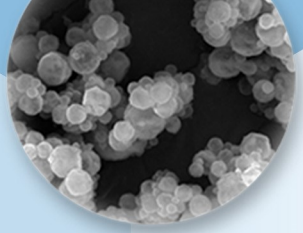
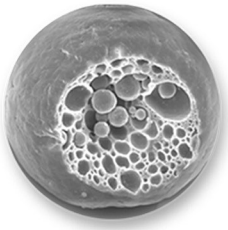
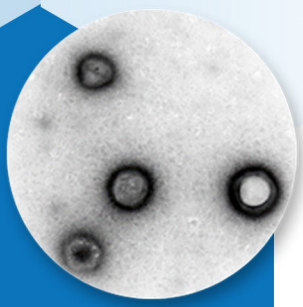
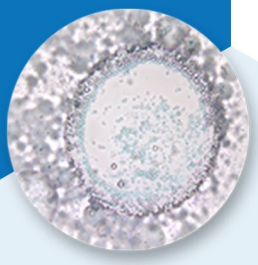



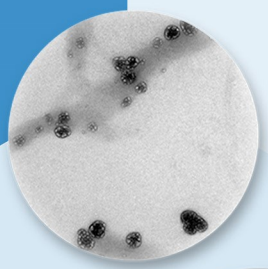
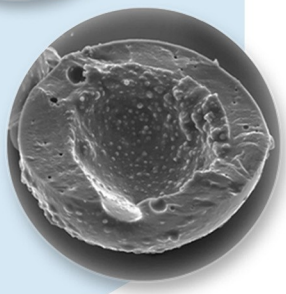
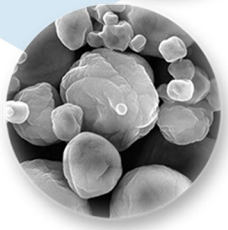
Formulation Days 2019

Advances in Formulation of Active Ingredients

January 10th and 11th, 2019

Lyon - France

Program

http://formulationdays2019.univ-lyon1.fr

UNIVERSITÉ DE LYON

Congrès Lyon 1

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LAGEPP
Laboratoire d'automatique, de génie des procédés, et de génie pharmaceutique.

SCF Société Chimique de France

Table of Contents

Welcome	4
Committees	5
Conference Venue	6
Gala Dinner	7
Program	8
Abstracts of invited speakers	12
Abstracts of oral communications	34
Posters list	59

Welcome

The national symposium "Journées de Formulation" is organized annually by the **Formulation Group** of the **Chemical Society of France (SCF)** to bring academic and industry experts and PhD and MSc students together to discuss the latest developments and ideas in the area of Formulation.

This year, as part of the 19th version of the "Journées de Formulation", **the Formulation Group of the French Chemical Society** and the **International Society of Drug Delivery and Pharmaceutical Technology (APGI)** have worked together to present a Europe wide conference entitled ***Formulation Days 2019: "Advances in Formulation of Active Ingredients"***. This event, located in Lyon, is dedicated to advances in the formulation of active molecules for applications in cosmetics and pharmaceuticals.

Locally organized by the Automation, **Process & Pharmaceutical Engineering Laboratory (LAGEPP)**, this conference aims to:

- be a forum where industry experts and universities researchers working in the field of formulations for health applications meet to exchange,
- allow students to meet the main actors in this field and develop their knowledge through access to presentations and poster sessions.

For this edition, the scientific program covers 4 themes:

- Materials & biomaterials
- Formulations for dermal administration or mucosal routes
- Nanoparticles: nanomedicine, toxicology & regulation
- Processes, characterization & modeling

Committees

Organization committee

Claire BORDES, LAGEPP, Université Lyon 1, Lyon
Sandrine BOURGEOIS, LAGEPP, Université Lyon 1, Lyon
Giovanna LOLLO, LAGEPP, Université Lyon 1, Lyon
Pierre LANTERI, ISA, Université Lyon 1, Lyon
Catharina KROLING, APGI, Châtenay-Malabry

