAB) model. Solubility was lower for films processed at high pressure (minimum of 46±2 % at 157 kN). All films were brown and colour parameters (L\*, a\* and b\*) increased when processing temperature decreased.

The results obtained are very promising, showing that it is feasible to obtain cohesive and environmentally friendly films with good physical properties from bovine hair.

Acknowledgments:

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Thanks are due to U.Porto /Santander Totta "Projectos Pluridisciplinares 2010" and to AdI for financial support to FILMEQUE project: Development of keratin films from waste poultry feathers and bovine hair (SI IDT – 5551/2009 AdI).



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# Quantitative image analysis of osteoblast behavior on SAMs of alkanethiols on gold: effect of surface wettability on adhesion and cytoskeleton organization.

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Surface chemistry and wettability are well known parameters affecting cell behavior of anchorage-dependent cells such as osteoblasts.

Image analysis is a powerful tool for acquisition and comprehension of data from cell images obtained by fluorescence microscopy. These data may be used to evaluate the effect of surface wettability on the adhesion and cytoskeleton organization of these cells, which was the major goal of this work.

The images analyzed in this study were of osteoblasts MC3T3-E1 adhered to self-assembled monolayers (SAMs) of alkanethiols on gold, with four different levels of hydrophilicity that were prepared using SH-(CH<sub>2</sub>)<sub>11</sub>OH and SH-(CH<sub>2</sub>)<sub>15</sub>CH<sub>3</sub>, at different concentrations in solution (0%, 80%, 90% and 100% of OH-terminated thiol).

The used images were from representative samples with reported concentrations. In these images, nuclei and cytoskeletons were represented by blue and green, respectively.

Initially, the images were manually edited with Photoshop to eliminate overlapping cells, allowing them to be automatically analyzed afterwards with MATLAB. With this software it was possible to separate the RGB channels to obtain distinct images of the nuclei and cytoskeletons. Morphological operations, such as dilations and erosions, and pre-processing operations, such as contrast adjustment, were applied to eliminate artifacts around the components of interest, enabling the calculation of cells' total area and roundness factor.

The obtained results showed that areas with higher percentage of functional group OH had cells with greater circularity factor, which lead us to conclude that these cells were longer and better adsorbed on the surface. Another fact with great biological interest is that cells with larger area tend to be more present in surfaces with higher percentage of OH, which reveals the higher affinity of these cells for surfaces with this functional group.

The quantitative automatic analysis using MATLAB can still be improved to provide results in larger scale and overcome difficulties such as those caused by overlapping cells.

# Study of the potential use of agar for biodegradable films and coatings production

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Currently, mainly non-biodegradable petroleum-based synthetic polymers are used as packaging materials for foods, because of their availability, low cost and functionality. However, biodegradable/edible films can be made from polysaccharides, proteins, and lipids without the environmental issues of petroleum-based polymers [1].

The use of edible coatings can work as an additional barrier to food spoilage and can help reducing the amount of packaging material needed.

In both cases, parameters such as appearance, texture, flavor, nutritional value and safety are determinant in the acceptability of the food products by the consumer [2].

Agar is extensively used in food and pharmaceutical industries as gelling and stabilising agent. Its use as film forming agent is less documented in published literature.

The main objective of this work is to evaluate the potential use of agar as a film forming agent for food coating and packaging. Commercial agar was used. *Gracilaria vermiculophylla*, a red macroalgae recently established at Ria de Aveiro, northwestern Portugal, as an invasive species threatening the coastal ecosystems equilibrium was also exploited as source of agar.

Agar films were made using a knife-coating system, with previous water solubilisation. A plasticizer was added. Coatings were made by dipping pieces of apples in the agar solution. Films were tested for visual appearance and mechanical properties, in a TA-XT2 Texture Analyser. Coated apples were tested for visual appearance, loss weight, texture and nutritional changes.

Strong, flexible, transparent films were obtained, with appropriate properties for food packaging applications. The presence of an agar coating led to sliced apples with better appearance than sliced apples with no coating. The other tested properties were statistically similar in both cases.

## Acknowledgments

Thanks are due to U.Porto /Santander Totta "Projectos Pluridisciplinares 2010".

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# Sucrose inversion by enzymatic catalysis

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This experimental study is part of the curriculum unit Práticas de Engenharia Química V, in which we executed some modifications in one of the experimental works already existing.

As the title indicates, we performed sucrose inversion by enzymatic catalysis in a tubular reactor with packed beads, immobilizing the invertase in calcium alginate particles.

Not achieving the primary objective of the study, we decided to do a diagnosis of the reactor and evaluate the sucrose adsorption on the particles by injecting a dye to characterize the flow inside the reactor. We verified that it wasn't uniform, occurring a phenomenon referred as "Fingering", in which the flow chooses preferred ways inside the reactor.

Additionally, it was found that the adsorption of the sucrose in the calcium alginate particles wasn't the same through the various sections of the reactor, this was proven by colorimetric analysis using GLUCOSE GOD PAP reagent, when in contact with glucose (one of the products of sucrose inversion) the particles gain the color pink.

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# The effects of hydrodynamic stress on the performance of a microbial fuel cell

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A microbial fuel cell (MFC) is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms [1]. A typical MFC consists of anode and cathode compartments separated by a cation specific membrane. In the anode compartment, organic substances are oxidized by microorganisms, generating electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, and the protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

In this work, a MFC previously described [2] was used to assess electricity production due to the consumption of organic substances from a synthetic wastewater. The MFC (mediator less) consisted of two separate chambers which were inoculated with microorganisms from a wastewater plant. These compartments, a 500 mL anodic section (operating under anaerobic conditions) and a 500 mL cathodic section (operating under aerobic conditions), were separated by a cation-specific membrane (Nafion<sup>®</sup> 112). The microorganisms, adhered onto a carbon brusher at the anode, oxidized the substrate. The resulting electrons and protons were transferred to a platinum-coated (10 mg Pt/cm<sup>2</sup>) carbon cloth colonized by microorganisms at the cathode through an external circuit (electrons) and the membrane (protons). The cathode consisted of a Perspex cylinder, allowing the formation of biofilms. The effects of hydrodynamic stress on MFC performance was assessed through the exposure of the cylinder plus biofilm to different rotation speeds (0, 100, 500, 1000 and 1500 rpm). The results obtained with the MFC operating continuously demonstrated the potential of this system to produce electricity and to treat a synthetic wastewater, for all the hydrodynamic stress conditions tested. The maximum power density obtained for each condition was 2.90 (0 rpm), 4.12 (100 rpm), 10.2 (500 rpm), 37.3 (1000 rpm) and 9.43 mW/m<sup>2</sup> (1500 rpm). The chemical oxygen demand removal of the synthetic wastewater was always higher than 80 %.

The results clearly demonstrate the influence of hydrodynamic stress on MFC energy production. A maximum energy production and coulombic efficiency (41.9 %) was obtained with the bio-cathode rotating at 1000 rpm. Also, the results propose that the optimization of processing conditions, particularly the hydrodynamic conditions, is of the utmost importance in the performance of a MFC.

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# Three-Dimensional Geographic Information Modelling in Urban Environment

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3D Urban Models have been playing a key role in several application areas such as Tourism, Urban Planning, Environmental Studies, Navigation and Simulation for Emergency Scenarios. With increasing urbanization worldwide, the sustainability of infrastructures has become a concern and one of the biggest challenges in local governance, but the emerging need for growth, development and change in quality of life may be widely valued and benefited with the use of 3D Urban Models: from preview of future projects to the analysis of existing infrastructure, allowing a more realistic view of the world around us, providing unprecedented flexibility and a plethora of information and solutions.

This paper aims to present some of the results reached within the author's research thesis [1], with the goal of identifying and evaluating techniques and applications of three-dimensional modeling in the urban environment and testing the application of two techniques in real life 3D modeling applications within urban planning.

Three case studies were developed and prepared according to two distinct methodological approaches: one directed at complex spatial analysis using the potential of 3D GIS platform, ArcGIS, and another oriented to photorealistic representation of urban space, using techniques of geometric and texturing on Google SketchUp platform and viewing on Google Earth platform.

The experience and knowledge gained in this project lead to the conclusion that the processes involved in 3D Urban Models generation are presented as a challenge in many aspects, such as costs related to data acquisition and work platforms, the type and data format to use, the level of detail adopted, and size and complexity of the models regarding their final application. Given the above, the final application of the models as well as the variability of the available information, determines both the potential and reliability of the models as alternative representations of urban reality.

In this context, 3D Urban Models are a valid and expeditious instrument of integration and provision of information, usable and adaptable to the needs in question. Although this is a project in development, it can provide the decision-making power with greater awareness of the potential of 3D Urban Models and a vision of future needs related to its implementation as part of the Infrastructure for Spatial Information.

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# UFSM's Biomass Potential Estimative and Feasibility Study Implementation of DG for Sustainable Energy Purposes

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“Biomass in general will achieve much greater importance as a primary carrier for energy supply in the near future” [1]. Its usage brings advantages beyond energy savings as example of know-how, overcoming dependencies on fossil fuels, reduction of high energy costs, implementation of isolated systems, reduction of environmental problems and less waste of natural resources or residues that might allow a closed circle process sustainable. Facts with will lead us to significant changes in the personal habits of people and in the agricultural cultivation, methods applied today [1], from industry to ours homes with the crescent Distributed Generation diffuses.

So due to operation, energy demand, vast area and large amount of organic waste, similar to industry that's make up the UFSM's campus a great exemple to quantify the potential of biomass, its usage experience and energy optimization.

Some needs to achieve that goal: raise the typical parameters of each substance and its quality, calorific values, disposal methods, processing or mainly the best clean energy generation alternative to justify and allow the project's feasibility, with were based on site inspections and some interviews with specialists from each mapped sector, resulting in the biomass composite defined by Fig.1 and subsequently on a thorough study of alternative sources utilization, using DG to improve which loads or schedules shall the proposed system serve. The interaction and analysis of collected data will be made in simulations software HOMER, RETScreen and Biogás at next steps; suing them under various technical scenarios or multicriterial variables.



Fig.1 – UFSM's area (yellow): 1.933,57 hec (A: Occupied Locations / B: Breeding)

Finally several profiles were produced, linked to their substance kind and situated efficiency of DG, as reference documentation for waste managers and procedures dissemination of biomass process, to realize the sustainable implementation of renewable energy potentials with raw materials at low costs and rapid investment return.

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# Vital Responder Data Logging

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The ultimate goal of the Vital Responder research project (CMU Portugal) is to evaluate human stress in real-time under adverse conditions by means of continuous online vital sign monitoring of first responders. The approach followed is to explore the synergies between innovative wearable technologies, scattered sensor networks, intelligent building technology and precise localization services to provide secure, reliable and effective first-response systems in critical emergency scenarios. The main objective of VR Logger is to gather data that will be used in decision support in firemen's management.

We use smart phones as the information center of each user. Each user carries a mobile device running the VR Logger application was developed under Android Platform [2]. This application collects information about the activity and movement from the accelerometer, the outdoor localization from the GPS (both embedded in the device) and also the Wi-Fi environment around the device to check the relative positions between devices. In the next step, we will add vital signals showing the stress and heart pace measured by wearable sensorised t-shirts.

Initial tests have been carried out in firemen exercises: when starting the mission, they take a device with the VR Logger application installed (Accelerometer + GPS Logger + Wi-Fi Scanner) and start the VR Logger application. At the end of the mission, the data is exported into the device's SD Card (through SQLite dump files and also Google-Earth format: KML). Later, the data is sent to the main Vital Responder database, where it will be accessible to all members of the team for offline processing of the data. At this time, we already have gathered real data from the firemen trials, as we can see in Fig.1.



Fig. 1: Accelerometer and GPS log in the map (screened on Google Earth). The colour shows the accelerometer activity: from green (low activity) to red (strong movements registered).

The next step consists in developing a real-time ad-hoc network with the Android devices to gather the data online in a server to support decision-making during firemen missions. The data gathered about the Wi-Fi environment is critical for this step, as it will be used to infer Wi-Fi connectivity patterns among the firemen in forest environment. That knowledge is critical for the design of the ad-hoc networking protocols and yet not previously researched.

## Wastes Treatment and Management

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This work is integrated in a project whose purpose is the design of Chemical Sustainable Laboratories an emergent research area of REQUIMTE / GRAQ - ISEP group. One of the key aspects of sustainable laboratory design includes wastes management [1], the objective of this project. The purpose is to design and develop software solutions [2, 3] to support the wastes treatments and management.

The solution includes three software components that will operate in an integrated way.

One component, information repository system, structure, organize and store the information about chemical wastes produced in the laboratorial activities, and also the know-how developed and acquired from research activities related to waste treatments in order to reduce the quantity of waste to be sent to final disposal. This information and knowledge is a very valuable asset in sustainable development.

