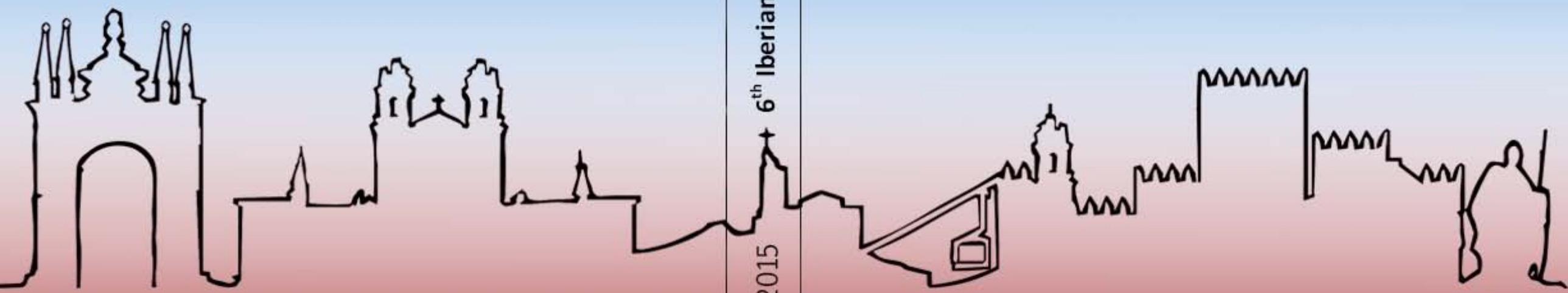
6th Iberian Meeting on Colloids and Interfaces

8th-10th July 2015
Guimarães/Braga, Portugal

RICI6



Abstracts

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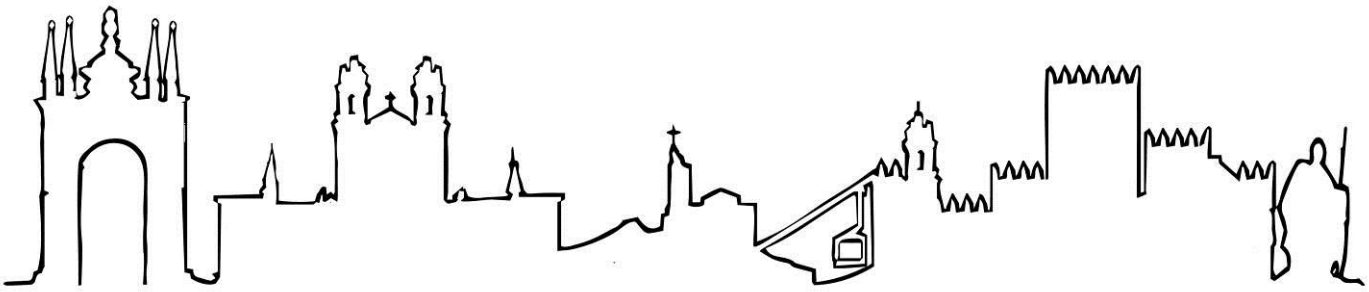
6th IBERIAN MEETING OF COLLOIDS AND INTERFACES - RICI6

BOOK OF ABSTRACTS



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Welcome to Guimarães/Braga and RICI6

We are pleased to welcome you to the **6th Iberian Meeting on Colloids and Interfaces, RICI6**. This time the RICI6 is held in the beautiful city of Guimarães, a world UNESCO Heritage site, from 8th to 10th of July, 2015.

RICI is now a well established and recognized Iberian conference, jointly promoted by *Grupo de Colóides, Polímeros e Interfaces (GCPI)* of *Sociedade Portuguesa de Química (SPQ)* and *Grupo Especializado de Coloides e Interfases (GECI) from the Reales Sociedades Españolas de Química y de Física (RSEQ e RSEF)*, an event which started in 2005 in Salamanca, followed by Coimbra, Granada, Porto and Donostia-San Sebastián.

One of the main goals of this joint effort has been to gather the scientific communities of both Iberian countries in order to exchange ideas and promote collaborations across the Iberian Peninsula and beyond, showing the latest research in the multidisciplinary field of Colloid and Interface Science.

This year's conference, the 6th in this series, pursues the same objectives of all others, which is to reach scientific and social success and bring people together, despite the difficult times in research and academia that we have been experiencing in our countries in recent years.

In this meeting, we decided to have five *Plenary Lectures* and five *Invited Lectures*, followed by two parallel sessions, thus aiming to increase the number of talks and stimulate discussions. In addition to the oral program, different poster sessions will run outside the lectures rooms.

As you will see over the course of the two and a half days of the meeting, the program will provide us with the opportunity to engage in a wide range of topics relating to this field and to exchange ideas, observe different perspectives and various type of experimental and theoretical approaches. The diversity and high scientific quality of the contributions of both senior and young Iberian and international researchers is a testimony of the vibrancy of the field. Following a good tradition in RICI meetings, the participation of young researchers in all contributions has been given priority.

Two awards for the best oral presentations among young scientists and three awards for the best poster presentations will be sponsored by *RSC Advances* and by the Wiley journal *Particle*, respectively. We take the opportunity to acknowledge all the institutional and private sponsors who have kindly joined us to make this meeting possible.

Last but not least, we thank you for your participation. We hope that you will enjoy the social program including the Welcome cocktail on the 8th evening at the City Hall of Guimarães, the Piano Recital at Fundação Martins Sarmento on the 9th evening and the city tour to Braga on the 10th followed by the conference dinner at the Hotel Golden Tulip at Falperra, Braga.

Once again, Bem-vindos/Bienvenidos/Welcome!

We wish you all a successful and enjoyable meeting.

M. Elisabete C. D. Real Oliveira (Chair)

Eduardo F. Marques (Vice-Chair)

Grupo de Colóides, Polímeros e Interfaces da Sociedade Portuguesa de Química

Jacqueline Forcada

Grupo Especializado de Coloides e Interfases de las Reales Sociedades Españolas de Química y de Física

WEDNESDAY, 8TH OF JULY	THURSDAY, 9TH OF JULY	FRIDAY, 10TH OF JULY
10:00-14:00 **REGISTRATION**	9:00-10:15 (Room A) **PLENARY LECTURE 2** **INVITED LECTURE 2**	9:00-10:15 (Room A) **PLENARY LECTURE 4** **INVITED LECTURE 4**
	10:15-10:45 **SESSION 4 (Room A)** **SESSION 6 (Room B)**	10:15-11:00 **SESSION 8 (Room A)** **SESSION 9 (Room B)**
	COFFEE BREAK **POSTER SESSION 1**	**COFFEE BREAK** **POSTER SESSION 2**
	11:45-13:00 **SESSION 4 (Room A)** **SESSION 6 (Room B)**	12:00-13:00 **SESSION 8 (Room A)** **SESSION 9 (Room B)**
14:00 **OPENING**	**LUNCH BREAK**	**LUNCH BREAK**
14:15-15:30 (Room A) **PLENARY LECTURE 1** **INVITED LECTURE 1**	14:30-15:45 (Room A) **PLENARY LECTURE 3** **INVITED LECTURE 3**	14:30-15:45 (Room A) **PLENARY LECTURE 5** **INVITED LECTURE 5**
15:30-16:15 **SESSION 1 (Room A)** **SESSION 2 (Room B)**	15:45-16:45 **SESSION 4 (Room A)** **SESSION 7 (Room B)**	15:45-16:15 **SESSION 8 (Room A)** **SESSION 9 (Room B)**
COFFEE BREAK **POSTER SESSION 1**	**COFFEE BREAK** **POSTER SESSION 2**	16:15-16:30 **CLOSING SESSION (Room A)**
17:00-18:45 **SESSION 1 (Room A)** **SESSION 2 (Room B)** **SESSION 3 (Room B)**	17:45-18:15 **SESSION 5 (Room A)** **SESSION 7 (Room B)**	17:00 **Braga city tour**
19:30-20:30 **Welcome reception** **Guimarães City Hall**	21:30 **Piano concert** **Fundação Martins Sarmento Guimarães**	21:00 **Conference dinner - Braga**

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9: Nanoparticles for Biomedical Applications

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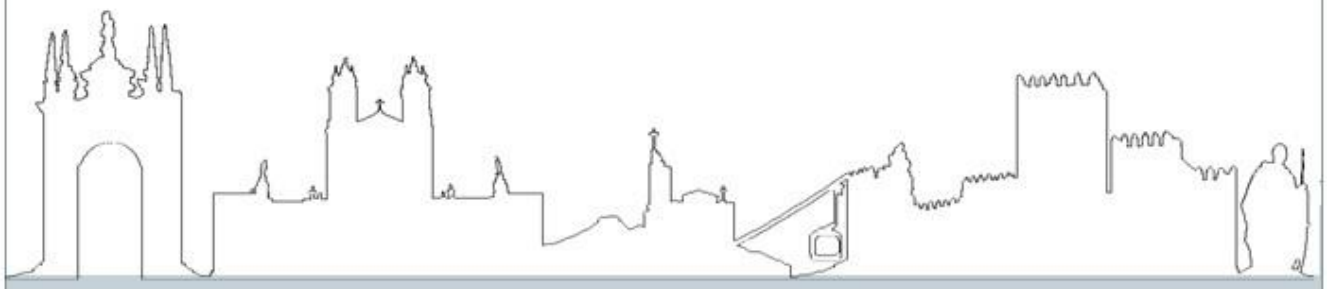

PLENARY LECTURES

Studying nanomedicine biobarriers by advanced fluorescence microscopy methods

Kevin Braeckmans[1,*]

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In the drug delivery field, intensive research is being carried out to develop 'intelligent' nanoscopic particles that are capable of efficiently delivering biopharmaceuticals to target cells. These nanoparticle formulations should fulfill several requirements including colloidal stability in biological media, protection against degradation of the pharmaceutical cargo, mobility in the extracellular space, efficient internalization and processing in target cells etc. Therefore, having detailed information on the physicochemical and biophysical properties of the nanoparticles during the various phases of the delivery process is required to achieve efficient optimization of their structure and composition.
In this presentation it will be discussed how different advanced fluorescence microscopy methods can be used for this purpose. First, in the context of intravenous administrations, methods will be discussed to measure colloidal stability of nanomedicines in blood. Next, the importance of investigating the extracellular mobility and the intracellular processing of nanomedicines will be considered. Ocular gene delivery will be used as a case study on how these biophysical evaluations may lead to a rational optimization of the structure of nanocarriers for drug delivery.

Smart water-based coatings from reactive polymer nanoparticles

José M. G. Martinho[1,*]

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The increasing pressure to reduce volatile organic compounds (VOCs) within the coatings industry has fostered the development of water-born systems. Water dispersed polymer nanoparticles (i.e., latex) have been successfully used to create polymer films that develop through three stages (Fig.1).
After evaporation and drying a densely packed agglomeration of polymer particles is formed (*stage I*). By the action of capillary, osmotic, and surface forces, a transparent void-free film composed of space-filling polyhedral is formed above the "minimum film formation temperature" (*stage II*). However, these films have weak interfaces between the particles. To strengthen them, polymer chain diffusion across the interface between adjacent particles has to be promoted (*stage III*) to form a mechanically rigid film.
Förster resonance energy transfer (FRET) has been used to follow interparticle chain diffusion during film formation. If the particles are labeled with a tiny amount (<1% mol) of a fluorescent dye (either a Forster energy donor or an acceptor), annealing of the film above the Tg of the polymers leads to colocalization of the dyes from different labeled particles in the interparticle regions with the consequent increase in the quantum efficiency of FRET. Since the dye distribution mimics the distribution of the polymer components, these experiments provide detailed information on the distribution of the labeled polymer components and the morphology of the nanostructure.
The strategies used to improve the properties of latex films to meet application specifications are revised, with special emphasis on reactive coatings (currently used to create polymer films with improved strength, hardness, and resistance to chemicals [1,2]) and smart latex dispersions with carry specific functionalities that either respond to particular changes in the environment (e.g. water content) or to external applied stimuli, like temperature, light and pH[3].

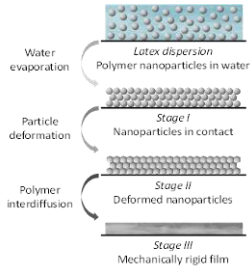

Figure 1: Film formation from a water dispersion of polymer nanoparticles.

Acknowledgements: *This work was supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) Project RECI/CTM-POL/0342/2012 and PEst-OE/CTM/LA0024/2013.*

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Materials for CO_2 capture and other industrial applications: synergies between molecular simulations and experiments

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As defined by the Brundtland Commission, sustainable development is the development that "*meets the needs of the present without compromising the ability of future generations to meet their own needs*". Sustainable development is of special relevance in the present situation, in which an explosive growth in energy consumption along with a rapid increase in population worldwide is observed. In this context, a great effort has been devoted in recent years to develop sustainable processes or improving existing ones, searching for a net positive impact in the environment. Hence, chemical industry needs drastic efficiency intensification of its processes in order to face these challenges and the search for an effective technology for separating and capturing CO_2 from a flue gas stream, as well as its storage or utilization, is becoming a very active area of research. Among the different possibilities, adsorption is seen as one of the most important physical processes for effective CO_2 capture and separation. The main interest in the research of adsorbent materials is given by their low energy requirement, high applicability and low equipment cost; hence, a wide range of adsorption materials are under development for this purpose [1].

The effective design of these materials requires a method that can relate the structure of the adsorbent to its performance. This level of understanding can be achieved by using molecular simulations, as they create a connection between what can be studied from a molecular perspective and what is observed in the macroscopic world by experiments, allowing a direct interpretation of the macroscopic behaviour of the system at the microscopic level. Hence, combining molecular simulations, at the right level of approximations and accuracy, with the required experimental data it is a powerful tool to advance in the design and optimization of these materials.

In this presentation we will first provide an overview of the present situation regarding CO_2 capture and utilization, briefly mentioning available technologies for CO_2 capture and their limitations [2]. The second part of the presentation deals with specific studies we are carrying out on materials to improve the CO_2 capacity for reversible absorption or permanent sequestration. The best material will depend on the specific process and conditions at which the gas needs to be separated from the stream, as well as the impurities coming with it. We will present and discuss very recent experimental and Grand Canonical Monte Carlo modelling results concerning the use of functionalized mesoporous silica adsorbents (as well as the process of functionalization) [3-4], zeolites and selected MOFs, as some of the most promising mid-term alternatives to achieve a viable process for CO_2 separation and capture at large scale. Results on the performance of these materials in specific processes, based on the molecular information, will also be provided.

Acknowledgements: *Contributions from S. Builes, C. Domingo, P. López-Aranguren and D. Bahamon are gratefully acknowledged. Financial support has been provided by the Spanish Government (CTQ2014-53987-R) and the Generalitat of Catalonia (2014SGR1582).*

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Role of metal clusters in the synthesis of anisotropic metal structures

Yasser A. Attia[1,2], David Buceta[3], Carlos Vázquez-Vázquez[3,*] and M. Arturo López-Quintela[3]

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Metal clusters are very tiny particles (< 1.5-2.0 nm in size) with well-defined structures and composed of a small number of atoms (below ca. 150) [1]. These clusters show a different than nanoparticles (with larger sizes) because their sizes become comparable to the Fermi wavelength of the electron (approx. 2 nm for Au or Ag).

Anisotropic metal structures and, in particular, gold nanorods (NRs) show unique optical properties and they are widely used in different areas, such as sensing, bioimaging, biodiagnostics, catalysis, optoelectronics, etc. From the several methods proposed for their preparation, the seeds-mediated method is the most widely used. However, till now the mechanism involved in this synthesis is still unresolved and the influence of some parameters (like the addition of silver ions) are controversial. Recently, we showed that Ag clusters formed during the synthesis are able to catalyse the anisotropic growth of the Au nanostructures [2]. The presence of these Ag clusters at the tips of the Au NRs could be identified due to their excellent photocatalytic properties. Moreover, the presence of such Ag clusters attached to the gold tips allows us to explain the observed Au photodissolution because of their semiconductor-like properties.

In addition, pursuing such idea we hypothesized that other clusters, like Au clusters, could also be used for such purpose because of their expected similar catalytic properties. Indeed, when using Au clusters it is possible to get the same level of control and tunability of the gold NRs formation as with Ag ions. Moreover, the seeds mediated method can be extremely simplified just by mixing two Au seeds solutions aged at different times. With this simple method one can produce Au NRs with high yield and large aspect.

Acknowledgements: *This work was supported by Obra Social "La Caixa" (OSLC-2012-007), European Commission through FEDER FP7 and H2020 programs (0681_InveNNta_1_E; FutureNanoNeeds, FP7-Grant 604602; Inspired, H2020-Grant 646155); MINECO, Spain (MAT2012-36754-C02-01), Xunta de Galicia, Spain (GRC2013-044, FEDER Funds).*

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Mechanisms of action and comparative toxicity of surfactants to eukaryotic and prokaryotic cells *in vitro*: insights into their use as antiseptic agents

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Surfactant use in disinfection and antisepsis is as old as the use of soap in personal hygiene. The use of non-soap surfactants as disinfectants and antiseptics dates to the early 20th century. The biocidal activity results from favorable partitioning of surfactants into biological membranes from the aqueous phase and subsequent perturbation of membrane structure and function. The perturbations range from induced changes in membrane area and curvature elastic energy, changes in surface charge and consequent membrane electrostatic potential, induction of inhomogeneities in the membranes with different length and time scales, increase in membrane porosity and, finally, dissolution of the membranes. Exactly which perturbations result are a function of the chemical composition and physical properties of the membranes as well as the chemical structure of the surfactant and its concentration in the membranes. Membranes of eukaryotic cells and prokaryotic organisms differ from each other in their chemical composition and, therefore, their physical properties. This difference may be useful in selection of surfactants as disinfectants and antiseptics. We have focused our attention in this area on the possible use of surfactants in prophylaxis of sexually transmitted bacterial infections and transmission of genital bacterial infections from parturient mothers to their neonates. Both are major health concerns, particularly in underdeveloped areas of the world where routine medical care leaves a lot to be desired. Our work so far has been limited to comparative *in vitro* toxicity studies on cultures of (squamous and columnar) epithelial cells and the relevant bacterial infective agents. The aim has been to define selective toxicity and the mechanisms that may be involved in this selectivity. A screening of all commercially available classes (non-ionic, zwitterionic, anionic and cationic) of surfactants showed that only cationic surfactants were more toxic to bacteria than they were to eukaryotic cells. All other surfactants were about equally toxic to bacterial and eukaryotic cells, the toxicity probably resulting from gross destruction of membrane barrier properties and/or membrane dissolution. In the case of cationic surfactants, the length of the apolar chain and the chemical structure of the polar head group were shown to be important parameters in selective toxicity. Shorter apolar chains are more selective and charge delocalization in the polar head group increases toxicity. Mechanistically, cationic surfactant toxicity to eukaryotic cells was shown to result primarily from damage to the mitochondrial system – its fragmentation and reduction in the efficiency of both electron transfer and oxidative phosphorylation. In bacterial cells the toxicity was shown to result from increased membrane porosity (at higher concentrations and shorter exposure times) and changes in membrane curvature elasticity and/or its surface potential that inhibit cell division and viability. The therapeutic index (ratio of concentrations that are toxic to human epithelial cells to concentrations that are toxic to bacteria) was shown to be between 10 and 100, which suggests that these commercially available, and cheap, cationic surfactants may be useful candidates for prophylactic uses in sexually transmitted infections and genital infections that may be transmitted from parturient mothers to their neonates.

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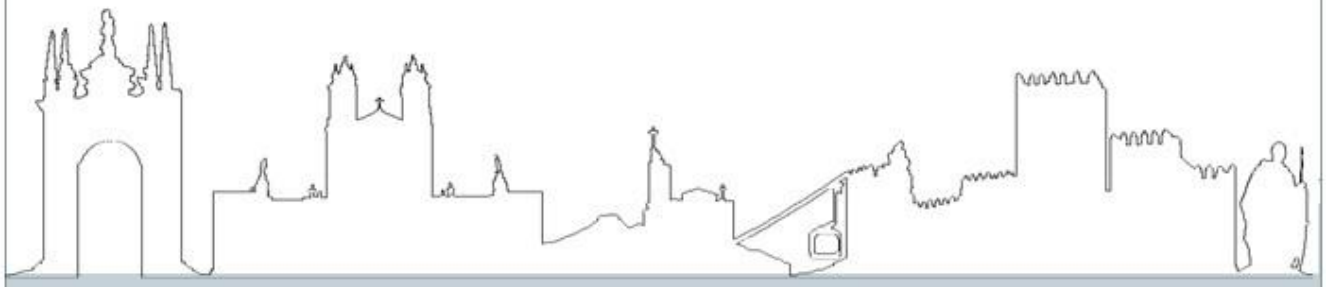

INVITED LECTURES

Interfacial mechanics of the apoptotic ceramide lipid

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Ceramide is a sphingolipid of eukaryotic cells that is formed on one of the membrane leaflets by enzymatic cleavage of sphingomyelin. Ceramide participates in some physiologically relevant process such as the cellular death, apoptosis. This talk focusses on the fundamental question about the compression and shear membrane elasticity of ceramide and sphingomyelin mixtures, which were investigated using oscillatory surface rheology of Langmuir monolayers [1-4]. Compared to the fluid parent sphingolipid, ceramide monolayers are univocally classified as 2D solids. This unusual behavior will be discussed in terms of the physiological signification of ceramide formation in biological membranes.

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Gene carriers and membrane interactions: How to defeat Nature with Nature's tricks

A.S. Jurado[1,2,*], A.M. Cardoso[1,2], C.M. Morais[1,2] and M.C. Pedroso de Lima[1,2]

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Nature observation has been a source of constant inspiration for humanity. The bioinspired materials are just an example where people learn from Nature and mimic their strategies to implement them at their own convenience. On the other hand, humans know that when someone has no way to fight the enemy, the best is to join him and use his own weapons against him. The employment of RNA interference (RNAi) technology to treat diseases such as cancer is a strategy emerging from this principle.

In this presentation, gene therapy strategies and tools to achieve control of target cell functioning by a genetic or epigenetic intervention will be addressed. Experimental examples from our laboratory and literature will profusely illustrate such strategies, with emphasis on those addressing cancer therapies, and the systems used to carry and deliver nucleic acids will be approached, essentially taking into consideration structure-activity relationships. An ideal nucleic acid carrier protects its cargo, preventing degradation or binding to non-target sites, and transports it to a specific intracellular compartment, where it is intended to act. Studies have been conducted in our laboratory with a great variety of compounds (e.g. surfactants, cell penetrating proteins and polymers), to approach the physical and structural properties that provide them with the capacity of achieving high cargo transfer efficiency, avoiding cytotoxic side effects and ensuring a successful biological activity of the delivered nucleic acids. Physical and biophysical properties of those molecules (including their ability to interact with membranes and the mode through which they modify membrane lipid organization and dynamics) have been studied in order to establish structure-activity relationships at three main steps in the delivery process: the passage through the cell membrane, the escape from the endosome and the arrival at the target site for the delivery (cytosol for siRNA and nucleus or mitochondria for plasmid DNA). Multiple questions can be raised in this context, such as the following: i. In cells presenting different endocytic pathways, which of them will be preferentially used for the internalization of different types of nucleic acid complexes, differing in size, composition and architecture? ii. For a defined nucleic acid/vector particle, whose cell uptake proceeds through different mechanisms, which of them will ensure the highest cargo transfer and the most efficient biological activity? iii. Which features might carriers to be provided with to warrant nucleic acid protection, avoiding premature cargo dissociation, and efficient delivery in the adequate intracellular compartment? Although some trends towards the success of carrier-mediated delivery have been identified with a certain level of consistency, mostly based on properties involved in cellular uptake, endosomal escape and rate of complex association/dissociation, differences in the efficiency of the biological response are often hardly correlated with all these issues.

Overall, data emerged from our work and others demonstrate that structural features of the materials used for nucleic acid delivery can be fine-tuned in order to modulate the ability of complexes to overcome biological barriers, which opposed to the success of these "trojan horse" inspired devices, thus representing a step forward towards the rational design of new nucleic acid delivery systems with widespread application in pre-clinical and clinical therapeutic approaches.

Acknowledgements: *This work was funded by Portuguese Foundation for Science and Technology and FEDER/COMPETE through the grants PTDC/DTP-FTO/0265/2012 and PEst-C/SAU/LA0001/2015 (CNC, University of Coimbra).*

Protein based micro-nanoemulsions

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Micro- and nano-scale systems have emerged as important tools for developing clinically useful drug delivery systems. In this tutorial review, we discuss the exploitation of biomacromolecules for this purpose, focusing on proteins, polypeptides, nucleic acids and polysaccharides and mixtures thereof as potential building blocks for novel drug delivery systems. We focus on the mechanisms of formation of micro- and nano-scale protein-based capsules and shells, as well as on the functionalization of such structures for use in targeted delivery of bioactive materials. We summarise existing methods for protein-based capsule synthesis and functionalization and highlight future challenges and opportunities for delivery strategies based on biomacromolecules.

Perspectives in the development of solid lipid nanoparticles for biomedical applications

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During the last two decades, solid lipid nanoparticles (SLN) have emerged as innovative drug carrier systems of great interest in the pharmaceutical field, mainly associated to their biocompatible and biodegradable nature. In addition, some limitations encountered in the stability of certain drugs may also be mitigated by the drug loading in SLN, with additional improvements in their bioactivity and bioavailability [1]. SLN are lipid matrices, composed of solid lipids and surfactants, which can entrap lipophilic or hydrophilic drugs, and according with the drug´s localization in the lipid matrix, the literature describes 3 different types (Figure 1). The lipids figuring in the SLN composition are of physiological nature, which provides several advantages regarding the safety and efficiency of these carriers [2]. The encapsulation of drugs could enhance their therapeutic properties to treat several chronic diseases and also to improve the barrier permeation and bioavailability of drugs [3]. SLN are being investigated for several administration routes e.g. oral, transdermal, ocular and/or nasal. We have reported the use of these systems for the delivery of antidiabetic and antioxidant drugs. To achieve the most promising SLN formulation, a factorial design study has been employed, by optimizing the physicochemical and biopharmaceutical parameters. The study also describe the most recent in vitro and in vivo results – with a particular emphasis for ocular administration - highlighting the potential use of SLN for drug targeting and delivery [3-5].

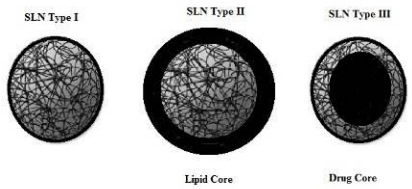

Figure 1: Schematic illustration of the different types of SLN.

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Magnetic suspensions as model colloidal materials

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Magnetic-driven suspensions are promising systems in Colloid Science because magnetostatic interactions can be tuned externally and are significantly much stronger than other "conventional" forces such as, for instance, purely electrostatics, DLVO and acid-base interactions. Magnetorheological fluids are a particular kind of magnetic-driven suspensions that involve essentially non-Brownian particles. In this case, the application of an external magnetic field magnetizes the particles and promotes the formation of directed self-assembled mesostructures that dramatically influence the mechanical (rheological) properties of the suspension itself. For large enough magnetic fields, a "liquid-to-solid" transition has been reported (so-called MR effect).
Magnetorheological fluids are traditionally constituted by polydisperse spherical carbonyl iron particles dispersed in a linear viscous fluid. In an attempt to improve their performance in comercial applications, our research group is currently studying composite colloidal materials -with special emphasis in their anisotropy, roughness, polydispersity and viscoelasticity- in shear and biaxial elongational flows. In this presentation we will briefly summarize some of the most recent achievements in this field.

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Guimarães2015

ORAL COMMUNICATIONS

1. INTERFACES, FILMS AND COATINGS

Physical-chemical investigation of newly synthesized cationic lipids with a peptide-like backbone for gene transfection in 2D and 3D systems

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The gene-therapy could be a promising way to cure human diseases like AIDS, cystic fibrosis or cancer. Therefore, cationic lipids were developed as a DNA carrier to create the so-called lipoplexes. The lipoplex should be able to cross the cell membranes and release the DNA near the cell nucleus. The delivery is depended on the structure of these complexes (cationic lipid and negatively charged DNA). For this reason new cationic lipids are permanently synthesized, and their physical-chemical properties have been characterized in 2D (monolayers at the liquid/air interface) and 3D (aqueous dispersions) [1]. To determine the properties of 2D monolayers, we use the pressure/area isotherms, infrared reflection absorption spectroscopy and x-ray methods like GIXD, Reflectivity and TRXF. For experiments in bulk SAXS/WAXS and QCM-D were used.

The various lipids have a similar basic structure in which the chain pattern differs by the unsaturation degree. Additionally, various head groups with different number of amine groups have been studied.

The increasing fluidity (double bonds in the chains) and the increasing size and charge of the head group have an influence on the phase state and the protonation degree of the lipids. We used TRXF to quantify the number of charges per molecule at different pH values. The phase state of the lipid chains has been measured by IRRAS. Furthermore, the adsorption of calf thymus DNA on the lipid monolayers has been quantified by IRRAS depending on the sub phase pH value and additionally in bulk by QCM-D. The results will be discussed as a function of the chemical structure of the lipids.

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Bioactive response of Ta-based surfaces

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Materials' surface properties are important parameters that should be taking into account for tissue engineering applications. According to the literature [1], moderately hydrophilic (≈70-60°), negatively charged and highly bioactive surfaces tend to quickly adsorb the adhesion-promoting proteins improving the biological response on hard tissue engineering practice [1]. Furthermore, dental implants present a number of limitations and their use is sometimes accompanied by failure [2], mainly of them associated with surface constraints related with bioactivity absence of titanium (Ti) [3]. Tantalum (Ta) is pointed as a potential material for bone ingrowth, once it has both more bioactive response and interesting chemistry valences than Ti, which will better promote the osseointegration process [4-6].
In this work Ta based coatings were deposited by DC reactive magnetron sputtering into Ti CP substrates in an $Ar+O_2$ atmosphere. In order to assess the osteoconductive response of the studied materials, contact angle and zeta potential measurements as well as *in vitro* tests of the samples immersed in Simulated Body Fluid (SBF) were followed. Structural results shows that the small increase of O content leads to a change of Ta phase from stable phase (α-Ta: bcc) to mixture with metastable phase (β-Ta: tetragonal) achieving the oxide phases with a large amount of O. Morphology images revealed that with increase of oxygen amount on the coatings a change in columnar to a featureless morphology was achieved. In-vitro test results demonstrate that Ta oxide surface shows higher wettability and surface energy, and consequently an increased affinity for apatite adhesion, when compared to Ti substrates, and moreover they showing higher apatite formation even for 14 days immersed in SBF solution.

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Protein behavior in presence of big hydrophobic ions

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Food allergies have increased significantly during the last years. One of the most common food allergies comes from cow milk, and the major causative allergens of the milk are proteins such as β-casein and β-lactoglobulin. This problem has carried out a significant interest in developing techniques that allow to extract the allergen proteins from the food and also the development of systems capable of detect the proteins as, for example, the use of biosensors. For both, the extraction of proteins from food and developing a biosensor, is crucial to know the properties of the proteins in solution and their interactions with other surfaces. Taking this into account, the goal of this work has been to study the adsorption properties of β-casein, β-lactoglobulin and BSA, the latter as a reference protein. For that, we have covered latex particles with proteins. And to analyze the behavior of the adsorbed proteins, we have used two big hydrophobic ions that is expected strongly interact with the proteins.
The mentioned ions are the tetraphenylborate anion (Ph_4B^-) and the tetraphenylarsonium cation (Ph_4As^+). Both ions have a very similar size, chemical structure and net electrical charge, the only significant difference is the sign of their charge. Despite this, it is well known that the anions cause a more intense effect over systems than the cations [1, 2] due to their different hydration capabilities, so in general the anion (Ph_4B^-) is more hydrophobic than the cation (Ph_4As^+) [3]. These monovalent ions present a high affinity to hydrophobic interfaces, and we have observed in previous studies that they are able to produce great charge inversions over colloidal systems at very small concentrations, when they act as counter-ions, specially the anion [4]. This effect is more pronounced when it deals with soft-matter systems, like proteins or polymers [5].
In order to determine the hydrophilic or hydrophobic global character of the proteins, we have carried out both electrophoresis and stability measurements of the mentioned systems. We have found that BSA and β-lactoglobulin, both globular proteins, have a similar behavior, whereas β-casein, which is a disordered protein and has more flexibility to exhibit their hydrophobic amino acids, presents a more hydrophobic character. This is reflected by the fact that the tetraphenyl ions feel more affinity toward this protein and in the case of the anion (Ph_4B^-), it occurs charge reversal, whereas we have not observed inversion charge phenomena for BSA and β-lactoglobulin. In addition, we can conclude that the use of tetraphenyl ions is a very suitable and easy method in order to determine the hydrophilic/hydrophobic character of colloidal systems.

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Graphene and its derivatives: a new platform for bio-imaging and bio-monitoring

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Graphene – the planar, hexagonal arrangement of carbon atoms forming a honeycomb structure and representing the 2-dimensional allotrope of carbon, is a novel fascinating material discovered only in 2004 [1].
Recently, graphene has also been recognized as unique two-dimensional plasmonics material that displays a wide range of extraordinary properties [2]. Excited fluorescent molecules in close proximity to graphene can efficiently excite graphene plasmons. A state of the art, Time Correlated Single Photon Counting (TCSPC) aided by a Fluorescent Lifetime Imaging Microscopy (FLIM), apparatus based on a femtosecond Ti:Sapphire Laser was employed to monitor the temporal change in the mean lifetime of fluorescent molecules immobilized on top of graphene at well-defined distances. This lifetime reduction by graphene induced quenching has a strong dependence on the distance to the graphene surface, varying sharply over a scale of ten nanometers. This is caused by resonant energy transferred that occurred between them. In particular, a long-range quenching of the fluorescence is predicted, which is unique to graphene [3]. Evaluating the quenching of the fluorescence the distance dependence is extracted and is observed to be in agreement with the theoretical predictions. This distance dependence extends over a longer scale than is typically observed between donor and acceptor molecules interacting via the more conventional mechanism of Förster resonant energy transfer (FRET). This effect could be developed into a new molecular ruler for larger distances.
Graphene can also be assembled into quantum dot structures (GQDs) which have a broadband fluorescence emission. This is their most notable optical property which is a somewhat unexpected consequence of their heterogeneous atomic and electronic structures [4]. The strong fluorescence emission from GQDs can potentially be used as a biomarker in bio-imaging applications.
In this talk, I will present some recent results in these two related areas obtained by our group at the Centre of Physics in the University of Minho (CFUM).