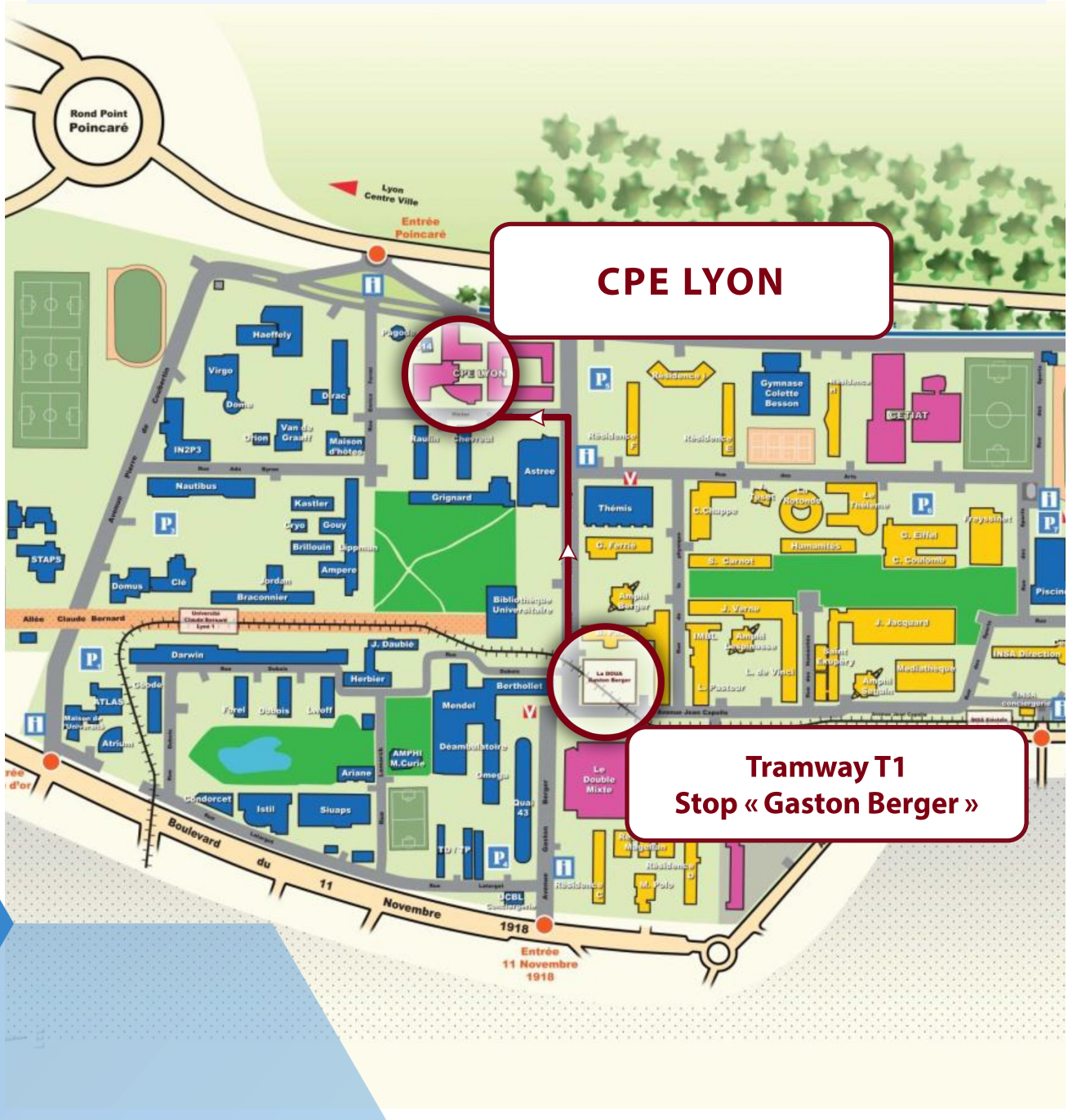
Scientific committee

Florence AGNELY, Institut Galien, Université Paris-Sud, Châtenay-Malabry
Amélie BOCHOT, Institut Galien, Université Paris-Sud, Châtenay-Malabry
Cécile BONNET-GONNET, IPSEN, Boulogne-Billancourt
Claire BORDES, LAGEPP, Université Lyon 1, Lyon
Sandrine BOURGEOIS, LAGEPP, Université Lyon 1, Lyon
Stéphanie BRIANCON, LAGEPP, Université Lyon 1, Lyon
Odile CHAMBIN, UMR PAM, Université de Bourgogne, Dijon
Alain DURAND, LCPM, Université de Lorraine, Nancy
Giovanna LOLLO, LAGEPP, Université Lyon 1, Lyon
Juergen SIEPMANN, APGI, INSERM U1008, Université de Lille, Lille

Conference Venue

École Supérieure de Chimie Physique Électronique de Lyon
Bâtiment CPE (Rue Victor Grignard)