Another system component, laboratory management, aims to improve the laboratory processes making them more visible and traceable. This component provides a decision support in the daily work of the laboratory. All this will contribute to the continuous improvement and to the achievement of a higher standard quality.

The third component is a Web Portal that allows divulgation of objectives, tasks, knowledge and work to the community [4]. This portal facilitates, also, the internal and external communication.

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## Lipid messengers and decidualization: Endocannabinoids mediate apoptosis through the generation of ceramide

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In rodents, decidual cells differentiate and proliferate during early pregnancy in response to implanting blastocyst. Later, decidual cells undergo a cycle of regression, which occurs mainly by apoptosis [1]. This is essential to support placental function and, consequently, conceptus growth/development. However, the exact mechanisms controlling decidual regression are not fully understood. The discovery of “endocannabinoids” (ECs), the endogenous cannabis-like compounds, highlighted a new “clan” of lipid mediators. Although ECs have been associated with various physiological processes, their involvement in reproduction is still very intriguing [2]. We have previously shown that ECs machinery operates on decidual cells and found that AEA, the main endocannabinoid, induced apoptosis in decidual cells through cannabinoid receptor 1 (CB1) [3-4]. Ceramide levels, a lipid second messenger, have been shown to mediate cannabinoid induced apoptosis in vitro and also in vivo [5]. In the present study we intend to investigate if ceramide may be involved in the apoptotic process observed during decidual regression. In that way, we have quantified its levels by HPLC-MS/MS after AEA treatment, using primary decidual cell cultures. Moreover, we also studied how cannabinoid receptors are coupled to the generation of ceramide. We found that AEA (10  $\mu$ M) induced a significant increase in ceramide levels, effect inhibited by the pre-treatment with the CB1 receptor antagonist. The results suggest that AEA- induced cell death of decidual cells could be mediated by ceramide and in that way it may impar normal pregnancy, effect dependent on the activation of CB1 receptor.

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# Lipophilic Cinnamic Cations: New mitochondria-targeted antioxidants for neurodegenerative disease therapy

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Neurodegenerative diseases has becoming a health concern to societies worldwide that can affect millions of people. Neurodegenerative diseases occur in middle and late life but the specific cause of these disorders remains unknown; however, increased production of ROS is a hallmark in early steps of these disorders. The matrix space in mitochondria is the intracellular compartment which is potentially the most active in production of reactive species (ROS and RNS) and is the most vulnerable to oxidative damaging effects. For this reason, modulating mitochondrial functions appears like a desirable target that could be an effective way to slow the neurodegenerative progression and consequently the ageing processes in the brain. In this context, the control of mitochondrial redox processes is an attractive perspective and the development of mitochondriotropic compounds is of great importance in such an effort.

Lipophilic cations, composed to antioxidant group, a linker and a penetrating cation (*e.g.* triphenyl phosphonium- TPP ), can pass directly through phospholipid bilayers without requiring a specific uptake mechanism and they accumulate substantially within mitochondria owing to the large membrane potential.

Benzoic and cinnamic acids found in diet (*e.g.* sinapic, ferulic and caffeic acids) have been often used as templates for the design and development of new antioxidants. However, the majority of natural antioxidants, studied until the date, had limited success. One of the reasons mentioned for this limitation is related to the fact that the majority of natural antioxidants are distributed throughout the body and only a small fraction penetrates into the target sites (*e.g.* the mitochondria).

The aim of our project is the synthesis of several hydroxycinnamic antioxidant derivatives harbouring the positive charges of TPP cations at physiological pH (hence capable of mitochondrial accumulation) that could be used as potent and selective agents throughout specific targeting the mitochondria. Some of the results obtained so far will be presented in this communication.

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# Microbiological and Biophysical studies of cecropin A-melittin hybrid peptides

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Tuberculosis remains one of the most deadly human diseases. The genus *Mycobacterium* includes not only *Mycobacterium tuberculosis*, the agent of tuberculosis, but other pathogenic and non-pathogenic species. *Mycobacterium avium* causes disseminated disease in immunocompromised hosts, especially in AIDS patients. The fact that these bacteria are intracellular, residing mainly in macrophages, and have an extremely complex and highly impermeable cell wall, contributes to the poor action of the antibiotics. Current therapy consists of a combination of antibiotics taken for at least six months. In the case of *Mycobacterium avium*, current regimens have an overall clinical success rate of no more than 60% in AIDS patients over one year [1]. Furthermore, mycobacterial antibiotic resistance is increasing worldwide, urging the need to develop novel classes of antimicrobial drugs.

Antimicrobial peptides (AMPs) are one potential alternative to fight infections and there has been increasing interest and research in this area. AMPs are present in most living organisms as part of their immune system. Several AMPs have been discovered from different sources, varying in length, sequence and structure. The mechanism of action of AMPs is still under debate, although in most cases it is thought to act by membrane disruption by a variety of mechanisms.

In this study, we combined Microbiology and Biophysical techniques for the study of two AMPs, namely a cecropin A-melittin hybrid (CA(1-7)M(2-9)) and its lysine N<sup>ε</sup>-trimethylated analog (K7) [2].

To test the antimicrobial activity of the peptides, *Mycobacterium avium* was grown in liquid medium with different peptide concentrations. The viability of the bacteria was assessed by Colony Forming Units (CFUs) and Resazurin reduction.

In an attempt to contribute to the understanding of AMP's mechanism, we characterized the structures obtained for mixtures of these peptides with Phosphatidyl Ethanolamine (PE) model membranes at critical ratios by Small Angle X-Ray Diffraction (SAXD) studies at different temperatures.

Our findings show that these two peptides have a direct inhibitory effect on *Mycobacterium avium*, indicating that AMP could be a promising alternative therapy. Further, the SAXD results provide a possible mechanism of interaction.

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# Morphometric evaluation of green tea and green tea extract effects on rat hippocampal CA3 pyramidal neurons during aging

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In this study we intended to analyze the effects of prolonged consumption of catechins present in large amounts in green tea and green tea extract on morphologic alterations induced by aging in rat hippocampal CA3 pyramidal cells.

Aging is associated with biochemical alterations related to oxidative stress that cause progressive cellular damage. The central nervous system, and particularly the hippocampal formation are very vulnerable to these degenerative events resulting in deleterious morphological modifications in several neuronal organelles such as mitochondria and lysosomes and increased lipofuscin accumulation in neuronal cytoplasm. Oxidative injury markedly compromises hippocampal formation functions affecting learning and memory. As catechins display, among several others, strong anti-oxidative and anti-inflammatory properties, we intended to investigate if prolonged consumption of catechin-rich beverages such as green tea or green tea extract could exert neuronal protective effects during aging in the rat hippocampal formation.

Ten male Wistar rats aged 12 months were treated with green tea (GT-19M;  $n = 5$ ) or green tea extract solution rich in catechins (GTE-19M;  $n = 5$ ) as the only liquid source until 19 months of age. Both groups of rats were compared with control groups of 19 month-old (C-19M;  $n = 5$ ) or 12 month-old (C-12M;  $n = 5$ ) to provide baseline data. Using unbiased stereological methodology in electron microscopy photographs of hippocampal CA3 pyramidal cells, the volume of lipofuscin granules, mitochondria, lysosomes and multivesicular bodies per neuron was quantified. The same neurons were also analyzed to estimate the number of pores in the nuclear membrane.

The volume of lipofuscin granules was increased significantly from C-12M to C-19M rats. Interestingly, cytoplasmic lipofuscin content was reduced by treatment with either green tea or green tea extract when compared with age-matched controls, to levels similar to those found in young controls. The same pattern of variation was evident in lysosomal volume. Furthermore, aging was associated with a significant increase in mitochondrial volume, but not in the number of mitochondria. However, neither of the treatments was able to impede the age-associated volumetric change in mitochondria. Finally, no differences were detected in the volume of multivesicular bodies or in the number of pores in the nuclear membrane when all groups were compared.

Our results show a remarkable protection of age-associated morphological changes on hippocampal CA3 pyramidal neurons. Previously demonstrated anti-oxidant and cell signaling-regulating properties of green tea polyphenols are likely to be involved. In our opinion, further studies are warranted to unravel the modes of action of these compounds and to ascertain the impact of the effects herein demonstrated on functional physiological and pathological processes involving the hippocampal formation.

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# Near infrared spectroscopy for polymorphism characterization in pharmaceutical solid dosage forms

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This work aims at developing high-throughput spectroscopic methods for polymorphism characterization in pharmaceutical solid dosage forms (SDFs) within the process analytical technology (PAT) framework. Near infrared spectroscopy (NIRS) was used as the analytical method since it can provide chemical and physical information in real-time, be implemented in-situ, is cost effective and robust enough for industrial usage. The main motivations for this project are: 1) the need for faster and effective tools for polymorphism characterization of SDFs by the pharmaceutical industry, 2) the lack of NIRS systematic studies of spectroscopic based methods for polymorphism characterization of pharmaceutical SDFs and 3) to take advantage of the existing leading-edge know-how for the benefit of the Portuguese pharmaceutical cluster growing sector [1]. The pharmaceutical production process of SDFs may result in changes in the crystalline structure of materials. Examples of severe consequences are known, as the sudden appearance of new polymorphs in pharmaceutical products during production [2]. These situations are expected to occur more often, since in the context of the drug discovery process, combinatorial chemistry generates increasingly larger molecules, which often show extensive polymorphism and poor aqueous solubility [3]. The cacao butter has four different polymorphic forms with a melting temperature range between 17°C and 37°C. Due to the large range of polymorphic forms and the need of better understanding and control of these forms the cacao butter was used as a case study for this analysis. NIRS spectra were collected from commercial cacao butter and after melting under different cooling conditions. Samples spectra were analysed using principal component analysis (PCA) with the objective of differentiating the different polymorphic forms aroused from the thermal treatment. Differential scanning calorimetry (DSC) was used as the reference method. The results showed that NIRS is able to distinguish between polymorphic forms and stable form of cocoa butter. This study revealed a positive outlook for the application of NIRS in the characterization of polymorphs of substances in solid forms.

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## Neonatal depletion of peptidergic nerve fibres reduces muscarinic receptor-mediated effects in the ileum, but not in atria of adult Wistar rats

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Both, the cardiovascular and the enteric nervous systems, are richly supplied by capsaicin-sensitive nerves containing substance P (SP) and calcitonin gene-related peptide (CGRP), which contribute to integrate their activity in response to pathophysiological conditions. Parasympathetic cholinergic nerves are important players in the regulation of cardiac and enteric performance through the activation of muscarinic M<sub>2</sub> and M<sub>3</sub> receptors, respectively. Direct evidences of the interplay between peptidergic- and cholinergic-mediated actions are still lacking. We, therefore, investigated how depletion of peptidergic nerve fibers caused by neonatal capsaicin administration affected muscarinic receptors expression and activity in the ileum and in atria of adult rats.

The experiments were performed at 37°C on longitudinal muscle-myenteric plexus (LM-MP) preparations of the ileum and on spontaneously beating atria of *Wistar* rats, which were isolated from a control group (C) and from a group of animals injected subcutaneously with capsaicin (CAP) in the neonatal period. Neonatal CAP causes extensive (>95%) degeneration of non-myelinated afferent fibers (Jancsó, 1985, *Neurosci. Lett.* **59**:209-214). Preparations were continuously superfused with Tyrode's solution, which was continuously gassed with 95% O<sub>2</sub> plus 5% CO<sub>2</sub>. Isometric muscle tension recordings were continuously monitored on a computer screen via a PowerLab data acquisition system (Chart 5, v.4.2 software; AD Instruments, USA). Co-localization experiments were performed by immunofluorescence using a laser-scanning confocal microscope (Olympus FV1000).

In the ileum, muscarinic M<sub>3</sub> receptors co-localize with SP-sensitive NK<sub>1</sub> receptors on intramuscular interstitial cells of Cajal (IM-ICC). Neonatal CAP-treated rats exhibit increased NK<sub>1</sub> and decreased M<sub>3</sub> receptors immunoreactivity on IM-ICC as compared to the control group. As expected, we found a reduction in the number SP and CGRP positive nerve fibres in neonatal CAP-treated rats. Muscarinic M<sub>3</sub>-receptor-induced contractions (Oxo, 1 µM) of LM-MP preparations were significantly ( $P<0.05$ ) reduced in CAP-treated animals ( $0.071\pm 0.028$  mN/mg of dry weight,  $n=5$ ) as compared to their control littermates ( $0.359\pm 0.096$  mN/mg of dry weight,  $n=4$ ). Blockade of muscarinic M<sub>3</sub> receptors with J107129 (6 nM) failed to attenuate Oxo (0.003-300 µM)-induced contractions of the ileum of CAP-treated rats ( $n=4$ ), but it significantly ( $P<0.05$ ) shifted to the right the concentration-response curve of Oxo in C rats ( $n=5$ ). In spontaneously beating atria, Oxo (0.01-3 µM) caused concentration-dependent negative chronotropic and inotropic effects, which were of a similar magnitude in both C ( $n=17-31$ ) and CAP ( $n=7$ ) rats. Blockade of muscarinic M<sub>2</sub> receptors with AF-DX 116 (10 µM) significantly ( $P<0.05$ ) shifted to the right the concentration-response curves of Oxo in both C ( $n=6$ ) and CAP ( $n=9$ ) rats.