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A combined TEM, SEM and AFM study on the nanostructures in asymmetric chain surfactants: from twisted/coiled ribbons to nanotubes

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Surfactants with chiral headgroups, such as amino acid-based surfactants, often self-assemble in water into a wealth of complex nanostructures due to the combined effect of their hydrophobic/hydrophilic balance (packing parameter), chirality and headgroup charge and H-bonding interactions [1, 2]. Thus, besides forming micelles, vesicles and liquid crystalline phases, they have the possibility of forming extended discrete assemblies such as fibers, ribbons and nanotubes. Parameters such as temperature, concentration and pH clearly influence the thermodynamic stability of a given structure over the others [3]. These aggregates with a high *d*/*L* aspect ratio have high potential as gelators and as stimuli-sensitive nanocarriers for biomolecules [1, 2]. In this work, we present a detailed microstructural investigation of the aggregates formed by double-chained anionic lysine-based surfactants, designated as 8Lys16, 12Lys12 and 12Lys16 [3]. All the surfactants below their chain melting temperature self-organize into tube-like "crystalline" structures, comprising twisted ribbons, coiled ribbons and nanotubes, which eventually induce gelation of the aqueous dispersion. Combined data from phase contrast light microscopy, TEM, SEM and AFM were used to obtain a consistent picture of the morphologies formed. The long-chained compound, 12Lys16, has the particularity of spontaneously forming stable vesicles once it solubilizes around 50°C, while the other surfactants form micelles [3]. The effects of the chain length mismatch on the type of structures (size distributions and polydispersity) formed and their evolution towards equilibrium will be presented and interpreted at molecular level.

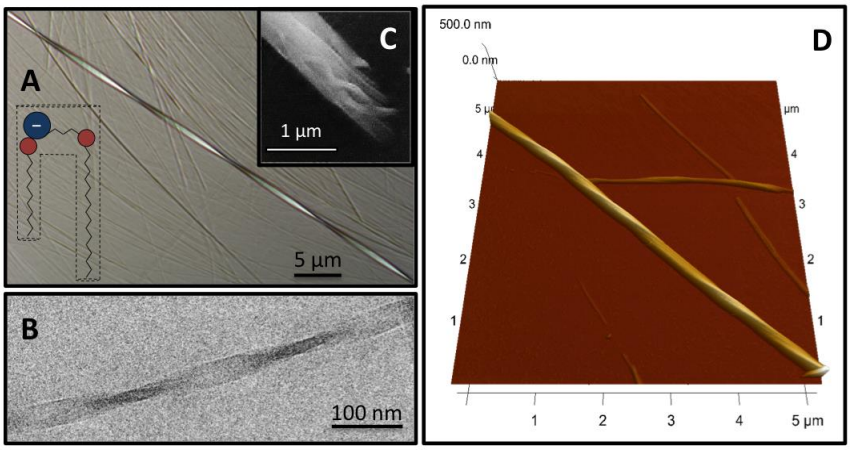

Figure 1: Twisted ribbons from 8Lys16 showing different length scales for the helical pitch, as observed by: A) phase contrast light microscopy; B) cryo-TEM; c) cryo-SEM; D) AFM.

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Biomimetic calcium phosphate coatings for enhanced titanium implant design – mixed and patterned self-assembled monolayers

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INTRODUCTION

Biomimetic hydroxyapatite coatings can be employed in order to promote bone growth on titanium surfaces used as biomaterials for fast healing effect. Metal modifications with organophosponate self-assembled monolayers (SAM) have the advantage of providing stability, better adhesion and biocompatibility when calcium phosphate layer is formed on this chemically-tailored titanium surface [1]. For improved biomaterial-host interactions, it is of interest to bring together the features of two grafting molecules (mixed SAM). The other option may be to vary surface properties by Direct Laser Patterning (DLP) of the chemically modified Ti (patterned SAM). Since wettability of a solid is conditioned by its chemical composition, contact angle (CA) measurements open the way for demonstrating the quality of a functionalized Ti surface.

EXPERIMENTAL METHODS

Commercially pure titanium (cpTi, ASTM grade II) was modified with four organophoshonate molecules with different terminal groups: $-CH_3$ and -COOH groups (DLP treated SAM), and $-PO(OH)_2$ and -OH groups (aqueous mixtures of two molecules for mixed SAM [2]). These surfaces, when modified with $-CH_3$ and -COOH functional groups as the protocol described here [3], were patterned by DLP. Both types of patterns were validated as for to biomimetic calcium phosphate layer formation. CA measurements were performed *via* tilted plate method with 40 µl Milli-Q water drops. Modified cpTi surfaces were also examined by AFM, XPS and SEM.

RESULTS AND DISCUSSION

We were able to successfully obtain mixed and patterned SAMs with organophosphonate molecules, as well as to evaluate them *via* biomimetic coating formation.

CONCLUSIONS

We were able to design mixed and patterned SAMs from organophosphonates on smooth cpTi. These biomaterials may be useful for directed cell growth in dental implantology.

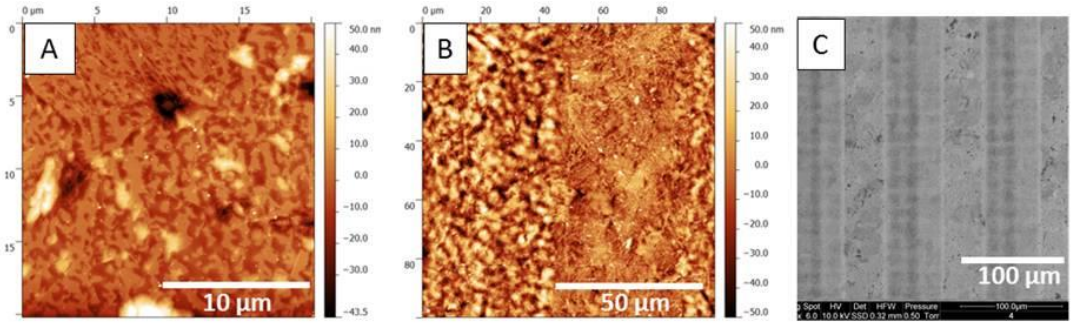

Figure 1: AFM images with mixed (A) and patterned SAM ($-CH_3$ functional group)(B). SEM photo of the patterned SAM formed with organophosponate having -COOH functional group (C).

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Chondroitin sulfate or fucoidan crosslinked with a sol – gel network as sorbents for metal cations

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Chondroitin sulfate (CS) and Fucoidan (Fd) are compounds of marine origin extracted from the cartilage and tissues of animals and seaweed respectively [1, 2]. Both are linear acidic polysaccharide, composed of repeating disaccharide units and modified with sulfate residues at different positions (Fig. 1).
The aim of this study took into account the valorization of marine resources by aiming at innovative applications. In this context, the reticulation of CS/Fd via a sol-gel process was explored with the purpose of preparing sorptive materials for metal cations such as Pb^{2+}, Cu^{2+} and Zn^{2+}. The same process was also attempted for the molecular imprinting of target cations ($Cu^{2+}$and Pb^{2+}) envisaging an increase in the selectivity of the adsorbents [3]. Simultaneously controls, corresponding to adsorbents without CS/Fd, were performed. All sorbents were structurally characterized and its efficiency in sorption of the cations under study was briefly assessed by solid phase extraction (SPE) and monitored by atomic absorption spectrophotometry (AAS).
The developed sorbents presented low surface areas (range of 4 - 6 m^2/g) and low pore volume (range of 0.003 - 0.004 cm^3/g) but the adsorbents with CS/Fd showed significant SPE retention of Cu^{2+}/Zn^{2+} (aprox. 90% and 84 % respectively) and Pb^{2+} (aprox. 70%), which demonstrated that the compounds of marine origin greatly benefit the adsorption of cations in study. Sorbents composed of CS showed better retention capacity to metals as compared with sorbents composed of Fd, however the later appears to retain more strongly the cations. The molecular imprinting did not increase the retention of the imprinted cation, however it increased selectivity for the metals used as templates.

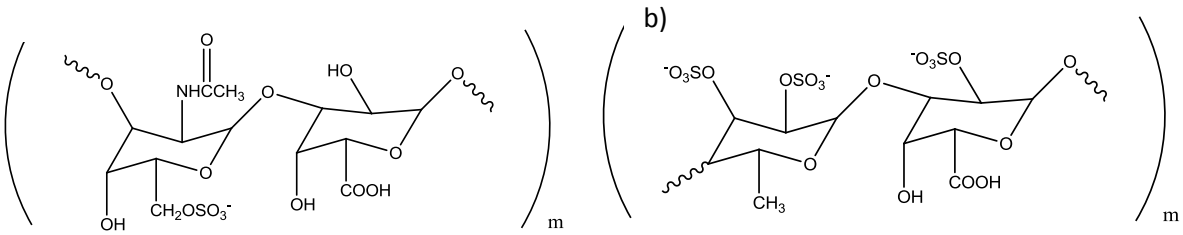

Figure 1: Chemical structure the a) Chondroitin sulfate and of b) Fucoidan

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Particles trapped at fluid interfaces: structure, phase behavior and dynamics

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Particles trapped at fluid interfaces are increasingly used for substituting synthetic surfactants in stabilizing dispersed systems such as emulsions and foams [1]. Furthermore, they can be used as model systems of 2D fluid and solid systems. Even though questions remain open about the interparticle interactions and the particle density dependence of the 2D system, the particle dynamics at the interface, and their use as probes for measuring the shear viscosity of surfactant and polymer monolayers is still rather controversial. The dynamics is very important because it is responsible of the response of emulsion droplets to external mechanical perturbations such as the collisions between droplets. It is also important to the response of the inter-bubble walls to the shear field due to liquid draining.

In this communication after a brief review of the structure and phase diagram of particles (from 1 to 5 μm) monolayers [2] and the role played by the three-phase contact angle [3], we will examine the dynamics of particles in these quasi-2D systems. In particular, the particle mean square displacement variation on going from dilute (fluid-like) monolayers to concentrated (solid-like) ones will be discuss, and these results compared with the dynamics of particles trapped in an optical tweezers system.

Finally, we will show that following the dynamics of a few particles embedded in a surfactant or polymer monolayer can be used to obtain information about the complex shear modulus of the monolayer [4], though the results are still controversial and a careful examination of the data must be performed for using particles as rheological probes.

Acknowledgements: *This work has been supported in part by MICIN under grants FIS2009-14008-C02-01, FIS2012-38231-C02-01, by UCM-Banco de Santander under grant GR3/14 and by U.E. under grant Marie-Curie-ITN "Co-Wet". E. Guzmán is grateful to MICIN for a Juan de la Cierva contract. We are grateful to Th.M. Fisher, R. Miller, and L. Liggieri for helpful discussions.*

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Films of smart colloidal nanoparticles for coatings with optimized properties

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Polymer networks are used in many industrial applications due to their strength and malleability. Poly(butyl methacrylate), PBMA, has a low Tg, yielding films with good viscoelasticity but poor resistance. Controlled crosslinking can improve this last property, providing that crosslinking reactions are slower than chain interdiffusion across the interfaces between nanoparticles.
In this work we encapsulated a sol-gel precursor, TEOS, inside PBMA colloidal nanoparticles. The resulting emulsion remained very stable at neutral pH for very long periods of time but, once spread over a substrate, the particles became reactive and originated films with excellent healing properties.
Two sets of PBMA colloidal dispersions were prepared, one formed by nanoparticles containing TEOS, T-NP, and the other by nanoparticles of PBMA only, NP. Each set of colloidal particles was dye labeled, half with an energy donor, half with an acceptor. Films casted from mixtures of dye-labeled colloidal particles were annealed between 80°C and 110°C. Förster Resonance Energy Transfer studies in the T-NP films demonstrated that chain interdiffusion was very fast at 80°C, but not at higher temperatures due to the increasing rate of crosslinking reactions. Apparent mean diffusion coefficients, <Dapp>, for T-NP and NP films were also determined and compared. Films were further submitted to chemical resistance essays and T-NP films clearly showed improved results.
We concluded that the sol-gel precursors acted as smart molecules, remaining quite unreactive in colloidal emulsion at neutral pH but recovering their reactivity when forming films. In the solid state, these molecules could either act as plasticizers - enhancing chain interdiffusion across the interfaces and contributing to films malleability - or react to form hybrid networks - enhancing films resistance, due to the formation of more cohesive interfaces. By tuning the annealing temperature, these two roles were balanced and films with optimized properties obtained.
These materials are very promising and we foresee very high commercial perspectives as binders with improved resistance for aqueous paints and coatings. [1]

Acknowledgements: *This work was supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) through project RECI CTM-POL 0342 2012.*

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Interfacial behavior of oppositely charged polyelectrolyte – surfactant mixtures

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In general, oppositely charged polyelectrolyte-surfactant mixtures lead to the formation of supramolecular complexes that strongly modify interfacial properties. These mixtures have a large number of technological applications (such as biocompatible coatings, stabilization of colloidal solutions and particle dispersions, flocculation process, surface treatments, cosmetics). Despite the intense research work done, the behaviour of these mixtures is not clear yet. In this work we present a detailed experimental study of the bulk properties (zeta potential, diffusion coefficient, binding isotherms) as well as interfacial behaviour (adsorption at the vapour/liquid interface, surface dilational rheology) of a polycation of cosmetic interest, poly(diallyldimethylammonium chloride) with three different surfactants. The adsorption isotherms showed the presence of a minimum equilibrium surface tension at low surfactant concentrations, which has been associated with the formation of polymer/surfactant complexes. This behaviour also correlated with the results of the binding isotherms. In addition, the dilational rheology is associated to the appearance of these complexes in the bulk. [1, 2]

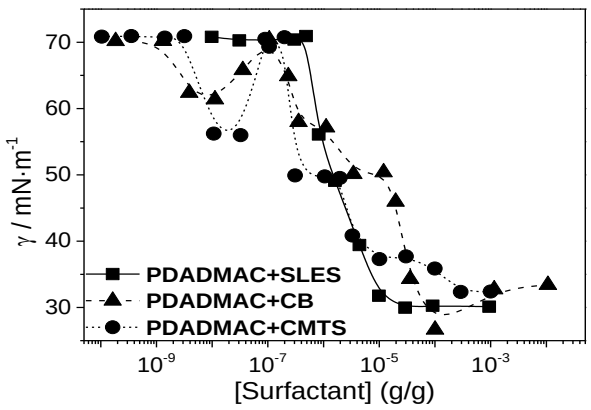

Figure 1: Equilibrium surface tension for poly (diallyldimethylammonium chloride) + SLES, ■, + CMTS, ●, and + CB, ▲, as a function of surfactant weight fraction.

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RIC16

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ORAL COMMUNICATIONS

2. COLLOIDAL SYSTEMS FOR GENE THERAPY

Monoolein-based liposomes for siRNA delivery – optimization and validation

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Delivery of small interfering RNA (siRNA) for the treatment of several diseases has received great attention since the discovery of the RNA interference (RNAi) mechanism [1]. But even though some successes in this field have been achieved, the ability to efficient deliver siRNA to the target cells is still a major obstacle for its therapeutic application. Our work has been focused on this specific issue of gene therapy – the nanocarrier system. We have selected a neutral lipid with great polymorphism – monoolein (MO) - and studied its combination with cationic lipids for the production of efficient and safe nucleic acid delivery systems [2,3]. Here we report the development, optimization and validation of MO-based liposomes for siRNA delivery. Liposomes formed by the cationic lipids dioctadecyldimethylammonium bromide (DODAB) or chloride (DODAC) and MO were used to encapsulate siRNA. Parameters as the cationic lipid counter ion (Br^- *versus* Cl^-), cationic lipid:monoolein molar fraction, and presence of poly(ethylene glycol) (PEG) were tested in order to optimize the MO-based liposomes as siRNA nanocarriers. Additionally, a careful characterization of the nanocarriers interation with biological interfaces was performed, including the avaliation of the cytotoxicity induced on different cell lines, silencing efficiency and cellular internalization of siRNA lipoplexes *in vitro*, as well as interaction with serum proteins. Our results showed that MO increased the fluidity of both DODAC and DODAB bilayers, although its distribution was dependent on the counter ion. We obtained small sized siRNA-lipoplexes with a highly positive surface charge that was reduced by the presence of PEG. Additionally, the pegylated lipoplexes could achieve a good stability in bodily fluids. Finally, the *in vitro* cellular evaluation demonstrated that presence of different counter ions and varying percentages of MO affected the silencing efficiency and associated cytotoxicity of the siRNA-lipoplexes. This work shows the potential of MO-based liposomes for siRNA delivery, and emphasizes the importance of the careful optimization and selection of the lipid components.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT (PEst-OE/BIA/UI4050/2014 (CBMA), PEst-C/FIS/UI0607/2013 (CFUM) and PTDC/QUI/69795/ 2006). Eloi Feitosa thanks FAPESP (2011/03566-0) and CNPq (303030/2012-7), and Renata D. Adati thanks FAPESP for scholarship (2011/07414-0). Marlene Lúcio holds a position of Researcher FCT (IF/00498/2012), and Ana Oliveira a FCT scholarship (SFRH/BD/68588/2010). K. Raemdonck is a postdoctoral fellow of the Research Foundation – Flanders (FWO-Vlaanderen).*

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Lipoplexes formed by Imidazolium oligo-oxyethylene based gemini cationic lipids and monooleinglycerol compact siRNA with cubic structures that improve gene silencing

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Gene therapy using exogenous small interfering RNAs (siRNAs) is an emerging area in nanomedicine allowing nucleic acid delivery into cells to knockdown or silence the expression of defective genes. In early days, this field was limited to plasmid DNA, but the discovery of siRNAs has prompted a remarkable progress in the search of not only new nucleic acid therapeutics, but also of non viral carriers with improved performances. Cationic lipids (CLs) are nowadays the most successful non viral gene vectors and, among them, gemini cationic lipids (GCLs) have given the best results in lipofection experiments. Their molecular structure, with two head groups linked by a spacer, offers a huge variety of potential modifications that can be made to improve the transfection, as it has been reported in previous works [1]. These findings have motivated major research efforts focused towards developing GCL-siRNA complexes as efficient gene silencing vectors [2]. Following these guidelines, the use of GCLs to compact and deliver siRNA to the cells may give excellent results in gene silencing. This study is focused on the biophysical characterization of the lipoplexes formed by a series of gemini cationic lipids $(C_{16}\text{Imidazolium})_2(C_2H_4O)_nC_2H_4$ (with n = 1, 2 or 3), the neutral lipid MOG (monooleinglycerol) and a commercial siRNA with around 22 base pairs. The biophysical characterization has been carried out with several techniques as zeta potential, cryo-TEM and small angle X-ray scattering (SAXS), while FACS and MTT experiments were done to determine the gene knockdown level and cell viability, respectively. It is remarkable that: i) cubic lyotropic phases (Ia3d and Pm3n) are present in these systems; and ii) the maximum gene knockdown levels have been found at charge ratios (+/-) of ρ = 6 and 8, while cell viabilities are higher at α = 0.12 and 0.15 molar ratios of the lipid mixture.

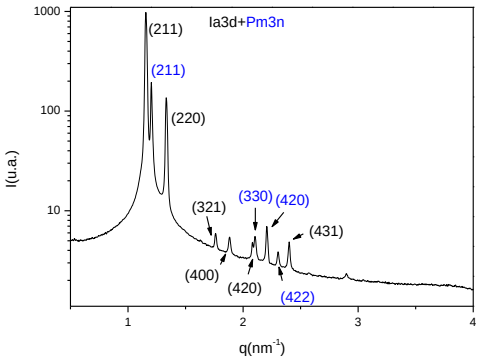

Figure 1: SAXS diffractograms showing a mixture of cubic phases and the corresponding CryoTEM image.

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Lipofection vectors based on serine-derived gemini surfactants and monoolein for therapeutic siRNA delivery

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Gene therapy based on gene silencing with small interfering RNA (siRNA) has evolved and gained great importance over recent years. However, a major challenge for the use of RNA interference-based therapies is the efficiency of the delivery through an appropriate vector. Serine-based gemini surfactants have been recently synthetized and characterized [1], with the ultimate goal of designing suitable vectors for intracellular gene delivery [2]. The inclusion of helper lipids is one of the possible strategies to potentiate the transfection efficiency of cationic surfactants [3]. Monoolein (MO) is known to be a strong promoter of inverted nonbilayer structures (e.g. inverted cubic mesophases) and to enhance the fusogenicity of the nanostructures, thus contributing to a higher lipoplex transfection efficiency [4]. In this work, we have focused on the development and characterization of novel vectors for siRNA delivery based in three different gemini amino acid-based surfactants derived from serine (Figure 1) with MO, as helper lipid. It will be shown that the inclusion of MO as helper lipid in liposomal or micellar formulations induces modifications on the morphology of the aggregates. Furthermore, the three gemini:MO systems tested have good ability to complex efficiently siRNA and they do not show significant levels of cytotoxicity. Lastly, the results obtained show high percentages of down regulations, indicating that these gemini:MO formulations are good candidates as lipofection vectors for RNAi-therapies.

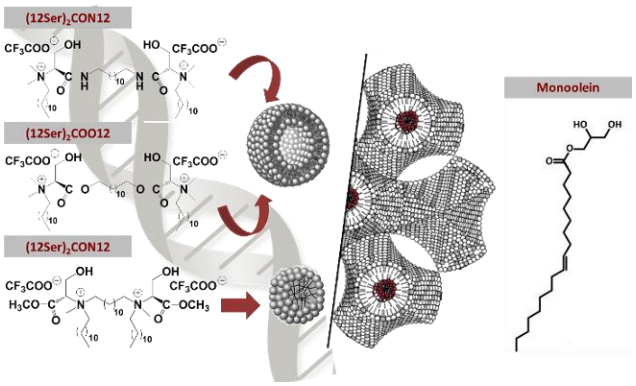

Figure 1: Chemical structure of serine-based gemini surfactants and the helper lipid monoolein (MO).

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The effect of DC-Cholesterol and MO on DODAX lipid systems for DNA delivery

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Gene therapy is one of the promising strategies concerning molecular therapy. In recent years, the development of cationic liposomes as non-viral vectors for gene delivery has been thoroughly researched. The combination of different cationic lipids is one of the strategies to improve the efficiency of liposomal nanocarriers for gene therapy, tuning the lipid membrane composition in order to obtain less citotoxic and more effective lipoplexes. In this study we included a third lipid component (β-[N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol hydrochloride (DC-Chol) in dioctadecyldimethylammonium halide (DODAX):monoolein (MO) mixed liposomes (X accounting for Br^- or Cl^- counterion) in order to optimize the lipoplex structural properties and consequently reach higher transfection efficiency [1,2].

The impact of DC-Chol on DODAC:MO and DODAB:MO bilayers was evaluated by differential scanning calorimetry (DSC). Specific combinations of the three lipids result in different liposomal membrane characteristics, affecting differently DODAB:MO and DODAC:MO bilayers, thus leading to different lipoplex formation. The fusogenic ability of the liposomes was also assessed by by a Förster Resonance Energy Transfer (FRET) assay, further showing differences between DODAB:MO:DC-Chol and DODAC:MO:DC-Chol liposomes.

DODAC-based liposomes were further evaluated regarding mean size, surface charge, and ability to complex DNA. All liposomes investigated were able to efficiently condense DNA, and small (< 150 nm) positively charged lipoplexes were obtained. Cytotoxicity and transfection efficiency of the lipolexes was evaluated on human embryonic kidney 293T cells, and correlated with the physicochemical and thermotropic properties of the liposomes. This work shows that the inclusion of DC-Chol on DODAC:MO liposomes is a promising strategy for DNA delivery.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the projects PEst-OE/BIA/UI4050/2014 and PEST-C/FIS/UI607/2013 and PTDC/QUI/69795/2006. Marlene Lucio holds a position of Researcher FCT with the reference IF/00498/2012 and Ana Oliveira holds scholarship SFRH/BD/68588/2010. This work is protected by Portuguese National Patent nº 104158-Refª DP/01/2008/10900-31/12/2008 and International Patent submitted: PCT/IB2009/05361-PPI nº40759/09.*

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Binding of DNA by a dinitro-diester calix[4]arene: Denaturation and condensation of DNA

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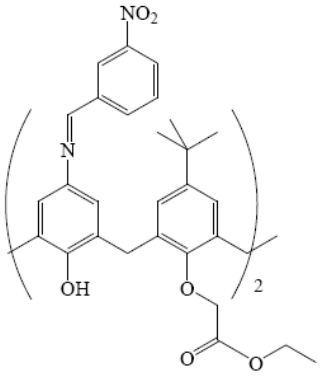
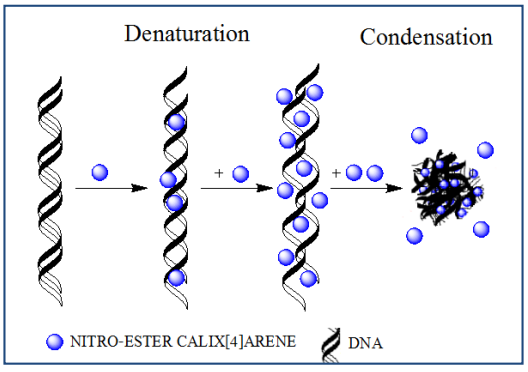
Calixarenes, macrocycles composed of phenolic units linked by methylene groups at the 2- and 6-positions, are among the most widely studied organic supramolecular hosts and have been described as having "(almost) unlimited possibilities" because they can be easily modified [1]. They exhibit a great ability to be used as drug delivery systems. Compared to other cyclic systems, calixarenes have a peculiar characteristic: they can adopt different conformations [2]. The number of conformations increases with the number of benzene units in the system. It also depends on the solvent. Calixarenes can be modified in order to favour their interactions with DNA. The introduction of cationic groups at the upper rim and of long hydrocarbon tails at the lower rim is the usual strategy. Large calixarene dimers have also been used as suitable tools for cooperative DNA complexation. The binding interactions calixarenes/DNA and the resulting DNA conformational changes depend on the structural characteristics of the macrocycle. In this work the interactions between a nitro-ester calix[4]arene, 5,17-(3-nitrobenzylideneamino)-11,23-di-*tert*-butyl- 25,27-diethoxycarbonyl methyleneoxy-26,28-dihydroxycalix[4]arene, with calf thymus DNA, ctDNA, were investigated using several techniques. The calixarene used has a terminal ester group at the lower rim and an aromatic ring at the upper rim that is able to bind to nucleic acids. The system was studied at various molar ratios X=[calixarene]/[DNA]. Results show that changes in the DNA conformation depend on the X value. At low macrocycle concentration, the calixarene binds to the polynucleotide. This interaction, mainly in groove mode, weakens the hydrogen bonds between the base pairs of the helix, inducing the denaturation of the double strands, as well as the condensation of the polynucleotide, from an extended coil state to a globular state. An opposite effect is observed at X molar ratios higher than 0.07. The de-condensation of DNA happens, that is, the transition from a compact state to a more extended conformation, probably due to the stacking of calixarene molecules in the solution.





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Guimarães2015

ORAL COMMUNICATIONS

3. COLLOIDAL SYSTEMS AND SELF-ASSEMBLY

Fluorescence quenching of 1-pyrene-carboxaldehyde by iodide ion in the presence of anionic and cationic micelles

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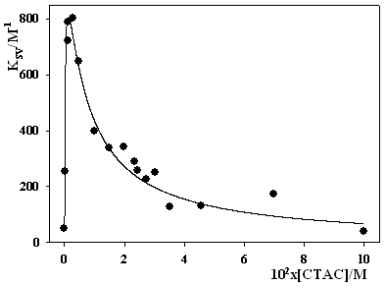
Chemical species can accumulate on the surface of some receptors (micelles, polymers, cyclodextrins, etc.) in solution. As a consequence of the favorable interactions between the species, S, and the receptor, M, a change in the chemical potential of both of them is observed. It can be shown that the activity coefficient of S is given by [1]:

$$\gamma_s = \frac{1}{1+K[M]} \quad (1) \qquad\qquad \Delta\mu_s = RT\ln\gamma_s \quad (2)$$

change in where K is the equilibrium constant corresponding to the process $S_F + M \rightleftarrows S_B$ and S_F and S_B are the free and bound (to the receptor) states of S. If S participates in a chemical or photochemical reaction, the process occurs under restricted geometry conditions. The changes in the Gibbs energy of S upon variations in the receptor concentration will imply a change in the reaction rate. These changes can be described by the Pseudophase Model. According to this model, if *S_F is in equilibrium with S_B*, the observed rate constant, for a unimolecular ground state process, is given by:

$$k_{obs} = \frac{k_F + k_B K[M]}{1+K[M]} \quad (3)$$

k_F and k_B being the rate constant corresponding to the reactions of S_F and S_B, respectively. An intriguing fact observed in relation to photochemical processes is that equation 3 seems to be adequate to describe changes in the rate of these processes by varying [M], *in spite of the fact that the equilibrium condition between free and bound reactants does not hold*. This situation has been considered by some of us, from a theoretical point of view, developing an original treatment for receptors such as cyclodextrins and polyelectrolytes (DNA). In the present work, this treatment has been used to rationalize the results corresponding to the quenching of 1-pyrene-carboxaldehyde by iodide in micellar solutions (SDS and CTAC). The agreement between the theoretical and the experimental data was not good and some modifications of the treatment were necessary in order to quantitatively explain the data.



Acknowledgements: *This work was financed by Consejería de Innovación, Ciencia y Empresa de la Junta de Andalucía (FQM-274 and P12-FQM-1105) and FEDER funds.*

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Solubilization of a copper dibrominated salen complex in homogeneous and micro heterogeneous P-123 and F-127 Pluronic™ surfactants

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Salens type compounds are being synthetized as starting materials in the synthesis of antiallergic, antiphlogistic, antibiotics and antitumor compounds [1]. Recently, a new compound, a copper dibrominated salen complex (Cu-SalenBr$_2$ – Figure 1-a), which presented good results against several kind of cancer cells was synthetized. Spectroscopic studies of [Cu-SalenBr$_2$] in homogeneous media showed that this molecule presents a very low solubility in aqueous media. An approach to overcome such drawback is the solubilization of the complex in an adequate carrier, thus allowing the improvement of its efficiency in practical applications [1]. In this work, we have decided to use nanostructured Pluronics™ micelles as carriers for the Cu-SalenBr$_2$ solubilization. The encapsulation of the Salen complex into bioavailable polymeric micelles of Pluronics™ P-123 and F-127 (Figure 1-b) were carried out by the dispersion solid method [2]. This method allowed the incorporation of the Cu-SalenBr$_2$, in a stable monomeric form (Figure 1-c). In this study, the entrapment efficiency and drug loading of the Salen complex in the presence of polymeric micelles, as well as the corresponding interaction mechanism, were studied by spectroscopy, diffusion ordered spectroscopy (DOSY – Figure 1-d) and dynamic light scattering (DLS). The experimental data are promising for future applications of Salen complexes in pharmaceutical and biomedical areas.

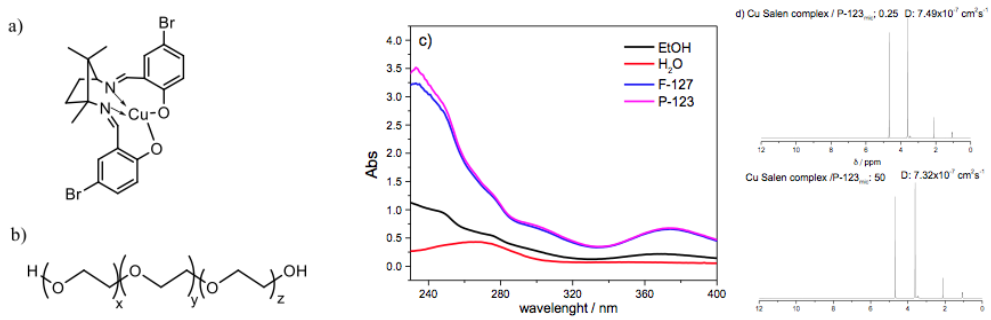

Figure 1: a) Molecular structure of the copper dibrominated salen complex (Cu-SalenBr$_2$ and b) Pluronic™ surfactants F-127 (x, z:100) and P-123 (z=20 and y=70). c) Absorption spectra of Cu-SalenBr$_2$ (2.2×10^{-5} molL^{-1})/Pluronic(8.0×10^{-5} molL^{-1}) mixtures in different solvents. d) ^{1}H NMR spectra of P-123 micelles (0.5% w/V) in different ratios Cu-SalenBr$_2$/P-123 and its diffusion coefficients at 25°C.

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Interactions between an anionic amphiphilic triblock copolymer and ionic surfactants

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Block copolymers have been widely studied in recent years due to their ability to self-assemble into nanostructures in aqueous solution, and also because of their potential applications in enhanced oil recovery, targeted drug delivery and sensor design [1,2]. Poly(*N*-isopropylacrylamide) (PNIPAAM) is a thermosensitive water-soluble amphiphilic polymer that exhibits a lower critical solution temperature (LCST) of about 32 ºC in aqueous solutions [3]. Below this temperature, PNIPAAM is water-soluble, whereas above LCST the molecules are in a collapsed state and the solution approaches macroscopic phase separation [4]. In this work, aqueous solutions of a thermoresponsive negatively charged triblock copolymer methoxy-poly(ethylene glycol)-*block*-poly(*N*-isopropylacrylamide)-*block*-poly(2-succinic acid-propyloxyl methacrylate) ($MPEG_{45}$-*b*-$PNIPAAM_{48}$-*b*-$PSAPMA_{20}$), in the presence of sodium dodecyl sulphate (SDS) surfactant or dodecyltrimethylammonium bromide (DTAB) surfactant, have been characterized at a constant concentration of polymer and various levels of surfactant addition. The objective of this work is to examine the interactions of the ionic surfactants with the polymer, resorting to zeta-potential, turbidity, small-angle neutron scattering (SANS), dynamic light scattering (DLS), and cryogenic transmission electron microscopy (cryo-TEM). The results for the neat block copolymer show that the zeta-potential increases with increasing polymer concentration. The presence of surfactant in the system generates intricate polymer-surfactant electrostatic interactions and the results point to a subtle interplay between those interactions and solubilisation of the polymer. The findings reveal that the behaviour depends on surfactant concentration and the temperature. In addition, cytotoxicity tests were carried out on cancer cells MDA-MB-231 and fibroblasts cells NIH-3T3, to investigate the potential of the systems for applications as drug delivery carriers. Results show that the cytotoxicity of polymr changes with surfactant, raising as the concentration of SDS increases but decreasing with increasing DTAB concentration.

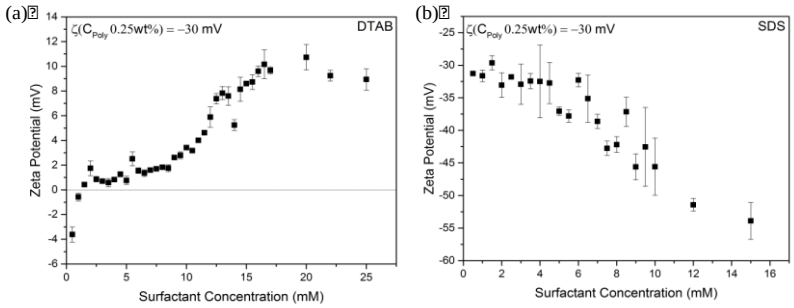

Figure 1: Zeta-potential measurements in aqueous mixtures of the triblock copolymer with different surfactants at 25°C: (a) DTAB (b) SDS.