Domaine scientifique de la Doua
3, rue Victor Grignard
69100 Villeurbanne
France

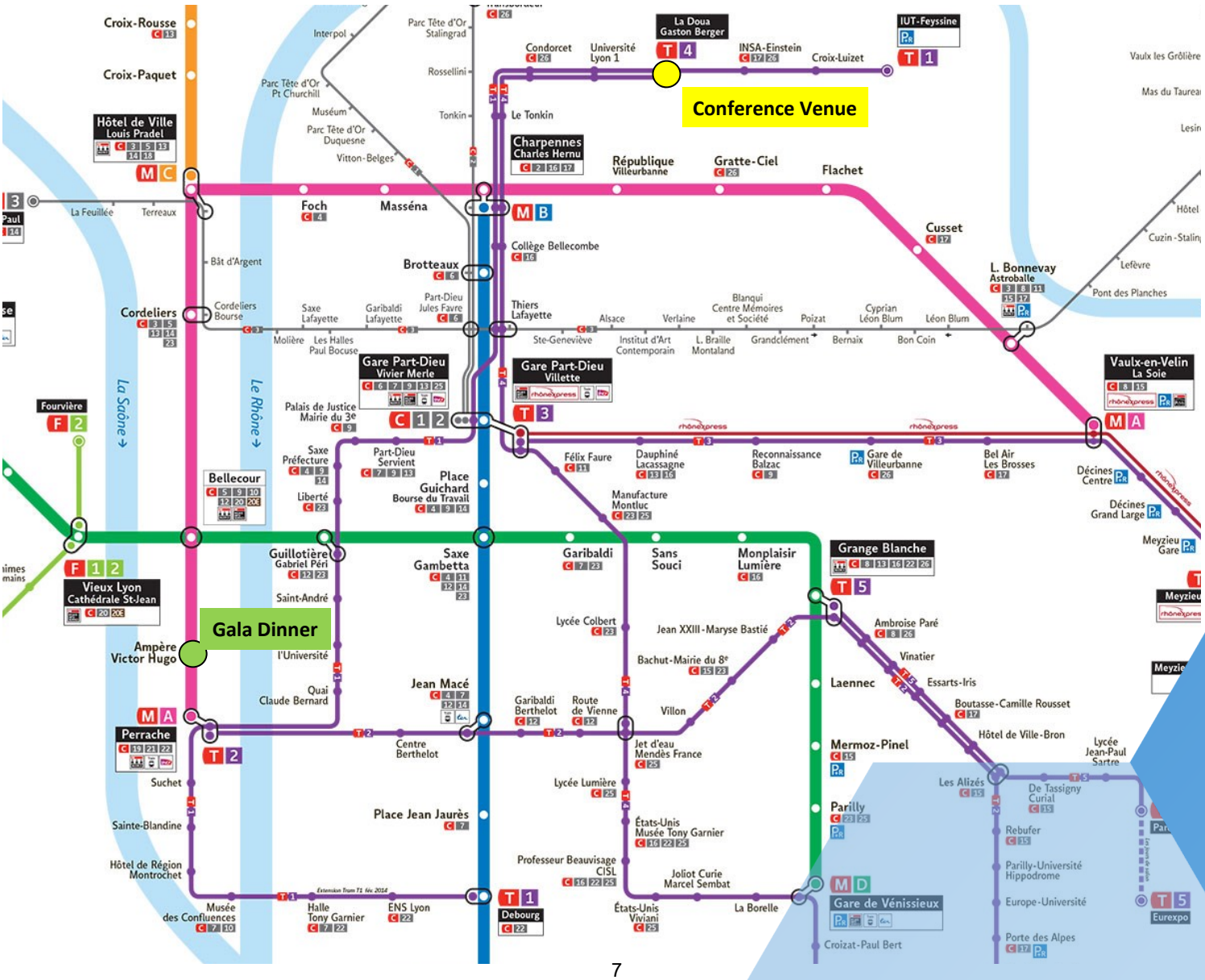


Gala Dinner

Restaurant Vatel Lyon
8 rue Duhamel
69002 Lyon

From the Conference venue with public transport : 35min

Take Tramway 1 to « **Charpennes** »
Take **Metro line A** to **Perrache** - Stop at **Ampère Victor Hugo**
Walk 9 min



DAY 1 : Thursday, January 10th 2019

8h30	*Registration*
9h00	Opening session - A. Durand (SCF), A. Bochot (SCF,APGI) and S. Briançon (Dir. LAGEPP)
	Session 1 : Materials, Biomaterials — ***Chairmen :*** **S. Briançon & A.Bochot**
9h30	**Pr S. Lecommandoux, University of Bordeaux, France - I.01**
	Biomimetic biofunctional and bioactive polymer-based vesicles
10h15	**Dr. C. Le Visage, INSERM, University of Nantes, France - I.02**
	Polysaccharide hydrogels and cell therapies: application to osteoarticular regenerative medicine
10h45	**F. Carton, University of Verona, Italy - O.01**
	Development of hyaluronic acid-based nano-complexes for pentamidine encapsulation
11h00	*Coffee break/ Posters and stand sessions*
11h30	**Pr F. Goycoolea, Procter Dpt of Food Science, University of Leeds, UK - I.03**
	New approaches to deal with antimicrobial resistance bacterial pathogens
12h00	**Dr L. David, University of Lyon, France - I.04**
	Physical chitosan hydrogels for biomedical applications
12h30	**S. Charaabi EBInnov, Cergy-Pontoise, France - O.02**
	Formulation of organosilicates particles complexed with bioactive molecules: adsorption and release studies
12h45	**J. Mahe, University of Bourgogne, France - O.03**
	Pectin modification by esterification with Octenyl Succinic Anhydride (OSA) to obtain new drug delivery systems
13h00	*Lunch / Posters and stand sessions*
	Session 2 : Formulations for cutaneous or mucosal administration — ***Chairmen:*** **C. Bonnet-Gonnet & F. Agnely**
14h30	**Pr M.A. Bolzinger and Pr Y. Chevalier, University of Lyon, France - I.05**
	New dosage forms for skin absorption
15h30	**Dr M. Eeman, Dow Corning, Belgium - I.06**
	Tuning Silicone Systems to Maximize the Release Rate and Skin Penetration of Actives
16h00	**E. Munnier, University of Tours, France - O.04**
	Core-shell encapsulation systems as a help to formulate active cosmetic ingredients and enhance their skin penetration
16h15	*Coffee break/ Posters and stand sessions*
16h45	**Pr. M. Lane, School of London, UK - I.07**
	Skin penetration enhancement - where do we go now?
17h15	**Dr O. Aubrun, L'Oréal, France - I.08**
	A new galenic for best sensory with high active concentration
17h45	**A.-C. Groo, University of Caen, France - O.05**
	Development of a nasal drug delivery composite nanosystem for Alzheimerâ€™s desease treatment
18h00	**M. Tarnowska, University of Lyon, France - O.06**
	Skin absorption of ions from thermal spring waters