Data indicate that depletion of peptidergic nerve fibers by neonatal administration of capsaicin affects predominantly muscarinic M<sub>3</sub>-receptor-mediated contractility of the rat ileum, without significantly affecting the negative chronotropic and inotropic tonus operated by M<sub>2</sub> receptors in spontaneously beating atria.

\*Both authors equally contributed to the work presented in this abstract.

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# Neural Stem Cells for Nervous System Regenerative Therapies: study of NS-5 neuronal differentiation profile

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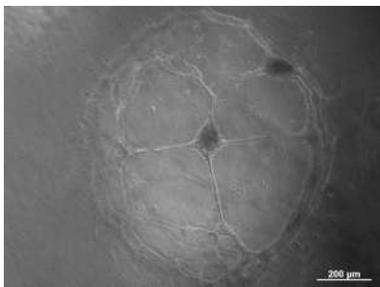
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With the emergence of neural stem cell (NSC) biology, the repair of the central nervous system (CNS) has become a more attainable challenge. In this project we aim at the design of an endothelialized chitosan scaffold seeded with neural stem cells (NSCs) embedded in a fibrin gel, to serve as a therapeutic platform to treat spinal cord injuries (SCIs). The use of this cell combination is expected to improve NSCs survival and differentiation since it aims to mimic NSC niches found in the adult CNS [1]. To achieve this goal, NSCs ability for neuronal differentiation and cell culture specific conditions should be studied to ensure a successful neuron replacement on the injured site. In this work we use a clonogenic NS-5 cell line derived from mouse ESCs under serum-free and adherent monoculture [2]. Here we report the NS-5 neuronal differentiation profile in 2D culture conditions and in a fibrin gel to create a 3D environment (3D culture). NS-5 cells were cultured in N2B27/Neurobasal media for 14 days. During the first 4 days of culture bFGF was added to promote NSC survival. NGF and BDNF were added from day 8 of culture on to sustain neuronal maturation.

Cell viability and morphology were assessed using Calcein AM/PI dual staining and phase contrast microscopy, respectively. Concerning 2D cultures, immunofluorescence analysis was performed to evaluate cell differentiation against the following phenotype markers: Nestin (NSC),  $\beta$ -III tubulin (early neuronal), GFAP (astrocytes) and O4 (oligodendrocytes). Results showed that cells are viable at day 14 of culture and are mainly  $\beta$ -III positive, which is consistent with their bipolar neuron-like morphology. Although in a lower number Nestin and GFAP positive cells were also observed. For culture in 3D conditions, NSCs were suspended within a fibrin gel in the form of neurospheres (Fig.1). Results showed cell sprouting from the neurospheres during the culture period, with retention of cell viability. Their differentiation behaviour is currently under study. In order to shade light into the endothelial cell-NSC cross-talk, the effect of the former in NSC differentiation will be subsequently assessed.



**Figure 1:** Neurosphere in 3D culture conditions.

**Acknowledgments:** This work was supported by FCT (FEDER funding, PTDC/SAU-BEB/65328/2006).

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## Neuregulin increased expression and its modulation by bosentan in pulmonary hypertension.

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The NRG-1 system is activated at the heart level in myocardial stress situations [1]. However, there are no studies regarding the activation of this system in pulmonary arterial hypertension (PAH) and in right ventricular HF. Given that bosentan, one of the approved therapeutics for the treatment of PAH, is an inhibitor of endothelin-1 receptors and that NRG-1 synthesis is stimulated by this peptide, our objective was to evaluate the NRG-1 expression in an experimental model of PAH and its modulation by chronic treatment with bosentan.

Male Wistar rats (180-200g) randomly received a subcutaneous injection of either 60mg/Kg of monocrotaline (MCT), in order to induce PAH, or an equal volume of vehicle. Two days after MCT injection, these animals were randomly assigned to receive a chronic treatment (21 days) with bosentan (200mg/Kg/day, *per os*) or with vehicle. The chronic study resulted in 3 experimental groups: control (n=10); MCT (n=10) and MCT+bosentan (n=7). Right ventricular samples were collected for molecular analysis, 25 to 28 days after MCT administration. NRG-1 mRNA quantification was performed by real time PCR and normalized to GAPDH and relatively to the CTRL group (Arbitrary Unit, AU).

An increase of NRG-1 expression ( $11.1 \pm 2.7$  AU) was observed in the right ventricle of MCT group animals. However in the right ventricle of MCT+bosentan group animals there was no significant change in the NRG-1 expression.

In the MCT-induced PAH animal model, right ventricle NRG-1 gene expression levels are increased. This alteration is not observed when endothelin-1 receptors are inhibited through chronic treatment with bosentan. These results suggest a relevant role of NRG-1 in the pathophysiology of PAH and right ventricular HF and suggest a new mechanism that might underlie the therapeutic action of endothelin-1 receptor antagonists.

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# NORADRENERGIC TRANSMISSION IN THE BRAIN CORTEX IS MODULATED BY ASTROGLIAL PURINERGIC RECEPTORS

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In the rat brain, neuron-glia signalling can be mediated by ATP released from astrocytes in response to noradrenaline (NA), which by activation of P2Y receptors modulates neuronal NA release [1]. Additionally, astrocytes express transporters that regulate the synaptic concentration of NE and are modulated by purinergic receptors [2]. The aim of this study was to identify the P2Y receptor-subtypes involved in the modulation of NA release from cortical brain slices and to investigate the influence of these receptors in the NA-uptake by cortical astrocytes.

Cortical occipital-parietal slices from male Wistar rats were incubated with 0.1  $\mu$ M of [<sup>3</sup>H]-NA, superfused with a physiological solution and stimulated with trains of 100 pulses at 5 Hz. The [<sup>3</sup>H]-overflow was used as an indicator of NA released evoked by electrical stimulation and effects of drugs are expressed as % change (increase or decrease) from respective controls as mean  $\pm$  S.E.M from *n* slices. Primary cultures of astrocytes were prepared from brain cortex of newborn Wistar rats (P0-P2). Confluent cultures with 17-20 day were used in NA-uptake assays. NA-uptake by astrocytes was calculated as pmol/mg protein and effects of drugs are expressed in % increase from respective controls as mean  $\pm$  SEM *n* cultures.

The nucleotides tested inhibited NE release up to 58% with the following order of potency: ADP $\geq$ ATP>ADP $\beta$ S>UTP $\geq$ UDP. The effects of nucleotides were attenuated by the selective A<sub>1</sub> receptor antagonist DPCPX (0.1  $\mu$ M). The effect of ADP (0.3 mM; 48 $\pm$ 2%, n=10) was attenuated by adenosine desaminase (ADA 2U/mL; 30 $\pm$ 5%, n=5; P<0.05), DPCPX (0.1  $\mu$ M; 15 $\pm$ 4%; n=5; P<0.05) and by the selective P2Y<sub>1</sub> antagonist MRS 2500 (1  $\mu$ M; 23 $\pm$ 4%; n=6; P<0.05) being abolished by the combination of DPCPX plus MRS 2500. ADP $\beta$ S (0.3 mM) caused an inhibition of 40 $\pm$ 1% (n=12; P<0.05) that was also abolished by DPCPX plus MRS 2500 but was not changed by ADA (2U/mL). The inhibitory effect of UDP (1 mM; 18 $\pm$ 2%, n=6) was abolished by 0.1  $\mu$ M DPCPX and by the selective P2Y<sub>6</sub> antagonist MRS 2578 (1  $\mu$ M) whereas the inhibition caused by UTP (1 mM; 26 $\pm$ 1%, n=5) was attenuated by 1  $\mu$ M MRS 2578 to 18 $\pm$ 1% (n=4; P<0.05). In primary astrocyte cultures, ADP $\beta$ S (0.1 mM), UDP (1 mM) and CGS 21680 (0.1  $\mu$ M) increased NE uptake up to 117%. The increase caused by ADP $\beta$ S (0.1 mM; 117 $\pm$ 15%; n=5) was attenuated by the selective P2Y<sub>12</sub> antagonist AR-C66096 (10  $\mu$ M; 62 $\pm$ 9%; n=5; P<0.05) and almost abolished by MRS 2500 (0.1  $\mu$ M) whereas the effect of UDP (1 mM; 96 $\pm$ 14%; n=5) was attenuated by MRS 2578 (1  $\mu$ M; 42 $\pm$ 4%; n=5; P<0.05). The facilitatory effect of CGS 21680 (0.1  $\mu$ M; 94 $\pm$ 13%; n=6) on NE-uptake was abolished by the selective antagonist of A<sub>2A</sub> receptors SCH 58261 (30 nM). In conclusion, the nucleotides tested inhibited NA release evoked by electrical stimulation through activation of P2Y<sub>1</sub>, P2Y<sub>6</sub>, A<sub>1</sub> receptors and eventually A<sub>1</sub>/P2Y<sub>1</sub> heterodimers [3]. Additionally, they increased NA-uptake by astrocytes upon activation of P2Y<sub>1</sub>, P2Y<sub>12</sub>, P2Y<sub>6</sub> and A<sub>2A</sub> receptors, in both cases, leading to an inhibition of the noradrenergic transmission in the rat brain cortex.

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# O-(Benzotriazol-1-yl)-N-N-N'-N'-tetramethyluronium tetrafluoroborate as an Efficient Coupling Reagent for the Synthesis of Chiral Xanthenes

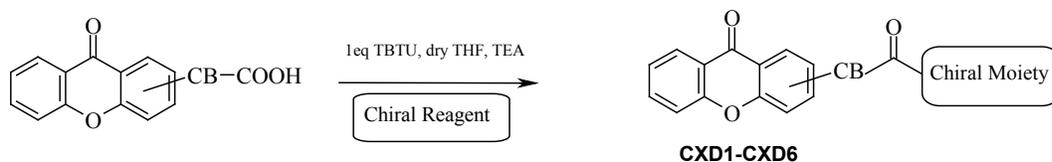
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The development of efficient methodologies for the synthesis of chiral compounds is becoming one of the most important tasks in Medicinal Chemistry [1]. Coupling reagents, such as O-(benzotriazol-1-yl)-N-N-N'-N'-tetramethyluronium tetrafluoroborate (TBTU), could be used for the synthesis of pharmaceutical peptides [2], amides and phenylhydrazides [3]. Therefore, chiral xanthone derivatives (CXDs) can be obtained by coupling a suitable functionalized xanthonic building block (carboxyxanthone derivative) to chiral ligands (primary amines). In this work, six new CXDs were synthesized by coupling both enantiomers, (*S*) and (*R*), of commercially chiral reagents: 2-amino-4-methyl-1-pentanol, 1-amino-2-propanol and 4-dimethylbenzylamine, to a carboxyxanthone derivative (Scheme 1). These coupling reactions were carried out with TBTU at room temperature, showed good yields (above 90%) and short reaction times. All the synthesized compounds were structurally elucidated by different spectroscopic methods (<sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and MS).



CB: chemical bridge; THF: tetrahydrofuran; TEA: triethylamine  
TBTU: O-(benzotriazol-1-yl)-N-N-N'-N'-tetramethyluronium tetrafluoroborate

**Scheme 1.** Synthesis of different CXD (1-6).

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## Organochlorine pesticides levels in human adipose tissue

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The organochlorine pesticides (OCP) are toxic, persistent, bioaccumulative, and lipophilic. If they enter into food chains, they are biomagnified, with all the halogenated Persistent organic pollutants (POPs) stored in the lipid for a long time resulting in great concentrations in the human body. Although a number of studies reported the contaminations of POPs in different environmental compartments, human body burdens of these pollutants can better reflect their toxicities on human health. The objective of this study was to quantify OCP in human visceral and subcutaneous adipose tissue collected from residents in Portugal to assess the levels of these pollutants in the population during the 2010.

The human adipose tissue samples were homogenized with n-hexane and the OCP ( $\alpha$ ,  $\beta$ ,  $\delta$ -hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), lindane, aldrin, endosulfan I, dieldrin, p,p'-DDE, endrin, endosulfan II, o,p'-DDT, p,p'-DDD and methoxychlor) were cleaned up using C-18E SPE (solid phase extraction) columns. Concentrations of these residues in adipose tissue were determined by gas chromatography-electron capture detector with 4,4'-dichlorobenzophenone as an internal standard. The recoveries were between 73.75 to 100.25 % for lindane and o,p'-DDT, respectively. The data obtained from the repeatability studies of the instrumental precision ranged between 0.21 % to 10.12 % for HCB and methoxychlor, respectively, and a mean value of standard deviation of 2.88 % which was considered acceptable given the limit of 15% set by the chromatographic methods validation rules for intra-day precision. The HCH, HCB, aldrin, dieldrin, DDT and methoxychlor were the most common pesticide detected in human adipose tissue obtained from North Portuguese people.