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Aqueous phase behavior and supramolecular aggregation of anionic lysine-based surfactants and cationic polymers

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Aqueous mixtures of surfactants and polymers are of great interest from a colloidal point of view, in particular regarding the effect of the composition and architecture of the polymer on the surfactant aggregate structure and stability, the type of short and long range interactions involved, and the possibility of formation of 3-D gel networks.[1] Besides, these mixtures are technically relevant for the encapsulation and molecular transport of biomolecules, and for the rheological control of soft materials.[1,2] In this work, we report on the characterization of polymer/surfactant complexes derived from the anionic lysine-based surfactant 16Lys12 and the cationic hydroxyethylcellulose (HEC) derivatives JR-400 and LM-200.[3,4] Both polymers establish strong electrostatic interactions with the surfactant and, in the case of LM200, also hydrophobic interactions due to the alkyl side chains grafted onto the polymer backbone. The polymer-surfactant phase behavior and microstructure were investigated by differential scanning calorimetry (DSC), video-enhanced light microscopy (VELM) and cryogenic scanning electron microscopy (cryo-SEM). Three different phase regions were identified: tubular/vesicular dispersions at surfactant charge excess, coacervate/precipitate at charge equimolarity and gels at polymer charge excess. Differences in the vesicular shape (spherical versus irregular), tubular morphology (linear versus bifurcated) and vesicle/tubule coexistence have been identified and correlated not only with the tubule-to-vesicle transition temperature of 16Lys12 at 44.1 °C, but also with the differences in contour length, cationic charge density and amphiphilic character of JR-400 and LM-200. Variations in the apparent gel microstructure were also characterized by cryo-SEM. Results are overall interpreted on the basis of electrostatic and hydrophobic interactions at play.

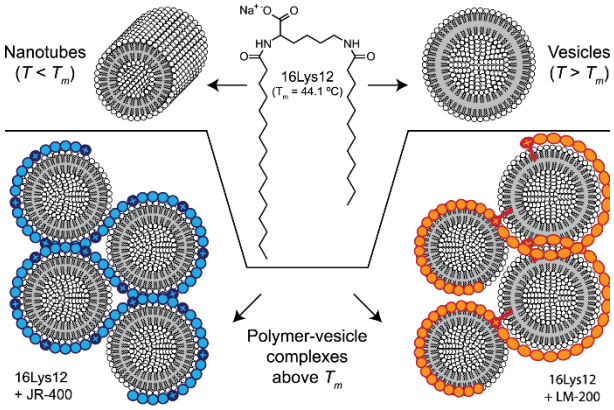

Figure 1: Schematic view of the polymer-vesicle complexes leading to gelation formed by anionic surfactant 12Lys16 and the two polycations JR400 and LM200.

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ORAL COMMUNICATIONS

4. GELS AND POLYMERS

Interaction between poly(vinyl alcohol) and conjugated polyelectrolytes in aqueous solutions

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Conjugated polyelectrolytes (CPEs) are advanced materials, whose applications include optoelectronics and sensing. Their aqueous solubility favours processing from solution, and may also facilitate self-assembly. Fluorene based systems, such as the cationic HTMA-PFP are excellent candidates for many of these applications because of their blue emission, high fluorescence quantum yield, and potential as charge transport and blocking layers [1]. They tend to aggregate in water, but it is possible to obtain homogeneous solutions by addition of cosolvents or surfactants [2]. We have recently found that aqueous poly(vinyl alcohol) (PVA) also dissolve these CPEs at the molecular level. Films based on this make interesting matrices for studying room temperature phosphorescence of conjugated polymers. We report a study of the interaction between PVA and both anionic and cationic CPEs in water using UV/Visible absorption and fluorescence spectroscopy, viscometry, electrical conductivity and small angle X-ray scattering (SAXS). Factors favouring these interactions will be discussed.

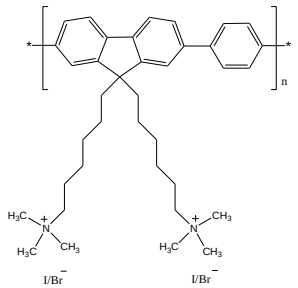

Figure 1: Structure of HTMA-PFP

Acknowledgements: *The authors thank the financial support of the Fundação para a Ciência e a Tecnologia to the Coimbra Chemistry Centre through the project UID/QUI/00313/2013.*

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Smart polymeric nanoparticles for use in boron scavenging

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Boron is beneficial to human health and agriculture in trace quantities, but becomes toxic at higher quantities.[1] Boron compounds are used in many industrial applications, including the fabrication of soaps and detergents, glass and ceramics, insecticides, fertilizers, semiconductors, flame retardants, high duress compounds, and pharmaceutical drugs. High boron contents in water might result from industrial discharges or leaching from rocks and soils containing borates and borosilicates.[1]
Boron is hard to detect [2,3] and remove from water,[4] a sometimes required step in the treatment of residual waters. We have synthesized thermoresponsive polymer nanoparticles containing vicinal diol groups for boron scavenging. The particles have a core of poly(methyl methacrylate) (PMMA) and a thermosensitive shell with a brush composed of a copolymer of N-isopropylacrylamide (NIPAM), 2-aminoethyl methacrylate (AEMH), and either D-gluconoamidoethyl methacrylate (GAEM) or monodiol methacrylate (MDM) boron-chelating diol-containing monomers. The nanoparticles revealed good boron chelation capacity, with removal of phenylboronic acid being more efficient than chelation of boric acid. The best results of boron scavenging were obtained in the particles with greater density of D-gluconoamidoethyl groups. At temperatures above ca. 35°C the particle shell collapses, inducing particle aggregation that facilitates particle separation.

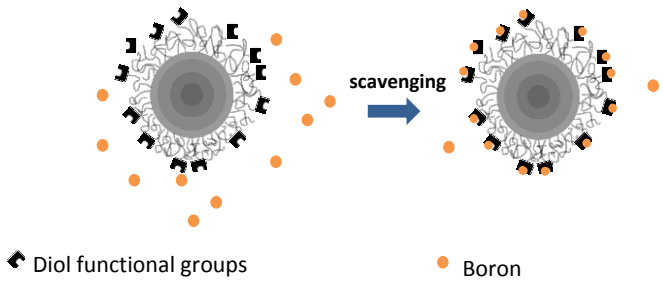

Figure 1: Schematic representation of the boron scavenging process.

Acknowledgements: *This work was partially supported by Fundação para a Ciência e a Tecnologia (FCT-Portugal), POCI 2010 and COMPETE (FEDER), projects PTDC/CTM-NAN/115110/2009 and PEst-OE/CTM/LA0024/2013. S. A. acknowledges a postdoctoral grant from FCT (SFRH/BPD/74654/2010).*

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Synthesis of thermoresponsive poly(N-vinylcaprolactam)-based nanogels using reactive cationic polymeric stabilizers

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Thermoresponsive nanogels dispersed in aqueous media are fascinating cross-linked polymeric colloids, which undergo a volume phase transition temperature (VPTT) in water. These nanogels proved to be suitable candidates for biomedical applications due to their capacity to uptake and release active species [1]. Moreover, the introduction of cationic functionalities into nanogels can offer the opportunity to complex DNA and facilitate cellular uptake [2]. The biocompatibility of Poly(N-vinylcaprolactam) (PVCL) combined with a LCST in the range 32-38°C, close to the physiological temperature, makes PVCL-based nanogels an interesting candidate for such applications [3].
The synthesis of PVCL-based nanogels by emulsion polymerization is generally conducted at low initial solid content (≤ 2 wt %) [1-4]. The use of a reactive macromolecular stabilizer to synthesize Poly(diethyl acrylamide) thermoresponsive nanogels by polymerization induced self-assembly (PISA) proved to be a suitable method to increase the initial solid content of the synthesis up to 20 wt % [5]. The present work highlights how the use of a reactive cationic polymeric stabilizer allows synthesizing for the first time stable cationic PVCL nanogels at high initial solid contents (up to 10 wt %). The synthesized thermoresponsive nanogels exhibit a sharp and reversible VPTT in the absence of hysteresis. The influence of different parameters (i.e. molar mass of the stabilizer, presence of a reactive chain end, concentrations of stabilizer and monomer) on the final colloidal features of the nanogels was thoroughly investigated.

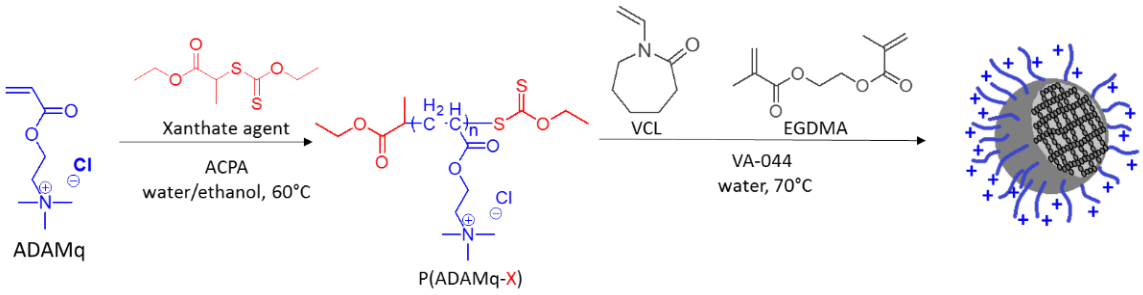

Figure 1: Synthesis of thermoresponsive PVCL-based nanogels using a reactive cationic stabilizer.

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Structural features of maltose-functionalized hyperbranched poly(ethylene imine) and their complexes with retinol in aqueous solution

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Hyperbranched polymers and dendrimers have attracted a great deal of interest as nanocarriers due to their well-defined structures and high density of surface groups.[1,2] Structure, internal density distribution, and size of hyperbranched poly(ethylene imine) (PEI) functionalized with various amounts of maltose (PEI-Mal) [3] in phosphate buffer were studied by small angle X-ray scattering (SAXS) and dynamic light scattering (DLS). The value of pH was varied in the range from 3 to 9. Virtually no effect of pH on the nanostructure was found in this interval. The SAXS results revealed a broad segmental radial density distribution, i.e. a "fluffy" globular structure rather than a distinct core-shell structure with a high-density compact core and a low-density corona. This suggests that the maltose units are rather evenly distributed both in the interior and on the surface of the species with a PEI-core of molar mass of 25 000 g/mol. In contrast to the core-shell model expected from the synthesis of the PEI-Mal architectures [4], the SAXS results reveal that this is not a realistic model. The DLS measurements showed that the overall size of the PEI-Mal derivatives increased as the number of maltose units in the PEI-Mal structures rises. In addition, the interaction of the hydrophobic model drug retinol with PEI or PEI-Mal derivatives was also investigated. The UV-visible spectroscopy results disclosed that the solubility of retinol in the phosphate buffer is very poor and it takes a very long time to solubilize retinol. Moreover retinol induces aggregation of dendritic glycopolymers where the growth of aggregates occurs continuously over several days and then remains virtually constant.

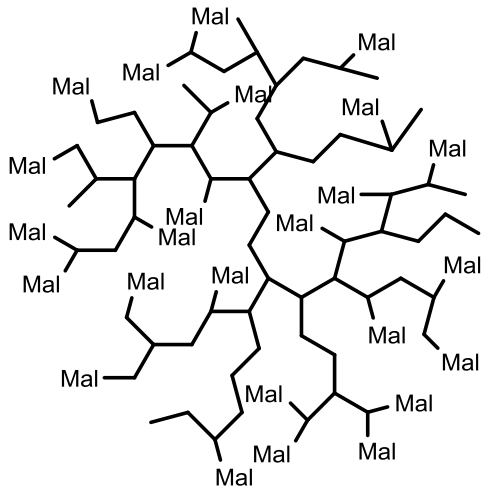

Figure 1: Schematic illustration of the PEI-Mal structures, showing that the Mal units are located both at the surface and in the interior of the hyperbranched PEI.

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Plasmonic nanostructures coated with microgels for multiplex immunophenotyping celular receptors and imaging tumour cells

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Detection technologies employing optically encoded particles have gained much interest toward clinical diagnostics and drug discovery, but the portfolio of available systems is still limited. The fabrication and characterization of highly stable surface-enhanced resonance Raman scattering (SERRS)-encoded colloids for the identification and imaging of proteins expressed in cells are reported. These plasmonic nanostructures are made of gold octahedra coated with poly(N -isopropylacrylamide) microgels and can be readily encoded with Raman active dyes while retaining high colloidal stability in biofluids. A layer-by-layer polyelectrolyte coating is used to seal the outer surface of the encoded particles and to provide a reactive surface for covalent conjugation with antibodies. The targeted multiplexing capabilities of the SERRS tags are demonstrated by the simultaneous detection and imaging of three tumor-associated surface biomarkers: epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), and homing cell adhesion molecule (CD44) by SERRS spectroscopy. The plasmonic microgels are able to discriminate tumor A431 (EGFR+/EpCAM+/CD44+) and nontumor 3T3 2.2 (EGFR−/EpCAM−/ CD44+) cells while cocultured *in vitro* (Figure 1).

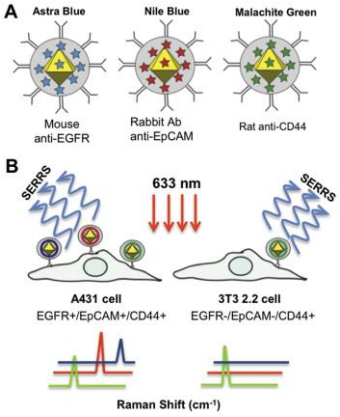

Figure 1: (A) Au@pNIPAM SERRS tags indicating their Raman codification and targeting entities. (B) Schematic representation of the SERRS immunophenotype detection of A431 and 3T3 2.2 cells.

Acknowledgements: *This work was supported by the European Research Council (PLASMAQUO, 267867) and by the Spanish MINECO (MAT2013-45168-R), by the Xunta de Galicia/FEDER (Grant GPC2013-006; INBIOMED-FEDER "Unha maneira de facer Europa"), V.M.-G. acknowledges FPU scholarship from the Spanish MINECO.*

Highly porous chitosan materials with controlled architecture by emulsion templating

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Macroporous polymer materials with controlled pore size can be obtained in highly concentrated emulsions [1], which have large volume fractions of disperse phase (> 74 vol%), surpassing the maximum packing of uniform undistorted spherical droplets [2,3]. Polymerization and/or crosslinking in the external phase of highly concentrated emulsions produces low-density solid foams. The present study describes the synthesis of macroporous foams made of chitosan [4,5], prepared using genipin, a natural crosslinker. Two types of emulsions were used: oil-in-micellar solution (O/Wm) and oil-in-liquid crystal (O/LC) emulsions, depending on surfactant concentration. These emulsions were characterized by visual observation, rheology and small angle X-ray scattering (SAXS) [6]. Chitosan materials were obtained by crosslinking in the external phase of these highly concentrated emulsions, washing and drying (Fig. 1). The results showed that the type of self-aggregate had a great influence on the porous texture. Chitosan macroporous foams were obtained in the O/Wm system, whereas porous chitosan fibrous structures with long fibers resulted from O/LC systems.

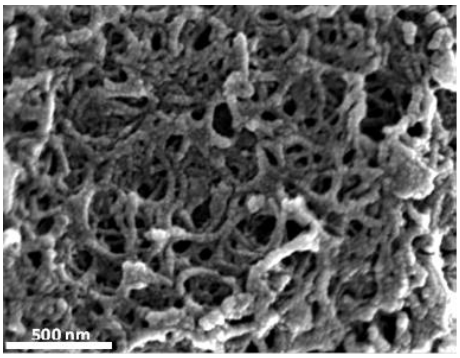

Figure 1: SEM image of a chitosan low-density material.

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Mechanically active hydrogels internally actuated by embedded motor proteins involved in bacterial cell division

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Soft active materials are needed for applications in medicine and nanotechnology focused on the engineering of hybrid artificial materials that reproduce the active and dynamic response of biological tissues. As an initial approach we embedded the self-assembling bacterial cytoskeletal protein FtsZ into a polyacrylamide hydrogel matrix to measure hydrogel deformation by rheology. The polymerization or self-assembly of FtsZ is very dynamic and FtsZ protein filament length, stability and reorganization is determined by the GTP exchange and turnover. The incorporation of the FtsZ monomers reinforces the hydrogel matrix, a structural change that is detected as an increase of the shear rigidity of the soft material. Specific triggering the FtsZ polymerization by the addition of catalytic magnesium to the hydrogel medium, causes a strong decrease or "softening" of the shear rigidity and viscosity of the hydrogel matrix, even below the basal value of the hydrogel matrix. These rheological results infer that the mechanical behaviour of the hydrogel is dominated by the reversible polymerization dynamics of the FtsZ filaments.

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Biocompatible and biodegradable thermo-responsive nanogels as 5-fluorouracil carriers

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The effectiveness of the conventional anticancer therapy is limited by the undesired drug accumulation in the healthy tissues, the low bioavailabiliy of the drug after administration and the difficulty to trigger the drug to the tumor tissues. To overcome these main drawbacks, anticancer drugs have been encapsulated in nanocarriers which are able to protect the drug and avoid its recognition by the reticuloendothelial system.[1] Among these nanocarriers, nanogels have been investigated for potential therapeutic applications in controlled and targeted drug delivery. These stimuli-responsive nanogels are cross-linked colloidal particles, which can undergo from a polymeric solution (swell form) to hard particles (collapsed form) when a physical (temperature, magnetic field), chemical (pH) or biochemical (enzymatic substrate) stimuli are present. [2]. The stimuli-responsive nanogels offer attractive features for anticancer therapy such as easy synthesis and functionalization, high encapsulation efficacy and the ability to drug targeting and deliver the cargo in a controlled fashion. The components employed in the synthesis of the nanogels determine the biocompatibility and biodegradability of these stimuli-responsive nanosystems.

In this context, temperature-responsive nanogels were synthesized employing N-vinylcaprolactam (VCL) as main monomer and a dextran-methacrylate (DexMA) as macro-cross-linker.[3] Biocompatible poly-(VCL) (PVCL) has a thermo-responsive nature with a volume phase transition temperature (VPTT) close to physiological temperature. The macro-cross-linker employed (DexMA) not only shows biocompatibility but also biodegradability since it can be enzymatically degraded by dextranases of the colonic microflora. An antitumor drug, 5-Fluorouracil (5-FU), considered one of the first-line treatments for colorectal cancer disease, was incorporated in the thermo-responsive PVCL and DexMA based nanogel. Different 5-FU/nanogel ratios were assayed and the formulations developed (composed of the nanocarrier and the drug) were characterized in terms of colloidal properties, encapsulation efficiency drug loading and in vitro release studies. Furthermore, different procedures were assayed to determine the highest encapsulation efficiency: (i) an aqueous dispersion of nanogel and an aqueous solution of 5-FU were mixed, (ii) lyophilized nanogel was mixed with an aqueous solution of 5-FU and (iii) a lyophilized 5-FU-loaded nanogel was dispersed with an aqueous solution.

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Transport and equilibrium properties of toluidine blue O-containing modified gum arabic hydrogels as a drug delivery vehicle

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Hydrogels are polymers, chemically or physically cross-linked which forms networks that can absorb large amounts of water and biological fluids [1]. Hydrogels constituted of modified gum arabic (GAm) is an interesting alternative for applications in controlled drug delivery systems, presenting favorable characteristics as, for example, non-toxicity and biodegradability [2]. Furthermore, GAM-hydrogels show a pH-responsive behavior. In this work, gum arabic hydrogels were post loaded with Toluidine Blue O (TBO), a phenothiazine compound used as a photosensitizer in photodynamic inactivation of microorganisms (PDI) and photodynamic therapy [3]. In this communication, adsorption kinetics and isotherms (Figure 1), and release kinetics of TBO from GAm-based hydrogels to mimetized biological media (gastric and intestinal fluids), will be reported.

Figure 1-A shown that the adsorption kinetic profile of TBO in GAm hydrogels achieves the equilibrium in 1000 minutes, showing an encapsulation of approximately 2.5x10-4 mol.L-1 of TBO in the hydrogel matrix. Additionally, it was verified that the system TBO/GAm hydrogels showed a Freundlich type adsorption mechanism, representative of the presence of multiple phenomena in the adsorption mechanism. The drug release studies showed that GAm hydrogels are promising pH responsive drug delivery systems for release of TBO.

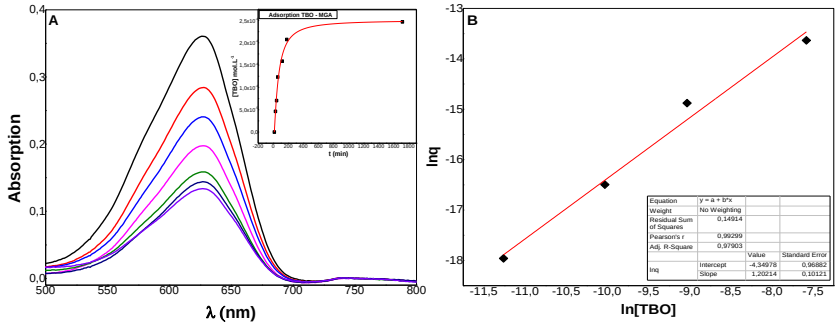

Figure 1: A) UV-visble spectra of TBO aqueous solutions used to obtain the TBO adsorption kinetics (see inset figure). B) Sorption isotherm of TBO to Gam-based hydrogels. The solid line represents the fitting of the Freundlich equation to experimental data. Experiments were carried out at 25.0°C.

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Biocompatible stimuli-responsive nanogels for antitumor drug delivery

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Recent advances in nanotechnology have stimulated the use of nanocarriers for drug delivery. Many antitumor drugs, such as Doxorubicin (DOXO), have limited efficacy because of its low solubility and the severe side effects caused by non-specific biodistribution in the body. Therefore, in order to overcome these drawbacks, the design of a suitable nanocarrier is necessary. In this sense, much attention has been directed to environmentally responsive cross-linked colloidal particles, known as nanogels, due to their ability to swell faster than macroscopic gels in a thermodynamically good solvent, responding to external stimuli such as temperature, pH, and ionic strength, among others. The advantageous properties of nanogels related to their small size, high porosity and capability to be functionalized make them very attractive to be used as nanocarriers in drug delivery [1].
Among different nanogels, special attention is focused on thermo-sensitive and pH-sensitive nanogels since different parts of the human body can have different temperature and pH levels, e.g., unhealthy tissues and cells have different pH and temperature from healthy ones [2]. In the case of thermo-sensitive nanogels, poly(N-vinylcaprolactam) (PVCL) based nanogels will be a good option thanks to their tunable volume phase transition temperature (VPTT) in the physiological range and biocompatibility [3]. On the other hand, in the case of pH-sensitive nanogels, those based on poly(2-(diethylamino)ethyl) methacrylate (PDEAEMA) result in appropriate candidates being biocompatible and presenting a volume phase transition pH (VPTpH) in the physiologically relevant pH range [4]. However, due to the complexity of the drug delivery systems, multi-responsive nanogels could be more effective as compared to single stimulus-responsive nanogels. Therefore, current developments tend to prepare nanogels that are able to respond simultaneously to various stimuli in order to obtain a higher control in drug delivery.
In this work, the synthesis and colloidal characterization of thermo-responsive PVCL-based, pH-responsive PDEAEMA-based, and thermo- and pH-responsive PVCL/PDEAEMA-based nanogels are presented. In addition, their potential application as cargo delivery systems has been studied using DOXO as a drug. The uptake of DOXO into the different nanogel particles and in vitro cytotoxicity tests of loaded nanogels in cervical cancer (HeLa) and breast cancer (MDA-MB-231) cell cultures have been carried out. Furthermore, their cellular uptake pathway has been studied by confocal fluorescence microscopy.

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Ionic permeation inside microgel particles: when theory meets simulations

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Ionic microgels are charged colloidal particles of gel dispersed in a solvent, formed by cross-linked polyelectrolyte chains. They can swell or shrink as a response to a wide variety of stimuli such as temperature, pH, solute concentration or solvent nature. [1] This feature is an advantage for a wide number of biotechnological applications. In particular, the design of microgels as drug transport and delivery systems is gaining attention in recent years, since a therapeutic molecule could be encapsulated inside the particle and be transported to different parts of the human body. With this aim, theoretical studies have been developed to study the permeation of ions and solutes inside microgels particles. [2-4] One of the most relevant results of recent works is that microgel-ion interaction is not exclusively controlled by the electrostatic forces, but also a steric interaction must be taken into account. It appears as an excluded volume repulsion exerted by the fibers of the polymer network that hinders ion penetration. In this work we focus on the effect that this microgel-ion steric repulsive interaction has on the permeation of counterions inside heterogeneous microgel particles. [5] For this purpose, we use a novel hybrid method that blends Ornstein-Zernike integral equations and Monte Carlo simulations. Two important properties have been obtained using both methods, namely the microgel effective net charge and the counterion radial distribution functions. On the one hand, the effective net charge strongly depends on the number of counterions that have penetrated inside the microgel particle, and represents an estimate of how efficiently the microgel bare charge has been screened. On the other hand, the calculation of ionic density profiles constitutes a novelty in the sense that we are able to predict not only the net permeation of ions inside the microgel, but also to determine the region where they become preferentially adsorbed.

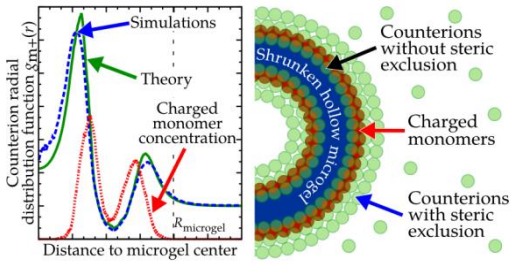

Figure 1: Radial distribution function of counterions inside and around a shrunken heterogeneous microgel. In the picture, green circles represent counterions and red circles are the charged monomers of the nanoparticle.

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ORAL COMMUNICATIONS

5. NANOPARTICLES AND TOXICITY

Zebrafish embryogenesis: a swift and reliable tool for *in vivo* toxicity assessment of non-metallic (lipid-based) nanoparticles

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Over the past 50 years, since the pioneering observation of Alec Bangham, liposomes evolved from just another exotic biophysical tool to highly competent biomedical nanodevices, tuned to enhance therapeutic efficacy and reduce toxicities of conventional delivery systems [1]. Multiple diagnostic and therapeutic applications are envisioned for these phospholipid bilayered nanostructures, but information on their long-term toxicological impact is underexplored - a paradox, given that a safety profile is a key feature for their validation as proficient nanovehicles for delivery of drugs or genetic material towards targeted cells and tissues. Therefore, the establishment of efficient nanotoxicity screening protocols is required. The zebrafish embryo toxicity (ZET) assay gained particular wide notice in the environmental health and safety sciences, with the majority of the studies been focused on (eco)toxicological effects of metallic nanoparticles [2]. Zebrafish embryogenesis is completed within 120 hpf [3], representing a swift and proficient *in vivo* system for evaluating vertebrate development pathways and nanotoxicity assessment than large-scale biocompatibility studies in small mammal models. Moreover, the external fertilization and embryos transparency pose reason for using as imaging-model of pathological processes, valuable for medical theranostics. The aim of this study was to investigate the *in vivo* nanotoxicity profile of monoolein-conjugated dioctadecyldimethylammonium (bromide and chloride) nanosystems using the ZET assay. Newly fertilized zebrafish eggs were exposed at different molar ratios of DODAC:MO and DODAB:MO nanoformulations, for 80 hpf. The following morphological and physiological developmental endpoints were assessed: mortality, development delay, phenotypic malformations, spontaneous movements, cardiac frequency and hatching rate. Results suggest that zebrafish embryos compose an informative, sensitive and reliable *in vivo* model to fast-track the biocompatibility of non-metallic (lipid-based) nanoparticles.

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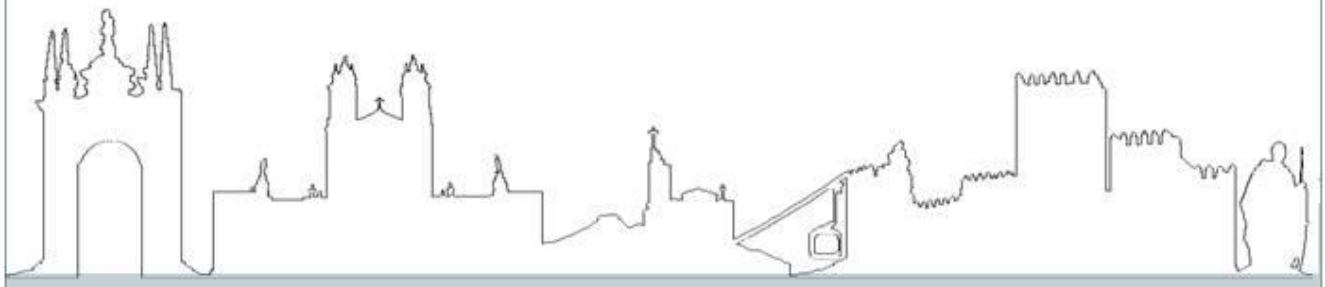

ORAL COMMUNICATIONS

6. BIOMIMETIC AND BIOINSPIRED SYSTEMS

Membrane interaction of acylated $S4_{13}$-PV analogs: understanding their delivery efficiency

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Cellular membranes act as barriers to hinder the access of therapeutic agents to their intracellular sites of action. The recognized ability of cell penetrating peptides (CPPs) to transpose membranes and condense nucleic acids, facilitating their cellular uptake, has been exploited in the gene therapy field. In this context, the CPP $S4_{13}$-PV, a dermaseptin derived peptide fused to the nuclear localization sequence of the SV40 large T antigen, and a family of $S4_{13}$-PV analogs have been used in our laboratory to evaluate its potential for nucleic acid delivery. In a previous work, we demonstrated that the attachment of five histidines to the N-terminus of $S4_{13}$-PV peptide (to generate H_5-$S4_{13}$-PV) allowed, in general, an improvement of plasmid DNA and siRNA delivery [1]. In the present work, we show that siRNA delivery and gene silencing mediated by $S4_{13}$-PV can be modulated through the incorporation of acyl groups at the N-terminus of the peptide. In this regard, the lauroyl group showed to be the most advantageous. Furthermore, the addition of this group to H_5-$S4_{13}$-PV peptide allowed a significant enhancement of delivered siRNA molecules and gene silencing in the presence of serum. Aiming at gaining insights into the molecular mechanisms responsible for the different behavior of the acylated $S4_{13}$-PV analogs in terms of membrane translocation and siRNA delivery, biophysical studies were performed to unravel how each peptide affects membrane physical properties, by using a set of membrane models and different experimental approaches, namely differential scanning calorimetry and assays of calcein release. We expect to contribute with our study for the establishment of structure-activity relationships, towards a rational design of new and efficient peptide-based nucleic acid delivery systems.

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In-vivo-like study of the excluded volume effects on the kinetics of enzymatic reactions

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The cell is a heterogeneously distributed and highly crowded medium in which a wide variety of physical and chemical processes take place. Until recently, each single process had been studied as an independent and isolated event, as close to ideality as possible. Still, this is quite unrealistic, both in terms of intermolecular interactions and in the fraction of occupied volume, which can be up to 300-400 g/L. Volume exclusion is one of the most relevant entropic effects occurring inside the cell, since it gives raise to steric repulsions, depletion forces and directly impacts on diffusion, interactions, kinetics and conformational equilibriums of biopolymers [1]. In the present work, the volume exclusion problem, also known as macromolecular crowding, has been applied to the field of enzyme kinetics. It has been approached by adding neutral, relatively inert polymers, which act as crowding agents or obstacles, in the media of given enzymatic reactions, monitored spectroscopically. The concentration and size of these obstacles have been changed systematically in order to obtain kinetic information about each reaction. The kinetic behavior of four differently sized enzymes has been studied in crowded media: α-Chymotrypsin (α-Chy, 25 kDa) [2], Horseradish Peroxidase (HRP, 42 kDa) [3], Alkaline Phosphatase (ALKP, 104 kDa) [4] and Lactate Dehydrogenase (LDH, 140 kDa) [5]; within a range of Dextran (D) polymers size from 5 to 500 kDa, at increasing concentrations up to 100 g/L. Results, in both experiment [6] and simulation [7], indicate that the performance of a certain enzyme depends on the amount of excluded volume, regardless of the enzymatic system. However, small, monomeric proteins behave with independence of the obstacle size, while large, oligomeric proteins display an obstacle size-dependent behavior. In this regard, the enzyme-crowding agent ratio can have a significant impact on the kinetics of a given reaction. Besides, it has been shown that such crowding can hinder diffusion to the extent of being capable of altering reaction control from activation to diffusion.

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Stimuli-responsive bionanocomposites for bio-applications

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Merging the intrinsic functionalities of the inorganic nanofillers and the biointerfaces of biopolymers, bionanocomposites are materials with unique responsive features that have raised increased attention in Nanomedicine and Biotechnology [1]. In this context, the incorporation of inorganic nanoparticles in polysaccharide hydrogels is currently being explored as a strategy to confer to the hydrogels novel functionalities valuable for specific bio-applications such as drug-delivery [2]. For example, an immediate benefit arising from the incorporation of magnetic nanoparticles into hydrogels is the magnetically driven drug transport which enables site specific drug delivery to be envisaged. The addition of nanostructures with photothermal properties (gold nanostructures or carbon nanotubes - CNTs) to thermosensitive hydrogels confers the ability of triggered release upon light exposure. The research communicated here aims to understand the role of nanofillers (Au, Fe_3O_4 and CNTs) on the structure and properties of the nanocomposites and the implications in the release of a model drug from thermosensitive hydrogel nanocomposites. Hydrogel nanocomposites comprising a thermosensitive biopolymer matrix and the dispersed nanophases above mentioned were prepared by encapsulation of previously formed NPs or by in situ generation of the NPs. [3,4] Nanogel particles of selected formulations of composites were prepared using nano-reactors (microemulsions) in order to adjust the final particles' size distribution. An integrated characterization approach, comprising thermal, rheological, morphological and vibrational spectroscopy measurements of bulk and nanosized bionanocomposites was carried out. The implications of the incorporation of nanoparticles into hydrogels as a route for the design of drug delivery systems with tailored and triggered release behavior will be discussed.