20h00 **Gala Dinner**

DAY 2 : Friday, January 11th 2019

	Session 3 : Nanoparticles: nanomedicine, toxicology and regulation	***Chairmen:*** **O. Chambin & G. Lollo**
8h45	**Pr E. Fattal, Institut Galien Paris-Sud, Châtenay-Malabry, France - I.09**	
	Nanotoxicology a crucial step in order to bring nanomedicines to the market	
9h30	**Dr K. Remaut, Lab. of General Biochemistry and Phys. Pharmacy, Belgium - I.10**	
	Effect of peritoneal fluids and nebulization on the colloidal stability and distribution of intraperitoneally administered nanoparticles ou Potential and pitfalls of ocular mRNA delivery for neuroprotection	
10h00	**J. Balegamire, University of Lyon, France - O.07**	
	Iodinated Polymer Nanoparticles as a Contrast Agent for Computed Tomography using a Spectral Photon counting	
10h15	**L. Séguy, University of Caen, France - O.08**	
	Nanoemulsions as a useful tool for drug development	
10h30	*Coffee break/ Posters and stand sessions*	
11h00	**Dr F. Dalençon, SANOFI, France - I.11**	
	New adjuvants for vaccines	
11h30	**Dr S. Acker, BASF, France - I.12**	
	Nanoparticles for Suncare application	
12h00	**K. Matha, University of Angers, France - O.09**	
	A new 5-FU derivative encapsulated in lipid nanocapsules : in vitro evaluation on 2D and 3D models.	
12h15	**Dr J. Richard, IPSEN, France - I.13**	
	Therapeutic Peptide Delivery : How Can Nanosystems Help Address Present and Future Challenges	
13h00	*Lunch / Posters and stand sessions*	
	Session 4 : Process Engineering, characterization, modelling	***Chairmen:*** **A. Durand et C. Bordes**
14h15	**Pr C. Marquette, ICBMS, University of Lyon, France - I.14**	
	Printing for life sciences : 3d.FAB platform activities.	
15h00	**Dr V. Faivre, Institut Galien Paris-Sud, Châtenay-Malabry, France - I.15**	
	Lipid-based drug delivery systems: Influence of excipient structural properties on their preparation processes	
15h30	**R. Ramsch, FORMULACTION, France - O.10**	
	High shear microfluidic rheology rheometer Rheology optimization of ophthalmic eye drops	
15h45	**F. Dormont, Institut Galien, Châtenay-Malabry, France - O.11**	
	Translation of Nanoparticle Formulations from Lab to Industrial Scale Synthesis: The Case of Squalene-Adenosine Nanoparticles	
16h00	**E. Akanny, University of Lyon, France - O.12**	
	Development of Gold nanoparticles for the characterization of different bacteria by Surface Enhanced Raman Scattering	
16h15	*Coffee break/ Posters and stand sessions*	
16h45	**Dr C. Nouvel, University of Lorraine, France - I.16**	
	Product engineering : a combined approach for the design and the processing of drug delivery particles	
17h15	**Dr I. Pitault and Pr C. Jallut, University of Lyon, France - I.17**	
	Mass transfer studies through biological membranes. A chemical engineering approach.	
17h45	Alain Foissy Award for best poster (Formulaction)	

Abstracts of invited speakers

Biomimetic polymer self-assemblies as innovative biomaterials

Sebastien Lecommandoux[1]

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Univ. Bordeaux, CNRS UMR 5629, Bordeaux-INP ENSCBP, Pessac, France

Keywords. Block copolymers, self-assembly, biomimetic, polypeptide, drug-delivery

We report here an overview on the self-assembly in water of amphiphilic block copolymers developed at LCPO into different nanomedicines, mainly focusing on polymer vesicles, also referred as polymersomes, and their applications in loading and controlled release of both hydrophilic and hydrophobic molecules and biomolecules.
We pay special attention to polysaccharide and polypeptide-based block copolymer vesicles and their development in nanomedicine.[1-5] Indeed, the field of synthetic polypeptides has seen many significant advances in recent years, including studies on block and hybrid copolypeptides that form vesicles, fibrils, and other structures with potential applications in medicine and materials chemistry. However, the development of glycosylated polypeptides has not kept pace, primarily due to the inability to readily synthesize glycopolypeptides in a controlled manner. Glycosylation of natural proteins provides diverse functionality such as mediation of recognition events, modification of protein conformations, ect, that may find interest and application in biomedical field. In this context, we developed over the last years synthetic strategies for the design of glycosylated polypeptides and polysaccharide-polypeptide biohybrids with controlled placement of sugar functionality. We were especially interested in designing amphiphilic copolymers able to self-assemble into well- defined micelles and vesicles that can advantageously be loaded with drugs and present a surface with multivalent presentation of bioactive saccharides or oligosaccharides. The ability of these nanoparticles for different biomedical applications, from drug-delivery to inhibitor, will be presented. We especially evidenced the particular benefit of nanoparticles and their multivalency toward the interaction with biological receptors.[6-7]
Finally, our recent advances in using “biomimicry approaches” to design complex, compartmentalized and functional protocells will be proposed. Such a system constitutes a first step towards the challenge of structural cell mimicry and functionality, and may act in the future as an autonomous artificial cell that can sense and cure *in situ* any biological deregulation.[8-12]

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Polysaccharide hydrogels and cell therapies: application to osteoarticular regenerative medicine

Catherine Le Visage

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Keywords. Stem cells, Cell encapsulation, Intra-articular injection, Immunomodulation, Osteoarthritis

Osteoarthritis, a degenerative and inflammatory joint disease, is a major cause of pain and disability in the elderly. It consists of abnormal remodelling of joint tissues induced by inflammatory mediators and is characterized by cartilage erosion, thickening of the subchondral bone, and synovial inflammation [1]. Despite its incidence and dramatic impact on patients' quality of life, there is no effective treatment to date, and only analgesics are used, providing pain relief at best.