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## **Painful diabetic neuropathy under hypertensive conditions: a study in streptozotocin-diabetic rats**

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Behavioral evidence of hypoalgesia and decreased neuronal activation of the spinal dorsal horn has been described under acute noxious stimulation in several models of hypertension. During chronic pain, however, the normal functioning of the overlapping systems modulating pain and blood pressure seems to be affected, and both hypo- and hyperalgesia have been described. Both hypertension and neuropathic pain are common complications of diabetes mellitus, an increasingly prevalent disease in developed countries. It is our aim to expand the studies of modulation of chronic pain during hypertension, using the streptozotocin (STZ)-induced model of painful diabetic neuropathy (PDN) in spontaneously hypertensive rats (SHR) and their normotensive controls, Wistar-Kyoto (WKY) rats.

PDN was induced in 12-weeks old SHR (n=8) and WKY (n=8) rats by injection of STZ (60 mg/kg, i.p.). All animals were trained to the experimenter and experimental conditions before the beginning of the experiment. Blood pressure (BP) was measured by the non-invasive tail-cuff method and behavioral responses to mechanical (von Frey) and thermal (Hargreaves test) stimuli were evaluated, before STZ-injection and 1, 2, 3 and 4 weeks later. At 4.5 weeks post-STZ, spontaneous pain-related behaviors were evaluated, following formalin injection (0.2%, s.d.) in the hindpaw, during 1h post-stimulus. Two hours after formalin injection, animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), perfused and fixated (4% paraformaldehyde). Collected spinal cords were cryosectioned and immunoreacted for Fos protein. Results were analyzed by 1- or 2-way ANOVA followed by SNK test, with a 95% significance level.

Systolic BP values of SHR, which were significantly higher than WKY's before STZ injection, showed a gradual decrease thereafter, reaching identical levels at 4 weeks post-STZ. Diabetic animals exhibited thermal hypoalgesia from day 7 onward, more pronounced in SHRs than in WKYs, and mechanical hyperalgesia, only on day 28, also more pronounced in SHRs than in WKYs. All animals developed marked hyperglycemia after STZ-injection, with no differences between groups. No behavioral differences were found between groups in the formalin test nor in Fos expression (neuronal activation maker) after formalin stimulus. Therefore, hypertension seems to affect differentially thermal, mechanical and chemical pain submodalities, in PDN.

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## Pathophysiological significance of nucleotide hydrolysing enzymes in human vasculogenic erectile dysfunction

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Stable adenine nucleotides exert a dual role on the human corpus cavernosum (HCC) (Faria *et al.*, 2008, *Nucleos. Nucleot. Nucleic Acids* **27**:761-68). Contractile responses to ATP are mediated by P2X<sub>1</sub> receptors, which undergo fast desensitisation upon increasing the concentration of the endogenous ligand. Conversely, relaxation of HCC strips is operated via ADP-sensitive adenylyl cyclase-coupled P2Y<sub>12</sub> receptors; we observed a yet unexplained shift towards the activation of phospholipase C-coupled P2Y<sub>1</sub> (and perhaps P2Y<sub>13</sub>) receptors in patients with vasculogenic erectile dysfunction (VED) (Faria *et al.*, 2010, *Purinergic Signal.* **6** (suppl 1):p10-9). Nucleotide effects may be cut-short by extracellular hydrolysis via the ectonucleotidase cascade. In this study, we investigated the pathophysiological relevance of the ecto-NTPDase pathway to probe its relevance to HCC hemodynamics in impotent men.

HCC specimens were collected from organ donors and from patients with VED at the time of penile prosthesis insertion (procedures approved by the Ethics Committees of ICBAS-UP and CHP-HGSA). Isometric muscle tension was measured from longitudinal strips of HCC pre-contracted with 1 µM phenylephrine. The kinetics of adenine nucleotides (30 µM) hydrolysis was evaluated by HPLC. Co-localization studies were performed by confocal microscopy (Olympus FV1000).

The extracellular ATP (30 µM) metabolism was slower in VED patients ( $t_{1/2}=26\pm 1$  min; n=3) than in controls ( $t_{1/2}=9\pm 2$  min; n=4). The metabolites detected in the bath were AMP (accumulation detected within the first min, reaching a maximum of  $6.8\pm 1.5$  µM at 15 min), adenosine and inosine, whose maximum concentrations ( $22.1\pm 3.8$  µM and  $8.9\pm 0.7$  µM, respectively) were reached at 45 min. Small amounts of ADP (<1.5 µM) were detected only after a 5-min incubation period. These results indicate HCC have a high NTPDase1/CD39 activity converting ATP directly into AMP, without a significant ADP formation. The activity of ecto-NTPDase 2, leading to significant ADP accumulation was observed in HCC only upon increasing extracellular ATP levels. Smooth muscle fibres of the HCC exhibit significant ( $p<0.05$ ) immunoreactivity against ecto-NTPDase 1 and 2; immunoreactivity against ecto-NTPDase 3 was detected only in a small number of helicine arteries. Inhibition of ecto-NTPDases 1 and 2 with POM-1 (100 µM) shifted to the left the concentration-response curve for the relaxing effect of ATP (0.3-3 mM) in HCC. In the presence of POM-1 (100 µM), as well as with the non-selective ecto-ATPase inhibitor ARL67156 (100 µM), relaxation caused by ATP (0.3-3 mM) was of similar magnitude in both control and VED patients.

Data indicate that extracellular ATP hydrolysis is slower in VED patients. Decreased ecto-NTPDase 1 and 2 activity lead to poor ADP and adenosine formation and, consequently, P2Y<sub>12/1</sub> and A<sub>2a/2b</sub> activation (Faria *et al.*, 2006, *JPET* **319**:405-413), thus contributing to explain why ATP relaxation of HCC is attenuated in VED patients.

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# Polymeric nanoparticles for the siRNAs delivery for antiapoptotic proteins

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The delivery and stabilization of RNAi with small-interfering RNAs (siRNAs) is a hurdle, since siRNAs are negatively charged hydrophilic molecules that show instability in biological fluids, leading to poor bioavailability and intracellular penetration.

Incorporation of siRNA in nanoparticulate systems grants a good protection against *in vivo* metabolization, because in this form they are completely isolated from the nuclease rich environment *in vivo*. Moreover, the vectorization of siRNA molecules on a carrier system, such as a nanoparticle, improves their intracellular delivery [1]. However, from a technical point of view, it is difficult to load smaller nucleic acid molecules like siRNA into PLGA nanoparticles, and at the same time, to attain high loading and encapsulation efficiency with the use of the classical preparation methods, like the double emulsion solvent evaporation method [2].

Aiming the incorporation of siRNA molecules in PLGA nanoparticles, preliminaries studies concerning the preparation of nanoparticle formulations using different techniques (solvent displacement and emulsification-solvent diffusion) were performed. Nanoparticle formulations were characterized regarding particle size and zeta potencial. Furthermore, the influence of several technological parameters (e.g. ratio of the cationic polymer, presence of bovine serum albumin), on nanoparticle characteristics was evaluated. When the solvent displacement technique was applied, nanoparticles with optimized characteristics for the incorporation of siRNA and for *in vitro* studies were obtained.

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# Population structure of nontyphoidal *Salmonella* causing human infections by Multi Locus Sequence Typing

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Salmonellosis is one of the most common foodborne infections in Europe and multidrug-resistant (MDR) *Salmonella* is emerging worldwide with increasing involvement of particular clones. Molecular typing methods are powerful tools in surveillance and outbreak investigation of *Salmonella* infections. In addition, Multi Locus Sequence Typing (MLST) is useful to obtain data on the evolution and population structure of contemporary *Salmonella* circulating from animals to food products and to humans in different regions and to increase the knowledge on the diversity of *Salmonella* epidemic lineages. The aim of our study was to assess genetic relationships between representative *Salmonella* isolates associated with human infections in different regions of Portugal and Brazil by using the technique of MLST with further analysis with the e-BURST software. The seven-locus scheme recommended in the *Salmonella* MLST database (<http://mlst.ucc.ie/>) was applied to 14 isolates from Brazil from different sources and 40 isolates from a large Portuguese collection (2002-2008), representative of MDR clones from human infections (n=22), food (n=14), and piggeries (n=4). The DNA was extracted using the InstaGene Matrix Kit™. PCR amplification was done with the AmpliTaq Gold® followed by purification. The Portuguese isolates studied represented 15 serotypes that accounted for 24 types of PFGE and 15 different STs. Most *S. Typhimurium* (n=13, 5 PFGE types) belonged to the worldwide spread ST19 (n=12) and to its SLV ST313 (n=1). They included the 3 most widespread clones in Portugal (DT104, OXA-30/CMY-2 and *sul3*) carrying different integron and plasmids types. The globally disseminated ST11 (n=4, 2 PFGE types) was identified in all *S. Enteritidis* isolates, including one carrying *qnrS* and belonging to a major clone. Isolates belonging to the emerging *S. Rissen* clone (n=5, 1 PFGE type) were assigned to the singleton ST469, only previously reported in European isolates of the same serotype. The remaining MDR isolates of different serotypes were assigned to STs belonging to already known clonal clusters (ST15, ST27, ST32, ST40, ST82 and ST334) or singletons (ST48, ST64, ST102, ST306 and ST358). Regarding the Brazilian isolates studied, they represented 5 serotypes which corresponded to 8 PFGE types and 7 different STs. As for the Portuguese isolates, all *S. Typhimurium* (n=5) and *S. Enteritidis* (n=2) belonged to worldwide spread clonal lineages, ST19 (n=3) or its SLV ST313 (n=2) and ST11, respectively. The remaining isolates of different serotypes were included in the already known ST10 (n=1), ST23 (*S. Oranienburg*; n=1), ST48 (*S. Panama*; n=2) and ST524 (*S. Give*; n=3). It is of note that most isolates of a particular serotype were assigned to a specific ST. This is the first study describing the population structure of nontyphoidal *Salmonella* in Portugal and Brazil. Our results confirm the prevalence of particular clusters enclosing major MDR clones that cause human infections, namely ST19 for *S. Typhimurium* and ST11 for *S. Enteritidis*. The selection and worldwide spread of particular STs might be influenced by the acquisition and horizontal transfer of specific resistance genes and genetic elements.

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# Preliminary study *in vivo* of the bone regenerative capacity of hydroxyapatite when associated with omeprazole in critical size defects

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The vacuolar-type H<sup>+</sup>-ATPases (V-ATPases) are abundant enzymes in the ruffled border of the osteoclast that have a fundamental role in the bone resorption process [1,2]. Consequently are considered crucial therapeutic targets in the treatment of lytic bone diseases [1,3,4].

In the present study, 80 mg of omeprazole, a gastric proton pump inhibitor (PPI), was evaluated *in vivo*, in calvarial critical size defects in Wistar rats, whether it increases bone regeneration when associated with the hydroxyapatite granules, Osteopatite<sup>®</sup>, and modified exoskeleton of shrimp (MES) membrane. In the same animal there were both test and control defects (paired sample design).

Although the results show slightly more bone regeneration and more Osteopatite<sup>®</sup> granules osseointegration in the test defects than in the controls, the results must be considered inconclusive. This issue will need further studies and to adequate the methodologies, in order to verify the omeprazole therapeutic effects as a potential local osteoclast function inhibitor.

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# Preservation Solutions for Liver Transplantation in Adults: Celsior (CS) vs. Custodiol (HTK). A systematic review and meta-analysis with an indirect comparison of randomized trials

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**Background:** The University of Wisconsin (UW) solution has been recognized as the gold standard of liver preservation; however, it possesses some limitations, and other solutions exist for organ preservation, including Celsior (CS) and Custodiol (HTK). The aim of this study was to compare the liver functions of transplanted grafts that had been stored in these different solutions.

**Methods:** We searched MEDLINE, EMBASE, LILACS, the Cochrane Central Register of Controlled Trials and SCIELO. We included randomized and quasi-randomized controlled trials that compared the efficacy and safety of Celsior and Custodiol with the Wisconsin solution for liver preservation in adults. The factors that were considered for analysis were their impacts on primary dysfunction, including primary nonfunction and initial poor function, ischemic-type biliary lesions and patient and graft survival rates. An indirect comparison of Celsior and Custodiol was also calculated.

**Results:** We identified three randomized controlled trials (RCTs) and one quasi-randomized controlled trial to pool in a meta-analysis of Celsior vs. Wisconsin solutions. The number of episodes of primary dysfunction was lower in the Celsior group (7.4%) than in the Wisconsin group (9.8%), but the difference was not statistically significant (RR=0.68; 95 %CI=0.22-1.97). The number of episodes of primary dysfunction was also lower in the Custodiol group (3.0%) compared to the Wisconsin group (8.4%), but the difference was not statistically significant (RR=0.36; 95% CI=0.08-1.70). An indirect comparison using data from the main analysis revealed no difference between the Celsior and Custodiol solutions (RR=1.88; 95 %CI=0.57-6.16).