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Reversibility of the interactions between a novel surfactant derived from lysine and biomolecules

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Cationic surfactants are known to interact with negatively charged biomolecules, such as proteins and nucleic acids.[1] The conformation as well as the biological function of the proteins can be modulated by surfactants and, consequently, the ability to control the protein-surfactant interaction is important in applications involving these systems. On the other hand, one of the most extensively studied applications of cationic surfactants regards to their use in gene therapy, as nucleic acid delivery systems. The practical use of cationic surfactants has a number of limitations, including cytotoxicity, environmental concerns and aquatic toxicity. Therefore, it is advantageous to develop biodegradable, eco-friendly and biocompatible surfactants and study their interaction with both proteins and DNA in aqueous solution. In this work the novel cationic surfactant derived from lysine (*S*)-5-acetamido-6-(dodecylamino)-*N,N,N*-trimethyl-6-oxohexan-1-ammonium chloride, LYCl, was prepared and its aqueous solutions characterized physicochemically. The binding of LYCl to bovine serum albumin, BSA, and to double stranded calf thymus DNA, ctDNA, was investigated using several techniques. Results show that LYCl binding to BSA is followed by a decrease in the α-helix content caused by the unfolding of the protein. LYCl association to ctDNA mainly occurs through groove binding and electrostatic interactions. These interactions cause morphological changes from an elongated coil to a globular structure (compaction of DNA).

Recovery of the initial conformation of proteins and polynucleotides is possible if the surfactant molecules can be stripped from the biomolecule. A representative class of molecules capable of modulating protein-surfactant and DNA-surfactant interactions is the cyclodextrins, CD, which can form inclusion complexes with surfactants. It was found that addition of β-cyclodextrin, β-CD, to the BSA-LYCl and ctDNA-LYCl complexes is followed by the refolding of BSA and the decompaction of ctDNA. This can be explained by the ability of β-CD to hinder BSA-LYCl and ctDNA-LYCl interactions due to the stronger and more specific β-CD-LYCl hydrophobic interactions. The stoichiometry of the β-CD:LYCl inclusion complex and its formation equilibrium constant were determined in this work. The reported procedure using β-CD is an efficient way to refold proteins and to decompact DNA, after the morphological changes caused in the biomolecules by their interaction with cationic surfactants.

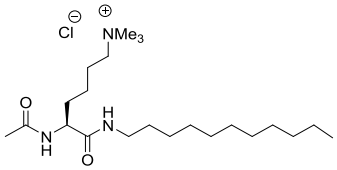

Figure 1: Molecular structure of the amino acid-based surfactant LYCl.

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Multi-stimuli-responsive magneto-nanogels for biomedical applications

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Demands on the design and production of stimuli-responsive nanogel particles are constantly evolving driven by the necessity of nanocarriers suitable for a wide range of pharmaceutical, therapeutical and biomedical applications. The beauty of environmentally-responsive cross-linked colloidal particles, known as nanogels, lies in their unprecedented ability to undergo large reversible volume changes in response to external stimuli such as temperature, pH, and magnetic field.[1] Moreover, the incorporation of magnetic nanoparticles into nanogels has received tremendous interest due to their extraordinary properties in presence of a magnetic field, such as guidance by magnet and heat dissipation in alternating magnetic fields. These merits make magneto-labeled nanogels a powerful tool for biomedical applications including magnetic resonance imaging (MRI), magnetically guidable drug delivery and hyperthermia cancer therapy. Thus, owing to both the ability to undergo reversible volume-phase transitions in response to environmental stimuli and magnetic properties, remarkable is the suitability of these hybrid nanogels for theranostics (diagnosis and therapy in a single system).
Based on the above, the main objective of this work was the incorporation of magnetic properties to the previously prepared multi-stimuli-responsive poly(2-diethylaminoethyl methacrylate)-based nanogels.[2] In this contribution, the synthesis and characterization of colloidaly stable multi-stimuli-responsive hybrid nanoparticles combining pH-, temperature- and magnetic response are described. What is more, a significant concern in development of a new nanomaterial for biomedical applications is its potential toxicity. Thus, their biocompatibility with cells of donated human blood was evaluated in vitro by studying the possible interference and undesired side effects in blood cells. These studies confirmed high biocompatibility of the hybrid nanogels with human blood cells. Owing to both the multi-stimuli-responsive swelling behavior together with the magnetic behavior of magnetic nanoparticles, the resultant hybrid nanogels exhibit such advantageous features to be used in magnetically controlled biomedical applications.

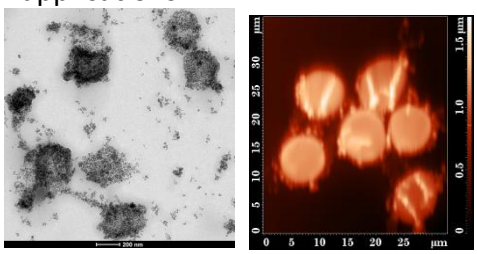

Figure 1: TEM microphotograph of some bare magneto-nanogels (left) and AFM microphotograph of magneto-nanogels attached to human red blood cells (right).

Acknowledgements: *The financial support by Basque Government (Predoctoral grant, BFI-2011-20) and Spanish Plan Nacional de Materiales (MAT2012-36270-C04-01) is greatly acknowledged.*

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Structural and mechanic effects of Daptomycin in model lipid membranes

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Daptomycin is a cyclic lipopeptide employed as an antibiotic for the treatment of infectious diseases caused by Gram-positive bacteria. Its mechanism of action is based on the irreversible bonding to the membrane of Gram-positive bacteria, where it is inserted. There, daptomycin oligomerizes in a process, which depends on calcium and other bivalent metallic ions and negative charged lipids, such as phosphatidylglycerol (PG). The antibiotic lipopeptide forms pores in the membrane that cause cell death as a result of the alteration of the potential of the membrane due to the loss of potassium ions. Model lipid membranes are usually used in order to conclude about structures and functions of real complicate biomembranes, as well as the effects that antibiotics have on them. In this work, Langmuir mixed monolayers of E coli Polar Lipid Extract (PLE) /daptomycin have been studied. The influence of the antibiotic on the structure and the mechanical response of the mentioned monolayers has been determined. The lipid monolayers were laterally dilated and compressed under the action of the Langmuir trough barriers while measuring its viscoelastic response. We study the stress response against deformations up to 10% of the initial area and using different frequencies in systems with linear and nonlinear behaviour. The results point out the dependence of the monolayer rigidity of the monolayer on its percentage of Daptomycin, which is evidenced to determine the mechanical response of the E coli lipid membrane.

Acknowledgements: *This work was supported by MINECO and CAM under grants FIS2012-35723 and S2013/MIT-2807 (to FM), respectively. ILM thanks to ERC for financial support under starting grant "MITOCHON" (ERC-StG-2013-883188) and to "Programa Ramon y Cajal" (RYC-2013-12609) from the Spanish Ministry of Economy MINECO.*

Blood flow dynamics around bioinspired microbots

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In the last decade, the interest in robots at micro scale has increased significantly, especially for biomedical applications to perform eye surgery, to remove blood clots or for cancer treatments [1]. Several works have been directed to the study of the fabrication, propulsion or control mechanisms of these microdevices. However, a detailed fluid flow characterization when they swim through the human body immersed in viscoelastic biofluids, i.e. blood, is still missing. In this work, we study the effect of the presence of the microbot prototype on the blood flow dynamics through a straight microchannel. To that end, four different simplified prototypes were considered: a cubic block, a sphere and two ellipsoids with aspect ratios ½ and ¼ respectively. Micro-PIV and pressure drop measurements were carried out using Newtonian and viscoelastic blood analogue fluids [2]. In general lines, the velocity profile is clearly affected by the presence of the prototype. As we move far from the prototype downstream of the microchannel this influence diminishes. In terms of the microbot's morphology, as expected, the square prototype provoked a bigger disturbance in the flow velocity in comparison with the sphere and the two ellipsoids. However, counterintuitively, at large *Re* and far away from the prototype, the ellipsoid ½ resulted in a better performance than the more slender ellipsoid. For the viscoelastic blood analogue, there is a higher disturbance in the flow velocity around the object even at low *Re* numbers in comparison with the Newtonian case, therefore considering exclusively Newtonian behavior of blood leads to underestimate the drag force that the real human blood exerts on the microbot.

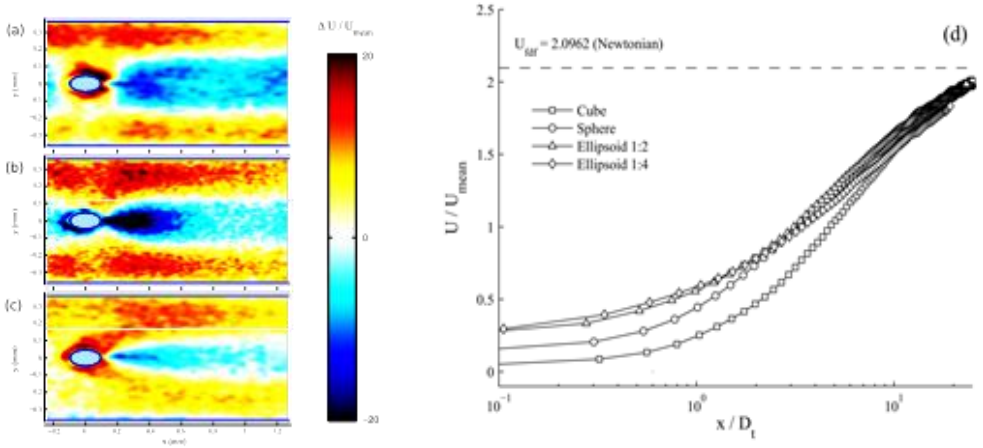

Figure 1: Effect of the elasticity on the velocity field ($(u_{visc} - u_{newt})/U_{mean}$) for the ellipsoid 1:2 at (a) *Re*=0.51, (b) *Re*=5.11 and (c) *Re*=51.1; and on the wake (d) for all the models at *Re*=51.1.

Acknowledgements: *Authors would like to acknowledge financial support from FCT, COMPETE and FEDER through projects PTDC/EQU-FTT/118716/2010 and EXPL/EMS-TRA/2306/2013 and grants IF/00148/2013 and IF/00190/2013.*

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ORAL COMMUNICATIONS

7. THEORY AND MODELLING

Interfacial properties of functionalized colloids on substrates

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Patchy colloids yield directionality of interactions being ideal building blocks for the rational development of self-assembled structures with novel physical properties. Studies of their equilibrium phase diagrams have revealed a myriad of possibilities as, for example, the capability of tuning the density and the temperature of the gas-liquid and sol-gel transitions. However, the kinetics of self-organization and the feasibility of assembling the predicted structures are still poorly understood.
We recently developed a stochastic model to study the irreversible adsorption of patchy colloids on substrates which allows simulating systems with more than one million colloids [1]. Using this model, we compared different mechanisms of mass transport (diffusion and advection) [2], analyzed the influence of the patches spatial arrangement [3], and explored the combination of different types of patches and selective interactions [4]. Our results suggest that the control of experimental conditions and the patch distribution may lead to interesting nonequilibrium interfacial and bulk properties.

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Particle-level simulations of magnetic suspensions in microchannels

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Magnetic micro-particles dispersed in a liquid carrier constitute a magnetic suspension whose rheological (mechanical) properties can be externally tuned under the application of magnetic fields. The influence of magnetic fields under a shearing flow kinematics has been thoroughly investigated in the past using torsional rheometry [1-2]. However, the case of microcapillary flows is not understood yet.

In this work we report a simulation study involving strongly confined magnetic micro-particle suspensions in pressure-driven flow mode through microtubes of different diameter. In the simulations, Brownian motion is neglected and hydrodynamic forces are approximated by Stokes drag, while magnetostatic interparticle forces are approximated in the point-dipole limit. The velocity field is determined by extending the method developed by Tamura and Doi [3] to three dimensions similarly to Pappas and Klingenberg [4]. Simulation results obtained are satisfactorily compared to preliminary experimental data obtained in PDMS microchannels and previous experimental works in the quiescent state [5].

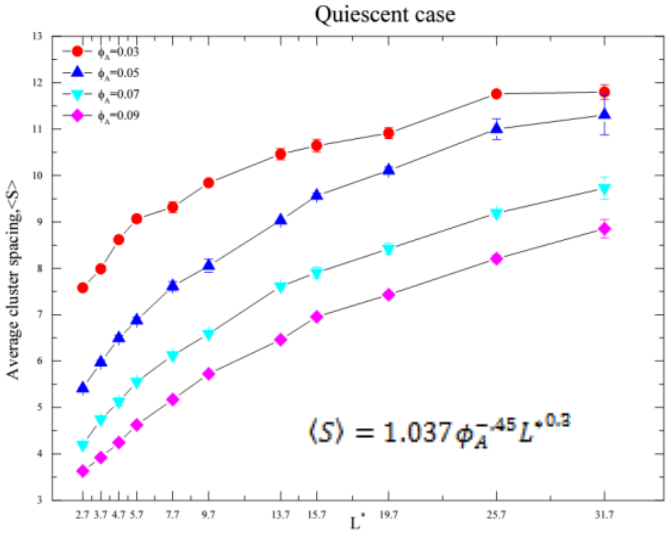

Figure 1: Effect of microchannel thickness on average cluster spacing.

Acknowledgements: *This work was supported by MINECO (MAT 2013-44429-R) and by Junta de Andalucía (P10-RNM-6630 and P11-FQM-7074) projects. E.C.-G. acknowledges the financial support by CONACYT (Ref #232347)*

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Liquid-vapour interfaces of patchy colloids

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We investigate the liquid-vapour interface of a model of patchy colloids. This model consists of hard spheres decorated with short-ranged attractive sites ("patches") of different types on their surfaces. We focus on a one-component fluid with two patches of type A and nine patches of type B (2A9B colloids), which has been found to exhibit reentrant liquid-vapour coexistence curves and very low-density liquid phases. We have used the density-functional theory form of Wertheim's first-order perturbation theory of association, as implemented by Yu and Wu [1], to calculate the surface tension, and the density and degree of association profiles, at the liquid-vapour interface of our model. In reentrant systems, where AB bonds dominate, an unusual thickening of the interface is observed at low temperatures. Furthermore, the surface tension versus temperature curve reaches a maximum, in agreement with Bernardino and Telo da Gama's mesoscopic Landau-Safran theory [2]. If BB attractions are also present, competition between AB and BB bonds gradually restores the monotonic temperature dependence of the surface tension. Lastly, the interface is "hairy", i.e., it contains a region where the average chain length is close to that in the bulk liquid, but where the density is that of the vapour. Sufficiently strong BB attractions remove these features, and the system reverts to the behaviour seen in atomic fluids.

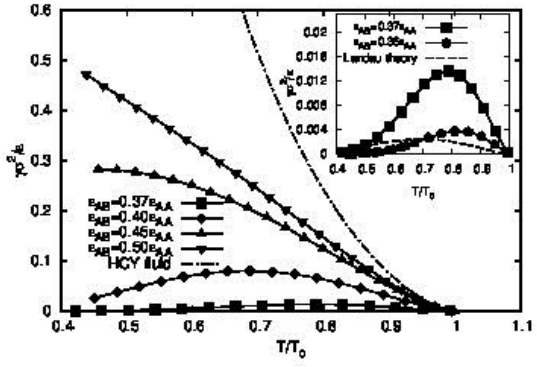

Figure 1: liquid-vapor surface tension vs reduced temperature T/T_c for 2A9B patchy colloids without BB attraction, ε_{BB}=0, and varying strengths of the AB attraction ε_{AB}.

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Molecular dynamics simulation of peptides interaction with lipid membranes

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The use of liposomes as carriers of biomolecules is an important step in vaccine production, drug-delivery systems, or carriers of diagnostic markers, among other applications. In this study, we focused on the interaction of several peptides corresponding to epitopes of proteins from the cell wall of Candida albicans with a lipid bilayer, using molecular dynamics simulations in high performance computing cluster. We used four different peptides, obtained from the proteins Enolase (Enol), Phosphoglycerate kinase (Pgk1), Methyltetrahydropteroyltriglutamate (Met6) and Fructose-bisphosphate aldolase (Fba) expressed on the wall of Candida albicans during disseminated candidiasis. The results suggest that the peptide membrane insertion is favoured in all cases, with the Enol peptide exhibiting a higher affinity for the membrane. The higher affinity of the Enol peptide with the membrane is an advantageous factor in the development of vaccines based on the use of liposomes, because it prevents leaking and early elimination

Nucleation and growth theory: Implications of sub-nanometric metal (0) clusters

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Subnanometric metal (0) clusters are particles of less than 100 atoms (under 1-2 nm). They represent a novel state of matter, located between the classical bulk (or nanoparticle) behavior and the different behavior of the corresponding atoms [1]. Contrary to the generalized opinion, they are very stable due to quantum size effects that appear due to its reduced size. In this talk it will be shown the general lines by which one can get clusters or nanoparticles (NP) -with a precise control- using the same synthesis method. Moreover, it will be shown how clusters can catalyze NP formation eliminating the inducing period commonly observed in their absence. The existence of stable clusters with different sizes, the possibility of getting a good control for their synthesis and the catalysis shown by clusters for NP formation, are observations that seem to contradict the thermodynamic principles usually used to explain the formation of NPs by the classical nucleation and growth theories (NGTs). For that reason we will review in this communication such theory and develop a qualitative approach that takes into account the cluster formation and their catalytic activity for the production of NPs, as it is schematically shown in Figure 1 [2].

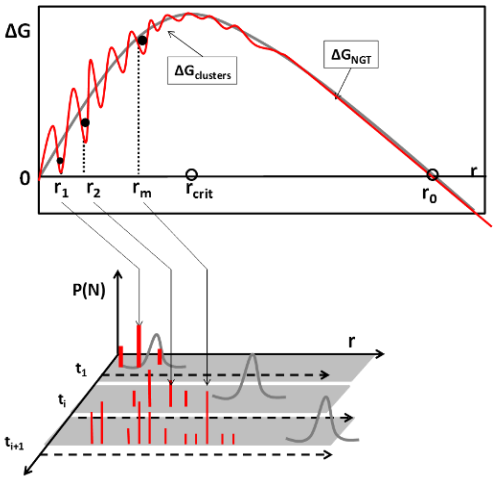

Figure 1: Variation of free energy during the chemical synthesis of metal nanoparticles following the classical NGT compared to the one modified including the AQC effect in the NGT. Size distribution for AQCs formation becomes a discrete magnitude that reflects the large stability of clusters. Reprinted from reference [2]

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CO_2 separation from multi-component mixtures by adsorption in MOF Cu-BTC and zeolite 13X

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There is an increasing interest on carbon emission reduction in coal or natural gas power plants via CO_2 separation from flue gas mixtures, being adsorption and membrane-based separation technologies the most advantageous for these problems, due to their low energy requirements compared to other conventional using aqueous amine solvents [1]. A variety of promising sorbents such as activated carbonaceous materials, microporous/mesoporous silica or zeolites, carbonates, and polymeric resins have been studied by different authors [2]; among them, Metal–Organic Frameworks (MOFs) have attracted significant attention in recent years due to their versatile structures and impressive high surface areas [3]. Moreover, the presence of open-metal sites in MOFs has a significant impact on the adsorption behavior since it strongly favors the direct interaction between metal and adsorbate.
For practical applications, selective adsorption and good thermal stability make these materials potential candidates for CO_2 adsorption separations [4], however the investigation of the effects from coexisting components or impurities in the flue gas, such as water, SO_2 and others is less explored [5, 6]. In this work, we perform a comparative computational investigation for CO_2 separation of multi-component mixtures with a composition typical of flue gas as adsorbed in Cu-BTC, a well-known MOF with open-metal sites, and zeolite13X. Grand Canonical Monte Carlo simulations were performed for a deep molecular understanding of the shapes of adsorption isotherms, the changes in adsorption density over the framework and in the isosteric heat distributions, all related to the interaction of the mixture with the adsorbing surfaces.

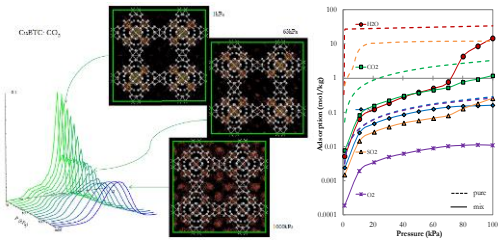

Figure 1: Isosteric heat distributions for CO_2 in CuBTC as a function of pressure, and adsorption isotherms for a five-component real mixture of a flue gas (15%CO_2/5%O_2/0.2%SO_2/1%H_2O/78.8%N_2) (T = 298K).

Acknowledgements: *Financial support from the Spanish Government (project CTQ2014-53987-R) and the Generalitat of Catalonia (project 2014SGR1582) is gratefully acknowledged.*

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ORAL COMMUNICATIONS

8. METALLIC AND MAGNETIC NANOPARTICLES

Metal nanoparticles@metal-organic frameworks NPs. Evidence of molecular diffusion beyond the aperture size limit through SERS measurements

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Composite nanomaterials are attractive for a diverse range of applications in catalysis, plasmonics, sensing, imaging, and biology. In such composite nanomaterials, it is desired and challenging the control of core-shell architectures involving metal nanoparticles and Metal-Organic Frameworks (MOFs) as core and shell, respectively. To address this challenge, we report a new concept of colloidal synthesis based on the surfactant mediated coating of metal nanoparticles with MOFs. The adsorption of cetyltrimethylammonium bromide or chloride (CTAB or CTAC) monomers on the surface of the metal nanoparticle core induces the nucleation and growth of the MOF shell leading to a core-shell architecture. We show that single metal nanocrystals are captured individually in crystalline MOFs. Furthermore, the synthetic procedure could be easily applied to surfactant stabilized gold nanorods, gold nanostars and Au@Ag nanorods (see Figure 1).
Additionally, large guests with molecular diameters 3-4 times the framework aperture size have been demonstrated to diffuse through the metal-organic framework (ZIF-8) shell. The effective diffusion through the MOF shell has been demonstrated by means of Surface Enhanced Raman Scattering (SERS) measurements where the guest molecules could only be detected if they are close to the metal nanoparticle surface. Kinetic studies showed that the molecule diffusion in ZIF-8 is dependent on the size of the guest molecule as well as the thickness of the MOF shell. A competition between dissociative and associative exchange mechanisms has been proposed as a possible explanation for molecule diffusion.

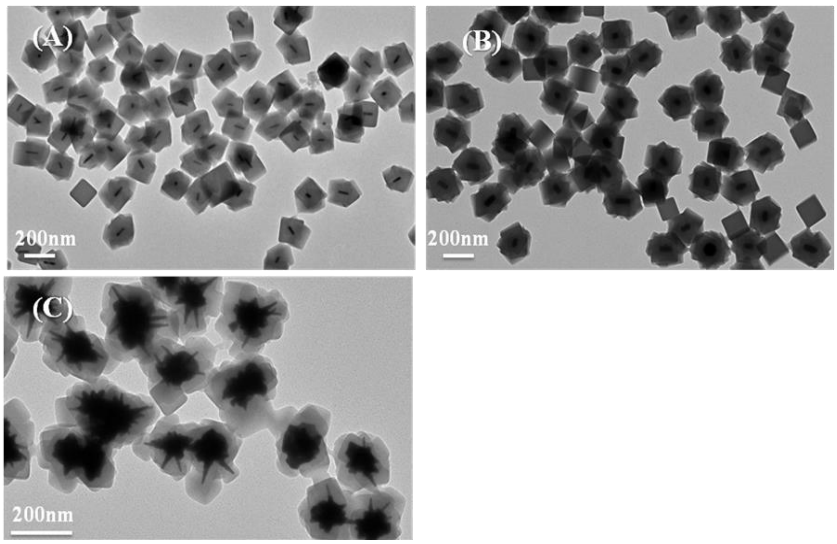

Figure 1: The TEM image of the as-prepared nanoparticles coated with MOFs. (A) Au nanorods @zif-8; (B) Au@Ag nanorods@zif-8; (C) Au nanostars@zif-8.

Gold nanoparticles assembly controlled via femtosecond pulsed laser irradiation

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The optical properties of gold nanoparticles are characterized by strongly localized electromagnetic near and far-field enhancements at the particle surface, which is related to the coherent oscillation of conduction electrons coupled to electromagnetic fields, also called Localized Surface Plasmon Resonances (LSPR). The strong dependence of the LSPR on the surrounding medium around each individual particle gives rise to new LSPR modes when gold nanoparticles are in close proximity and depends on the aggregation number, the interparticle distance and the geometry of the aggregate[1,2]. In this context, the assembly of gold nanoparticles requires surface modification and usually leads to uncontrolled final products [3]. We propose a new synthetic methodology where the interaction between monochromatic and coherent light and the LSPR of gold nanorods is used to control the assembly of the latter [4]. Irradiation with a femtosecond pulsed laser at selected wavelengths during the assembly process offers a novel plasmonic approach to control interparticle interactions, favoring selected species over the rest (see figure 1). Experimental conditions, such as the morphology of the gold nanoparticles (nanorods), the characteristics of the laser pulse (femtosecond laser) and the chemical reactivity (reaction times) allow an outstanding control of the assembly process.

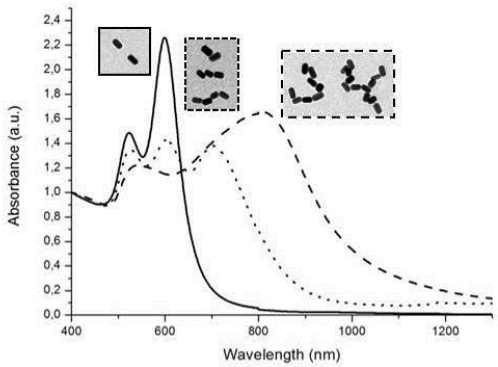

Figure 1. UV-Vis-NIR spectra and representative TEM images of (34±4) nm width and (60±5) nm length gold nanorods (solid line), and their assemblies obtained with 800 nm femtosecond laser irradiation (dotted line) and without irradiation (dashed line).

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Multiresponsive gold nanohybrids for multimodal therapy using LBL technique

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Small interfering RNAs (siRNAs) have gained interest because of their potential clinical applications [1]. The role of siRNA lies in silencing or interrupting the activity of specific genes involved in tumoral cells or neurological diseases like Alzheimer´s. However, siRNA therapeutics is hindered by poor intracellular uptake and limited stability in blood. The interest is focused on the creation and design of suitable nanocarriers for its effective delivery on cell cytoplasm. In this regard, gold nanoparticles have been used as suitable nanovehicles for in vitro and in vivo delivery of different cargos such as small drugs, proteins, oligonucletides [2-5] or antibodies [6-7] due to their low inherent toxicity and stability in biological environments.

In this work, we use a layer-by-layer (LbL) strategy to electrostatically attach negatively charged siRNA to positively charged gold nanorods (GNRs) [2,8,9] with the objective to release the cargo under near infrared (NIR) light stimulation, by exploiting the plasmonic properties of the metallic nanoparticles, and degradation by proteases. Our GNRs absorbs light at 800 nm [10], within "tissue optical window" (700 -1200 nm) where most biological soft tissues have relatively low light absorption. The absorbed energy modulates the attraction between the GNR and the charged cargo molecules, resulting in the release of the siRNA on demand [11]. We used poly-L-lysine (PLL) as the cationic layer to achieve an electrostatic association with the negatively charged of siRNA. We additionally modulate, thereby, the siRNA release by the action of cytoplasmatic proteases, including lysosomal cathepsin B (which is often up-regulated in cancer and inflamed cells) or trypsin [9], which slowly degrades the cationic PLL backbone inside the cells to ensure, if required, a very sustained and prolonged gene-silencing effect. Furthermore, the LBL method allowed us to configure a nanocarrier able to exert a multitherapeutic action by, for example, simultaneously carrying anticancer drugs, like doxorrubicin, and the oligonucleotide chains.

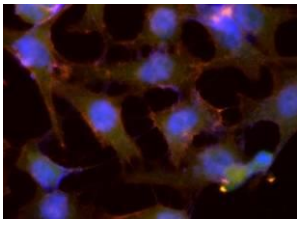

Figure 1: Confocal fluorescence image of HeLa cells with internalised GNRs. Nuclei were stained with DAPI (in blue), cytoplasm with Bodipy Phalloidin (in red). The release of FITC-stained siRNA inside cells can be observed (in green)

Acknowledgements: Authors thank MINECO and Xunta de Galicia for research projects MAT2013-40971-R and EM2013-046, respectively. Eva Villar thanks MECD for her FPU fellowship.

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Magnetic properties of clusters of nanoparticles of $Mn_xFe_{3-x}O_4$ for bio-related applications

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Nanoparticles in direct contact and grouped in clusters offer an alternative interesting route to study and control the spinel oxide magnetic properties, since they include a three-dimensional (3D) interface whose extent depends directly on the size of the nanoparticles, resulting in an increased percentage of atoms located precisely at this interface. These atoms undergo important modifications in their electronic orbitals, and accordingly, the interfaces established in this way offer a combination of effects. Herein we present the study of three samples of clusters of nanoparticles (CNPs) of $Mn_xFe_{3-x}O_4$ with different Mn/Fe ratios that combine superparamagnetism with high magnetization, two of the most desired magnetic characteristics for bio-related applications. The nanoparticulated clusters have been structural and magnetically characterized in order to understand the influence of composition, cation distribution and morphology in the final behavior of the system, revealing enhanced values of saturation magnetization and effective magnetic anisotropy with consequent improvement in their hyperthermic efficiency.

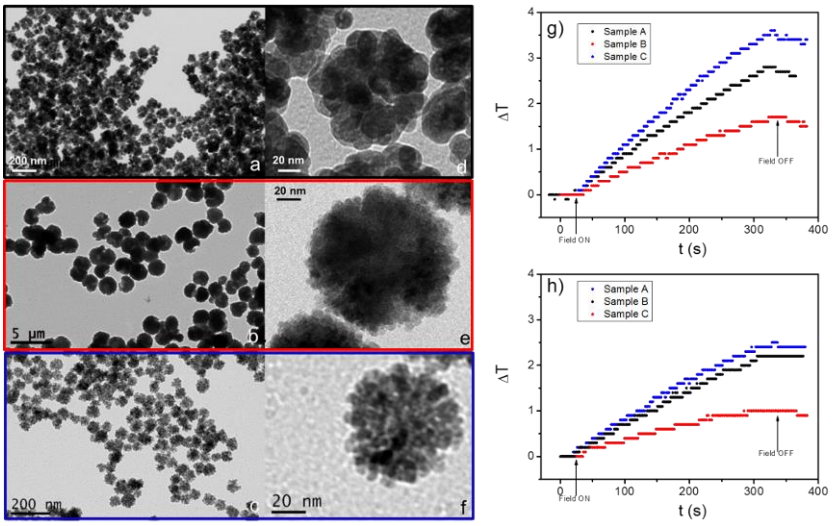

Figure 1: TEM images of samples **A** (a, d), **B** (b, e) and **C** (c, f) with a content of Mn (x) of 0, 0.3 and 0.6 respectively. Selected temperature kinetics of water dispersions of samples **A** (black dots), **B** (red symbols) and **C** (blue symbols), before (g) and after (h) the silica coating, during the application of an alternating magnetic field (17 kA/m, 183 kHz, 5 min).

Acknowledgements: *R. Otero-Lorenzo and V. Salqueiriño acknowledge the financial support from the Xunta de Galicia (Regional Government, Spain) under projects EM2014/035 and InBioMed.*

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Magnetoliposomes based on manganese ferrite nanoparticles for guided transport of antitumor drugs

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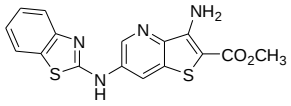
Guided transport of biologically active substances, most of them toxic and with systemic side effects, can focus the active molecules to specific sites in the human body and overcome systemic toxicity problems, allowing a lower drug dosage and a more efficient treatment. Magnetoliposomes (liposomes entrapping magnetic nanoparticles) are of large importance, as they can be used in guided transport of drugs by external magnetic field gradients and used in cancer treatment by hyperthermia [1,2].

In this work, manganese ferrite nanoparticles with size distribution of 46 ± 17 nm and superparamagnetic behavior were synthesized by coprecipitation method. These magnetic nanoparticles were either entrapped in liposomes, originating aqueous magnetoliposomes (AMLs), or covered with a lipid bilayer, forming solid magnetoliposomes (SMLs) (Fig. 1A). Membrane fusion between AMLs and SMLs and giant unilamellar vesicles (GUVs), used as models of cell membranes, was confirmed by FRET (Fig. 1B).

A promising fluorescent antitumor thienopyridine derivative [3], compound **1** (structure below), was successfully incorporated in both AMLs and SMLs, pointing to a promising application of these systems as nanocarriers for antitumor drugs.



Compound **1**

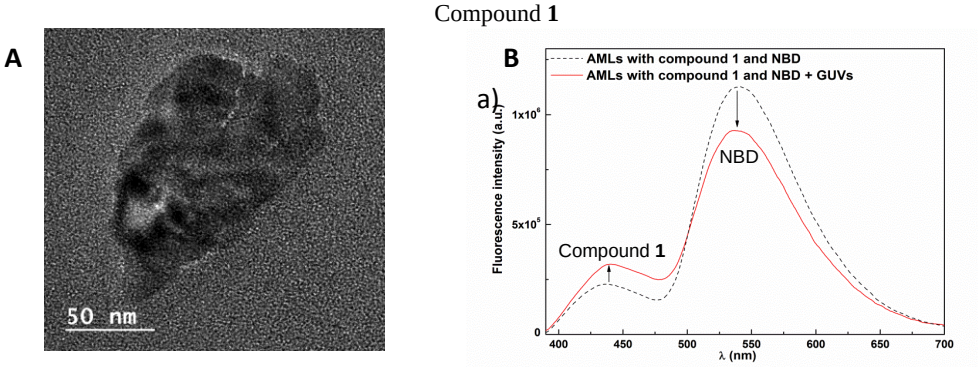


Figure 1 - A. TEM image of SMLs containing $MnFe_2O_4$ NPs. **B:** Fluorescence spectra of AMLs loaded with compound **1** and labeled with NBD-PE, before and after interaction with GUVs.

Acknowledgements: *MAP-Fis PhD Programme, FEDER, COMPETE/QREN/EU for financial support to CFUM (PEst-C/FIS/UI0607/2013) and FCT and POPH/QREN for PhD grant (SFRH/BD/90949/2012).*

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Exchange bias effect in $CoO@Fe_3O_4$ core-shell octahedron-shaped nanoparticles

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Increased coercivity and tunable exchange bias field values were measured in hybrid $CoO@Fe_3O_4$ core-shell octahedron-shaped nanoparticles considering two different average edge length of the antiferromagnetic cores and two different thicknesses of the ferrimagnetic shell. The magnetic hardness attained after growing epitaxially the magnetite shell onto the CoO {111} surface facets just underlines the different parameters playing a role in the type of interface established and the consequent tunability of the exchange bias effect registered.

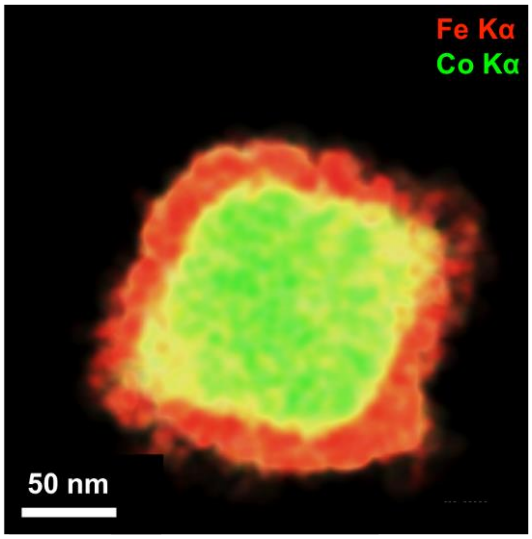

Figure 1: HAADF-STEM image and EDS elemental mappings showing the distribution of the Co, Fe and O within the particles.