Mesenchymal Stromal Cells (MSCs), derived from bone marrow or fat tissue, have recently been proposed as a relevant therapeutic approach to prevent joint degeneration. Indeed, MSCs have the ability to secrete immunomodulatory, anti-fibrotic and anti-inflammatory factors. Despite encouraging chondroprotective effects in models of arthritic mice and rabbits, intra-articular injections of MSCs have important limitations: (i) massive cell death and (ii) a risk of cell leakage outside the joint space. Cell protection in biocompatible and permeable hydrogels[2] has been envisioned as a way to i) create a biomaterial barrier that permits the exchange of oxygen, nutrients, and prevent the infiltration of immune cells, and ii) enable a controlled local delivery of biological factors synthesized by the encapsulated cells. In OA context, one could expect that cell encapsulation could therefore protect cells from death, avoid cell effusion outside the articular space, and provide a suitable 3D microenvironment supporting the biological activity of MSCs.

First, we will briefly review conventional microencapsulation approaches with natural polymers (hyaluronic acid, alginate)[3] as well as droplet-based microfluidics ones[4], that have been reported for cell encapsulation. We will then present recent studies that we have performed using some of these techniques. We have demonstrated that alginate particles, obtained through a dropwise method, support human hMSCs viability and bioactivity[5]. Nevertheless, these particles remained too large (1.5±0.2 mm of diameter) to be injected into the joint space of small animals that are relevant models to assess the regenerative strategy.

We then investigated millifluidics as well as a micromolding method of cell encapsulation to obtain microparticles compatible with intra-articular injection through an appropriate needle (26G). A T-junction geometry device combined with a gelation in a w/o emulsion was used to generate monodispersed and reproducible particles, and the viability of encapsulated cells was estimated at 70% after 14 days. However, we experienced technical difficulties in the sorting/collection of particles, and the removal of the oil continuous phase. Polydimethylsiloxane (PDMS) chips containing circular micromolds (150µm of diameter) were then manufactured and human adipose stem cells (hASCs) were encapsulated in alginate. After manufacture, microparticle size and shape were assessed using phase-contrast microscopy and digital imaging. We obtained cylindrical alginate particles with a diameter of 150±0.7µm. Mechanical characterization was performed using a Microsquisher (CellScale), where microparticles were submitted to a compressive force. The particles presented a Young Modulus of 3.7(± 0.9) kPa, regardless of the initial cell density. To evaluate cell viability, cell number and cell metabolic activity were determined using CyQUANT and Presto blue assays, respectively. We demonstrated that cell number and metabolic activity remained stable for 7 days after encapsulation, and injection through a 26G needle had no impact on cell viability. Finally, we confirmed that encapsulated cells could sense and respond to a pro-inflammatory environment (TNF-α, INF-γ, pathological synovial fluids) by secreting prostaglandin E2 and indoleamine2,3-dioxygenase. These encouraging results highlight the therapeutic potential of encapsulated MSCs in OA treatment.

Presenting author: Le Visage, Catherine, catherine.levisage@inserm.fr

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Presenting author: Le Visage, Catherine, catherine.levisage@inserm.fr

New approaches to deal with antimicrobial resistant bacterial pathogens

Francisco M. Goycoolea[1,2]

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[2] University of Münster. IBBP, Schlossplatz 8, Münster, 48143, Germany

Keywords. Antimicrobial resistance, quorum sensing, nanocapsules, nanoparticles, chitosan

Antimicrobial resistance (AMR) is a global rampant human health and animal production problem that has been placed at the top of the public health agendas worldwide. AMR calls for urgent and novel multidisciplinary approaches that lead to reduce the use of antibiotics to tackle infections. This challenge entails developing effective alternative treatments and "soft" nanotechnology approaches can be promising. To this end, in a series of collaborative studies, we have addressed the quorum sensing (QS), antiadhesion and biofilm inhibitory activity in Gram-negative bacteria for biopolymer-based nanoparticle and nanocapsules formulations. The average hydrodynamic diameter of these systems ranges from d.~150 – ~270 nm and the ζ-potential ~+20 – ~+50 mV. To this end, we have examined both drug-free and loaded systems. We have demonstrated experimentally and using computational simulations, that drug-free chitosan-based oil-core nanocapsules bind "stoichiometrically" to the bacterial envelope of an *E. coli* Top 10 GFP reporter strain [1,2], as the consequence of bacterial aggregation while reducing the QS activity [2]. Also, ionotropic gelled chitosan nanoparticles stabilised by a covalently crosslinked shell, exhibit a similar bioactivity. Different type of lipophilic phytochemicals, namely cinnamaldehyde [1,3], quercetin [4,5] and baicalein [4], have been loaded in nanocapsules and nanoparticles. In general, these systems associate >~60% of the active payload and remain stable against aggregation in bacterial culture media. Our results consistently show that the association of different type of bioactive phytochemicals results in modulation of the anti-QS and enhancement of the anti-biofilm activity [4]. Under a completely different approach, we have developed a formulation of nanocapsules loaded with curcumin and comprised by dextran sulphate (d.~227 nm, ζ-potential ~-50 mV). We have shown proof-of-concept of the *in vitro* activity of this formulation to inhibit the adhesion of *Helicobacter pylori* to AGS stomach cells in a physiologically relevant context. The translation of these findings to conceive novel approaches that contribute to fight AMR with relevance in pharmacy, veterinary and food production, is yet to be fully realised.