**Conclusions:** The Celsior and Custodiol solutions performed similarly to UW as preservation solutions in liver transplantation clinical settings. Adequately powered RCTs are required to evaluate the real effects of these solutions.

Keywords: liver transplantation, Celsior, Custodiol, University of Wisconsin solution, Primary dysfunction (PDF), Primary nonfunction (PNF), Initial poor function (IPF), Ischemic-Type Biliary Lesions (ITBL), indirect comparison.

# Production of a novel canine norovirus nucleocapsid protein in yeast for use in serological studies

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Human noroviruses (NoVs) are recognized as the most frequent cause of foodborne outbreaks of acute gastroenteritis and the most common cause of sporadic enteric illness [1]. Though the most important modes of transmission are person-to-person contact and consumption of contaminated food, zoonotic transmission has been recently suggested [2]. Therefore, it will be important to study NoVs in other animal species, especially in pets which are in close intimate contact with humans, to monitor emerging viral strains and to determine their genetic relationship to human.

In 2007, studies focused on the search of NoV in dogs led to the discovery of a novel canine NoV. This constituted a relevant achievement in the emerging field of the canine NoVs, compelling to explore the ecology and biology of this new virus, and a possible zoonotic transmission. Since this is a new virus, with no commercial Virus-Like Particles (VLP)-based Enzyme Immunoassay (EIA) available, the development of an in-house EIA will be crucial. To establish this assay, it will be necessary a substantial amount of VLPs to be used as an antigen. The yeast *Saccharomyces cerevisiae* has been extensively used for the expression of viral proteins, including viral core and nucleocapsid proteins [2,3].

Hence, the aim of this work is to use yeast cells, as a cost-effective and high-level expression system, to produce sufficient amounts of the novel NoV capsid protein, VP1 (which self-assembles into VLPs), to be used in the EIA. With this goal, the following working plan is underway in our laboratory: i) construction of the yeast expression plasmid encoding VP1 fused to a 6His tag; ii) transformation of the yeast *S. cerevisiae* with the plasmid constructed; iii) assessment of VP1 expression in yeast by Western blot; iv) extraction and protein purification using purification columns highly selective for histidine-tagged proteins; v) development and validation of the EIA.

Together, this work will allow the development of an in-house EIA for diagnostic tests and seroepidemiological studies of this novel canine NoV.

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# Protective Effect of Physical Activity on Dissatisfaction with Body Image in Children - A Cross-sectional Study

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An accurate body image perception is crucial for the way children and adolescents develop a healthy relationship with their bodies and food intake behavior [1]. Some evidence also exist showing that regular physical activity and sport have a beneficial effect on the way children and adolescents perceive their body image [2, 3]. Nevertheless, the association between physical activity and body image dissatisfaction has not been yet properly quantified. The aim of the present work was to evaluate the frequency of dissatisfaction with body image among secondary school students and to examine the association between the physical activity and Body Mass Index (BMI) with image dissatisfaction.

This is a cross-sectional study conducted in a Porto high school, in which 234 children of both sexes, aged between 10 and 17 years were evaluated. Dissatisfaction with body image was assessed by Collins' Child Figure Drawings for pre-adolescents and adolescents [4]. The degree of dissatisfaction with body image (FID) was calculated as discrepancy between the "ideal figure" and the "perceived figure". The *Baecke* questionnaire was applied to determine the Habitual Physical Activity Index (HPAI) of each participant [5].

Girls were more dissatisfied than boys with their body image (68.1% vs. 52.9%). A high proportion of students of both genders and age groups presented a distorted body image stating that their "perceived figure" was bigger than the "ideal figure". A moderate correlation was found between the FID and BMI percentile in both genders (boys:  $r = .698$ ,  $p < .001$ , girls:  $r = .582$ ,  $p < .001$ ). High levels of physical activity were associated with a protective effect on dissatisfaction with body image,  $OR = .38$ , 95% CI [.16, .87] for 3<sup>rd</sup> vs 1<sup>st</sup> quartile of HPAI;  $OR = .29$ , 95% CI [.12, .86], for 4<sup>th</sup> vs 1<sup>st</sup> quartile of HPAI, both adjusted for BMI and gender.

In conclusion, body image dissatisfaction increases directly with BMI percentiles in both genders and physical activity has a protective effect on distortion of body image.

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## Protein Nitrogen Fraction of Commercial Milk Samples: registered brands *versus* store brands

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Milk is, unquestionably, one of the most important foods in human diet, all around the world. Currently, the financial crisis experienced worldwide, has implications in feeding patterns of societies. Like other foods, milk is not an exception and in recent times we have witnessed an increasing replacement of registered brands against white-label products (store brands). In our days all the consumers make the same questions: is there any difference between nutritional contents in these milks? Is it worth to pay more for registered brands?

Kjeldahl procedure accurately measures total Nitrogen content of milk. Typically, it is converted in protein content using a 6.38 factor [1-2].

The purpose of this study was to assess the content of protein nitrogen fraction in milk. A total of 10 milk brands of Portuguese pasteurized milk obtained from supermarkets in Porto region were analyzed. Different milk fat contents were considered, as well as registered brands and store brands. All samples were analyzed in duplicate.

Protein nitrogen contents of pasteurized milk samples are in the range 2.85 – 3.33 g/100 g. According to the milk labels inscription, the levels of protein are in a range of values between 2.90-3.40 g/100g. Statistical tests were performed using a confidence level of 95%. The difference between medians was not significant. Thus, regardless of brand and fat content, protein content in milk is similar and the white-label products seem to give the same guarantees provided by registered brands, regarding to protein content.

Despite the currently crisis in milk sector, we can conclude that producers are not neglecting the nutritional quality of milk, at least in what concerns to protein content. Thus, consumers can be assured that the labels are consistent with the levels truly presents in milk, so they can enjoy the many benefits that milk proteins potentiate in the health of all of us.

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# Purinergic modulation of high-affinity gaba and glutamate uptake into synaptic plasma membrane vesicles of the brain cortex

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Na<sup>+</sup>-dependent high-affinity transporters assure the control of neurotransmitter signaling by promoting clearance from the synaptic cleft. Yet, there is little information on the modulation of these transporters by endogenous neuromodulators acting to adapt protein function to the synaptic environment. Purines are ubiquitous modulators that regulate synaptic function according to neuronal excitability that depends on the balance between GABAergic and glutamatergic inputs. ATP is co-released with classical neurotransmitters at both peripheral and central synapses and may act directly on P2 receptors or after being catabolized into adenosine (ADO) through P1 receptors. ADO acts through the activation of four distinct G-protein-coupled receptor subtypes (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>). ADO is considered an “endogenous anticonvulsant” mainly by activation of A<sub>1</sub> receptors that decrease glutamate release.

In this project, we aimed at investigating the modulatory role of purines on Na<sup>+</sup> dependent high-affinity GABA and glutamate (Glu) transport into synaptic plasma membrane (SPMs) vesicles isolated from synaptosomes of the rat brain cortex. GABA and Glu uptake reaction was assayed in a medium containing: NaCl 150mM, HEPES-Na 10mM, EGTA 50μM, (pH=7,4) supplemented with [<sup>3</sup>H]-GABA 0.5μM (0.25μCi/mL) or [<sup>14</sup>C]-Glu 10μM (0.25μCi/mL). Reactions were initiated by SPMs (0.5 mg/mL) addition and stopped at various time intervals by quick filtration. The amount of radiolabeled neurotransmitter retained in filters was quantified by liquid scintillation spectrometry.

The uptake of [<sup>3</sup>H]-GABA and [<sup>14</sup>C]-Glu by SPMs of the rat brain cortex was fast, reaching a maximum at 2 min of incubation and then declined gradually during the following 40 min. Preliminary results show that both GABA and Glu accumulation by SPMs were decreased in presence of extravesicular ATP (140 μM). This may be due to an inhibitory effect of ATP (acting through P2 receptors) or of adenosine resulting from extracellular ATP catabolism (estimated at 30 μM) on GABA and Glutamate transporters. The involvement of A<sub>1</sub> and A<sub>2A</sub> receptors is currently under investigation. The use of SPMs allows elucidating the involvement of downstream activation of protein kinases, as well as protein phosphatases, by purine receptors. Normal function of the modulatory mechanisms found will be addressed in tissues from Human patients with mesotemporal lobe epilepsy as compared with control individuals.

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# PURINERGIC MODULATION OF NMDA-EVOKED RELEASE OF NORADRENALINE FROM RAT BRAIN CORTEX

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In the rat brain cortex, noradrenaline (NA) release can be stimulated by neurons originated from the *locus coeruleus* and by activation of local NMDA receptors, which are modulated by P2Y<sub>1</sub> receptors [1]. The aim of this study was to investigate whether release of NA evoked by NMDA receptor-activation was modulated by purinergic receptors P2Y receptors as shown for the NA release is evoked by electrical field stimulation [2].

Cortical occipital-parietal slices from male Wistar rats were incubated with 0.1 μM of [<sup>3</sup>H]-NA, superfused with a physiological solution and stimulated by two bolus (S<sub>1</sub> and S<sub>2</sub>) of 30 μM NMDA applied by 2 min. In most of the experiments Mg<sup>2+</sup> and/or Ca<sup>2+</sup> were omitted from the physiological solution being replaced by equimolar concentration of NaCl. The [<sup>3</sup>H]-overflow was used as an indicator of NA released evoked 30 μM NMDA and effects of drugs were calculated as S<sub>2</sub>/S<sub>1</sub> ratios and expressed as % change (increase or decrease) from respective controls as mean ± S.E.M from *n* slices.

Tritium overflow evoked by 30 μM NMDA was abolished by omitting Ca<sup>2+</sup> from the medium and by blockade of voltage-sensitive Na<sup>+</sup> channels with 0.3 μM tetrodotoxin, indicating that tritium overflow is dependent on vesicle exocytosis. Adding 1.2 Mg<sup>2+</sup> or blocking NMDA receptors with the selective antagonist MK 801 (50 μM) also abolished the overflow of tritium evoked by 30 μM NMDA.

The nucleotides ATP, ADP, ADPβS, UTP and UDP, all at 0.1 mM concentration, inhibited tritium overflow up to 38 %. The inhibitory effect of ATP (0.1 mM; 38±2 %, n=5; P<0.05) was attenuated to 18±7 % (n=5; P<0.05) and that of ADP (0.1 mM; 33±5 %, n=5; P<0.05) was abolished to 3±6 % (n=5) by the selective antagonist of adenosine A<sub>1</sub> receptors DPCPX (0.1 μM). The inhibitory effects of ADPβS (0.1 mM; 38±6 %, n=3; P<0.05), UTP (0.1 mM; 32±5 %, n=5; P<0.05) and UDP (0.1 mM; 28±2 %, n=4; P<0.05) were not changed by DPCPX (0.1 μM) but the inhibitory effect of UTP was abolished by the selective P2Y<sub>6</sub> receptor antagonist MRS2578 (1 μM). Neither DPCPX (0.1 μM), nor MRS2578 (1 μM) or the non-selective P2 receptor antagonist reactive blue 2 (10 μM) changed the tritium overflow evoked by NMDA 30 μM, except the non-selective P2 receptor antagonist PPADS (10 μM), which caused an attenuation of 40±5 % (n=4; P<0.05). The selective A<sub>1</sub> agonist CPA (1 μM) and the selective A<sub>2A</sub> agonist CGS 21680 (0.1 μM) inhibited tritium overflow by 44±7 % (n=6; P<0.05) and 31±5 % (n=5; P<0.05), respectively. The effect of CPA was abolished by DPCPX (30 nM) whereas that of CGS 21680 was abolished by the selective A<sub>2A</sub> receptor antagonist SCH 58261 (30 nM). The results indicate that P2Y and adenosine A<sub>1</sub> receptors inhibited NA release evoked by 30 μM NMDA. The P2Y<sub>6</sub> receptors mediated the effect of UTP, most likely due to its metabolism into UDP. Interestingly the adenosine A<sub>2A</sub> receptors inhibited rather than enhanced NA release, suggesting that this effect may be due to a regulation of NMDA receptor channel, as demonstrated in some striatal neurons [3].

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# Recent trends in Gleason grading of prostate cancer

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**Introduction:** Histologic grading remains the most useful tissue-based predictor of prognosis of prostate cancer. The Gleason system is now the only grading system recommended by the World Health Organization (WHO) for prostatic carcinoma. In recent years, there has been a gradual shift of how the Gleason grading is applied in practice with unknown implications. Our objective was to evaluate the variation of the Gleason score in the last five years in all patients submitted to prostate biopsy at S. João Hospital.