Acknowledgements: *V. S. acknowledges funding from the Xunta de Galicia Regional Government (Spain) under project EM2014/035 (Emerxentes) and InBioMed.*

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Biofunctional Au core@shell colloid prepared via RAFT assisted emulsion polymerization and click chemistry

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Gold nanoparticles (Au NPs) have been highly explored in biosensing applications due to their unique optical properties. However surface modification and functionalization are required in order to obtain functional and stable Au NPs in physiological medium. In turn, the use of reversible addition-fragmentation chain transfer (RAFT) polymerization offers the possibility of full control over the composition and architecture of polymeric shells. The use of RAFT polymerization for surface modification allows preserving the optical properties of Au NPs and biofunctionalization aiming for specific biotargeting [1-3]. In this work, tailor-made macroRAFT agent (MR) based on 2-(dodecylthiocarbonothioylthio)propionic acid (TTC-A) have been synthesized via RAFT polymerization and used for the encapsulation of Au NPs (*d*=15 nm). The hydrophilic MR containing acrylic acid (AA) and poly(ethylene glycol) methyl ether acrylate (PEGA) was adsorbed on the NP's surface and then used to mediate the growth of meth(acrylate) chains following a *grafting from* strategy based on RAFT assisted emulsion polymerization. Preliminary results regarding the biofunctionalization of P(AA_2-*b*-$PEGA_{40}$)-*b*-(MMA-*co*-BA)-TTC@Au nanostructures using carbodiimide and/or click chemistry procedures will be presented. Figure 1 shows the strategy followed in this work to prepare biotinylated copolymer@Au colloid.

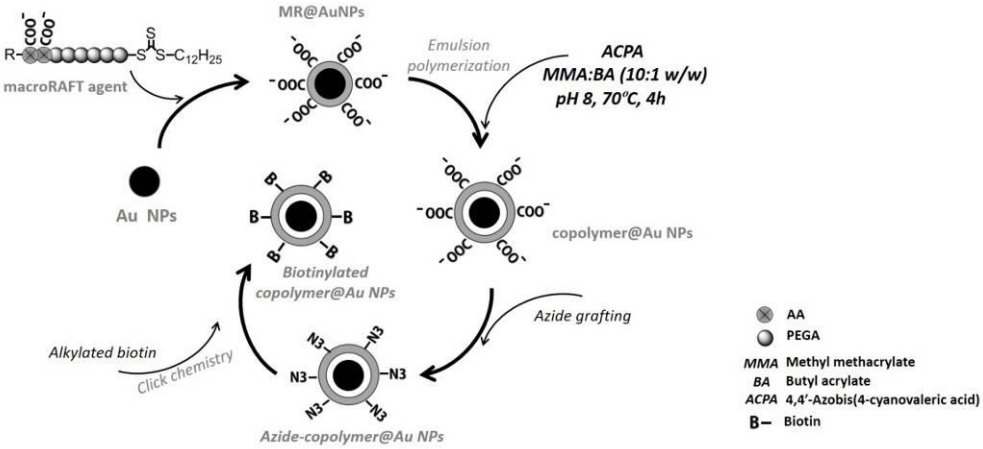

Figure 1: Strategy to prepare biotinylated copolymer@Au colloid.

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Synthesis and applications of confined plasmonic nanoparticles in hollow structures

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The synthetic architectures of complex nanostructures, including multifunctional hollow capsules, are expected to play key roles in many different applications, such as drug delivery, photonic crystals, nanoreactors, and sensing. Implementation of novel strategies for the fabrication of such materials is needed because of the infancy of this knowledge, which still limits progress in certain areas. We report herein the design of plasmonic hollow nanoreactors capable of concentrating light at the nanometer scale for the simultaneous performance and optical monitoring of thermal-activated reactions. These reactors feature the encapsulation of plasmonic nanoparticles on the inner walls of a mesoporous silica capsule. A Diels-Alder cy-cloaddition reaction was carried out in the inner cavities of these nanoreactors to evidence their efficacy. Thus, it is demonstrated that reactions can be accomplished in a confined volume without alteration of the temperature of the bulk solvent while allowing a real time monitoring of the reaction progress. Additionally, these plasmonic nanoprobes have been shown as an advanced intracellular hybrid SERS sensor for relevant signaling molecules (NO). After their inner functionalization with a NO chemoreceptor, the sensor is quantitative and can perform in-situ, real-time monitoring of the dynamics of intracellular NO in living cells while remains fully biocompatible. Its sophisticated design prevents the interaction of cytosolic macromolecules within the active optical material and the enzymatic degradation of the sensor. It additionally facilitates the diffusion of small molecules between the interior and exterior thanks to the plasmonic thermal gradients generated upon their illumination.

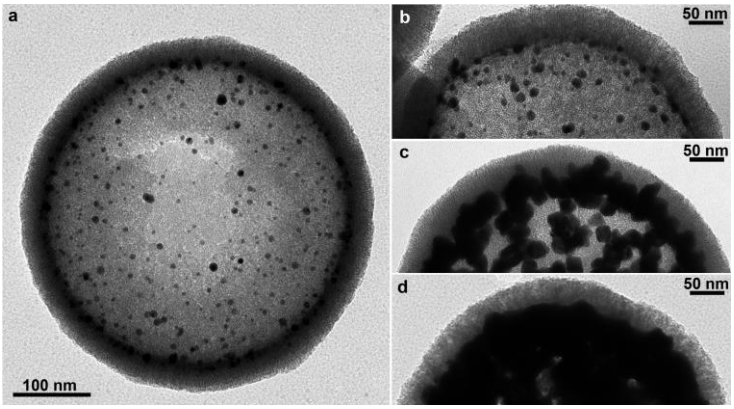

Figure 1: Typical TEM images of the plasmonic nanoreactors (a, b) before and (c,d) after the controlled growth of the inner Au nanoparticles.

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Superparamagnetic iron oxide nanoparticles as multifunctional systems for biomedical applications

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Superparamagnetic iron oxide nanoparticles (SPIONs) have been extensively studied in several fields during the last years due to their various remarkable properties. In particular, the use of SPIONs has attracted wide interest for biomedical applications such as drug delivery or magnetic resonance imaging (MRI). The characteristics that make them highly attractive in biomedicine are mainly their ability to form colloidally stable dispersions in aqueous media, the absence of toxicity, their easy surface functionalization and their superparamagnetism: they respond to externally-applied magnetic stimuli but the effect of the magnetic field disappears completely once the field is removed. This magnetic behavior is essential for biomedical applications in order to diminish the risk of particle aggregation. The nanoparticles must remain unchanged towards effects in dilution, temperature, and ionic strength to ensure stability in biological environments. For this purpose, several strategies can be followed to provide the nanoparticles with an appropriately functionalized surface. One of the most common and attractive ways is the use of different biocompatible polymeric ligands, i.e. polyethylene glycol and polyacrylic acid, based on the efficient surface interaction between the iron atoms from the SPIONs and the oxygen atoms from the capping agent. Here, we report on SPIONs synthesized by hydrothermal method and surface stabilized with polyacrylic acid as coating polymer. Stability tests as a function of dilution, temperature, and ionic strength will be shown. Aggregation issues in cell culture media will also be discussed in the context of contrast agents for MRI.

Figure 1: The basic requirements for SPIONs to be used as contrast agents for MRI.

Acknowledgements: *This work is funded by POCTEP (Operational Programme for Cross-border Cooperation Spain-Portugal) and co-financed by the ERDF (European Regional Development Fund) under a grant for the InveNNta Project.*

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Guimarães2015

ORAL COMMUNICATIONS

9. NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

Fully filled membranar nanoparticles: a new approach for paclitaxel entrapment and biophysical properties improvement

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Paclitaxel is a taxane with a wide spectrum of antitumor activity [1]. The drug is highly lipophilic, with a Log Kp of 3.5 in n-octanol/water system, which limits its administration. Castor oil and ethanol have been use for paclitaxel solubilisation in aqueous solutions. However, this vehicle itself has significant toxicity and it is responsible for most of the side effects reported, such as hypersensibility reactions and neurotoxicity, limiting the administration of higher doses [2]. Liposomes are employed to solubilize lipophilic drugs in order to reduce the toxic side effects of cytostatic drugs without hampering their efficacy and preventing the use of organic solvents [3]. Moreover, the encapsulation of drugs in liposomes can result in pharmacodynamic and pharmacokinetic profiles improvement.
The combination of a cylindrical-shaped lipid and an inverted cone-shaped lipid has resulted in a new liposomal formulation, fully filled with membranes - without the conventional aqueous core [4]. The extra membrane content on the liposome has been used to achieve higher encapsulation efficiencies for a lipophilic drug such as paclitaxel.
Dynamic Light Scattering, Electrophoretic Light Scattering and Cryo-SEM images were used for physical and chemical characterization of fully filled membranar nanoparticles; paclitaxel was quantified spectrophotometrically to determine encapsulation efficiency, maximum drug content and drug release.
The encapsulation of paclitaxel by hydration method resulted in large unilamellar liposomes (~100 nm), positively charged. It was achieved an encapsulation efficiency higher than 90% and the maximum drug encapsulated was 6 mol%. The shelf stability studies performed over 6 weeks indicated that the formulations are stable. The dissolution profiles show that paclitaxel release is faster at pH 5 than at pH 7.4, reporting selectivity for tumour microenvironment/endosome. Finally, the biological activity of the developed formulation was evaluated in breast cancer cell lines.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the projects PEst-OE/BIA/UI4050/2014 and PEST-C/FIS/UI607/2013 and PTDC/QUI/69795/2006. Marlene Lucio holds a position of Researcher FCT with the reference IF/00498/2012. This work is protected by Portuguese National Patent nº 104158-Refª DP/01/2008/10900-31/12/2008 and International Patent submitted: PCT/IB2009/05361-PPI nº40759/09.*

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PLGA nanoparticles for Vitamin D3 delivery to human cancer cells

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Poly (d,l-lactic-co-glycolic acid) (PLGA) polymer have been widely studied as an efficient drug delivery system due to its well established clinical safety and its ability to enhance drug therapeutic benefits [1]. In this work, we propose PLGA nanoparticles (NPs) for the delivery of calcitriol. This active metabolite of Vitamin D3 is a potential anticancer agent but exhibits several drawbacks [2]. PLGA nanoparticles with controlled sizes and properties were synthesized using the single emulsion solvent evaporation technique. The PLGA NPs were physicochemically characterized in terms of size, shape and zeta potential. The attained systems for calcitriol delivery showed mean diameters smaller than 200 nm, encapsulation efficiency of 57% and a loading capacity of approximately 6%. The PLGA NPs remained stable at storage conditions for several weeks and they were lyophilized to assess their behaviour in terms of shelf-life. The in vitro release studies showed a biphasic pattern with an initial burst release of the surface-adsorbed vitamin, followed by a slower and controlled release of the calcitriol entrapped inside the NPs' matrix. The cytotoxic effect of calcitriol encapsulated in PLGA NPs was evaluated on a human lung adenocarcinoma cell line, A549. The in vitro studies demonstrated that bare PLGA NPs are biocompatible and the antineoplastic effect of calcitriol against human cancer cells is enhanced by the nanoparticle formulation. Flow cytometry studies demonstrated that calcitriol entrapment in PLGA NPs enhanced the growth inhibition by inducing the cell cycle arrest at the G1-S transition. From the attained results, it was possible to conclude that PLGA NP formulation is a suitable nanocarrier for calcitriol, since it was shown a clear efficacy in the therapeutic effects.

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DODAB:monoolein liposomes as a novel delivery system with adjuvant capacity

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Liposomal formulations of cationic lipids are effective delivery systems and have been shown to be immunostimulatory [1]. Here, we describe the preparation and characterisation of DODAB: Monoolein-based liposomes loaded with *C. albicans* cell wall surface proteins (CWSP) and demonstrate their adjuvant potential and use in antigen delivery. These liposomes assemble as stable, negatively charged spherical nanoparticles with a mean size of 280 nm. High loading efficiency (91.0 ± 9.0 %) is attained with high lipid concentrations. The nanoparticles obtained are non-toxic, avidly taken up by murine macrophages and accumulate in membrane rich regions within 20 minutes. In addition, these nanoparticles caused significantly higher activation of APCs than CWSP alone, as revealed by enhanced expression of co-stimulatory molecules (CD80 and CD86) and of antigen-presenting MHC class II molecules.

BALB/c mice were immunized subcutaneously thrice with DODAB: Monoolein-CWSP, CWSP alone or empty liposomes. DODAB: Monoolein –CWSP immunized mice displayed strong humoral and Th1/Th17 cell-mediated immune responses. Furthermore, immunization with DODAB: Monoolein-CWSP prolonged survival of BALB/c mice challenge with *C. albicans.*

In conclusion, DODAB:MO-based liposomes loaded with *C. albicans* proteins have an excellent immunogenic potential and can be explored for the development of an immunoprotection strategy against Candida infections.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the projects PEst-OE/BIA/UI4050/2014, PEst-C/FIS/UI0607/2013 (CFUM) and PTDC/QUI/69795/2006, while Catarina Carneiro holds scholarship SFRH/BD/69068/2010. We acknowledge NanoDelivery-I&D em Bionanotecnologia, Lda. for access to their DLS.*

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Lipid based nanocarriers for delivery of the bioactive compound resveratrol

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Resveratrol is a phenolic compound produced naturally by 72 different plant species, particularly grapevines, pines and legumes [1]. This compound has powerful anti-oxidant, anti-inflammatory and anti-cancer effects, which indicates that it may be a valuable chemoprotective agent [2,3]. However, its fast metabolization and reduced solubility in biological fluids impairs its bioavailability. Therefore, it is essential to obtain a suitable carrier to achieve an effective therapy. Liposomes are great candidates as delivery systems since they present high biocompatibility and protection and controlled release of the drug.

In the present study, plain and resveratrol loaded DODAB:MO liposomes (1:2) were prepared by extrusion. Three different methods were used to encapsulate resveratrol – incubation, hydration and direct mixing – to perceive the most effective method. Liposomes were characterized over time for size, surface charge and polydispersity index by Dynamic Light Scattering (DLS) to obtain information about the liposomes shelf stability. To understand the impact of resveratrol in the system's phase transition temperature (Tm) and phase transition cooperativity (B), the DLS technique was also employed. Also, encapsulation efficiency and coefficient partition assays were performed by derivative spectrophotometry and fluorimetry. Moreover, the effect of free and encapsulated resveratrol in the growth of a yeast culture was determined, as well as its protective effect against hydrogen peroxide induced oxidative stress.

Results obtained show that incubation and hydration methods are suitable for resveratrol encapsulation since the encapsulation efficiency is about 75%. Also, liposome size, polydispersity index and ζ-potential values were adequate for drug delivery purposes. Results regarding the system's phase transition temperature and cooperativity show that the resveratrol molecules are unevenly distributed in the lipid formulation and that they are mostly located in the rigid portion of the vesicles, therefore diminishing the system's microviscosity by disturbing the lipid packing. The partition coefficient of the drug in the liposomal system indicated that resveratrol has a lipophilic character, which suggests that it has a preferential partition into the liposome's matrix instead of remaining in the aqueous media. Also, it is possible to observe a pronounced bathochromic shift in the derivative spectra, which is an indication that the drug is being displaced from a polar environment to a non-polar environment. Regarding the effect of resveratrol in the yeast cultures, it is possible to observe that resveratrol has no negative or positive influence in the culture's growth and that, after incubation with this phenolic compound, the cells were somehow protected against the oxidative stress induced by hydrogen peroxide.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the project PEST-C/FIS/UI607/2013. Marlene Lucio holds a position of Researcher FCT with the reference IF/00498/2012. This work is protected by Portuguese National Patent nº 104158-Refª DP/01/2008/10900-31/12/2008 and International Patent submitted: PCT/IB2009/05361-PPI nº40759/09.*

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Monoolein-based nanocarriers for therapeutic siRNA delivery in colorectal carcinoma treatment

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Colorectal cancer (CRC) is a leading cause of cancer related mortality in the Western World. RNA interference (RNAi) therapy has been established as a new therapeutic alternative approach to conventional drugs. Preto et al. [1] showed that individuals with sporadic microsatellite instability (MSI) coloretal cancer harboring BRAF but not KRAS mutations are good candidates to be treated with specific BRAF inhibitors. BRAF is crucial for proliferation and survival of (MSI) CRC with BRAFV600E [1]. Nevertheless, the challenge of RNAi therapy remains a challenge for the development of safe and effective delivery vectors. Cationic liposomes have been extensively used among the nonviral methods used for gene delivery, being MO-based liposomes established as efficient systems for RNAi delivery [2,3].

This work aims to design a novel liposomal nanocarrier based on the mixture of the neutral lipid monoolein (MO), cationic lipids of the dioctadecyldimethylammonium (DODA) family, and DC-Cholesterol (DC-Chol) for the delivery of specific BRAF- siRNA into colorectal cancer cells.

DLS measurements and lipid mixing/fusion ability were performed to characterize the liposomal formulations. The physical-chemical properties of the lipids mixtures will be also characterized by Langmuir-Blodgett technique. Their efficiency in vitro will be evaluate by cytotoxicity, cellular uptake and siRNA transfection assays.

Our results demonstrated that all MO-based liposomal formulations were able to efficiently encapsulate siRNA. Stable lipoplexes of small size (100−160 nm) were obtained with a positive surface charge (>38 mV). DODAC-based liposomes exhibit higher fusogenic ability compared with DODAB-based liposomes but more cytotoxicity in the CRC derived cell line RKO. Although further studies are needed, our preliminary results suggest that DODAB:MO:DC-Chol-BRAF-siRNA nanocarriers are efficient in silencing BRAF expression in CRC cells.

In conclusion, the DODAB:MO:DC-Chol lipoplexes developed in this work might be promising nanovectors for siRNA delivery as a therapeutic approach for gene silencing in CRC.

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Liquid lipid nanocapsules protected with a cross-linked protein shell

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Liquid lipid nanocapsules (LLN) constitute a new generation of nanoparticulate drug-delivery systems able to load drugs, vaccines, diagnostics, and nutraceuticals to be administered by different routes (topical, oral, pulmonary or parenteral). LLN are colloidal systems with a core-shell structure and a high degree of biocompatibility and versatility, since they are composed by a liquid lipid core (usually natural oil, like olive oil in our case) that acts as a reservoir for the drug, and a protective shell-like polymeric wall. Oily core nanocapsules feature some important advantages such as high drug loading capacity, prevention from drug degradation, and reduced burst release, thanks to the shell. We have formed this shell with human serum albumin (HSA, the most abundant protein in blood), and curcumin has been encapsulated into the nanocapsules. Curcumin is a lipophilic model drug with important properties such as antioxidant, anti-inflammatory and anticancer.

In this work we describe how the protein layer may become more rigid and protective by covalently linking the HSA molecules with glutaraldehyde. Crio-TEM visualization revealed that a kind of "membrane" is formed around the particle. A comparison between the electrophoretic behaviour of LLN with and without glutaraldehyde shows that the former present a higher surface charge and, consequently, an increase in colloidal stability. On the other hand, curcumin liberation studies suggest that LLN treated with glutaraldehyde retarded the release of the drug. These experiments also showed that the curcumin loss percentage increased with the initial encapsulated amount.

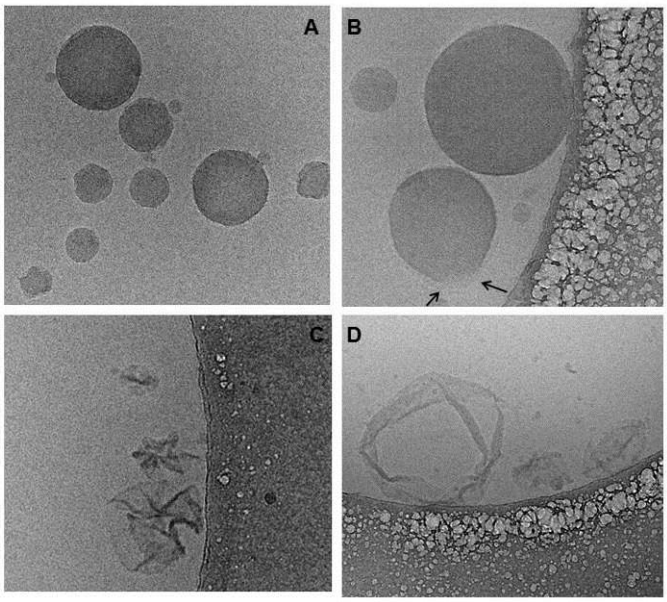

Figure 1: LLN Crio-TEM images.

MO filled core liposomes as a strategy to enhance anticancer drugs encapsulation

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Doxorubicin (DOX) is considered one of the main "first-line" anticancer drugs for a broad spectrum of tumor types, but this drug has the disadvantage of being toxic for other healthy organs and tissues. The use of liposomes as carriers of DOX is thus very appealing to counteract this disadvantage and protect the healthy tissues from contact with the DOX toxicity. Despite several liposomal formulations were already proposed for the delivery of DOX, the majority uses "active loading" methods and the small number of liposomal formulations that use "passive loading" methods achieve small encapsulation efficiency (EE) of the drug. The "active loading" methods are used to increase DOX amounts in the nanocarriers, but have however the disadvantage of drug precipitation and formation of dimers for which the therapeutic value is yet to be proved [1].
In this work it is proposed a nanocarrier system of Dioctadecyldimethylammonium Bromide (DODAB) and 1-oleoyl-rac-glycerol (Monoolein (MO)) (1:2) that has previously been studied as a system with great potentiality of encapsulating drugs, not only at the DODAB enriched bilayer level, but also at the inverted non-lamellar MO-enriched phases at the vesicle interior [2] that increase the payload content even by a passive encapsulation.
Three methods of DOX passive encapsulation in the formulation DODAB:MO (1:2) were tested and characterized measuring the size and zeta potential of the liposomes overtime by dynamic and electrophoretic light scattering and measuring DOX EE (evaluated through UV/Vis spectrophotometry). EE studies revealed high encapsulation values of DOX (87 %) turning the developed formulation in a very promising nanocarrier system for DOX. The study of the partition coefficient of DOX has confirmed that it is highly distributed in the lipid formulation. The biophysical effects of DOX in the formulation indicated an increase in the cooperativity of the phase transition confirming DOX distribution at the membrane level. Cytotoxicity assays were also performed in a cancer cell line and it was concluded that the formulation with DOX encapsulated in DODAB:MO (1:2) has a better cytostatic effect than the free drug, confirming the potentiality of the developed formulation to be used in cancer treatment. Finally controlled release assays were carried out in media with different relevant physiological pH values (5 and 7.4) to predict the pharmacokinetic behavior of the drug when loaded in the developed nanocarriers.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the projects PEst-OE/BIA/UI4050/2014 and PEST-C/FIS/UI607/2013 and PTDC/QUI/69795/2006. Marlene Lucio holds a position of Researcher FCT with the reference IF/00498/2012 and Ana Oliveira holds scholarship SFRH/BD/68588/2010. This work is protected by Portuguese National Patent nº 104158-Refª DP/01/2008/10900-31/12/2008 and International Patent submitted: PCT/IB2009/05361-PPI nº40759/09.*

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Neutrophil elastase inhibitor-loaded starch-based nanocapsules for skin targeting: *in vitro* and *in vivo* studies

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Psoriasis and atopic dermatitis diseases have an excessive amounts of elastase in peripheral blood neutrophils and epidermal plasminogen activator. The high levels of this enzyme inactivate the endogenous inhibitor barrier thus, the search for new human neutrophil elastase (HNE) inhibitors are required. This work presents a novel HNE inhibitor which was carried on a novel nanoparticulate system. The present study aims at developing a novel starch-based nanoparticulate carrier system (StNC) for skin delivery of HNE inhibitor (ER143) and studies its influence on skin delivery.

The StNC were prepared by emulsion-solvent evaporation method, using Miglyol® 812 as the lipid component, Tween®80 and cetrimide as surfactants and modified starch as a polymer. The StNC was characterised in terms of particle size analysis (Malvern Mastersizer 2000 coupled with a Hydro S accessory) and the surface charge that was determined by measurements of the ζ potential (Zetasizer Nano Z in water, at 25°C, Malvern).. Permeation studies were performed using vertical Franz diffusion cells with porcine skin. Water: ethanol (70:30 w/w) were used as receptor phase for ER143. Data was expressed in cumulative amount of ER143 permeated per cm^2 in order to time. Tape stripping was performed 24h after in vitro permeation studies. Stratum Corneum was separated from the epidermis and dermis using 20 tapes. An ER143 solution was used as a control. The drug content was analyzed by fluorescence methods for all of the experiments. In vivo anti-inflammatory activity was accessed using the croton oil-induced ear inflammation model in mice and StNC formulation was used as a control.

The particle size obtained for StNCER143 was between 200-250 nm and showed a positive ζ potential. In vitro permeation studies thought porcine skin showed that the StNC were suitable for the delivery of ER143. After 24 h the amount of ER143 permeated was 573.2±92.7 ng/cm^2 and 248.6±50.0 ng/cm^2 for StNC ER143 and ER143 solution, respectively. The tape stripping assay showed that 22.7±3.9 % and 5.14±0.8 % of the drug was detected on the SC for StNC ER143 and ER143 solution, respectively, and 10.6±1.2 % and 2.2±0.5 % in epidermis and dermis for StNC ER143 and ER143 solution, respectively. Hence, StNC formulation contributed for both higher skin retention and permeation profiles of ER143possibly due to the presence of skin permeation enhancers as well as lipid content. In vivo results showed that erythema and edema were attenuated in 98% and 69% by the local application of StNC ER143 and StNC formulations, respectively, revealing a synergic effect between placebo and ER143-loaded StNC.

These StNC nanocarriers are suitable for a deeper skin penetration and retention. Here we proved that StNC are useful as topical delivery systems, with promising in vivo results.

Evaluation of nanoparticle antimicrobial properties in surgical gauze

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Nanoparticles (NPs) have the potential to be used in fabrics for medical infection control. Namely antibacterial properties for use in burn wounds, where one could predict the need for a non-reusable highly antibacterial structure. This study, investigated the potential for antibacterial activity properties of different nanoparticle systems. Through sol-gel process, silver, silica, titania, Ag-TiO_2 and Ag-SiO_2 nanoparticles, with sizes ranging from 10 to 140 nm, were fabricated. Later, were successfully incorporated in 100% cotton surgical gauze on dip condition sample textile at room temperature for five minutes. Nanoparticle characteristics such as size and morphology have been evaluated by STEM and SEM. Qualitative antimicrobial properties against *E.Coli*, were evaluated by halo test.

RIC16

Guimarães2015

POSTER PRESENTATIONS

The 2D properties of cardiolipin monolayers in single-component systems and binary mixtures

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As reactive oxygen species have a serious influence on the properties of biological membranes their investigation has to be promoted. To determine the effects of reactive oxygen species (ROS) on model membranes in 2D systems (monolayers at the air/liquid interface), different techniques can be applied, e.g., infrared reflection absorption spectroscopy (IRRAS). The target substance to be investigated is the phospholipid tetramyristoyl cardiolipin (TMCL) containing four saturated fatty acid residues. TMCL is analyzed in single-component monolayers as well as in a mixed system containing dimyristoyl phosphatidylcholine (DMPC).

The systems were firstly studied by surface pressure versus molecular area isotherms coupled with IRRAS. The phospholipids are dissolved in chloroform and spread on a phosphate buffered saline subphase at pH 7.4. The TMCL/DMPC mixed membranes are characterized at different mixing ratios and at different temperatures. Brewster angle microscopy has been applied to visualize the domain formation in first-order phase transition regions.

The investigation of TMCL/DMPC mixtures showed that the conformation and orientation of the fatty acid chains are influenced by the different molar ratios. By adding DMPC, the lift-off point in the isotherms is shifted to lower areas. Additionally, increasing amount of DMPC shifts the phase transition pressure from the liquid-expanded to the liquid-condensed phase to higher lateral pressures. Increasing temperature has the same effect - the phase transition pressure increases with increasing temperature for TMCL as well as for the different mixtures. The chain conformation has been analyzed by IRRAS. The band of the CH_2-stretching vibration changes from higher to lower wavenumbers during the phase transition. The dichroic ratio determined along the isotherm indicates a perpendicular orientation of the chains even at the lowest surface pressure.

In near future, the influence of ROS on the TMCL/DMPC systems will be investigated.

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Carbon nanotube thin films as precise volumetric sensors

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Determination of accurate droplet size have become crucial in a wide range of applications. There are different techniques available for measuring dosed quantities and procedures for measuring individual drop volumes in nanoliters. However, these methods may be based on very complex measurement technology and advanced instrumentation non-practical for real-time purpose. Carbon nanotubes (CNTs) have shown great promise as sensing elements in nanoelectrical sensors. Upon exposure to water, changes in their electrical properties can be detected by various methods. In the present work we propose a novel oxidized carbon nanotube/cellulose fiber based sensor for effortless and cost-effective droplet volume measurement based on highly stable but sudden surface conductivity changes. Its analytical theory has been established and a working prototype has been characterized and tested. To this end, a complex nanodroplet deposition platform has been developed, providing a method for electrical characterization of the sensor behavior upon water exposure while been in a temperature and humidity controlled environment.

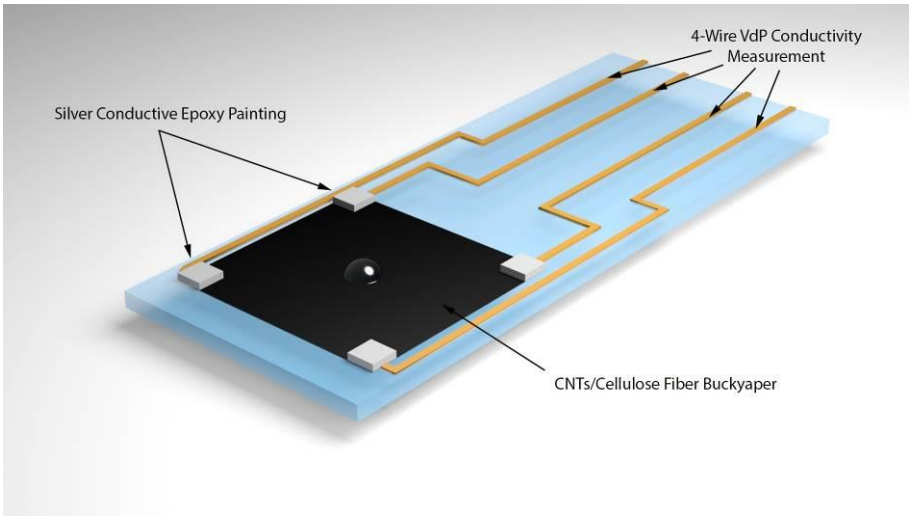

Figure 1: CNT Droplet Volume Sensor Structure.

Arginine based gemini surfactants. monolayer - antimicrobial activity relationship

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Continued use of antibiotics results in a high bacterial resistance. Thus the design and development of new antibacterial agents should be undertaken. Arginine based surfactants have proved to show a noticeable antimicrobial behaviour. Moreover they are non toxic and biodegradable. Therefore these surfactants are candidates to be further studied.
In this work we report on the study of the interaction of three cationic gemini arginine based surfactants with phospholipid monolayer. The surfactants considered belong to the NαNω - bis(acyl-L-arginine) family (see figure for structure). Each surfactant was tested against four different phospholipid monolayers: DPPC, PE, PG and E. coli polar lipid extract.
π-A isotherms resulting from the test carried out show that both interaction and antimicrobial activity strongly depend on the compound spacer chain. Given that the monolayer model used in this work is rather simple, we plan to conduct further research with more elaborated models.

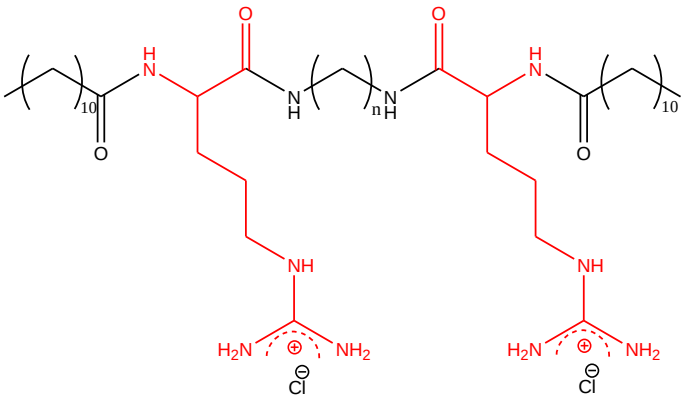

Figure 1: Arginine based gemini surfactants. n = 3, 6 and 9.

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Interfacial behavior of natural products inhibiting lipolysis

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A study on the interfacial behavior of different extracts of natural products inhibiting lipolysis has been carried out by using a pendant drop tensiometer with a subphase exchange device [1]. There is a growing interest on fat digestion (lipolysis of emulsified fat) since a rational control of this process would be an alternative approach to different diseases (obesity, cardiovascular, eating problems…). It is well known that the rate of the lipolysis is controlled by enzyme (lipase) ability to access the interface of its emulsified substrate [2-5]. By controlling this interfacial process it is possible to enhance or inhibit the lipase activity. In order to reduce fat intake in the diet usually an inhibition of lipase activity is induced by using different commercial drugs. However, this inhibition can oftentimes lead to side effects. Using natural extracts with inhibiting properties would be an interesting alternative to avoid the negative effects. At the UGR we have designed a novel methodology to assess lipolysis and inhibition with the pendant drop technique. We present an interfacial lipolysis study of different natural products. By monitoring the interfacial (oil/aqueous solution) tension in the presence of a natural extract with lipase in the medium it has been possible to determine the inhibition capacity of each natural product. These behaviors are compared with those exhibited by lipase and lipase/Xenical® (a known inhibitor commercial product) under-human duodenal conditions. This study reveals the great potentiality of the pendant drop technique to study the phenomena involved in the lipolysis process and its inhibition.

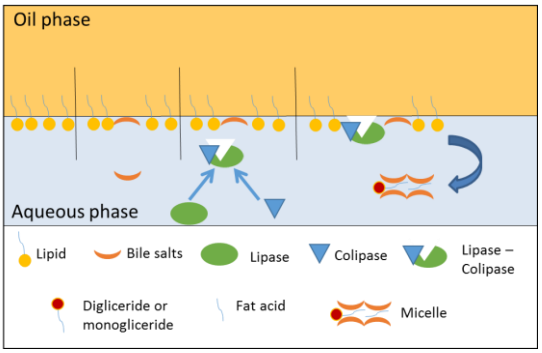

Figure 1: Scheme of the interfacial lipolysis process.