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Chitosan Physical hydrogels for male fertility preservation

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Keywords.
Compartmented hydrogels, chitosan, germ cells, ex vivo spermatogenesis

The design and processing of materials for cell culture is a fertile field of research for tissue engineering or more fundamental cellular biology studies. In particular, hydrogels are particularly attractive since they partly mimic the high hydration level and the mechanical properties of the natural extracellular matrix. A classical strategy is to tune the cell/hydrogel interactions to induce proper cell response and phenotype (Higuchi 2012): substrate surfaces become a tool to control cell response through the control of the topology and surface chemistry. Several recent reviews highlight the interest of using different type of hydrogels (natural, synthetic, or mixes) for culturing cells on planar surfaces (2D) or entrapping cells within the hydrogel (3D cell cultures). Several hints can be used to functionalize the hydrogels (RGD grafting, incorporation of trophic factors) or process property and concentration gradients or structural gradients within the hydrogel structure. The processing of hydrogels with 3D printers also offers new opportunities for the fabrication of new cellularized systems with full control of the cell seeding topology, when the processing route is compatible with cell survival.

In this context, we proposed the new concept of hydrogel microbioreactors for cell culture within 3D template architectures (Ladet 2008), *i.e*. chitosan physical hydrogels with internal compartments (such as multimembrane hydrogels or alternatively tubes sealed at the ends) to host cells or tissue fragments. Applications in the tissue engineering of cartilage were particularly attractive (Ladet 2008; Ladet 2011) and lately microbioreactors were successfully optimized and used for germinal cell maturation, yielding complete human, monkey and rat ex-vivo spermatogenesis from testicular tissue (Perrard, Durand, David 2013; Perrard 2016). Such achievements have been performed in collaboration with the Kallistem company (http://www.kallistem.com), IGFL ENS Lyon and IMP CNRS 5223.

We hypothesize that within such hydrogel bioreactors, cell may survive, amplify or differentiate thanks to the highly ‘porous’ structure of hydrogels (such multiscale structure was investigated in details, see Sereni 2017) ensuring diffusion across the hydrogel membranes. In addition, the chemical structure of the hydrogel is partly mimicking the glycosaminoglycans of the ECM, and the stability and biocompatibility of Chitosan physical hydrogels is compatible with long culture times in cell culture media (up to 8 months). Most important, the isolation of the cells from the liquid culture medium flushing maintains higher levels of intra and intercellular signaling and thus induces stronger maturation effects (human complete spermatogenesis and folliculogenesis are not possible in standard 2D or 3D conditions). For this reason, the use of compartmented bioreactors represents a breakthrough in cell culture and tissue engineering in vitro, but may also open new ways for the design of new glandular organs with in vivo applications when the bioreactors are designed as implants.

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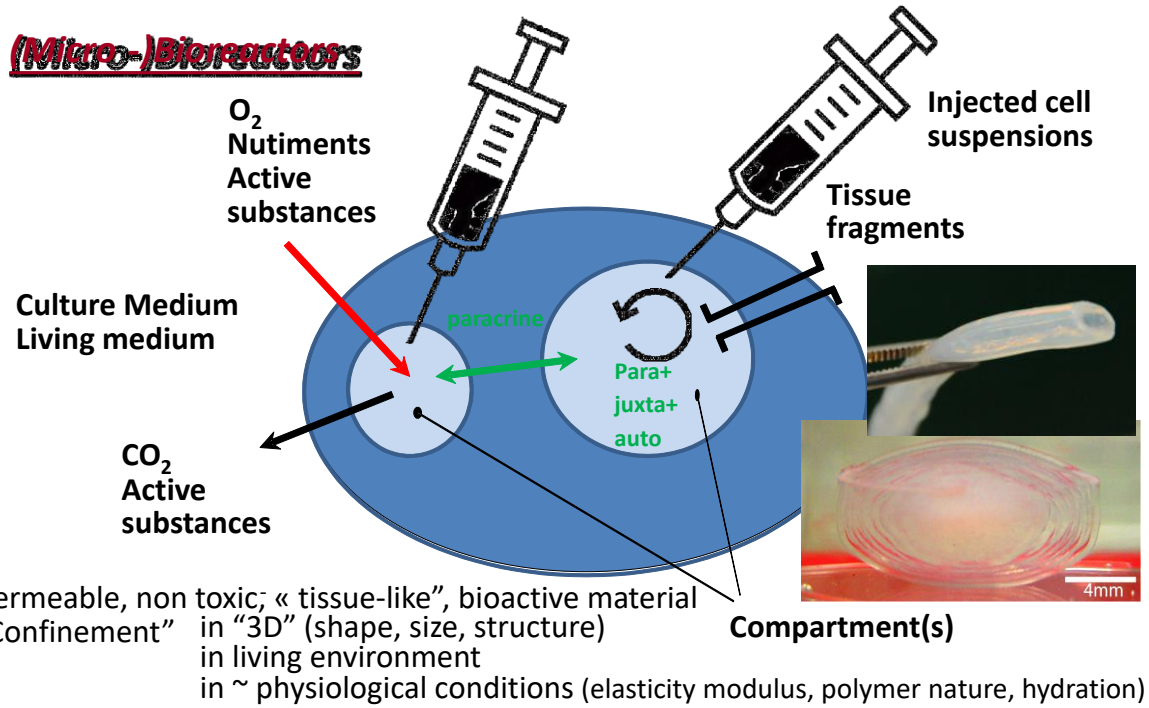

Figure 1. General scheme of the compartmented micro-bioreactors. Cells of different types can be inserted in the different compartments (Ladet 2011). The hosting system must preserve the diffusion of metabolites, oxygen, nutrients and the biomolecules implied in autocrine, juxtacrine, paracrine and cell messaging's. Such signalling is hypothesized to be enhanced due to a 'confinement effect' within bioreactors leading to specific maturation or differentiation effects.