**Methods:** Between January 2005 and June 2010 we consecutively enrolled 1817 candidates referred to ultrasound guided trans-rectal prostate biopsy, on the basis of abnormal rectal examination and/or elevated total Prostate Specific Antigen (tPSA) levels, in the Department of Urology of S. João Hospital. Prostate pathology and the prostate cancer cases Gleason score were defined by biopsy results. All prostatic biopsies were reviewed by two different pathologists that were blinded to the patients' characteristics. Gleason scores results were compared between groups using chi-square test.

**Results:** Patients had a median age of 67 years and a median tPSA of 7.05ng/mL. Prostatic biopsies revealed prostate cancer in 719 cases (39.6%). Among patients with prostate cancer there were 0.3%, 17.1%, 55.4%, 16.2%, 10.3% and 0.6% with Gleason 5, 6, 7, 8, 9 and 10 in the biopsy, respectively. The percentages of patients with Gleason score of 7 and 8 or higher were 42.7% and 27.1%, 59.8% and 27.3%, 60.0% and 28.3%, 57.2% and 29.7%, 53.7% and 27.3%, 58.2% and 19.4% from the year 2005 to the year 2010, respectively (p=0.017).

**Conclusion:** Gleason grade results have varied in recent years in patients submitted to prostate biopsy, but without a clear trend. Urologists and Pathologists should be aware of these variations. This study is limited by the small time frame and possible confounder effect of patient tendency.

# Salt amounts in Oporto ready-to eat soups

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Soup is an indispensable food in a balanced diet, being an important vehicle for the ingestion of vegetables, whose health benefits remain unquestionable. The consumption of soup outside home is increasing, being regarded as healthy complements to fast meals taken at the lunch breaks, in snack-bars, coffees or restaurants. Based on the consumer patterns in the Oporto population<sup>1</sup>, almost 60% of the adults inquired consume soup at least once a day. Soup, however, is also recognized as an important source of sodium, due to the generalized use of salt (NaCl) as major seasoning, and to the relatively high volumes ingested per day.

The objective of the present work was to obtain real figures associated with the current sodium content in “non-homemade” soups, in order to address its representativeness to the daily ingestion of sodium in this population. Soups consumed in canteens were not included in this study, based on the principle that these are further controlled and adjusted to the nutritional recommendations. Also, only total sodium was under scrutiny, without distinction between added salt and the amount of sodium naturally present in the soup ingredients. A total of 83 soups were purchased, being the sodium evaluated by flame-photometry, using an analytical method developed and validated for the purpose.

Mean amounts of  $297 \pm 76$  mg/100mL were obtained for Na content, equivalent to  $0.75 \pm 0.20$  g/100 ml of NaCl. This average value is slightly above those reported in the Portuguese Composition Table<sup>2</sup> ( $241 \pm 9$  mg/100mL) but the variability in the present study is comparatively higher. Indeed, the Na amounts ranged from 126 mg to 523mg per 100 mL. Taking into account an average dose of 250 mL per soup, the values on Na range from 315 to 1283 mg, or from 0.80 to 3.26 g when expressed as NaCl per dose. Based on a recommended Tolerable Upper Level Intake of 2300 mg of Na per day<sup>1</sup>, these mean values represent around 37% DDR, for a single dose. When these results are transposed to the daily intake of soup, where several persons ingest two servings per day, including children and older people frequently with associated health issues, these figures are of extreme concern.

Based on these findings, and aware of the recommendation to reduce salt ingestion, soup consumed outside home can be regarded as an important source of Na, being mandatory to implement strategies for its control or standardization, as well as generalized recommendation for food producers and effective sensorial alternatives.

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# Salt content of soups served in school canteens from Porto and Alfândega da Fé

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Introduction: Portuguese traditional diet is often high in salt, consumption of salty foods, salted foods, and salt itself. Portugal is also known as the third largest consumer of soup.<sup>1,2</sup> The consumption of vegetable soup (a traditional preparation that includes high amounts of low energy-dense and high-dietary fiber, micronutrient rich vegetables), has a high satiating power, and is associated with decreased levels of obesity. However, soup can also be rich in added salt which may decrease its nutritional value. Sodium is essential for the proper and normal functioning of the body, but in excessive amounts it may favour cardiovascular diseases and certain types of cancer. WHO recommends a daily intake of 5 g of salt, or 2 g of sodium.<sup>3</sup>

The purpose of this study is to quantify the salt /sodium intake through soup consumption in high school meals (a school in the district of Bragança and the city of Porto).

Methods: Salt composition of soups was determined by Flame Photometry. Flame Photometry is widely used in clinical analysis and quality control of food when we want to quantify ions of alkali metals and alkaline earth metals such as sodium, potassium, lithium and calcium. We obtained 27 samples of a school from Alfândega da Fé (AF) and 39 samples of a school from Porto (P), between November and December 2010.

Results: Mean ( $\pm$  sd) salt content in the all samples of soup was 1.63 g ( $\pm$  0.268)/200 g, being 1.452 g ( $\pm$  0.19 g) / 200 g for AF and 1.760 g ( $\pm$  0.243 g) / 200 g for P. Percentiles 25, 50, and 75 of salt content from soups arising from the all samples were, respectively, 1.478 g, 1.665 g and 1.802 g; salt content ranged from 1.150 g to 2.180 g per 200 g of soup.

Discussion: If we consider that only 30% of the total daily salt intake should be consumed at lunch, the entire meal should have no more than 1.5 g of salt, already considering the added salt. According this theoretical cut-off, the described values of salt may be considered relatively high for salt and sodium consumption.

Conclusions: The soup represents an important source of salt intake at school meal. Given its high nutritional value and important at meals, it is necessary to establish guidelines for food processing and preparation in school canteens and caterers, in order to define the optimal levels of added salt in soup preparation.

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## Searching for small molecule modulators of caspase family members using yeast cell-based approaches

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Caspase 3 and 7 are key effectors of apoptosis and therefore promising pharmacological targets in anticancer therapy [1]. Several flavonoids showed to inhibit the survival of tumour cell lines through activation of a caspase-dependent apoptotic pathway [2]. It was also suggested that prenyl side chains increased the antiproliferative effect of flavonoids in human tumour cell lines [3].

In order to search for selective modulators of caspase 3 and 7, the yeast *Saccharomyces cerevisiae* expressing human caspase 3 or 7 was used. Expression of human caspase 3 or 7 in yeast was confirmed by Western blot. Effects of expression of caspase 3/7 in yeast were evaluated by analysis of cell growth, plasma membrane integrity, DNA fragmentation and reactive oxygen species production. As described for human cells [1], we showed that expression of caspase 3/7 in yeast causes a marked growth inhibition due to the induction of an apoptotic cell death. The validation of the yeast assay to search for small molecule modulators of caspase 3 and 7 was carried out using the procaspase activating compound-1 (PAC-1) and the inhibitor Ac-DEVD-CMK that increased and reduced, respectively, the caspase 3/7-induced growth inhibition. Using this yeast assay, the effect of prenylated flavonoids, obtained by the CEQUIMED-UP group and with cytotoxic effects on several human tumour cell lines, was analysed on human caspase 3 and 7. The results obtained showed that some of the flavonoids tested are potential activators of human caspase 3 and/or 7.

Together, this work establishes an efficient first line screening approach to search for small molecule modulators of human caspase 3 and 7. Additionally, using this yeast approach, potential activators of human caspase 3 and 7 were indentified. These compounds might represent promising small molecules to be further explored as anticancer drugs and/or as lead compounds of more potent and selective activators of caspase 3 and 7. We thank to REQUIMTE, CEQUIMED-UP (I&D 4040/2007) and U.Porto/Santander Totta for financial support. I. Coutinho (SFRH/BD/36066/2007) and M. Neves (SFRH/BD/21770/2005) are recipients of FCT fellowships.

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# Self-Concept Evaluation on the Bariatric Patient

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**Background:** Obesity is a public health problem that affects an increasing number of subjects and morbid obesity's prevalence has been increasing. There is a greater prevalence of psychological and psychiatric morbidity among obese subjects when compared to normal-weight ones [1]. Today, Bariatric Surgery's considered, in the long-term, obesity's most effective therapeutic option, resulting in significant improvements on the individual's physical, psychological and social health [2]. Research in this field has focused on the diseases' psychological aspects, however, Self-Concept (perception that the subject has of himself) has been drawn down.

**Objectives:** Evaluate Self-Concept on the subject with morbid obesity, about to undergo bariatric surgery, and characterize it's variation following the surgical procedure.

**Methods:** This study included patients from a central hospital, who have undergone adjustable gastric banding. They were evaluated before, 1 and 3 months after surgery at a Nutrition follow-up appointment. Anthropometric data were collected and Self-Concept was evaluated using ICAC.

**Results:** In the studied sample a significant ( $p=0,046$ ) increase in mean physical activity levels was observed between pre and post-surgical evaluations. After surgery, significant ( $p<0,05$ ) reductions on mean body weight, BMI and waist and hip perimeters were found. Self-concept's total score lowered a mean of 1,5 points between the two evaluation moments. A variation in the associations for total self-concept and their factors was observed, however none of the associations showed statistical significance.

**Discussion:** Studies are needed with samples of adequate size and with periodic follow-up superior to understand the importance of assessing the ICAC in the framing of obesity with specific clinical indication for bariatric surgery.

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# SEM/EDX analysis of GSR persisting on the hands of a shooter firing a 6.35 mm pistol

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**Introduction:** Gunshot residues (GSR) are particles produced during the discharge of a firearm and can be organic or inorganic in nature. Inorganic (metallic) components originate predominantly from the primer mixture and are generally considered to be the best choice for analysis in firearm discharge related incidents. SEM-EDX is a powerful tool, providing morphological and analytical information for single particles in a non-destructive way and allowing *maximum specificity* in GSR detection. Most researchers agree that the time up to which GSR may be reasonably expected to be detected on the hands of a shooter ranges between 2 to 4 hours, but in no previous studies was a 6.35 mm *Browning* pistol used (one of the most common firearms encountered in the Portuguese setting).

**Materials and methods:** Experimental shots were fired in a controlled environment, mimicking an intermediate range shot (50 cm) and using a 6.35 mm *F.N. Browning*® handgun (Mod. 1906) and *Speer Lawman*® ammunition (Total Metal Jacket). A total of 24 shots were fired, of which 20 were taken into consideration for this study (4 other shots were fired in a preliminary study). Carbon coated stubs were used to collect samples from the hands of the shooter (one stub for the back and one for the palm, for each discharge), immediately after the firearm discharge, and then at 1, 3 and 6 hours after shooting. Samples were analyzed at the Materials Research Center of the University of Porto (CEMUP), on a FEI Quanta 400FEG ESEM equipped with a EDAX Genesis X4M EDS, in low vacuum (30 Pa), with a working distance of 10 mm and accelerating voltage of 25 keV. The automation software used was the FEI gunshot residue analysis package (*GSR-Magnum*®). Data were analyzed with descriptive statistics methods.

**Results and discussion:** High variability in particle counts from shot to shot was found, whether considering both samples of the hand collected at a given post-firing time or just the sample from a single surface (spatial distribution analysis appears to be of limited practical value). There was a clear count drop related to post-firing sampling time, with the largest loss occurring after the first 3 hours (93.7% of all the analyzed particles were detected within this time period), which is consistent with previous published studies. Only 37.3% of the particles were spheroid (although most authors claim that 70% of GSR particles have this shape). Of all the particles found, 67.2% were below 5µm, which is consistent with previous published studies. *Ba-Sb* particles were the most commonly found (59.7%), which may be explained by the jacketed ammunition used (TMJ), which reduces the *Pb* contribution to GSR particles.

**Conclusion:** Given the number of variables that can be accounted for in firearm discharge incidents, a *case-by-case* analysis, always taking into consideration the gun and ammunition used, is clearly preferable to a more formal approach (i.e. a strict analysis and interpretation protocol).

# Signalling transduction pathways involved in Urocortin 2 induced relaxation of the iris sphincter muscle

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## Introduction

Urocortin 2 (UCN2) is an endogenous peptide of the CRF family. At the ocular level, UCN2 is expressed in the retinal pigmented epithelium and has a protective effect in retinal pos-ischemic degeneration. Also in the eye, UCN2 has a vasodilator effect in the retinal circulation.

UCN2 plays a role in muscular tissues, specifically in skeletal, cardiac and smooth muscles. The subcellular pathways involved in the effects of UCN2 may be, among others, nitric oxide release, prostanoids production and cAMP release.

We intend to characterize the effect of UCN2 in another smooth muscle tissue, the iris sphincter muscle, and add UCN2 to a group of substances that change iris sphincter muscle kinetics and consequently are responsible for the neurohumoral regulation of the anterior segment of the eye. The receptor and subcellular pathways involved in that phenomenon were also be studied and described.