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New insights into DMPG-Chitosan-DNA interactions using isothermal titration calorimetry

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Several techniques have been applied to characterize lipid-polycation-DNA lipopolyplexes, including zeta potential measurements, dynamic light scattering, electron spectroscopy, fluorescence correlation spectroscopy and microscopy. Isothermal titration calorimetry (ITC) is a powerful technique for analyzing interactions of biomolecules in solution because it does not require a reporter probe and it is not susceptible to solution turbidity [1, 2]. Recently, it has been used to determine the binding constant, enthalpy formation and the stoichiometry of binding of DNA with cationic polymers. ITC has also been used to study the effect of solution pH on the DNA-polycation complexation. We have used chitosan as a polycation, phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) as a lipid.

In this study, the interaction of chitosan/DNA, chitosan/DMPG and (chitosan/DNA)/DMPG were investigated as a function of pH, charge molar ratio between chitosan/DNA and the molecular weight of the chitosan, using isothermal titration microcalorimetry. ITC data were analyzed with specific interaction models.

The interaction between DMPG with chitosan occurs until a charge molar ratio of 1.0, while the (Chitosan/DNA)/DMPG interaction occurs until a charge molar ratio of 0.2. This is due to the interaction of polyplex with DMPG can only occur through free charges of chitosan, confirming the existence of a lipopoliplex.

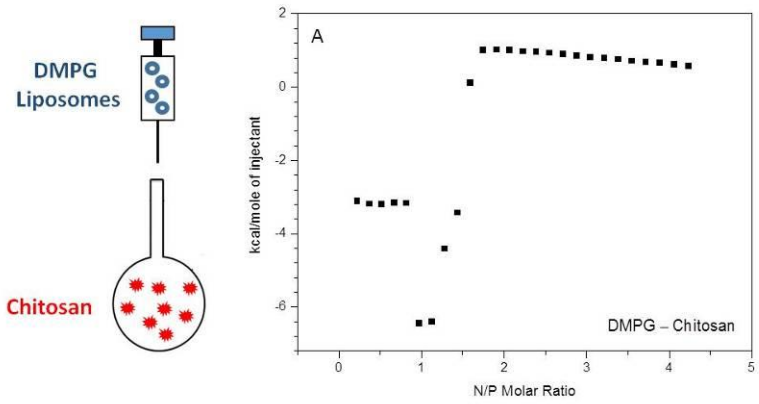

Figure 1: Integrate heats of interaction from calorimetric titrations of DMPG into chitosan.

Acknowledgements: *Spanish "Ministerio de Economía y Competitividad" (Project MAT2011-26330), by the European Regional Development Fund (ERDF).*

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Co-encapsulation of siRNA with non-coding pDNA or Poly-*L*-glutamic acid in DODAB:MO (2:1) liposomes for enhanced gene silencing

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RNA interference (RNAi) mechanism is a critical pathway naturally used by cells to control gene expression. This pathway can be used as a gene therapy approach by using synthetic short interfering RNA (siRNA) molecules to promote the silencing of target genes [1]. Recent studies from our group have established a novel liposomal formulation for siRNA delivery, based on the cationic lipid dioctadecyldimethylammonium bromide (DODAB) and the *helper* lipid monoolein (MO). This liposome formulation has promoted efficient gene silencing in a human non-small cell lung carcinoma cell line (H1299) [2].

Here we aimed to improve the silencing efficiency of DODAB:MO (2:1) liposomes, by promoting the co-encapsulation of siRNA with additional anionic components. Poly-L-glutamic acid of different molecular weights (PG1 or PG2) or non-coding plasmid DNA (pDNA) were added to siRNA solution and encapsulated within DODAB:MO (2:1) liposomes. Lipoplex mean size diameter and surface charge were characterized in order to understand the physical differences caused by addition of the anionic components. Cellular internalization, cytotoxicity and silencing efficiency of the system were observed in two different cell lines (293T and MDA-MB-468). The results suggest that, although the addition of either pDNA or PG molecules to siRNA/DODAB:MO lipoplexes results in systems with similar size and surface charge, some improvements in siRNA encapsulation efficiency, cellular internalization and cytotoxicity were observed for these systems. Moreover, the addition of an anionic cargo led to an improvement in EGFP gene silencing efficiency, suggesting that the presence of an anionic cargo can indeed enhance the efficiency of the systems.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT (PEst-OE/BIA/UI4050/2014 (CBMA), PEst-C/FIS/UI0607/2013 (CFUM) and PTDC/QUI/69795/ 2006). Ana Oliveira holds a FCT scholarship (SFRH/BD/68588/2010).*

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Characterization of the interfacial and aggregation properties of threonine-based surfactants and catanionic vesicles thereof

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There is a continuous quest for environmentally friendly surfactants, with respect to aquatic toxicity, biodegradability and bioaccumulation. In parallel, there is need for surfactants with higher performance levels. From a biomedical and pharmaceutical viewpoint, amphiphiles with low cytotoxicity are of special interest for the design of drug and gene delivery formulations. In this context, amino acid-based surfactants offer several advantages, from higher biocompatibility, higher biodegradability, enhanced interfacial performance and varied self–assembly patterns [1]. In recent years, we have addressed the synthesis and physicochemical characterization of a variety of ionic amino acid-based surfactants [2-4]. Here, we report the interfacial properties of a newly synthesized homologous series of single-chained surfactants derived from threonine (8, 10, 12, 14 and 16 hydrocarbon chains). The compounds are anionic and their micellization parameters have been compared with those from commercial anionic amphiphiles. The aggregation properties of catanionic mixtures based on 12-chained threonine derivatives (12Thr) and selected gemini surfactants, dicationic quaternary ammonium salts (12-s-12, with s = 2, 5 and 12), have also been studied by video-enhanced light microscopy, Cryo-SEM and dynamic light scattering with aim of probing the type of colloidal self-assembled structures present in solution. The conditions that yield spontaneous vesicle formation, as well as an overall discussion of possible vesicle stabilizing mechanisms at stake, will be presented. Versatile catanionic vesicles, in terms of size, charge and pH, which have also long-term stability, are of great interest for a number of applications [4].

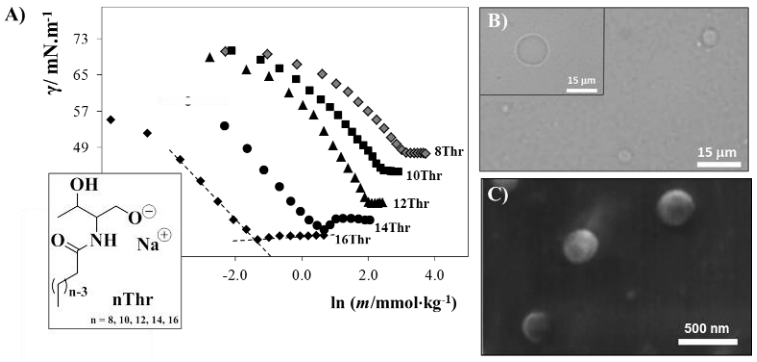

Figure 1: A) Surface tension curves for threonine derivatives. B) Light and C) Cryo-SEM micrographs of 12Thr/12-2-12 liposomes (x_{12Thr} = 0.75).

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Di-cationic gemini surfactants as dispersants of carbon nanotubes: the role of molecular structure

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Carbon nanotubes (CNTs) are a quasi-one-dimensional nanomaterial with unique electrical, optical, mechanical and thermal properties and hence with a high potential for a variety of applications, e.g. composite reinforcement, energy storage, molecular electronics, drug delivery and catalysis [1]. However, due to the strong van der Waals interactions established between them, CNTs are prone to agglomeration into big bundles, which makes their handling and usage rather difficult. Most current applications require, though, the dispersion and isolation of individual CNTs. One of the most common procedures to suspend and separate this material in water is using non-covalent dispersion methods, which involve the adsorption of amphiphilic molecules (such as surfactants, lipids, some polymers and proteins) on the surface of CNTs through hydrophobic interactions, and colloidal stabilization by electrostatic and/or steric repulsions provided by the surfactant headgroups [1 -3]. In this work, we have carried out a systematic study of the dispersion ability of a set of di-cationic gemini surfactants of the bis-quat type, with variation of main chain length (*n* = 12 and 16), and spacer length (*s* = 2, 6 and 12), cf. Fig. 1a). The exfoliation and de-bundling of the nanomaterial was achieved by a dual ultrasonication-centrifugation procedure, followed by accurate quantification of the concentration of dispersed by CNTs by thermogravimetric analysis and UV-Vis absorption spectroscopy. Single and multi-walled carbon nanotubes (SWNT and MWNT, Fig. 1b) were employed, covering a wide range of surfactant concentration, below and well above the critical micelle concentration (*cmc*). The dispersion curves obtained allowed us to define and extract parameters such as critical surfactant concentration for dispersion, maximum dispersed CNT concentration, and respective surfactant concentration at that point, dispersion efficiency and performance index for each surfactant. The effects of the gemini surfactant structure (e.g. charge separation, spacer length and chain conformations) and critical micelle concentration in the dispersion process are presented and critically interpreted.

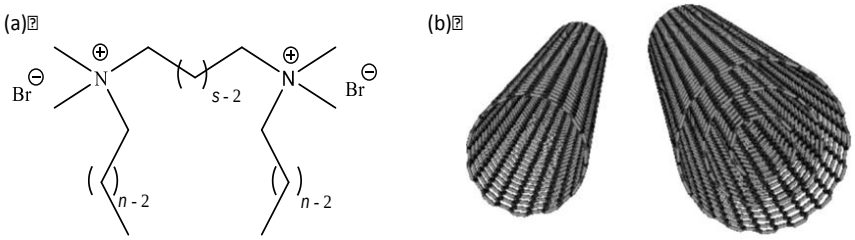

Figure 1: a) Molecular structure of the used gemini surfactants (*n* = 12, 16; s = 2, 6, 12); b) SWNT and MWNTs.

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Aggregation of supramolecular surfactants based on calixarenes

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Contrary to conventional surfactants, supramolecular surfactants are amphiphilic compounds made of non-covalent interactions [1]. One of the different existing ways to obtain supramolecular surfactants is the use of host-guest supramolecular systems due to the opposite hydrophilicity of the host and the guest. Calixarenes are macrocycles particularly attractive in the construction of surfactants because of their relatively simple chemical modification [2]. In this work, the process of autoaggregation has been studied by determination of the critical micellar concentration (*cmc*) of the mixed system formed by an anionic amphiphilic calixarene, tetrasodium 5,11,17,23-tetrasulfonato-25,26,27,28-tetrakis(*n*-propyl)-calix[4]arene (SC4TP, which has the capacity to form aggregates on his own at a *cmc* of 10.5 mM), and a series of cationic surfactants with different lengths of hydrocarbon chain (Figure 1) *via* fluorescence emission of the pyrene. The collected data point to the formation of inclusion complexes between the calixarenes and the conventional surfactants, resulting in supramolecular surfactants with amphiphilic properties and a higher tendency to aggregate, with the possibility of observing a decrease in the *cmc* of more than 350 times.

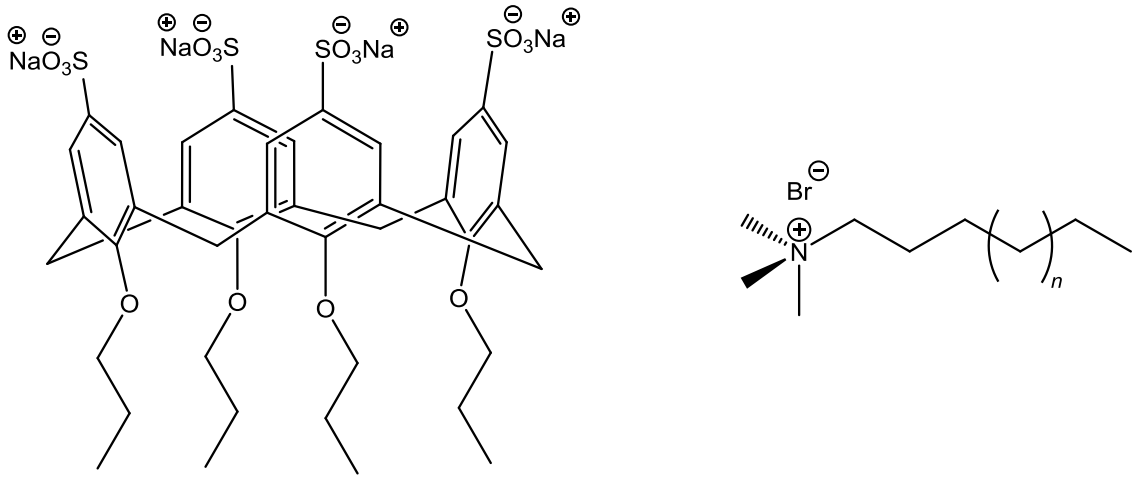

Figure 1

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Self-aggregation of cationic dimeric surfactants in water-ionic liquid binary mixtures

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The solution properties of surfactants can be modulated by controlling temperature, pressure and/or by addition of different modifiers. Ionic liquids, ILs, are a class of organic electrolytes, which are composed of an organic cation and an inorganic or organic anion, that melt at temperature lower than 100°C [1]. ILs are considered environmentally friendly and have been widely used in organic synthesis, catalysis, nanomaterial separation, chemical separation, etc. The surfactant-water-IL three component systems are particularly interesting because ILs can behave not only as co-solvents, but also as background electrolytes and as co-surfactants, their main role depending on the IL structure. This gives the opportunity of tuning the physicochemical properties of the surfactant aggregates formed in water-IL binary mixtures. Bearing this in mind, the micellization of four dimeric cationic surfactants derived from *N*-dodecyl-*N,N,N*-trimethylammonium chloride was studied in pure water and in water-ionic liquid (IL) solutions by a wide range of techniques. In order to minimize organic ion pairing effects as well as the role of the ionic liquids as potential co-surfactants, ILs with inorganic hydrophilic anions and organic cations of limited hydrophobicity were chosen, namely ethyl, butyl, and hexyl-3-imidazolium chlorides. The spacer nature hardly affects the micellization process, neither in water nor in water-IL solutions. However, it does influence the tendency of the dimeric surfactants to form elongated micelles when surfactant concentration increases. In order to have a better understanding of the ternary water-IL surfactant systems, the micellization of the surfactants was also studied in aqueous NaCl solutions, in water-ethylene glycol and in water-formamide binary mixtures. The combined results show that the ionic liquids play a double role in the mixed systems, operating simultaneously as background electrolytes and as polar organic solvents. The IL role as organic co-solvent becomes more dominant when its concentration increases, and when the IL alkyl chain length augments.

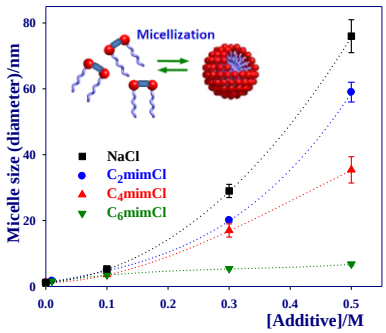

Figure 1: Dependence of micelle size on IL concentration in water-IL and water-NaCl solutions

Acknowledgements: *This work was financed by Consejería de Innovación, Ciencia y Empresa de la Junta de Andalucía (FQM-274 and P12-FQM-1105) and FEDER funds.*

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Double chain surfactants from arginine: aggregation behaviour, antimicrobial activity and cytotoxicity

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Cationic double chain surfactants have attracted much interest because they can give rise to cationic vesicles that can be used in biomedical applications. Using a simple and economical synthetic approach, we have synthesized five arginine-based cationic surfactants, one single chain derivative (LAM) and four double chain surfactants with different alkyl chain lengths ($LANHC_x$) (Figure 1). The critical aggregation concentration of the double chain surfactants is at least one order of magnitude lower than the CMC of LAM and the solutions prepared with the $LANHC_x$ contain stable cationic vesicles. These new arginine derivatives show low hemolytic activity and weaker cytotoxic effects than conventional dialkyl dimethyl ammonium surfactants. In addition, the surfactant with the shortest alkyl chain exhibits good antimicrobial activity against Gram-positive bacteria. The results show that a rational design applied to cationic double chain surfactants might serve as a promising strategy in the development of safe cationic vesicular systems.

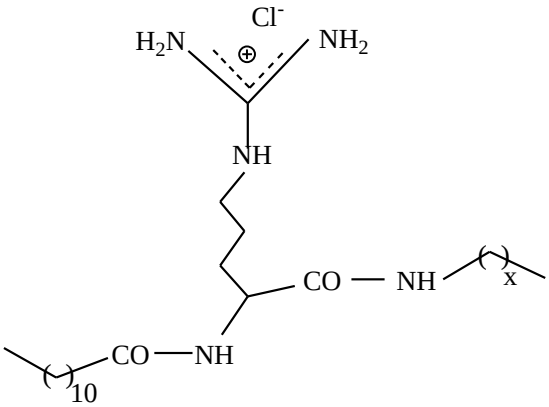

Figure 1: Chemical structure of double chain arginine surfactants $LANHC_x$

Acknowledgements: *Authors acknowledge the financial supports by the National MINECO grants MAT 2012-38047-CO2-02 and CTQ2013-41514-P. Also thanks to Unidad Asociada CSIC-UB "Interacción de tensioactivos con membranas celulares".*

A quality by design approach to optimize a novel w/o emulsion for topical application

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Pickering emulsions differ from classical emulsions because they are stabilized by solid particles instead of surfactants. This type of emulsions has been widely investigated in pharmaceutical and cosmetic fields since they present less adverse effects than the classical emulsions.
In the present work we describe the successful optimization study of an innovative starch-stabilized Pickering w/o emulsion using a quality by design approach and its rheological behaviour. w/o emulsions stabilized by starch (ST) were prepared using a modification of a cold emulsification process, as described elsewhere [1]. The continuous phase consisted of paraffin and the solid particles were ST granules at different concentrations. A computerized image analysis device coupled to an optical microscope (Olympus BX51, Germany), was used to determine emulsions droplet size. The emulsions were optimized using a Central Composite Design (CCD). The independent variables were the percentage of the aqueous phase (AQ) and the percentage of ST relatively to the aqueous phase. Data was analysed using MOODE® software (Umetrics, Sweden) and statistical analysis was considered significant when $p<0.10$. Rotational viscosity was determined using a C35 mm cone geometry, with an angle of 1º. Dynamic viscosity measurements were carried out between 1 and 1000 Pa on a logarithmic increment. Oscillation frequency sweep tests were performed at frequencies ranging between 0.01 and 1 Hz.
Concerning the optimization results, it can be inferred that a decrease in the AQ as well as an increase in the ST will produce an emulsion with smaller droplets, promoting a long-term stability. The formula key parameters that were proven to affect emulsions quality were used to construct the Design Space (Figure 1). Regarding the rheological studies, it was observed that, for each formulation tested, G' increases with ST content. This indicates that the structure of the emulsions becomes more robust with higher content of ST. The system maintained the gel-like properties (G'>G'') due to the presence of the stabilizer agent, which assured stability during the stress tests and exhibited long-term storage stability.
The results obtained suggest that ST-stabilized emulsions are therefore an attractive, promising, simple and a novel template for the production of pharmaceutical and cosmetics vehicles.

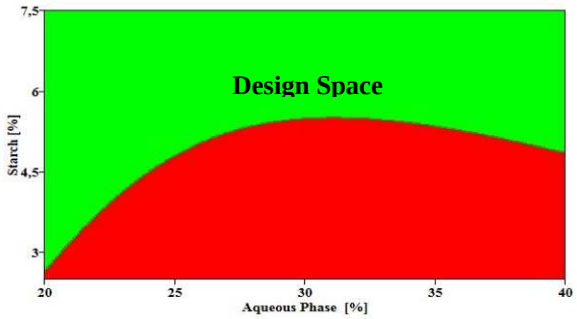

Figure 1: Overlay plot evidence the Design Space for the formula.

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Interaction between conjugated polyelectrolytes and Metal ion complexes of 8-Hydroxyquinoline-5-Sulfonate in micellar solutions

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Recent studies have shown that the complexation of Al(III), Zn(II) and Ga(III) metal ions with 8-hydroxyquinoline-5-sulfonate (8-HQS) is accompanied by marked changes in the UV/visible absorption and fluorescence spectra of 8-HQS.[1-3] Additionally, it was also shown that sensitivity in the fluorescence measurements of 8-HQS/Al(III) complexes can be markedly increased in the presence of the cationic surfactant cetyltrimethylammonium bromide. Here we report the interaction of 8-HQS/Al(III), 8-HQS/Zn(II) and 8-HQS/Ga(III) metal complexes with cationic conjugated polyelectrolyte, poly{9,9-*bis*[6-*N*,*N*,*N*-trimethylammonium) hexyl]fluorene-*co*-1,4-phenylene} dibromide (HTMA-PFP), in aqueous and micellar solutions. Complex formation and the possibility of energy transfer from the HTMA-PFP to the 8-HQS/metal complex were investigated, through absorption, fluorescence and NMR spectroscopy. These systems have important applications in optoelectronic devices and sensing.

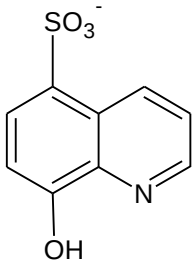

Figure 1: Chemical structure of 8-hydroxyquinoline-5-sulfonate (8-HQS)

Acknowledgements: *Financial support of the Coimbra Chemistry Centre from the FCT through project PEst-OE/QUI/UI0313/2014 is gratefully acknowledged. T.C. acknowledges FCT for the postdoctoral grant (SFRH/BPD/47181/2008).*

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Catanionic crude oil emulsifier mixtures with high potential use in the oil industry

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Oil-in-water emulsions have outstanding roles in the petroleum industry, especially in extraction, transport or storing. Asphalt emulsions have unquestionable advantages when compared to asphalt cements, being the main one the considerable lower temperatures of application. They are employed in road construction and roof water-proofing. Generally, either a cationic or an anionic emulsifying agent is used. Each type of emulsion has a different mechanism and speed of breaking. The objectives sought when evaluating an asphalt emulsion to be used in a pavement is to seal the road from moisture intrusion and to provide a new skid resistance surface while procuring the opening to traffic as soon as possible without the loss of aggregate.

Polyvalent cations, such as Ca^{+2} and Mg^{+2}, present in basic stones such as calcareous ones, react with anionic surfactants producing uncharged insoluble soaps. On the other hand, cationic surfactants are electrostatically adsorbed on the negatively charged siliceous surfaces. This means that the kind of emulsion to be used is ruled by the nature of the soil substrate.

Using an anionic-cationic surfactant mixture as emulsifier will have both the advantages of cationic and anionic emulsions. However, cationic-anionic surfactant mixtures have the general tendency to precipitate in some proportions. In a previous work we have studied a catanionic mixture that does not precipitate in any proportion [1].

Sodium oleate (NaOl)- hexadecyltrimethylammonium bromide (HTAB) mixtures are soluble at all proportions. The crude oil emulsifier properties of this aqueous system have been studied at different proportions of the surfactants. NaOl-HTAB mixtures have shown to be good O/W emulsifiers. The system having mole fraction of HTAB, α_{HTAB}, 0.75 gave the largest volume of emulsion having a narrow unimodal size distribution of small droplets. This emulsion has a relatively high viscosity. All the studied emulsions were stable on ageing and when temperature was risen. The breaking speed and impregnation capabilities of the emulsions were tested with quartzite stones from the Pigüé quarry (Argentina), selected because of their poor performance to produce pavements with commercial asphalt emulsions (their treatment with a commercial asphalt emulsion resulted in an incomplete coverage of the stones' surface, leaving the pavement vulnerable to water penetration). These properties make the studied system very attractive for multiple applications in the petroleum industry, for instance in fuels and pavement production and oil transport.

Acknowledgements: *ENS and EPS are assistant and adjunct researchers, respectively, at Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). This research was supported by a grant of Universidad Nacional del Sur.*

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A ternary mixture of surfactants for medical purposes. Analyzing the thermodynamic of micellization models

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Surfactant mixtures are used in most surfactant applications instead of pure amphiphiles because the mixtures often have enhanced properties when compared to the sum of properties of the pure components (synergism). The aqueous tricomponent surfactant mixture dodecyltrimethylammonium bromide (DTAB), sodium 10-undecenoate (SUD), sodium dodecanoate (SDD) has been studied over the complete triangular phase diagram and a coacervate (coexistence of two immiscible liquid phases having the same components and different concentrations) domain has been delimited. The mixtures do not prepicipitate in any proportion, a desired feature for many useful applications. In general the system behaves non-ideally, presenting a positive deviation of the critical micelle concentration CMC in a wide region. The Multicomponent Regular Solution Theory (MRST) [1] significantly subestimates the experimental CMCs and thus the applicability of the MRST is discussed. The biocide properties of DTAB and SUD against microorganisms, the high CMCs that ensure high concentrations of biologically active monomers and the possibility of having a system that remains liquid in any proportion, make the present system attractive for the design of bactericide and antifungal preparations for medicine, food and cosmetic industries.

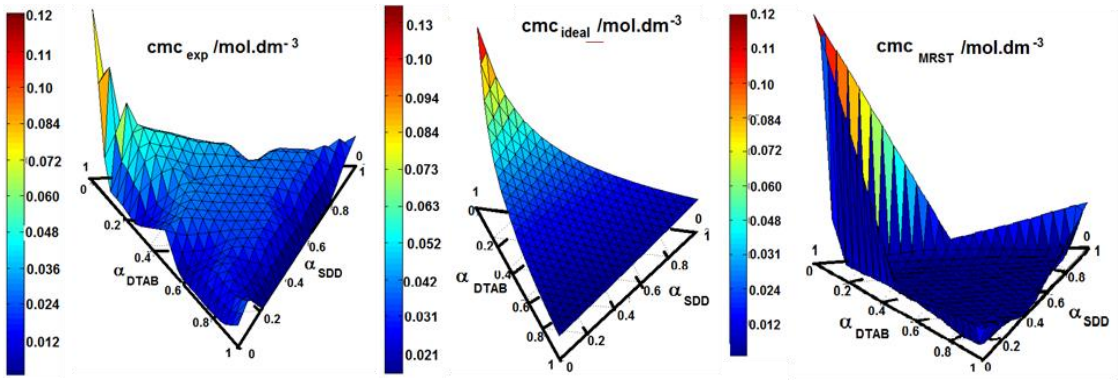

Figure 1: The experimental, ideal and computed (with MRST) critical micelle concentration of the mixtures.

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Thermodynamic study of bile salts micellization

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Bile salts, BS, are biosurfactants important in the digestion process by humans [1]. They are produced by the liver and stored in the gallbladder. Bile salts solubilize apolar material such as cholesterol, lipids, fatty acids, monoglycerides and fat soluble vitamins. The basic structure of bile salts consists of a rigid steroid backbone with a hydrophobic and a hydrophilic face to which a short and flexible tail is attached. The hydroxyl groups are generally located on one face and the methyl groups on the opposite face. As a consequence of this planar polarity, bile salts form smaller micelles than conventional surfactants, in the region to 2-9 molecules [2], because it is difficult to form large aggregates and maintain contact between water and all the hydrophilic faces.
In this work, the aggregation process of the bile salts sodium cholate, NaC, sodium deoxycholate, NaDC, sodium glycocholate, NaG, sodium deoxyglycocholate, NaDG, sodium taurocholate, NaT, and sodium taurodeoxycholate, NaDT in aqueous solution has been investigated at several temperatures, in the absence and in the presence of NaCl 0.15 M, using isothermal titration calorimetry, ITC. Results show that both a decrease in the number of ring hydroxyls and an increase in the length of the side chain favor micellization, in the absence as well as in the presence of salt. These observations can be explained by considering that the hydrophobic effect is the driving force for the self-association process of BS. This is in agreement with the $\Delta_{mic}C_p^o$ values, which point out that the self-aggregation process leads to a diminution of the hydrophobic surface of the BS molecules exposed to water. The presence of NaCl 0.15 M in the aqueous phase favors micellization by decreasing the cmc due to the decrease of the electrostatic repulsions between the negatively charged groups of the BS molecules forming the micelles. However the presence of the background electrolyte has no substantial effect on either the micellar ionization degree or the enthalpy of micellization. The thermodynamic magnitudes indicate that BS micellization is entropy driven.

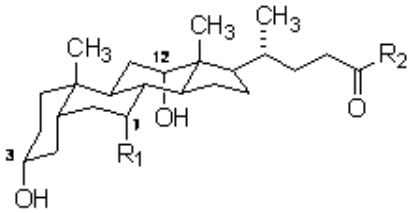
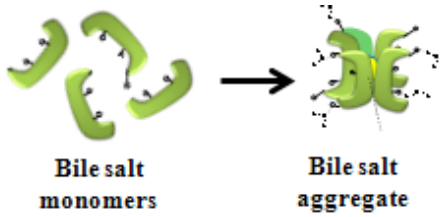


Figure 1: Chemical structure of bile salts



Self-aggregation process of bile salts

***Acknowledgements**: This work was financed by Consejería de Innovación, Ciencia y Empresa de la Junta de Andalucía (FQM-274 and P12-FQM-1105) and FEDER funds.*

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New biocompatible peptide-based hydrogels as drug nanocarriers

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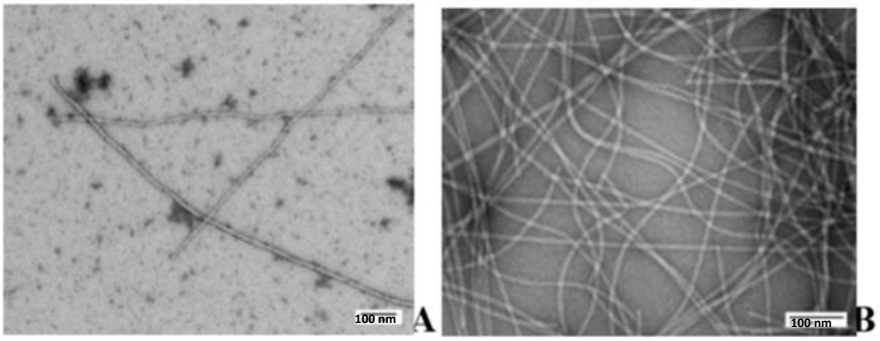
The biocompatibility of peptide-based hydrogels make them ideal for biomedical applications such as drug delivery, biosensing, tissue engineering and wound healing [1,2]. However, the enzymatic hydrolysis of these materials can be regarded as a serious disadvantage. One way to increase the biostability of this type of hydrogels consists in using non-proteinogenic amino acids. In this work, several new hydrogelators were developed, containing a Naproxen or a Naphthalene group, and their critical aggregation concentrations were determined by fluorescence. The influence of pH on the aggregation of these molecules was also investigated. TEM images revealed that these hydrogels contain entangled nanofibers, with width ranging from 9 nm to 18 nm (Figure 1).
The ability of these hydrogels to act as nanocarriers for antitumor drugs was investigated. FRET (Förster Resonance Energy Transfer) assays were performed between the several hydrogels (acting as energy donors) and a new antitumor fluorescent thienopyridine derivative [3] (acting as energy acceptor). Donor-acceptor distances between 2.5 nm and 3.5 nm were determined. The results obtained confirm that the peptide-based hydrogels can be used as drug nanocarriers.
As the antitumor compound tested is especially active against human melanoma cell lines (GI50=3.5 μM) [3], these results are promising to the development of hydrogel formulations for topical application.



TEM images of two hydrogels: Npx-Phe-ΔPhe-OH (A) and Npx-Phe-ΔAbu-OH (B).

Acknowledgements: *Foundation for the Science and Technology (FCT, Portugal), FEDER and QREN for financial support to the Research Centers, CFUM [PEst-C/FIS/UI0607/2013 (FCOMP-01-0124-FEDER-037291)] and CQ/UM [PEst-C/QUI/UI0686/2013 (FCOMP-01-0124-FEDER-037302)]. FCT is also acknowledged for the PhD grant of H. Vilaça (SFRH/BD/7265/2010).*

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Synthesis and characterization of β-cyclodextrin-containing chitosan/modified pectin hydrogels

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Pectin is an anionic polysaccharide present in the cell wall of plants. It consists primarily of D-galacturonic acid and its methyl ester linked by (1-4) glycosidic bonds [1,2]. Pectin may be modified by appropriate preparative conditions where the degree of swelling and release profiles can be tunned [2]. Chitosan is a natural chitin derivative comprising glucosamine and N-acetylglucosamine. Like pectin, chitosan shows good properties, such as, biocompatibility, non-toxic nature and film-forming properties [3]. Cyclodextrins (CD) are a family of natural oligosaccharides formed by 6, 7, or 8 α-(1,4) linked glucopyranose units, denoted as α-, β-, or γ-cyclodextrins, respectively . As a result of glucopyranose units in chair conformation, CD exhibits the form of a truncated cone or torus. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges. The nonbonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the cavity, producing a high electron density and lending it some Lewis base character. As a result of this spatial arrangement of the functional groups in the CDs molecules, the cavity is relatively hydrophobic while the external surfaces are hydrophilic. As a consequence of this structure, CDs are able to form inclusion and non-inclusion complexes by interacting with a large variety of compounds [4,5].

In this work, we describe the preparation and characterization of hydrogels based on modified pectin and chitosan with potential applications as adsorbents biodegradable and selective in wastewater treatment. The pectin was initially modified with beta-cyclodextrin (Pec-BCD) under specific reaction conditions as will be described in this communication. Hydrogels of chitosan/modified pectin were then prepared by using a water/oil high emulsion [6]. One of the main advantages of this technique, when compared with the conventional ones, is the high yield in the preparation of physical gels. The Pec-BCD had amorphous characteristics and improved solubility in water as compared to the unmodified pectin (PEC). The obtained hydrogels were characterized by swelling degree, thermogravimetry (TG), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

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Morphological effects on SERS activity of gold/κ-carrageenan hydrogels

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The controlled release of pesticides using hydrogel vehicles is an important procedure to limit the amount of these compounds in the environment, providing an effective way for crop protection.[1] A key-step in the formulation of new materials for these purposes encompasses the monitoring of available pesticides in the gel matrix under variable working conditions.[1,2]
On the other hand, silver and gold nanoparticles (NPs) coated with polymers have been used as efficient SERS (Surface-Enhanced Raman Scattering) substrates.[3,4] In this work, we report gelatin nanocomposite incorporating Ag nanoparticles (NPs), that can be used as a surface enhanced Raman scattering (SERS) platform for the detection of diethyldithiocarbamate (EtDTC), a pesticide model.[5] In particular, this research shows that EtDTC in these biocomposites has a SERS signal dependent on the gel strength, which in turn can be controlled by varying the amount of biopolymer. We believe that the findings reported here can prompt the development of multifunctional biopolymer platforms for the qualitative analysis by SERS by employing the adequate modifications.
We have now extended this research to hydrogels of k-carrageenan and Au nanoparticles with different morphologies. Several strategies were employed in order to vary the size and the morphology of the Au NPs, which include Au nanospheres and Au nanorods with different sizes. In particular, we will communicate strategies to tune the SERS sensitivity of the hybrid hydrogels by varying the gel strength of the biopolymer together with morphological effects arising from the Au NPs. Furthermore, attempts to correlate the analytical enhancement factor observed in the SERS experiments with the gel strength of the hydrogels will be presented and discussed.