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Organic and inorganic nanoparticles as Pickering emulsion stabilizers and their use for skin delivery of drugs

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Keywords. Pickering emulsions, Skin absorption, Cosmetics, Dermopharmacy

Pickering emulsions

A short presentation of the physical chemistry of Pickering emulsions is given first [1]. The importance of wetting conditions by water and oil at the surface of solid particles is outlined. The control of droplet size and the rheological behavior of Pickering emulsions are explained. Finally the benefits of working with Pickering emulsions instead of classical surfactant-based ones are highlighted.

Skin absorption from Pickering emulsions stabilized by inorganic nanoparticles

Pickering emulsions show specific behavior regarding skin penetration and transport of encapsulated drugs. *In vitro* drug penetration into skin has been assessed for both o/w and w/o type Pickering emulsions in comparison to classical emulsions stabilized by emulsifier.

O/w Pickering emulsions containing a hydrophobic drug (retinol) cause higher storage of the drug inside *stratum corneum* than the surfactant based [2].

W/o Pickering emulsions containing a hydrophilic drug (caffeine) improved cutaneous absorption to the deep layers of skin. Mechanistic aspects were investigated from measurements of release rate from emulsion droplets, adhesion energy of water droplets to skin surface, skin penetration of silica particles, and adsorption of caffeine to silica surface. The major contribution disclosed from measurement of adhesion energy of water droplets to skin surface turns being the higher affinity of Pickering emulsion droplets for the skin surface [3].

So o/w and w/o emulsions can be stabilized by Pickering emulsions; and their skin absorption behavior is quite different of surfactant-based emulsions [4].

Skin absorption from Pickering emulsions stabilized by organic nanoparticles

Biocompatible and biodegradable polymer nanoparticles and Pickering emulsions stabilized by such nanoparticles have been developed for their application to formulation and evaluated regarding their ability to deliver active substances to skin.
Using biodegradable nanoparticles decrease the toxicity risks observed with a lot of common chemical surfactants and inorganic particles, and it is expected to provide controlled release of drugs incorporated either inside the polymeric nanoparticles or in the oil phase of emulsions. It is well-suited for both cosmetic and pharmaceutical applications.

Polymer nanoparticles made of the block copolymers poly(lactide)-*block*-poly(ethylene glycol) (PLA-*b*-PEG) or poly(ε-caprolactone)-*block*-poly(ethylene glycol) (PCL-*b*-PEG) are micelle-like nanoparticles of 30 nm diameter that can be loaded in their PLA or PCL core with hydrophobic drugs. Pickering emulsions are stabilized by such solid particles in place of emulsifier; they are emulsifier-free emulsions [5].

Polymer nanoparticles and Pickering o/w emulsions loaded with all-trans retinol as a model hydrophobic drug have been assessed for topical administration as new dosage forms. The release profile of retinol and its distribution within the skin layers, *stratum corneum*, viable epidermis and dermis, was investigated and related to the partition of retinol between polymer nanoparticles and oil.

The behavior of different formulations: aqueous suspension of PLA-*b*-PEG and PCL-*b*-PEG block copolymer nanoparticles, surfactant micelles as reference, Pickering emulsions stabilized by block copolymer nanoparticles containing or not retinol, and a surfactant-based emulsion as reference, were compared in order to assess the block copolymer nanoparticles with respect to surfactant micelles [6], and their effects in Pickering emulsions as new dosage forms for topical application [7]. Such

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comparative investigation was performed *in vitro* on excised pig skin in Franz diffusion cells. The penetration of all-trans retinol contents in each skin layer was evaluated after 12 hours and 24 hours exposure to the formulations.

The permeation rate and storage of all-trans retinol in the different skin layers allows the evaluation of the benefits of the new dosage forms based on block copolymer nanoparticles and Pickering emulsions [8].

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Tuning Water-in-Silicone Systems to Maximize the Release Rate and Skin Penetration of Actives

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Keywords: Active delivery, Silicone ingredients, Water-in-silicone formulations, Anhydrous systems

The stratum corneum (SC), the skin's outermost layer, is the principal component of the cutaneous barrier acting as a physical barrier controlling the percutaneous absorption of external substances. The SC is composed of stacked corneocytes embedded in an extracellular matrix mainly constituted from ceramides, cholesterol and fatty acids. This matrix represents the sole continuous region of the SC and the preferential pathway to deliver cosmetic actives into the skin.

Although the diffusion of a molecule through the SC depends on its concentration, physicochemical properties, degree of solubility, interaction with skin components, potential metabolic and photochemical transformation within the skin, the composition of a topical formulation influences greatly both the bioavailability and release rate of the active from the formulation.