## Methods

The effect of increasing concentrations of UCN2 ( $10^{-10}$ - $10^{-6}$ M) on carbachol-precontracted ( $10^{-6}$ M) rabbit iris sphincter muscles ( $n = 7$ ) was tested. The effect of UCN2 was also evaluated in the presence of: (i) L-nitro-L-arginine, a nitric oxide synthase inhibitor (L-NA,  $10^{-5}$ M,  $n = 9$ ), (ii) indomethacin, a COX inhibitor (INDO,  $10^{-5}$ M,  $n = 8$ ); (iii) antisauvagine-30, a CRF<sub>2</sub> selective antagonist (ASV,  $10^{-6}$ M;  $n = 7$ ); (iv) chelerythrine, a PKC inhibitor (CLT,  $10^{-5}$ M,  $n = 6$ ) and (v) SQ 22,536, an adenylate cyclase inhibitor (ACI,  $10^{-6}$ M,  $n = 8$ ).

## Results

UCN2 promoted a concentration-dependent decrease on active tension of the precontracted iris sphincter muscles, with maximal effect at  $10^{-7}$ M ( $19,25 \pm 7,96\%$ ,  $p < 0,05$  vs control). This effect was preserved with L-NA ( $10,14 \pm 5,20\%$ ,  $p < 0,05$  vs control). The relaxing effect of UCN2 was blunted by indomethacin ( $-0,24 \pm 3,10\%$ ); antisauvagine-30 ( $-1,35 \pm 2,45\%$ ); chelerythrine ( $1,45 \pm 3,72\%$ ) and SQ 22,536 ( $0,83 \pm 4,69\%$ ).

## Conclusion

UCN2 is a new neurohumoral factor that promotes the relaxation of iris sphincter muscle. This effect is mediated by prostaglandins and is independent of nitric oxide production. CRF<sub>2</sub> is the receptor involved in this relaxant effect, activating the PKC and adenylate cyclase subcellular pathways.

## **Soya isoflavones, genistein and daidzein: Biological effects in a ER-positive breast cancer cell line.**

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In recent years the relationship between soya foods and breast cancer has become controversial due to the estrogen-like properties of soya-derived isoflavones. Epidemiological studies suggested that there is a low incidence of breast cancer in countries with high soya intake.

Soya contains the isoflavones daidzein and genistein, which are responsible for the protective properties, although their exact effects have not yet been clarified. Some of them are related to apoptosis and inhibition of cell growth such as reduction of Bcl-2/Bax ratio, activation of caspases and inhibition of tyrosine kinase [1]. Daidzein and genistein are described as selective estrogen receptor modulators (SERM). They act as estrogen receptor agonists at lower concentrations, but as antagonists at higher concentrations.

In this study we evaluate and compare the biological effects of a soya extract biotransformed by *Aspergillus Awamori* (SBE) and standards of the major isoflavones, genistein (G) and daidzein (D), on MCF-7aro, an estrogen-dependent breast cancer cell line stably transfected with the aromatase gene.

The results showed that cell viability decreased significantly after 48 hour treatment with SBE, G and mixture of D and G in a dose-dependent manner. In addition, it was also observed a decrease in cell proliferation evaluated by the thymidine assay. Morphological studies, using Giemsa and Hoechst staining, demonstrated chromatine condensation and the presence of some vacuoles in cytosol. Interestingly genistein concentration used to achieve similar levels of cell viability or proliferation inhibition was much higher when compared to the concentration of genistein in SBE. Although D effect, by itself, was not significant, the mixture of D and G provided higher inhibition than G. These results suggest that daidzein may potentiate the effects of genistein. On the other hand, it cannot be discarded the possibility that other substances may be present in SBE. Further studies are required to clarify which compounds are responsible for these effects.

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# Synthesis of a triprenylated mangiferin inhibitor of breast cancer cell growth

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Many naturally occurring and synthetic xanthenes have been found to possess interesting biological and pharmacological activities [1]. We have focused our attention on the synthesis of xanthone derivatives and their capacity to inhibit the *in vitro* growth of some tumor cell lines, especially MCF-7 ER(+) (breast adenocarcinoma estrogen receptor positive) [1]. The nature and/or position of the different prenyl substituents were associated to the growth inhibitory effect of xanthenes, being found that some of these derivatives were not only potent growth inhibitors but also selective against the MCF-7 ER(+) cells [2].

It was based in this last premise that we proceeded with the prenylation of the bioactive xanthonoid, mangiferin (Fig. 1).

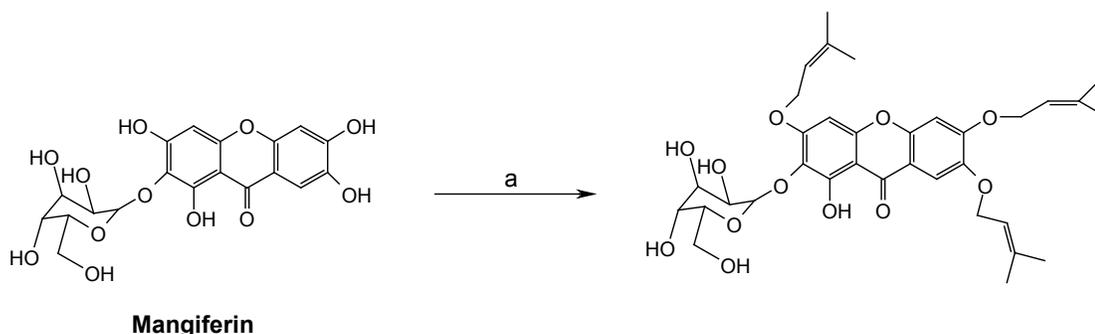


Fig. 1. Prenylation of mangiferin: (a) prenyl bromide, K<sub>10</sub> clay, CHCl<sub>3</sub>, MW (200 W/108°C/ 60 min).

The expected triprenylated mangiferin structure was proposed based on IR, <sup>1</sup>H, <sup>13</sup>C NMR, HR-MS data. This compound will be further investigated.

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## Synthesis of Flavonoids with Potential Antitumor Activity

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Prenylated flavonoids have long been recognized for their significant spectrum of pharmacological activities and have been extensively studied for their potential antitumour activity. Considering their mechanisms of action, their antitumor effect has been attributed to the interference with several biological processes. In particular, flavonoids have been described as interesting agents concerning apoptosis induction involving caspase activation [1]. The aim of this work was to synthesize novel compounds with antitumour potential, based on previous results from our group [2].

Several prenylflavonoids were synthesized by molecular modification of baicalein and 3,7-dihydroxyflavone. The ability of prenylflavonoids to inhibit the *in vitro* growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and A375-C5 (melanoma), was evaluated using the SRB assay [3]. The more active compounds were further evaluated for apoptosis induction in the NCI-H460 cell line, with the Human Annexin V-FITC/PI apoptosis Kit. Finally, in order to study the possible involvement of caspase-3 (one of the caspases involved in the apoptotic pathway), the effect of the monogeranylated derivative of baicalein was evaluated using a yeast system overexpressing human caspase-3.

Results from the *in vitro* screen of the compounds for cell growth inhibitory activity revealed that the presence of one prenyl or geranyl group was associated with an increase in the growth inhibitory activity. Using the NCI-H460 cells it was possible to observe that the effect of the monogeranylated derivative of baicalein on cell growth could be attributed, at least in part, to induction of apoptosis. The obtained results using the yeast system further revealed that the monogeranylated derivative of baicalein behaved as an activator of human caspase-3.

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# Targeting latent EBV infection using small molecules: from *in silico* screening to *in vitro* antiviral activity

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Epstein Barr virus (EBV) infects more than 95% of the human population. Following primary infection, EBV persists in B lymphocytes in a latent state usually without symptoms. Nevertheless, latent EBV infection has been associated with several cancers, such as Burkitt Lymphoma (BL). To date, there are no available anti-EBV therapies and the existing broad spectrum antiviral drugs are mainly active against lytic viral infection. Therefore, there is a strong need for new anti-EBV drugs. Xanthenes and flavonoids have been previously described as having potential antiviral activity [1, 2].

In order to search for potential anti-EBV molecules, an *in silico* virtual screen of a focused library of xanthenes and flavonoids was carried out using computational molecular docking (software eHiTS 2009). Structures of EBV proteins, namely EBNA-1, Zta and BHRF1 were used for docking. *In vitro* studies were performed in the BL cell line Raji, in order to assess cellular viability (trypan blue exclusion assay), EBV DNA load (semi-quantitative PCR) and viral and cellular protein expression (Western Blot).

Based upon the docking score results, three sulfated small molecules (**DS**, **MS2**, and **XGS**) were chosen to be tested *in vitro* in the Raji cell line. All the compounds decreased viral DNA content and LMP1 (latent protein) expression. None of the substances greatly affected cellular viability or induced viral reactivation. Results indicate that *in silico* screening is a good approach for the development of new anti-EBV agents, and we identified three novel sulfated small molecules capable of decreasing EBV levels in a BL cell line.

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# The Cholinergic Transmission in the Retrosplenial Cortex of the Epileptic Rat

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Recently it has been described a possible role of cholinergic neurotransmission system in epilepsy. The main purpose of this study is to assess if the pilocarpine-induced status epilepticus (SE) and the electroconvulsive shock (ECS) models of epilepsy are associated with changes in the cholinergic transmission in the retrosplenial cortex granular b (Rgb), a cortical area strongly interconnected with limbic structures well known to be involved in epilepsy, like hippocampal formation (HF).

SE was induced by treating rats with pilocarpine (350 mg/kg). Brief seizures were induced by ECS. Two months later the brains of the animals were processed for immunostaining for vesicular acetylcholine transporter (VAcHT) and the densities of cholinergic varicosities stained for VAcHT were estimated.

SE produced a statistically significant increase in the densities of cholinergic varicosities in Rgb layers I (C-  $64 \pm 21$ , SE-  $143 \pm 74$ ,  $p < 0.0499$ ), II/III (C-  $18, 5 \pm 7.8$ , SE-  $40 \pm 13$ ,  $p < 0.0370$ ), IV (C-  $67 \pm 17$ , SE-  $113 \pm 37$ ,  $p < 0.0483$ ) and VI (C-  $20 \pm 5$ , SE-  $36 \pm 14$ ,  $p < 0.0478$ ). In ECS group there was a non-significant increase in the densities of cholinergic varicosities in all layers of Rgb.

On the one hand, these findings suggest to an involvement of Rgb in epilepsy. On the other hand, it has been shown that the cholinergic dysfunction associated with epilepsy, previously described in the HF, is found too in Rgb cortex. These data support the hypothesis of cholinergic dysfunction in the initiation and/or propagation of epilepsy. In conclusion, our results show that SE is associated with an increase in the densities of varicosities immunoreactive to VAcHT in Rgb, while this did not happen with ECS.

# The effect of xanthohumol-supplemented beer on angiogenic and inflammatory *in vivo* assays

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Angiogenesis is a process by which new blood vessels are formed from pre-existing ones and can occur in adulthood during tissue regeneration. This process is closely related with inflammatory conditions [1,2].

Due to their notable biological activities, phenolic compounds have an important role in nutrition and human health. Special attention has been given to xanthohumol (XN), a compound present in hops and beer [3].

Our purpose was to evaluate the effects of a XN-supplemented beer on angiogenesis and inflammation, in a rat skin-wound healing process.

Six week old Wistar male rats drank water, 5% solution ethanol, stout beer or stout beer supplemented with 10 mg XN/L, during 4 weeks. Then, two incisions were created on the dorsal skin. Animals continued beverages consumption for 7 days. The number of vessels in the incision area (vWF staining), NO release, NAG activity and IL-1 $\beta$  content in serum were measured. Analyses of serum biochemical markers of hepatic function (AST, ALT and ALP activities) and of metabolic status (glucose, triglycerides, cholesterol, VLDL, LDL and HDL) were evaluated. GSH/GSSG plasma levels were measured in plasma by HPLC. Statistical differences were evaluated by ANOVA followed by the Bonferroni test. Differences were considered significant whenever  $p < 0.05$ .

The consumption of XN-supplemented beer led to decreased number of vessels in the wound area, and decreased NAG activity, NO and IL-1 $\beta$  content in the serum when compared to stout beer. Plasma biochemical markers of hepatic function and metabolic status did not change with the distinct beverages consumption. GSH/GSSG ratio increased with ethanol and beer consumption.

Altogether, these findings suggest that gastrointestinal administration of XN-supplemented beer may affect the wound healing process, in what concerns inflammation and angiogenesis, without hepatotoxic effects. Ethanol consumption seems to improve plasma anti-oxidant protection. These health-promoting properties of XN can be interesting to the brewing community.