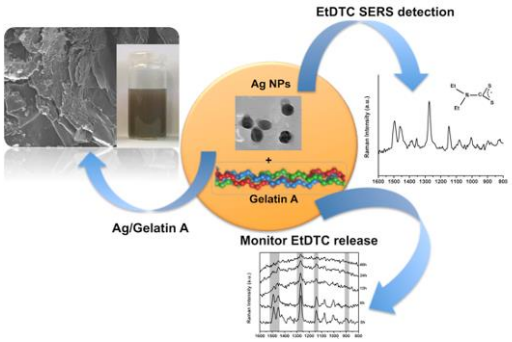

Figure 1: Ag/Gelatin nanocomposites prepared by ex situ method and applied as SERS platforms for monitor the release of pesticides in water

Acknowledgements: *S. Fateixa thanks Fundação para a Ciência e Tecnologia (FCT) for the Grant SFRH/BPD/93547/2013. This publication was financially supported by FEDER through Programa Operacio- nal Factores de Competitividade – COMPETE and national funds through FCT within CICECO project – FCOMP-01-0124- FERDER-037271 (FCT Ref. Pest-C/CTM/LA0011/2013) and project PTDC/CTM-NAN/120668/2010.*

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Colloidal systems in bone regeneration. Is the size important?

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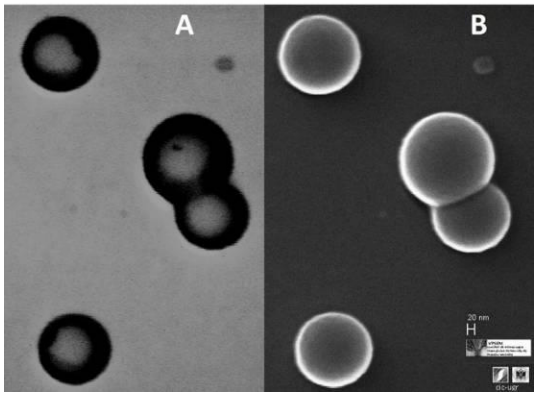
Poly lactic-co-glycolic acid (PLGA) is one of the most widely used synthetic polymers for development of delivery systems for drugs and therapeutic biomolecules. Its properties and versatility make it a reference polymer in the manufacturing of nano and microparticles to encapsulate and deliver a wide variety of hydrophobic and hydrophilic molecules, including biomolecules such as proteins or nucleic acids that must be released in a controlled way [1].

Delivery of growth factors such as bone morphogenetic proteins, and specially BMP-2, is an attractive therapeutic strategy for bone tissue engineering. However, their administration is problematic due to their short biological half-lives, localized action and rapid clearance. Consequently, its clinical use requires high doses far exceeding its physiological concentration which implies possible side effects and high costs. These barriers might be overcome by developing new delivery systems which allow a better control of the release rate in order to achieve the desired concentrations in specific site and time [2].

With this aim, in this preliminary study we have synthesized PLGA particles with different diameters, from nano (200 nm) to micro scale (12.5 µm) via double emulsion procedure, in order to study the influence of size in the release profile of lysozyme, which has been selected as an appropriate model for BMP2. A physico-chemical characterization of the particles was done, followed by a complete study on the encapsulation efficiency, cumulative protein release and bioactivity of the released enzyme with and without co-encapsulated bovine serum albumin, a protective biomolecule that can prevent protein instability during emulsification process. Additionally, fluorescently labeled lysozyme was used to study the protein distribution and the influence of particle size on the in vitro cellular uptake.



(A) STEM/ (B) SEM micrographies of PLGA/poloxamer188 blend nanoparticles.

Acknowledgements: *The authors wish to express their appreciation for the financial support granted by the "Ministerio de Educación y Ciencia" (MEC, Spain), project MAT2013-43922-R, and Research Groups #FQM-115, #CTS 583 (Junta de Andalucía, Spain).*

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Sensor manufacture based on two-dimensional block copolymer lithography

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In recent years different studies [1] have shown that ordered (quasi)arranged pattern composed of anisotropic shape metal nanoparticles provide significant increases of the Raman signals of analytes due to the enhancement of the electromagnetic field at certain regions within/between the interacting nanostructures [2] (the so-called ''hot spots''[3]). Hence, there substrates are good candidates for be used as SERS (bio)sensors. In order to build up these sensors, the use of block copolymer (BCP) self-assembly techniques into well-defined morphologies are very attractive because of the spontaneous organization of the BCP in the nanoscale allows the parallel large-scale production of periodic metallic nanostructures at low cost and very efficiently.
Thus, this work has been focused on obtaining well-ordered nanostructured systems by the "in situ" synthesis of anisotropic star-like gold nanoparticles using BCP thin films as templates or scaffolds. In this manner, we obtain quasi-hexagonal ordered arrays of star-shaped gold nanoparticles with controllable density and monodispersity at the nanometre scale to improve their potential application as plasmonic biosensors for SERS. The substrates characterization was performed by scanning electron microscopy (SEM), UV-VIS and Raman spectroscopy.

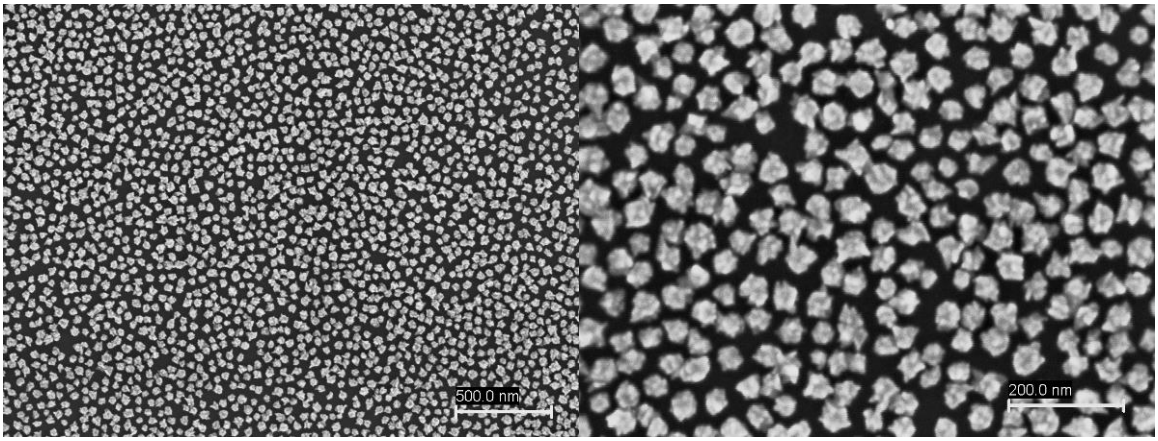

Figure 1: SEM micrographs of quasi-hexagonal ordered arrays of star-shaped gold nanoparticles at different magnifications.

Acknowledgements: *Authors thank MINECO and Xunta de Galicia for projects MAT 2013-40971-R and EM 2013-046, respectively.*

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Synthesis and application of plasmonic nanocapsules as SERS platforms for detection of diclofenac

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Existing water treatment plants are not designed for the detection of pharmaceuticals that are introduced into water sources through sewage. Thus, these chemicals have become an emerging concern to the public due to their potential to reach drinking-water. In this work, a surface-enhanced Raman scattering (SERS) sensor has been designed for the detection of diclofenac, a commom anti-inflammatory drug included in the watch list of substances that require environmental monitoring in the EU member states. With this aim, a mesoporous silica capsule containing closely spaced gold nanoparticle and therefore, a dense collection of hot spots have been fabricated. These nanoparticles are functionalized with an organic chemoreceptor whose SERS spectrum shows characteristic spectral "fingerprints" that selectively report on the presence of this contaminant. The obtained results show that these hybrid plasmonic nanocomposites act as a robust and highly SERS-active sensing platforms.

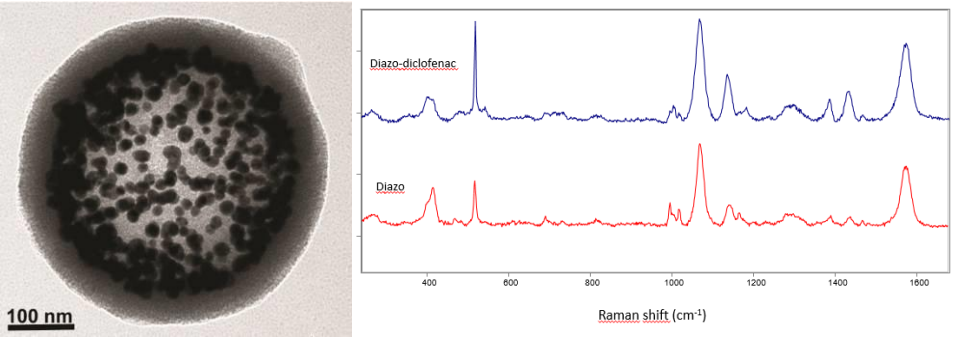

Figure 1: TEM image of a porous silica-based Au-containing plasmonic nanocapsule (left); SERS spectrum of the diazo chemoreceptor before and after contaminant attachment (right).

Exploring polymeric premicelles for improved drug uptake: lipophilic nanocarriers in the submicellar regime

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An investigation on the self-assembly behavior of a biocompatible polymer in the high dilution regime is reported herein. The obtained results reveal the existence of premicellar structures that may further extend the efficiency of traditional polymeric micelles as drug-delivery vehicles. Such an expansion in the excipient capacity arises from (i) the increased drug retention of submicellar assemblies due to their higher resistance to dilution and therefore to their improved circulation time and (ii) the superior carrier permeability of these premicellar aggregates as a result of their smaller size, which makes these drug vehicles more effectively targeted to the tumors through the so-called enhanced permeability and retention effect. The uptake ability of the polymeric premicelles described in this work has been tested through the use of a model drug with a lipophilicity similar to that of potent chemotherapy agents, and microenvironment-sensitive fluorescence properties relevant for localization purposes. Thus, it has been found that an efficient drug encapsulation can be achieved under conditions well below the normally required critical micelle concentration. These results may constitute a promising strategy in order to develop new and more efficient polymeric formulations in drug delivery technologies.

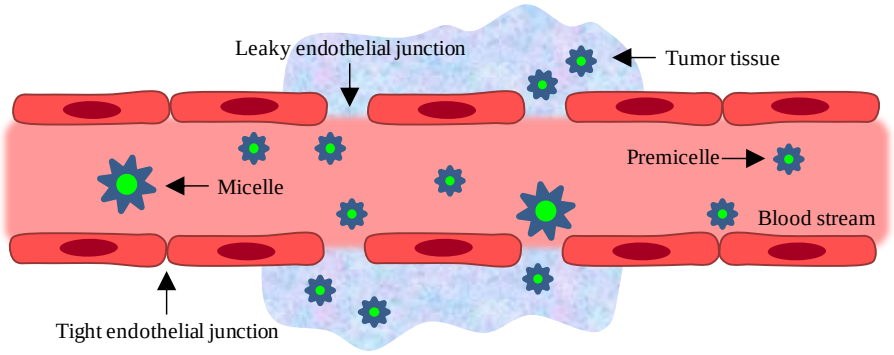

Figure 1: Schematic of the permeation of API-loaded premicellar polymeric carriers through the leaky endothelial junctions of a blood vessel.

Biophysical screening of safety and efficiency of Paclitaxel encapsulated in fully filled membranar nanoparticles

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The rationalization of the drug development process is nowadays a requirement both in pharmaceutical industries, and in academic research laboratories. The development of nanodelivery systems as therapeutic carriers is an exceedingly complex and demanding enterprise. Although the drugs encapsulated were already studied, the encapsulation in a new formulation changes completely its characteristics and *in vitro* screening assays are required to measure the so called drug property profile. The drug property profile consists on measuring fundamental physical-chemical and biophysical properties of drug in the formulation, which determine higher-level properties, such as pharmacokinetics. In this context, the *in vitro* screening assays can be used to predict aspects related with the absorption, distribution and toxicity of the drug in the body (ADME) [1,2]. Paclitaxel (PTX) is a wide spectrum anticancer drug. However, the highly lipophilic nature of paclitaxel turns it difficult to be administrated, also promoting its bioaccumulation in adipocytes preventing therapeutic concentrations at tumour environment. Liposomes are biodegradable, biocompatible, and able to carry both lipophilic and hydrophilic molecules aimed to improve drug pharmacokinetics. The aim of the current work is the assessment of an anticancer drug - paclitaxel (PTX) property profile on DODAC:MO (1:2) liposomal formulations that are being developed as PTX carriers. PTX property profile will be achieved by: determination of partition coefficient by derivative spectroscopy [3] at physiological conditions (37 ˚C at pH 7.4 and pH 5.5 in order to simulate healthy and tumour/endosome microenvironment respectively); location prediction of the compound on the membranes, evaluation of binding to plasma proteins by fluorescence quenching studies and determination of biophysical effects of the compounds by dynamic light scattering (DLS) and/or fluorescence anisotropy studies. The results obtained will permit *in vitro* screening of PTX-liposomal system properties and establish *in vivo* correlations to predict with confidence aspects related with the absorption and distribution of this drug as well as its security and therapeutic efficiency.

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In vivo toxicity validation of protein nanoemulsions using the ZET assay

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Owing to their attractive biological properties, nanoparticles have emerged as promising biomedical tools to enhance therapeutic efficacy and reduce toxicities of conventional delivery systems [1]. Several theranostic applications are envisioned for these nanoscale structures, but information on their safety profile following long-term exposure is lacking. Given that the evaluation of time-related toxicological effects of functional nanovehicles is crucial for their validation, there is an urgent requirement to establish efficient methods for nanotoxicity testing. Zebrafish, *Danio rerio* Hamilton (1822) has been demonstrated as a correlative *in vivo* vertebrate model for assessment of the ecotoxicological impact of engineered nanomaterials [2]. Taken into account that transparent embryos develop promptly into larvae within 5 dpf[3], permitting an *in vivo* fast-track of the morphological and physiological modifications, and given that *in vivo* evaluation of the nanotoxic effects of non-metallic nanoparticles is particularly underexplored [4], the zebrafish embryo toxicity (ZET) assay was applied to investigate the *in vivo* biocompatibility of protein nanoemulsions as a non-animal (according to European Directive 2010/63/EU on the protection of animals used for scientific purposes) 'intermediate' system, positioned amid the cultured cells and mice or rats models [5]. Zebrafish zygotes within 2 hpf were exposed at different concentrations of albumin nanoemulsions, with and without a PEGylated surfactant, for 80 hpf. The following developmental endpoints were assessed: mortality, development delay, phenotypic malformations, spontaneous movements, cardiac frequency and hatching rate. Results suggest that the ZET assay allow for a swift, informative and reliable *in vivo* method to evaluate the toxicity of non-metallic (protein-based) nanoemulsions.

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Profiling pharmacokinetic parameters of resveratrol-liposomal formulations for nanotherapy purposes

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Resveratrol (RSV) is a natural occurring phenolic compound with great anti-oxidant, anti-inflammatory and anti-cancer effects, indicating that this chemical may be a valuable chemoprotective agent [1, 2]. The drug-like property optimization is an important area of drug discovery advancement which captures the concept that certain properties of compounds are most advantageous in their becoming successful drug products. This optimization consists in examining the structural properties that affect the physicochemical properties which, in turn, affect the absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) of the drug, and how these structures can be optimized [3].

In the present study, the drug-like property profile of the bioactive compound RSV on DODAB:MO (1:2) liposomal formulations was assessed by: (1) determining the partition coefficients at different temperatures and at interesting physiological pH values (pH 5 and pH 7.4) by derivative spectroscopy; (2) predicting the location of the compound on the membranes and its binding to plasma proteins by fluorescence quenching studies; (3) determining the biophysical effects of the compound in the liposomal formulation by dynamic light scattering (DLS) and fluorescence anisotropy studies.

The results obtained led to an *in vitro* screening of the RSV-liposomal system properties and were combined with *in vivo* correlations, which made possible to predict with confidence aspects related with the absorption and distribution of this compound.

Acknowledgements: *Acknowledgements: This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the project PEST-C/FIS/UI607/2013. Marlene Lucio holds a position of Researcher FCT with the reference IF/00498/2012. This work is protected by Portuguese National Patent nº 104158-Refª DP/01/2008/10900-31/12/2008 and International Patent submitted: PCT/IB2009/05361-PPI nº40759/09.*

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Magnetic bio-hybrid nanosorbents for the uptake of organic pollutants from water

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Biopolymer-silica hybrid materials merge the properties of the respective organic and inorganic components to provide improved properties and as such, they have *drawn attention* as promising *materials* for several applications including water decontamination.[1] Among the available sorbents, magnetic materials are of potential interest for certain applications because the adsorbed pollutants may be removed by the application of a magnetic gradient.Keeping these features in mind, the present investigation aimed the development of magnetic nanosorbents based on organic-inorganic coated magnetite (Fe_3O_4) particles for the uptake of organic pollutants from water. Here, we report a new approach for the surface modification of Fe_3O_4 particles with biopolymer-silica hybrid shells. The method comprises first the synthesis of a biopolymer-silica hybrid precursor using the polysaccharide κ-carrageenan (SiCRG). The coating was then performed by alkaline hydrolysis/condensation of a mixture of SiCRG and an alkoxysilane in the presence of Fe_3O_4 particles. The present communication describes preliminary research on the use of the resulting materials (Fe_3O_4@SiO_2/SiCRG) as sorbents for the removal of an organic dye (methylene blue - MB) dissolved in water samples, by using a laboratorial neodymium magnet (NdFeB). The maximum MB adsorption capacity onto the magnetic hybrid particles was found to be 529.6 mg/g (25°C, pH 9). With such high adsorption capacity value these hybrid particles are among the most efficient MB adsorbents reported so far.[2] The high adsorption performance of these nanosorbents can be ascribed to their high surface area combined with the sulfonate groups available arising from κ-carrageenan incorporation, which have high affinity for MB molecules.[3]

Acknowledgements: *This work was financially supported by FEDER through Programa Operacional Factores de Competitividade – COMPETE and national funds through FCT within CICECO project – FCOMP-01-0124- FEDER-037271 (FCT Ref. Pest-C/CTM/LA0011/2013) and project PTDC/CTM-NAN/120668/2010.*

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Dissolution of nanomaterials in the context of safety assessment studies. Relevance of dynamic and equilibrium speciation techniques.

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The experimental determination and modelling of solubility is a very important issue from the point of view of risk assessment of emerging nanomaterials (NMs) [1]. Solubility is determined by the hydrochemical reactivity of the NMs and the concomitant release of ionic species, resulting in a fraction of material that is transformed to completely different species with non-nano characteristics. This dissolved fraction can, therefore, be treated as "conventional" chemical species with regard to regulation. The OECD guidelines for the safety testing of chemicals explicitly address the necessity of measuring solubility in water (Test Guideline 105), although the evaluation of this protocol for its adequacy to the specific features of NMs is currently under way.

So far, most regulatory documents refer to the determination of the total dissolved fraction. However, for the assessment of the (eco)toxicological impact of a relatively soluble (metal-based) nanomaterial, it is not enough to know how much it dissolves, but also how the dissolved fraction is distributed among the different soluble species (free ions, inorganic complexes, chelates such as EDTA, metal complexes with aminoacids, pH buffers, proteins, polysacharides, humic substances, inorganic colloids, etc.). This idea follows from a widely accepted paradigm among the trace metal toxicologists (toxic effect being related to speciation), which is the main assumption of *e.g*. the Free Ion Activity (FIAM) and Biotic Ligand (BLM) models. Actually, in several cases the acute toxicity of NMs has been related specifically with the concentration of free metal ion species released by dissolution [2].

In this work, we present an overview of the potential of trace metal speciation techniques such as Absence of Grandients and Nernstian Equilibrium Stripping (AGNES) [3] or Diffusive Gradients in Thin Films (DGT), in the experimental study of speciation in metal or metal oxide NM aqueous dispersions, without the need for an off-line solid/liquid separation step. The results shown here are focused in the case of ZnO nanoparticles.

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Size effects on the removal of aqueous Hg(II) using functionalized magnetite particles

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Progress on methods of synthesis and surface functionalization of diverse nanomaterials has provided opportunities for the fabrication of selective and efficient sorbents to remove toxic metal pollutants. Mercury is a heavy metal of primary concern due to its toxicological and biogeochemical behavior, namely its persistence in the environment and bioaccumulation. Taking advantage of the high chemical affinity between mercury and sulphur, colloidal magnetite (Fe_3O_4) particles functionalized with silica shells modified with dithiocarbamate moieties have been recently reported as efficient sorbents to remove Hg (II) from water [1]. In this case, a one-step sol-gel method was used to obtain materials coated with sulfur enriched siliceous shells by applying alkaline hydrolysis of TEOS in the presence of a siloxydithiocarbamate compound (SiDTC).
In this research, the effect of the Fe_3O_4 particles size on the chemical functionalization step and subsequent use for water remediation procedure was evaluated. Therefore, superparamagnetic Fe_3O_4 nanoparticles with an average diameter of about 15 nm were prepared by a chemical *co-precipitation* method [2], while ferromagnetic nanoparticles with an average diameter of 50 or 80 nm were synthesized by hydrolysis of $FeSO_4$ [3,4]. The ensuing colloidal nanoparticles, with distinct average sizes, have been coated as described above in order to obtain SiO_2/SiDTC coated magnetite particles (Fe_3O_4@SiO_2/SiDTC). Finally, a series of experiments have been carried out to inquire about the removal efficiency of the distinctly sized nanosorbents for Hg (II) in water, by applying magnetic separation methods.

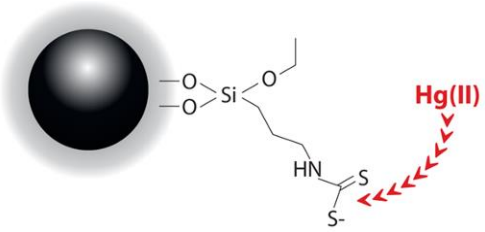

Figure caption: Scheme illustrating the capture of Hg(II) by Fe_3O_4@SiO_2/SiDTC nanosorbents.

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Phosphatidylcholine/phosphatidylserine/sphingomyelin/cholesterol mixtures as model membrane to study peptide-lipid interactions

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It is known that virus, with a capsid such as HIV and GBV-C, penetrate into the cells through a process involving fusion with cell membrane [1]. A preference toward cholesterol (CHOL) and sphingomyelin (SM)-rich composition was identified. These lipids form lipid microdomains (*lipid rafts*) in membranes which usually are involved in HIV entry [2]. Our group has been studied interactions of GBV-C peptides as inhibitors of HIV-1 FP using PC/PS (3:2) as model membrane [3, 4] which was chosen because of phosphatidylcholine (PC) is the major component of the outer leaflet of uninfected cells, and phosphatidylserine (PS) is a hallmark of programmed cell death, it is expressed at elevated levels in HIV-1-infected T cells or macrophages because of the association of apoptosis with the progression of AIDS [5]. In this work, the properties of mixed lipid/sphingomyelin/cholesterol (LIP/SM/CHOL) systems were studied in order to know the possible lipid ordered domains formation (*lipid rafts*). LIP was the mixture of phosphatidylcholines indicated above (PC/PS 3:2. Langmuir films, Langmuir-Blodgett films and giant liposomes in combination with fluorescence microscopy and AFM were used. These experiments allowed someone to perform thermodynamic analysis of the interactions between molecules, to observe domain structures and phase coexistence in every system. It was considered the length and unsaturation of acyl chains of LIP and different SM and CHOL content.

The results suggested that the LIP-1/SM/CHOL (1:1:1) (LIP 1: DOPC:DOPS 3:2) can serve as raft-like mixtures. At these composition cholesterol molecules associate mainly with SM, due to strong affinity of cholesterol to SM molecules and raft-like domains are formed. Figure 1 shows confocal images of giant liposomes (LIP-1/SM/CHOL 1:1:1) containing 1% of NBD-PC or DIL C20:0 fluorescent probes with rafts clearly seeable (green area in figure 3-C). On the other hand, at higher sterol concentration, additionally sterol/PC interactions appear, so the composition LIP-1/SM/CHOL 1:1:1 was selected to study lipid-raft implication in the union of possible HIV-1 FP inhibitors to membrane.

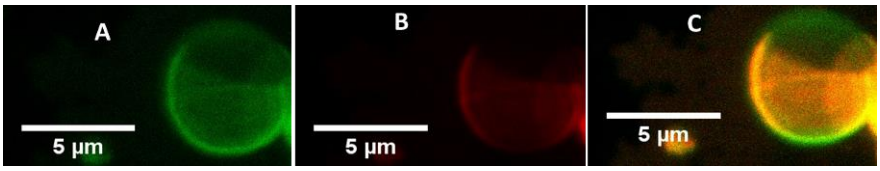

Figure 1: Confocal images of giant liposomes. Composition: LIP1/Chol/SM (1:1:1) containing A: 1% NBD-PC, B: 1% DiL C20:0 and C: 1% NBD and 1% DIL C20:0. LIP1: DOPC/DOPS (3:2), Chol: cholesterol, SM: sphingomyelin.

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Interaction of cytotoxic and cytoprotective bile acids with model membranes: influence of the membrane composition

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To understand the role of bile acids on the cell function, many authors [1,2]investigated their effect on biomembrane models which are less complex systems, but there are still many open questions. The present study aims to contribute for the deepening of the knowledge of the interaction between BAs and model membranes, in particular focusing the effect of BA mixtures. The cytotoxic deoxycholic acid (DCA), the cytoprotective ursodeoxycholic acid (UDCA), and the equimolar mixture (DCA+UDCA) were investigated. Monolayers and liposomes were taken as model membranes with two lipid compositions: lipid rafts-like (equimolar mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), sphingomyelin (SM), and cholesterol (Chol)) and equimolar POPC/SM mixture. The experimental techniques used were: Langmuir trough (lipid monolayer); Quartz Crystal Microbalance with Dissipation (supported liposomes); Differential Scanning Calorimetry and Phosphorus Nuclear Magnetic Resonance (liposomes in suspension). The obtained results showed that DCA causes fluidization of monolayers and bilayers, leading to the eventual rupture of POPC/SM liposomes, at high concentration. UDCA may provide stabilization of POPC/SM membranes by interaction with phospholipid headgroups, but has a negligible effect on the raft-like liposomes. In the case of the mixture DCA/UDCA, the interactions depend not only on the lipid composition but also on the design of the experiment. The BA mixture has a greater impact in monolayers than the pure BAs, suggesting a cooperative DCA–UDCA interaction that enhances the penetration of UDCA in both POPC/SM and POPC/SM/Chol monolayers. In the bilayers, the presence of UDCA in the mixture decreases the disturbing effect of DCA.

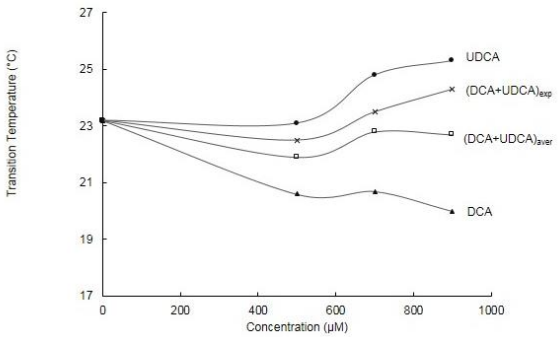

Figure 1: Transition temperature of POPC/SM liposomes as a function of concentration of DCA, UDCA and DCA/UDCA (1:1). The open squares represent the predicted transition temperature calculated from the average contribution of DCA and UDCA

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Interaction of Alzheimer Aβ(25-35) peptide with model membranes

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Alzheimer's disease is characterized by the presence of amyloid plaques in the brain. The main components of these plaques are the Aβ(1−40) and Aβ(1−42) peptides and the disease progression correlates with soluble oligomeric species of Aβ that are responsible for neuronal toxicity [1]. The Aβ(25−35) sequence is the most frequently studied fragment because it possesses the structural characteristic of Aβ and maintains the toxicity of the full-length peptide [2]. In the present work, the interactions of the amyloid Aβ(25-35) peptide with model membranes were investigated, taking into consideration the aggregation state of the peptide. Monolayers and liposomes were taken as model membranes with two lipid compositions: an equimolar mixture of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), sphingomyelin (SM), and cholesterol (Chol), and an equimolar mixture of POPC with SM. Lipid monolayers were investigated in the Langmuir trough, with the supported liposomes being characterized in a Quartz Crystal Microbalance with Dissipation, and the suspended liposomes by Differential Scanning Calorimetry. Nanoparticle Tracking Analysis was used to determine the aggregation of peptide solutions. The interaction of Aβ(25−35) with the monolayers depends on the size of the peptide aggregates which, in turn, is determined by their concentration and pH. The large aggregates that are formed in the bulk solution have a weak interaction with the lipid monolayers. In contrast, the monomers or dimers interact with the monolayers, more intensely in the presence of Chol. The results obtained led to the proposal of a three step mechanism for the interaction of Aβ peptide with the monolayers: *Adsorption* – monomers or dimers adsorb at the polar region of the lipid monolayer, by electrostatic interactions; *Nucleation* – adsorbed peptides act as nucleation sites of higher aggregates; and *Penetration* – these aggregates, that form near the polar heads of lipids and become more hydrophobic, insert the hydrophobic region of the monolayer, leading to the increase of the area per lipid molecule. The interaction of Aβ(25−35) with both types of liposomes is very small, independently of the peptide concentration.

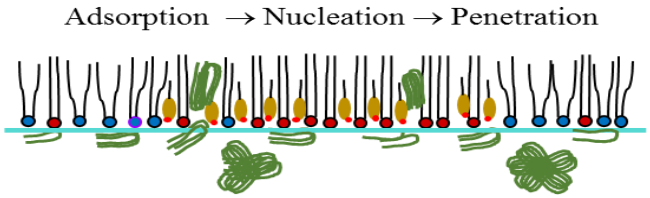

Figure 1: Scheme of the interaction of Aβ(25-35) peptide with a raft-like POPC/SM/Chol lipid monolayer

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Crowding effects on oligomeric enzymes: Kinetic analysis of the ALKP-catalyzed hydrolysis

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Studying enzymatic reactions in a medium that models the excluded volume inside the cell using synthetic polymers, provides us an insight on how metabolism is altered by the high concentrations of neighboring macromolecules surrounding any reaction. This issue is commonly referred as macromolecular crowding [1].
Kinetic behavior of Alkaline Phosphatase (ALKP) [2] and cooperative phenomena arising from it have been studied in dextran crowded media. A simple model to explain cooperativity, based on the Michaelis-Menten formalism, has been proposed. It allows us to discern how macromolecular crowding affects the cooperative behavior of the homo-dimer of ALKP. Results suggest that the effect of macromolecular crowding on this enzyme is both excluded volume and size-dependent, in accordance to what has been reported for other oligomeric enzymes such as Lactate Dehydrogenase (LDH) [3] or Malate Dehydrogenase (MDH) [4].
In particular, it has been found that v_{max} in crowded media is always lower than in dilute solution, regardless of the concentration (from 25 to 100 g/L) and size of the crowding agent (from 60 to 500 kDa). The maximum enzyme function decrease, and thus the maximum effect of excluded volume, is found for crowding agents of a size similar to the enzyme.

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Peptide-induced permeation of model membranes by antimicrobial peptidomimetics

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Antimicrobial peptides (AMPs) usually interact with cellular membranes, disrupting their integrity, and so, as a consequence of their target, the development of resistance to AMPs is much less likely to occur. Thus, the search for new membrane-active peptides is a current thrust in research and AMPs can be considered lead compounds for the development of a new class of antibiotic pharmaceuticals. Peptaibols are a family of naturally occurring AMPs that bear α,α-dialkylglycines such as Aib, Iva and Deg in their sequence [1-5]. These tetrasubstituted amino acids give peptides more defined conformations and more resistant to biodegradation as they are not recognized by hydrolytic enzymes [6].
The shortest member of the peptaibol family, Peptaibolin (Ac-Leu-Aib-Leu-Aib-Phol), has been the subject of recent *in silico* studies that suggest that its membrane affinity might be increased by replacement of Aib by other α,α-dialkylglycines, more structurally constrained and hydrophobic [7].
In the present communication, a set of Peptaibolin and several peptidomimetics incorporating unnatural α,α-dialkylglycines (Deg, Dpg, Ac_6c) were studied for their ability to interact and permeate model membranes from phosphatidylcholine/cholesterol, in different ratios. The permeation activity was monitored by fluorescence spectroscopy, following the release of encapsulated 6-carboxyfluorescein. The collected data suggested a relationship between the structure of the unnatural α,α-dialkylglycines (bearing longer and bulkier side chains) and the capacity of the corresponding peptidomimetic to permeate the model membranes.

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Coupling of conformational and ionization equilibria in a linear polymer. The site binding/rotational state (SBRIS) model

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The conformational and ionization properties of linear poly(ethylenimine) are studied by combining the site binding model (SB) with the rotational isomeric state (RIS) model, developed by Flory to calculate the conformational properties of neutral linear molecules [1]. The resulting approach (the SBRIS model) is used to rationalise the experimental poly(ethylenimine) titration curves. By fitting the experimental macroconstants, conformational and binding parameters are obtained. The obtained values are consistent with previous binding and structural information. The emergence of triplet interaction between protonated sites is explained as a natural consequence of the coupling between binding and conformational equilibria. When only nearest neighbour interactions are considered, transfer matrix techniques are used in the calculations. In order to account for excluded volume and long-range electrostatic interactions, Monte Carlo simulations are performed. The results indicate that at high ionic strengths, long-range interactions have a very limited impact on the titration curves. However, for long chains, they have a significant influence on the radius of gyration.

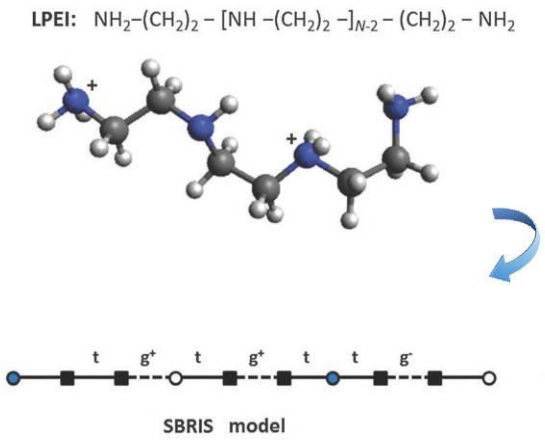

Figure 1: Possible ionization and the conformational state of a LPEI oligomer with four amine groups. In the SBRIS description, only three angles, those corresponding to energy minima of the bonds (i.e trans, gauche+ and gauche-), are considered. A possible roto-microstate is depicted

Acknowledgements: *This work was supported by grants from the Ministerio de Economia y Competitividad (CTM2009-14612 and CTM2012-39183) and the Generalitat de Catalunya (2009SGR465 and XRQTC)*

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New insight into the mixed micelles thermodynamics modelling

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Surfactant mixtures have wide applicability in fields such as cosmetics, biology, and pharmacology. The Regular Solution Theory (RST) [1,2] is the most frequently applied model for interpreting the behavior of surfactant mixtures. One of the major problems is to define the molar fraction of each surfactant in the aggregate since its value is fixed by the partition equilibria of the species between the aggregate and the surrounding medium, and only the total composition of the micellar solution is accessible to the experimenter. When the micelle is considered as a pseudo-phase and the activities of the micelle components are known for several mixtures, the Gibbs–Duhem relation allows the corresponding compositions of the aggregate to be determined. The regular solution approximation assumes that the excess entropy of mixing is zero. The classically employed RST applies the symmetric Margules-type formulations [3] in order to model excess molar thermochemica1 properties. Moreover, the extended version employed for multicomponent systems assummes that the ternary o higher order interactions can be described only by binary interactions [4,5]. This modelling approachs fails in representing many systems [6,7].
In the present work we present a novel approach based on the global minimization of the free energy constrained by the thermodinamics of mixed micellization, which simultaneously solves the whole model for all the compositions in the phase diagram. This procedures assures the application of the Gibbs-Duhem relation. Besides, we consider asymmetric Margules-type formulations which is a more general case than the symmetric ones. Within this frame, multicomponent systems are much better respresented considering higher order interactions. We used a well-known, state of the art, tool/language to solve the algebraic model, GAMS (General Algebraic Modelling System) [8], which provides the interface for the solving packages BARON and DICOPT [9].

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$CoO@MnFe_2O_4$ octahedron-shaped hollow nanoparticles

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Hollow and pierced magnetic nanoparticles, called magnetic nanocages, have large surface area, low density and peculiar magnetic properties. This makes Fe_3O_4 nanocages promising candidates for further applications in catalysis, hyperthermia, sensing and biodetection [1]. Nanocages were obtained using pre-obtained octahedron-shaped CoO nanoparticles as templates to first deposit a 3 nm magnetite (Fe_3O_4) layer [2-3]. After that, a solid-state reaction transforms the Fe_3O_4 into $MnFe_2O_4$, and the former solid nanoparticles into $CoO@MnFe_2O_4$ nanocages. Interestingly, TEM results show the octahedron shape is maintained along the process, despite the formation of large holes piercing the facets and the volume of the nanoparticle (Figure 1).

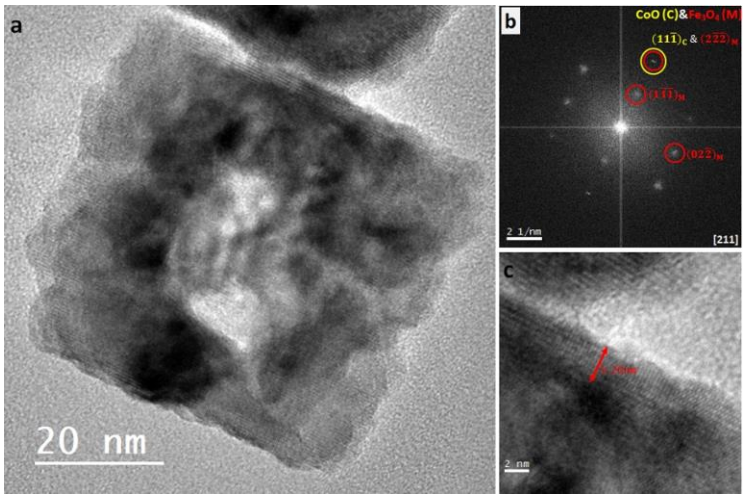

Figure 1. (a) TEM image of a $CoO@MnFe_2O_4$ nanocage in the [211] zone axis. (b) Fourier transform of the nanocage showed in (a) displaying spots corresponding to CoO and Fe_3O_4 aligned. (c) Image showing the thickness of the $MnFe_2O_4$.

Acknowledgements: *V. S. acknowledges funding from the Xunta de Galicia Regional Government (Spain) under project EM2014/035 (Emerxentes) and InBioMed.*

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Mechanism of formation of octahedron-shaped cobalt oxide nanoparticles and role of the Kirkendall effect

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Octahedron-shaped cobalt oxide nanoparticles can be prepared following the thermal decomposition of cobalt acetate as precursor.[1] Once synthesized, these nanoparticles undergo a kinetically controlled process, known as the Kirkendall effect, by which hollow nanostructures are produced.

The Kirkendall effect is a phenomenon based on the unbalanced inter-diffusion between two materials, produced by the difference in the diffusion rates of the elements or compounds involved. The direct consequence of the Kirkendall effect is the formation of voids in the bulk of the component with the faster diffusion rate, [2] consequence that can be exploited as a very effective approach for the preparation of hollow nanostructures.[3]

Then again, the Kirkendall effect can be coupled with many different chemical/physical processes, offering alternative routes for the synthesis of more complex nanostructures, on which studying unique properties, particularly magnetic, to improve performances for certain applications.

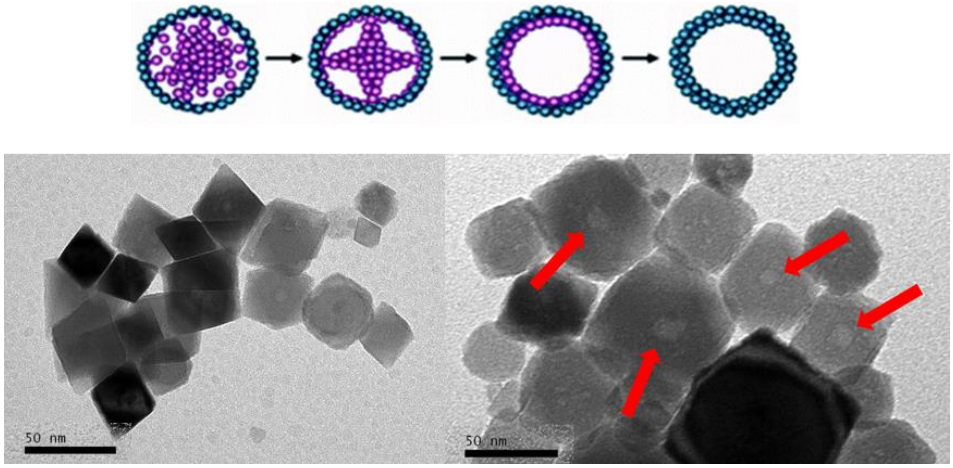

Figure 1: Schematic representation of the Kirkendall effect and TEM images of CoO nanoparticles with a 40 nm-average edge length at two different stages of this process (cavities shown by the red arrows).

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Nonhomogeneous silica promotes the biologically induced delivery of metal ions from silica-coated magnetic nanoparticles

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Herein we report the endocytosis of magnetic nanoparticles of two different transition metal ferrites, which are coated with silica. The variation in the cytotoxicity results, which correlate with the metal ions from the magnetic cores, stems from the inhomogeneity of the silica shell and consequent partial degradation of the nanoparticles once loaded into the endosomes of Caco-2 cells.

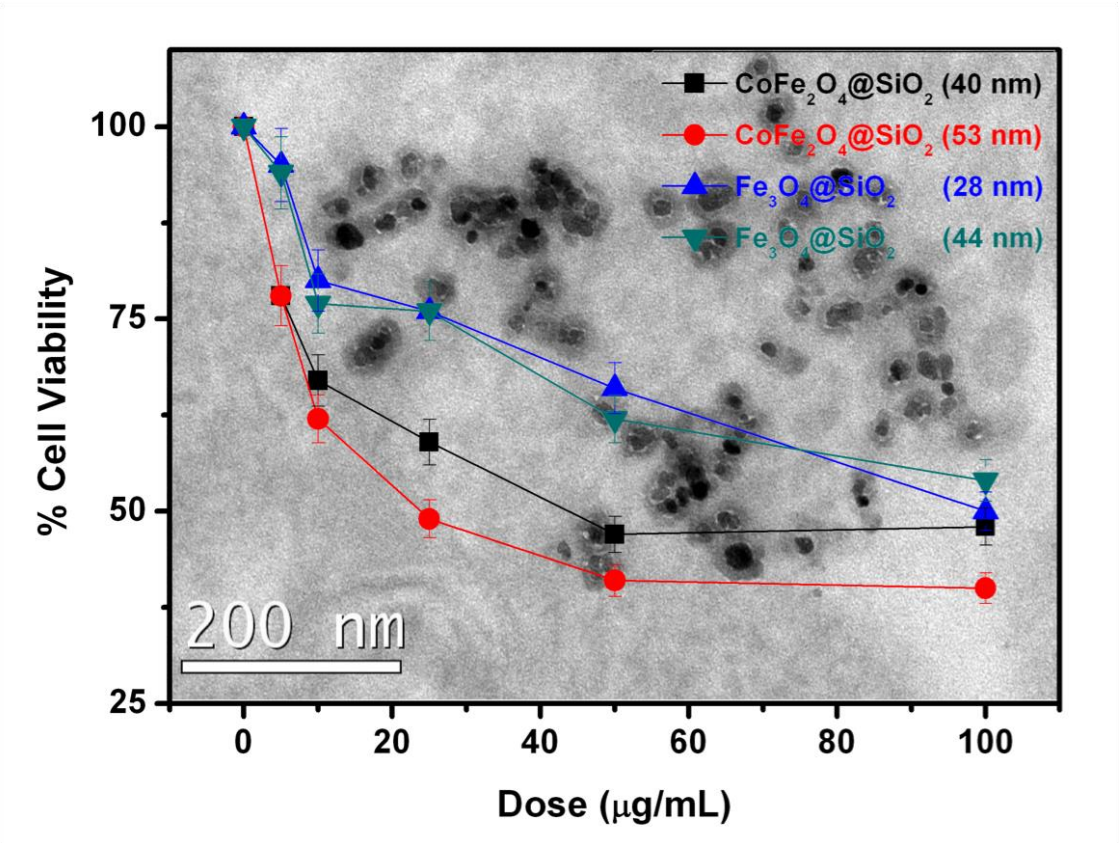

Figure 1: Comparison of cell viability for the silica-coated transition metal ferrite nanoparticles.

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Carbon nanotube-based magnetic nanocomposites as recyclable supports for enzyme immobilization

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In the last few years, the application prospects of enzymes as biocatalysts have been expanded beyond traditional industries into many new fields.[1] However, the use of free enzymes is often hindered by their short lifetimes and the difficulty of recovery and recycling. In this regard, their immobilization have been shown to improve their operational features, such as pH tolerance as well as heat and functional stability.[2-4] With this aim, the synthesis of a carbon nanotube-based magnetic nanocomposite has been accomplished in order to benefit from the high loading capacity and reduced diffusion limitations of this system together with its easy recycling when exposed to a magnetic field.[5] The obtained results show a remarkable stability and reusability of the biocatalysts through this architecture.

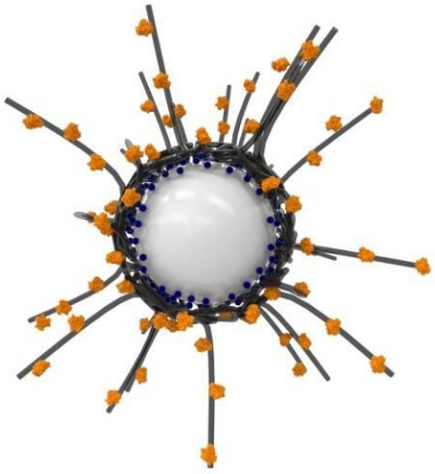

Figure 1: Schematic representation of the enzyme-loaded carbon nanotube-based magnetic hybrid nanocomposite synthesized herein.

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SERS study of corroles at the surface of colloidal metal particles

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The knowledge about vibrational and electronic properties of corroles is an important issue regarding their selection towards specific applications, such as, catalysis, photosensitization and photochemistry, and their use in assembly processes towards new nanomaterials.[1] Vibrational spectroscopy of non-symmetric macrocycles can become a very sensitive probe of their structure depending on the environment . However, the low oscillator strengths of vibrational transitions in corroles make IR and Raman measurements difficult for low concentrations in the analyte. Surface-enhanced Raman Scattering (SERS) spectroscopy might overcome this problem by recording the enhanced Raman signals upon adsorption of such molecular species at the surface of certain metals (typically gold and silver).[2] Thus, the adsorption of corrole onto the metal surface becomes a crucial point in the interpretation of the SERS spectra of corrole-containing systems. Various factors can affect the molecular adsorption on metal surfaces, such as the structure of the molecules and the mode of interaction between the adsorbates and the metal surfaces. In this way, the SERS behavior of a corrole precursor (TPFC) and, of a corrole containing a thiol function (TPFCSH) has been investigated, using colloidal gold and silver nanoparticles as the substrates. These results provide additional information that will complement our current studies on the development of corrole functionalized nanomaterials for photodynamic therapy, either as nanoparticles or their assemblies.

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Development of SERS substrates based on metal nanoparticles and natural fibers

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Since its discovery, surface enhanced Raman scattering (SERS) has been used as a tool to study certain organic molecules adsorbed at metal surfaces, typically of Au and Ag.[1] The development of high sensitive SERS substrates has been an important aspect in this context, which coupled to recent advances on portable equipment, can lead to important detection methods in multiple domains, such as in the detection of analytes of environmental interest. Our own interest in this field led us to develop efficient SERS substrates based on bionanocomposites aiming trace level detection [1-3]. This research describes our recent studies on new nanocomposites based on textile fibers and metal nanoparticles aiming the SERS detection of methylene blue (MB), a compound frequently used in textile dyeing and that might be found in aqueous wastes. First, citrate capped Au and Ag colloidal NPs of distinct particle size distributions (14-86 nm) have been prepared. Then, the deposition of the particles at the fibers' surfaces was accomplished by blending both components or by previously modifying the fibers with polyelectrolytes (layer-by-layer assembly method). In this research, several strategies were employed in order to assess the SERS activity of the ensuing bionanocomposites, such as the type of textile fibers (cotton, linen and silk), the metal nanophases (Au, Ag), particle size distribution and instrumental parameters such as the excitation source used for SERS. The SERS detection of MB using these bionanocomposites will be discussed by taking into account the properties of the substrates and analytical conditions, as provided by several techniques such as scanning electron microscopy (SEM), optical absorption measurements and confocal Raman microscopy.

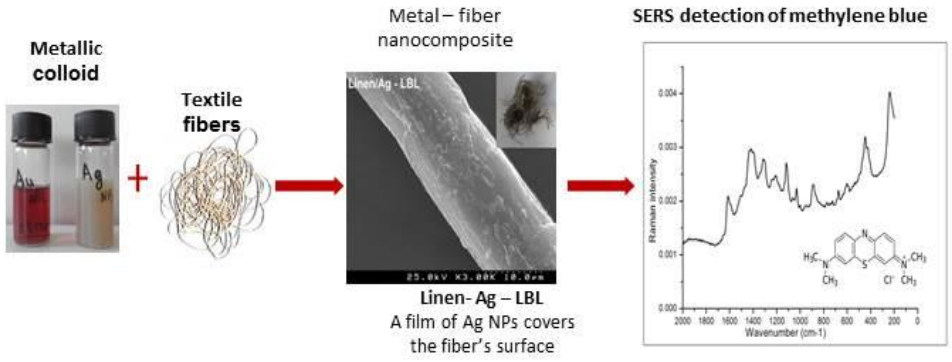

Figure 1: Synthesis of metal-fiber nanocomposites and SERS detection of methylene blue

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Synthesis of highly sintering-resistant silica-encapsulated sub-2nm gold clusters for catalytic applications

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Small gold nanoparticles dispersed on solid supports are well-known for being highly active and selective catalysts in a wide range of reactions.[1-4] Nonetheless, in spite of their outstanding performance their application at moderate-to-high temperatures constitutes a major challenge given the tendency of Au nanocatalysts to aggregate into fewer and larger particles. This work reports on the preparation of highly sintering-resistant silica-embedded gold nanoclusters in a SiO_2/AuNPs/SiO_2 configuration. The obtained results indicate that this architecture prevents nanoparticles movement and aggregation even at temperatures as high as 800 ˚C. The preservation of catalytic activity attained through this architecture makes of this methodology a rather general approach for the fabrication of metal nanocatalysts stable under realistic technical conditions.

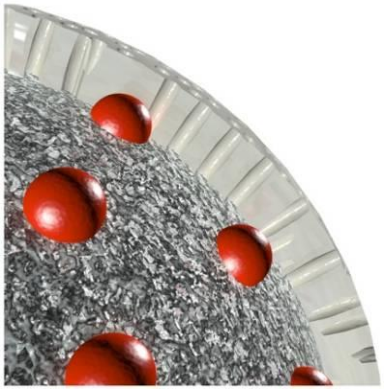

Figure 1: Schematic representation of the SiO_2/Au clusters/SiO_2 architecture which endows metal nanocatalysts with high thermal stability.

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Fabrication of taylor-made magnetic colloids using electrodeposition and soft lithography techniques

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Magnetorheological fluids are traditionally formulated by dispersion of spherical iron microparticles in a carrier liquid. However, recent work in this field demonstrates that the magnetic field response (e.g. apparent yield stress), sedimentation stability, and redispersibility of the colloid dramatically increases when magnetic particles are anisotropic in shape [1-3]. In this communication we prepare novel non-spherical magnetic particles using two different approaches. On the one hand, we employ Soft Lithography techniques (MicroTransfer Molding) to fabricate microcubes (25 × 25 × 25 microns) and microparallelepipeds (25 × 25 × 250 microns) (see Figure 1a-b). The idea is to form microparticles from an elastomeric mold using a colloidal magnetic suspension as a precursor which is solidified using UV radiation. On the other hand, we employ chemical template-based electrodeposition techniques to synthesize magnetic nanowires of controllable length (Figure 1c). In this case, the nanowires are fabricated by reducing Fe, Co and Ni cations in an electrolyte solution within the pores of an anodized aluminum oxide nanoporous membrane. This allows the formation of nanowires having 200 nm thickness and a controllable length up to a maximum of 60 microns.

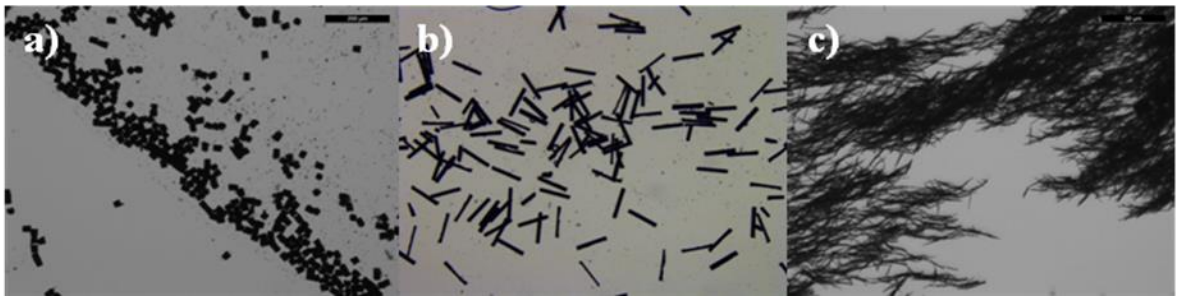

Figure 1: Typical optical microscopy pictures of some of the magnetic particles prepared in this work using Soft Lithography (a and b) and electrodeposition techniques (c).

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Synthesis and characterization of magnetite nanocubes formed by thermal decomposition

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In last decades, magnetic nanoparticles have been intensively studied due to their potential technological and biomedical applications [1]. Many of the synthetic techniques used to obtain magnetic nanoparticles have serious limitations in terms of costs and versatility, being thermal decomposition [2] one of the more robust and reproducible methods to obtain nanoparticles with a high purity and crystallinity while simultaneously achieving a great control over their shape and size. This work presents the synthesis and characterization of cubic magnetite nanoparticles obtained by thermal decomposition following the method proposed by Hyeon et al. [3] with important modifications. Iron (III) acetylacetonate was used as precursor, oleic acid as the stabilizing agent and benzyl ether as the liquid medium. We studied different parameters of the synthetic process such as the time and temperature of reaction, the heating rate, the molar ratio between the stabilizing agent and magnetic precursor and/or the presence of an oxidizing atmosphere, and their subsequent effects on nanoparticle formation and characteristics. In this manner, we have achieved an optimization of the synthetic process of cubic nanoparticles with full control over their size and shape. For example, slower heating rates and longer reaction times than those previously established result in well-defined and greater cubic nanoparticles spatial arrangements. Moreover, varying the molar ratio of stabilizing agent and magnetic precursor resulted in the production of particles with hexagonal and rhombic morphologies that can also have interest in future bio-applications, given their different structural and magnetic characteristics. The characterization of the obtained particles was carried out by scanning and transmission electron microscopy (SEM and TEM), vibrational sample magnetometry and X-ray and infrared spectroscopy.

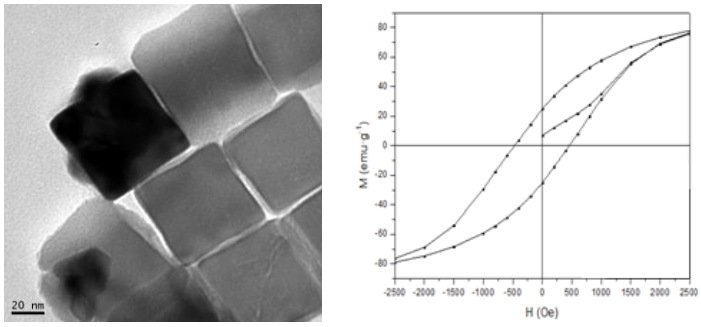

Figure 1: TEM image and magnetization-magnetic field curve at 5 K of magnetite nanocubes

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Hydroxyapatite: a novel material for the biofunctionalization of gold nanoparticles

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The use of nanoparticles (NPs) in biotechnology has experienced an extraordinary growth during the last years due to the importance of these novel materials in the diagnosis and treatment of different diseases. Actually, the design of more complex and sophisticated NPs is at the origin of the development of new fields of research like i.e. nanosensorics, hyperthermia or drug delivery. Nevertheless, the use of inorganic NPs for biological applications requires their stability in biological media for long periods of time both, in vivo and in cell culture. This requisite can only be accomplished after the successful surface functionalization of the original NPs with materials that prevent coagulation and ensure high biocompatibility in the desired biological media.
Here, we introduce the use of hydroxyapatite (HA) as an ideal candidate for the coating of Au NPs. This material is a natural occurring mineral that can be found in teeth and bone tissues in the human body, ensuring therefore a high degree of biocompatibility. In this work, we demonstrate that a continuous and homogeneous shell of HA can be grown surrounding Au NPs, and its thickness and crystallinity can be tuned by varying the temperature and reaction time.

Preparation of gold nanospheres using seed-mediated growth method

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Metallic nanoparticles show properties different from those of their corresponding bulk properties. Different metals ranging from noble to transition ones have shown interesting properties in different fields such as optics, catalysis, sensor, magnetisms, and others. The most common morphologies are nanorods [1]. Didodecyldimethylammonium bromide (DDAB,) is a twin–tailed cationic surfactant which in water forms lamellar liquid crystal at low concentration. This lamellar mesophase is preceded at very low concentration by formation of unilamellar vesicles which grow with concentration forming multilamellar liposomes. No micelles were detected. Its phase behavior has been studied in detail [2]. The synthesis of gold nanoparticles was performed following the technique proposed in reference [1]. The morphology of the nanoparticles (NP) was studied by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The formed structures were spherical with diameters between 10 and 20 nm, with some of 100 nm, which corresponds to the size of DDAB vesicles and liposomes at the working concentration. Separation of nanoparticles by centrifugation gave clusters of nanospheres glued by the remnant surfactant, while dialysis gave separate nanoparticles. Since liposomes are the precursors of lamellar liquid crystals, it was supposed that some nanolamellae might also form, and some of them were also observed. Both separation techniques have shown high reproducibility.

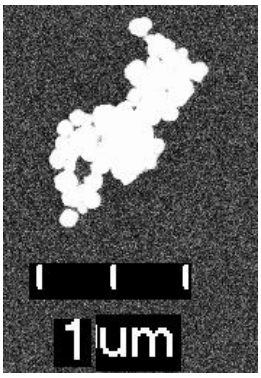

Figure 1: SEM of luster of gold nanospheres.

Acknowledgements: *ENS is an assistant researcher of the Argentine National Council of Scientific and Technical Research (CONICET). This work was supported by a grant from the German Scientific Exchange System, DAAD.*

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Development and characterization of DODAB:MO (1:2) liposomes encapsulating Bovine Serum Albumine (BSA) for targeted drug delivery

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The development of suitable carriers to enhance the bioavailability and therapeutic effect of drugs has been the focus of intense research in the recent years. Liposomes were among the first lipid nanoparticles to make a successful passage from concept to clinical application. The design of liposomes can be improved by tuning membrane properties and adding other components like bovine serum albumin (BSA), in order to achieve an improved loading capacity. BSA is a widely studied protein and a natural carrier due to its binding properties. It has a pH dependent charge and its drug binding abilities are also dependent on the pH of the medium [1] providing reversible sites for drug binding and releasing. DODAB:MO liposomes have been previously studied and have achieved successful results on DNA and RNA delivery to cells [2,3]. This work focused on the development and characterization of Dioctadecyldimethylammonium Bromide (DODAB) and 1-oleoyl-rac-glycerol (Monoolein (MO)) liposomes (DODAB:MO ratio 1:2) encapsulating BSA for drug delivery purposes.
Firstly, BSA was studied by Ultraviolet/visible spectrophotometry and fluorescence at relevant biological pH values (5.5 and 7.4), given the proteins pH-dependent properties. The stability of different BSA/lipid ratios was evaluated by Dynamic Light Scattering (DLS) and zeta potential assays. Ultraviolet/visible spectrophotometry and fluorescence were used to assess the encapsulation efficiency. The stability and encapsulation efficiency are different for each BSA/lipid mole ratio. The liposomes with less protein proved to be the most stable protein/lipid ratio with the highest encapsulation efficiency. The different BSA/lipid molar fractions also influenced the location of the encapsulated BSA molecules in different parts of the liposome, as assessed by the BSA partition coefficient determination and DLS and Zeta potential measurements. Also, circular dichroism revealed that BSA functionality was not affected by its encapsulation in the liposomes since secondary structure is maintained in an α-helix conformation.

Acknowledgements: *This work was supported by FEDER through POFC – COMPETE and by national funds from FCT through the projects PEst-OE/BIA/UI4050/2014 and PEST-C/FIS/UI607/2013 and PTDC/QUI/69795/2006. Marlene Lucio holds a position of Researcher FCT with the reference IF/00498/2012. This work is protected by Portuguese National Patent nº 104158-Refª DP/01/2008/10900-31/12/2008 and International Patent submitted: PCT/IB2009/05361-PPI nº40759/09.*

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Starch-based nanocapsules as potential carriers for topical delivery

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In recent years there has been an increased interest in developing improved delivery systems and also exploring new ways of using approved excipients, such as starch [1, 2]. The present study aims to develop a novel starch-based nanoparticulate carrier system for topical delivery of hydrophobic drugs. The role of the different factors that affect starch nanocapsules (StNC) particle size distribution prepared by the emulsification–solvent evaporation method was assessed using a quality by design approach, including a proposal for a design space.
The StNC were prepared by emulsion-solvent evaporation method, using Miglyol® 812 as the lipid component, Tween®80 and cetrimide as surfactants and modified starch as a polymer. Particle size analysis was performed using a Malvern Mastersizer 2000 coupled with a Hydro S accessory. The surface charge was determined by measurements of the ζ potential carried out with a Zetasizer Nano Z in water, at 25ºC. The process and the formula of the emulsions were optimized using a 3-factor Central Composite Design. For the process optimization, the independent variable was the stirring time and for the formula the % Tween®80 and the amount of lipid were the independent variables analysed. The data were analysed using the MOODE® software (Umetrics, Sweden) and statistical analysis was considered significant if the obtained $p < 0.05$.
This study describes the development of a new drug nanocarrier consisting of an oily core surrounded by starch shell (Figure 1). The rationale behind the design of this carrier was as follows: the oily core is intended to allocate significant amounts of lipophilic drugs whereas the external polymer shell is expected to improve the stability of the encapsulated drug. The starch was chosen as coating polymer because of its low cost and biocompatibility properties. Concerning the optimization results, the concentration of Miglyol® 812 had a distinct influence on the particle size distribution. Particularly, d(50) increases with the Miglyol® 812 concentration and decreases with an increase of % Tween®80 ($p<0.05$). However, for high lipid concentrations, an increase in the emulsifier amount is required because a decrease of o/w interfacial tension is related to a decrease in the size of the capsules. For d(50) and d(90) a negative correlation was observed for interaction between lipid content and percentage of surfactant which is in accordance with literature. All StNC formulations presented a ζ potential of ca. +33.6±6.7 mV, indicating that the nanocapsule suspensions are physically stable.
The design planning methodology has clearly shown its usefulness in this optimization formula, and is crucial for the understanding of StNC formation process.

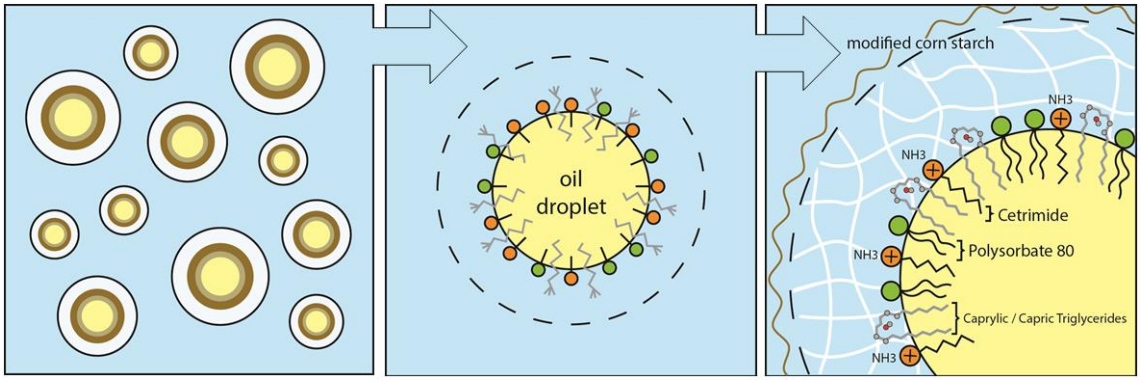

Figure 1: Structure of the proposed model for Starch-based nanocapsules.

Anisotropic hybrid nanoparticles for multimodal imaging and therapy

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One of the areas of nanotechnology that has captured great interest by scientific community worldwide is the development of nanoengineered multifunctional systems, which may be potentially used in a clinical strategy that simultaneously combine a (multi)diagnostic test and single or combined therapies based on the test results, the so-called nanotheranostic devices [1]. In this work, we present different hybrid nanoplatforms either with an inorganic or an organic core with anisotropic shape recently developed by our research group which are able to combine different elements in their structure to provide several simultaneous imaging (magnetic resonance (MR) and fluorescence imaging) and therapeutic (photothermal (PTT), photodynamic (PDT), chemo- and/or silencing therapies) capabilities in a single nanodevice. These nanodevices can be passively accumulated or targeted to specific receptors by suitable functionalization and are obsevred to be extensively accumulated in cancerous cell and tumors, exerting an enhaced dual imaging contrast and cytotoxic functions as observed in vitro and in vivo.

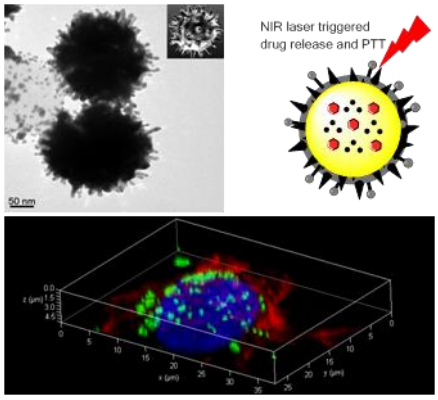

Figure 1: TEM image (left) and scheme of one of the proposed anistropic theranostic nanodevices. Below a 3D reconstructed fluorence image of nanohybrid localization (in green) inside a HeLa cell is shown.

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Gold nanoparticle dimers for plasmonic biosensing of proteins

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The development of biosensors is essential for application in clinical diagnosis, drug discovery, forensics, food inspection and pollution monitoring. Among the several possible targets for biosensing, there is particular interest in protein detection for biomedical applications because some diseases may be diagnosed from the presence of specific protein markers in blood, urine or body tissues [1]. Plasmonic metal nanostructures are interesting platforms for label-free detection of non-absorbing proteins using optical methods [2]. Here, we report on the preparation of dimers of spherical gold nanoparticles with high purity using DNA hybridization for particle assembly. Through this approach, it was shown that is possible to control the interparticle gap width for distances below 20 nm [3, 4]. Such narrow gaps are on the resolution limit of lithography techniques, but are accessible to self-assembly bottom-up approaches as shown here. The narrow gap widths are important to achieve large nearfields in the gap region, which will provide hot-spots for enhanced plasmonic biosensing.

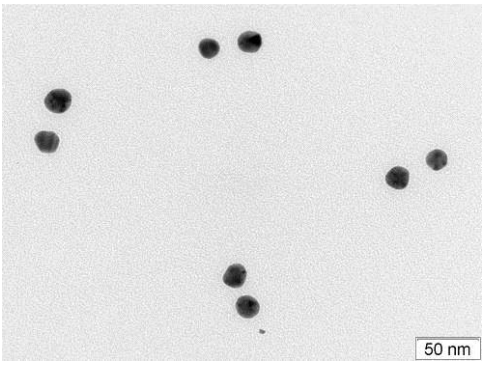

Figure caption: Gold nanoparticle dimers obtained from the assembly of particles with a size of 20 nm using a thiolated DNA with 60 base pairs.

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Folate-target nanodevices to activated macrophages for rheumatoid arthritis

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Methotrexate is the first line of treatment of rheumatoid arthritis. Since many patients become unresponsive to methotrexate treatment, only very expensive biological therapies are effective and increased methotrexate tolerance strategies need to be identified. In a previous European project NANOFOL, we performed the encapsulation of methotrexate in a new liposomal formulation using a hydrophobic fragment of surfactant protein conjugated to a linker and folate to enhance their tolerance and efficacy. We evaluate the efficiency of this system to treat rheumatoid arthritis, by targeting folate receptor β present at the surface of activated macrophages, key effector cells in this pathology. The specificity of our liposomal formulation to target folate receptor β was investigated both *in vitro* as *in vivo* using a mouse model of arthritis (collagen-induced arthritis in DBA/1J mice strain). In both systems, the liposomal constructs were shown to be highly specific and efficient in targeting folate receptor β. These liposomal formulations also significantly increase the clinical benefit of the encapsulated methotrexate *in vivo* in arthritic mice. A new project, called FOLSMART, will perform the preclinical development and the phase I clinical trials of this new liposomal formulation.

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