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## The importance of H-RAS 81T-C polymorphism in cellular behaviour

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Aneuploidy is one of the hallmarks of cancer, which is often correlated with high-grade, invasive tumours [1]. The RAS oncogenes have been associated with genomic instability by inducing gene amplification [2], generating aberrant chromosomal content and inducing chromosome missegregation, through the MAPK pathway [3], and through induction of centrosome amplification [4]. Recently, our group showed that the H-RAS 81T-C polymorphism is associated with alterations in the ploidy status in follicular thyroid tumours and in nodular goitres, being the C allele associated with patients harbouring aneuploid tumours.

The main goal of this work is to study the importance of the H-RAS 81T-C polymorphism in aneuploidy and tumorigenesis. In order to achieve this we stably transfected the HEK 293 cells with two versions of an H-RAS mini-gene, one carrying the T allele and the other carrying the C allele, in the position 81, and further analysed the importance of this alteration in cellular behaviour.

We found the H-RAS 81T-C polymorphism to be associated with modifications in the alternative splicing and, consequently, with alteration in the p21H-RAS/p19H-RAS isoforms expression ratio. We demonstrated that the H-RAS C allele, in comparison with the T allele, is associated with increased chromosomal instability, increased cell growth, decreased apoptosis and increased cell migration suggesting an increased *in vitro* transforming ability of the H-RAS C allele.

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# The role of bone marrow in the course of infection with *Mycobacterium avium*

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Members of the *Mycobacterium avium*–*intracellulare* complex (MAC) cause pulmonary disease indistinguishable from tuberculosis. Immunocompromised patients, namely HIV-infected individuals, are the main targets of infection by *M. avium*. Our group demonstrated that IFN- $\gamma$  produced during infection of C57Bl/6 (B6) mice with a highly virulent strain of *M. avium* (ATCC 25291 SmT) induced profound CD4<sup>+</sup> and CD8<sup>+</sup>-T and B cell depletion in the spleen and a profound thymus atrophy [1, 2]. In this study, we attempted to determine if thymus atrophy and peripheral lymphopenia is related to a bone marrow dysfunction during *M. avium* 25291 infection.

The determination of bacterial load was done in the thymus, spleen, liver, lung and bone marrow in B6 infected with *M. avium* 25291 and *M. avium* 2447 (less virulent strain) at different time-points of infection. Further, we have quantified the number of viable and dead cells in the bone marrow during infection. In order to determine if IFN- $\gamma$  has also a role in a potential alteration in bone marrow, we have determined the bacterial load in thymus and bone marrow of 25291-infected B6 and B6.IFN $\gamma$ <sup>-/-</sup>. The quantification of viable and dead cell numbers in the bone marrow was also determined.

The bacterial load increased over the course of infection in B6 infected with both strains of *M. avium*, being this increase more significant in *M. avium* 25291. The viable cell number in the bone marrow from 25291-infected B6 significantly decreased after day 50 post-infection, compared to 2447-infected B6. No significant difference was observed in the bone marrow and thymus bacterial load from 25291-infected B6 or B6.IFN $\gamma$ <sup>-/-</sup>. However, the viable cell counts significantly decreased at day 50 post-infection in 25291-infected B6 mice compared to 25291-infected B6.IFN $\gamma$ <sup>-/-</sup>. No differences were observed in dead cell counts. The data show that bone marrow is affected by infection and the loss of cells is also dependent on IFN- $\gamma$  production.

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# The role of p63 isoforms in the regulation of *CDH3* gene and in the malignant phenotype of breast cancer cells

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P63 encodes multiple protein isoforms with both transactivating and transcriptional repressor activities that can regulate a wide spectrum of target genes. P63 plays an important role in limb development and differentiation of stratified epithelial tissues such as epidermis, breast and prostate [1]. This transcriptional factor is also implicated in tumor formation and progression, with evidence for both tumor suppressive and oncogenic properties, which has been lead to controversy [2]. Recently, it was demonstrated that *CDH3*/P-cadherin gene, was a transcriptional target of p63 in a human limb bud and hair follicle model [3]. It is described that P-cadherin is associated with poor prognosis breast cancer. And, in *in vitro* model, it was also showed that P-cadherin overexpression by breast cancer cells promotes their invasive and migratory capacity [4,5,6]. Interestingly, Carrol *et al.* suggested that p63 play an important role in the modulation of gene expression programs involved in cell adhesion [7]. To clarify the association between p63 and P-cadherin we performed luciferase assays using MCF7.Az/Mock cells co-transfected with recombinant plasmids with the *CDH3* promoter, and the various isoforms of p63. We demonstrated that different p63 isoforms are able to differently modulate the activity of *CDH3* promoter in breast cancer cells, being the truncated isoform DeltaNp63 the one which induce greater gene activity. We also performed immunohistochemistry for p63 in series of breast cancer cases already characterized for P-cadherin. We observed a direct association of p63 expression with more proliferative and aggressive tumours, high grade lesions and low differentiated breast tumours. These preliminary results of reinforces the indication that there the differential effect of p63 isoforms in *CDH3* should be explored. Here, we developed a strategy in order to assess if p63 has any regulatory effect on the expression of P-cadherin, and to evaluate whether or not this regulation is directly via genomic. Additionally, we aim to study if the different p63 isoforms have an important role in the functions mediated by P-Cad, particularly invasion and motility of breast cancer cells. The results obtained will be important to better understand the relationship between p63, P-cadherin and it putative importance in the malignant phenotype of breast cancer.

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# Trace element status in hemodialysis patients: Results from a monitoring study on a Portuguese population

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Because hemodialysis therapy may lead to depletion of biologically essential trace elements or body burden of toxic trace elements, it is widely recognized that patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis therapy are at risk for trace element imbalances (deficiency of essential trace elements and/or excess of toxic trace elements) [1,2].

Therefore, and considering both the well known toxic effects of several trace elements (aluminum being the most notable case) and the biological importance of other trace elements, it has also been suggested that the impressive morbidity and mortality in hemodialysis patients may be at least in part associated with imbalances in their trace elements status [3].

Based on this background, we have been conducting a monitoring study (starting in July 2009) of several trace elements blood levels (Cu, Zn, Se, Mn, Pb) on a Portuguese population of hemodialysis patients (ca. 50 persons).

Compared to commonly accepted reference intervals (“normal values”) and results obtained from “controls” (non-hemodialysis subjects), the data obtained so far showed: *i*) Cu levels (serum) in the middle of reference interval; *ii*) Zn levels (serum) below the limit to consider Zn deficiency (i.e., < 80 µg/dL) [4]; *iii*) Mn levels (whole blood) near or even higher than the upper limit of the reference interval, and significantly higher than in controls; *iv*) Pb levels (whole blood) not significantly different from the normal population. Typically, the patients also presented no signs of aluminum overload, with most of patients presenting Al levels lower than 15 µg/L.

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# Understanding the damaging effects of chemical straightening of human hair

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Hair straightening is increasingly becoming a popular hairstyle. The hair straightening chemical process normally requires the use of an alkaline reducing agent that cleaves disulphide bonds of hair fibres. Then the hair is mechanically straightened using a comb, and bonds are reformed using an oxidizing agent. This procedure is recognized to cause hair damage<sup>[1]</sup>. In this work, virgin hair samples were analyzed with differential scanning calorimetry (DSC) and textural analysis in order to understand the damaging effects of the chemical treatment performed with the reducing agent ammonium thioglycolate (ATG). The influence of ATG concentration (0, 3, 5 and 7 % w/v) and application time (10, 20, 30 min for 7% ATG) was studied. Virgin hair samples in tress form were washed with lauryl ether sodium sulphate 10 % solution to remove impurities, prior to straightening treatment. pH was adjusted to 9.3 with ammonia and hydrogen peroxide (2.5 %, 5 min) was the oxidising agent. The hair was combed at the middle and at the end of the established application time of the reducing solution.

Thermal analysis was conducted from 30°C to 350°C, under a nitrogen flow rate of 40 mL.min<sup>-1</sup> at a heating rate of 10 K.min<sup>-1</sup> (200 F3 Maia<sup>®</sup> - NETZSCH, Germany). Single hair fibres were tested in a Texturometer (TAXT2i, Stable Micro Systems, UK) using a tensile test. The test speed was 0.5 mm.s<sup>-1</sup> and the extent of deformation was 40 mm. Ten measurements were performed for each sample. Hair colour was also assessed with a Colorimeter (CR400, Minolta, Japan) and the parameters L\*a\*b\* were obtained. The results were evaluated statistically with oneway ANOVA ( $\alpha=0.05$ ).

The application time did not influence the hair colour while for concentrations higher than 5 % an increase in lightness (L\*), a\* and b\* chromatic coordinates (related to red and yellow colour, respectively) was observed. Breaking strength was unaffected by the concentration of the reducing agent and by the application time. On the other hand, hair extensibility increased for all ATG concentrations when compared to untreated hair but no differences were observed between the various concentrations used. This parameter was only significantly increased for 30 min application time. Increasing both application time and ATG concentration cause a decrease on  $\alpha$ -keratin fusion enthalpy which was more pronounced for 30 min application time. Taken together these results suggest that the chemical straightening treatment performed with ATG presents a damaging effect to human hair which is influenced both by the ATG concentration and application time.

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## Using yeast cell-based assays to search for small-molecule activators of p53 and inhibitors of p53-MDM2 interaction

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The p53 tumour suppressor has a central role in the regulation of cell cycle and apoptosis. Activation of p53 has been recognized as a promising strategy for cancer treatment. The activity and stability of p53 are regulated by the endogenous negative regulator, MDM2. Hence, activators of p53 and inhibitors of p53-MDM2 interaction are considered promising drugs for cancer therapy [1].

Due to the high complexity of the p53 pathway in mammalian cells, yeast assays have been developed for a first-line screening of activators of p53 and inhibitors of p53-MDM2 interaction. With this goal, yeast cells expressing only human p53 and co-expressing human p53 and human MDM2 were used as cell models. Effects of small molecules and MDM2 on p53 activity were evaluated by analysis of yeast cell growth (by colony forming unit counts), cell cycle progression and cell death.

Our previous work showed that expression of human p53 in *Saccharomyces cerevisiae* induced yeast growth inhibition associated with S-phase cell cycle arrest [2]. Using this yeast p53 phenotypic assay, we analysed the effect on p53 activity of several flavonoid and xanthone derivatives, some of them synthesised by the CEQUIMED-UP group. The results showed that some of the compounds tested significantly increased the p53-induced yeast growth inhibition and DNA fragmentation without interfering with the plasma membrane integrity. These compounds behaved as potential p53 activators. Concerning MDM2, though expression of human MDM2 did not interfere with the yeast growth, its co-expression with p53 significantly reduced p53-induced growth inhibition and S-phase cell cycle arrest. The use of this yeast approach to search for inhibitors of p53-MDM2 interaction was validated by testing known inhibitors of p53-MDM2 interaction, as RITA and Nutlin-3a [1]. Also, with this yeast assay, a promising inhibitor of p53-MDM2 interaction was identified among the tested xanthone derivatives.

In conclusion, in this work, a p53-MDM2 interplay similar to that reported in mammalian cells was reconstituted in yeast. The developed yeast assays revealed to be efficient drug screening approaches to identify activators of p53 and inhibitors of p53-MDM2 interaction. Finally, the small-molecules identified as activators of p53 and as inhibitor of p53-MDM2 interaction may have potential applications as anticancer drugs and/or lead compounds.

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## Variance of bioimpedance analysis measurements with physical activity and ingestion of food and water

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**Introduction:** Bioimpedance Analysis (BIA) is a method used to assess body composition based on the measure of body segments. BIA is relatively simple and fast, and also safe, non-invasive, cheap and uses portable equipments, that makes it widely used in clinical practice. However, certain factors may affect BIA's reliability in assessing body composition. This work aims to examine how the effect of physical activity and the ingestion of food and drinks may affect BIA's analyses.

**Methods:** 27 volunteer (24 females) university students were enrolled in this study. The measurements were taken in three non consecutive days with four evaluations each day and consisted on the Body Mass Index (BMI), and percentage of fat mass (%FM), measured by two equipments. The influence of water ingestion and practice of moderate physical activity were studied in the second and third days, respectively.

**Results:** On the first day we observed a decrease in the percentage of fat mass throughout the day. The BMI showed a tendency to increase throughout the day, with a decrease in the late afternoon. The interclass correlation coefficient (ICC) was higher for the BMI than the ICC for the %FM of both equipments (BMI: ICC=0.999, p<0.001; Equipment A: ICC=0.990, p<0.001; Equipment B: ICC=0.912, p<0.001). On the second day the participants who drank water had a tendency to have an higher %FM in the *before lunch* measurement. An opposite tendency was found in the control group. However, these results are not statistically significant. On the third day the physical activity had a tendency to have an opposite effect to the water ingestion (however not statistically significant).

**Discussion:** Body weight has daily changes that arise from the ingestion of food and beverages and physical activity. In particular, it decreases during the day, having a peak after lunch. The BIA analysers appeared to be more affected by those variations, since BMI had an higher internal consistency of measurements than the %FM measured by both equipments.

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