The present work aimed at demonstrating that a formulation can be tuned around different classes of silicone-based excipients to maximize the release rate and skin penetration profiles of anti-ageing actives formulated into water-in-silicone and anhydrous systems.

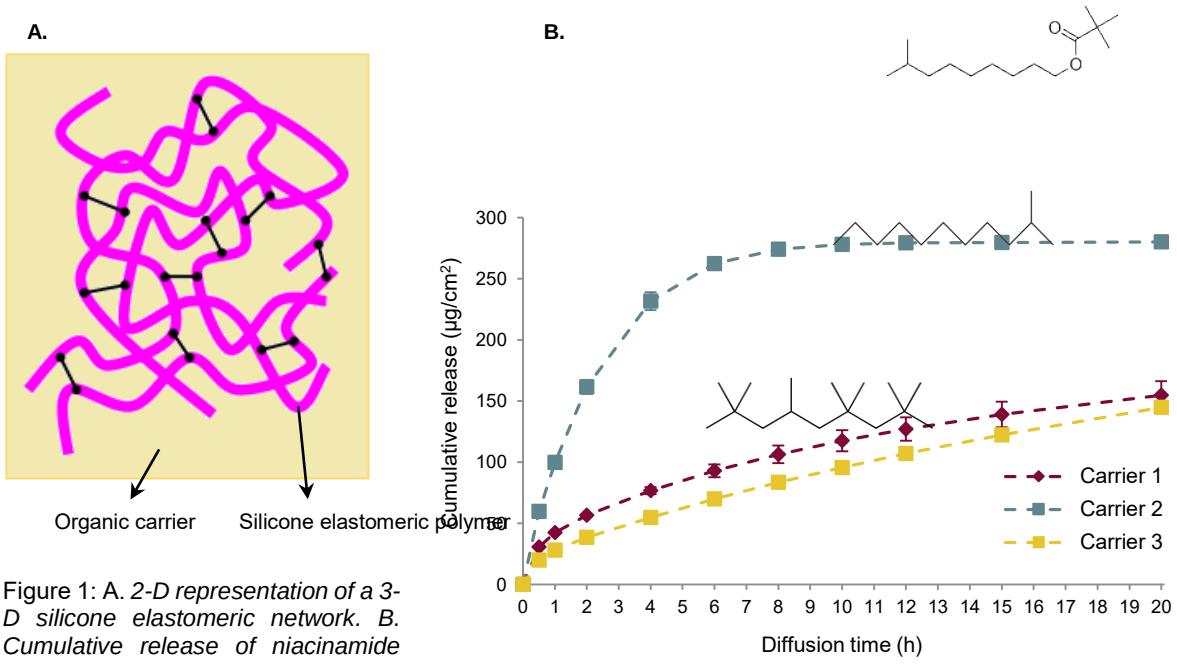

Figure 1: A. *2-D representation of a 3-D silicone elastomeric network. B. Cumulative release of niacinamide formulated into three water-in-silicone systems varying by the nature of the organic carrier used to disperse a silicone elastomeric polymer.*

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Skin – Mission Impossible?

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Keywords. Skin, formulation, penetration, enhancement, excipient

The skin has evolved to keep water in and other xenobiotics or foreign substances out. The outer layer, the stratum corneum, is a unique membrane that is about a sixth of the thickness of a piece of paper. It is composed of dead cells that are filled with keratin and are very dense in nature. Today we understand that it is this thin membrane that is the major barrier to effective targeting of actives from cosmetic formulations. The subject of this presentation is the ability of skin formulations to target skin.

Targeting of actives to specific regions of the epidermis and dermis is the 'holy grail' for a topically applied formulation. However skin penetration of most actives does not exceed more than 2-4% of the applied amount. Partly this reflects a lack of focus on the vehicle and a lack of awareness that the fate of the active is inextricably linked to the carrier components of the formulation. Skin formulations have also suffered from the concept that there is a generic "do-all formulation". However cosmetic actives vary considerably in water solubility and lipophilicity. No single formulation can address the competing requirements of such an array of diverse compounds. Instead, consideration must be given to the solubility of the active in the vehicle components as well as the residence time of those vehicle components in the skin. Rational formulation design is predicated on identifying those vehicle components which are optimal for a specific active. We have recently demonstrated the utility of this approach for niacinamide using both *in vitro* and *in vivo* studies.

Because of the nature of diffusion it is challenging to target specific strata of the skin. Diffusion occurs as a result of a concentration gradient and therefore it is difficult to modify a formulation such that the active will be concentrated in the viable epidermis or dermis. Highly lipophilic materials will concentrate in the stratum corneum because of the low partition into the underlying tissue which is predominantly aqueous in nature. Where the vehicle is volatile in nature there is also the risk of inadvertent "stranding" of the active in its crystalline form in the outer layers of skin. Such an event means that the active is not available for further movement though the skin and nor is it able to exert a biological effect as it is no longer in solution in the skin. Although there may be slow dissolution of the active in skin lipids it is far more likely to be lost through desquamation than to achieve meaningful effects at its intended target site.

Nanotechnology continues to revolutionise many aspects of modern life. However, the role of nanotechnology in actual skin penetration of actives from cosmetic formulation has not been fully exploited to date. Mathematical models confirm that the rate of diffusion of particles of diameter >1nm is negligible. Promising applications of these colloidal systems are in improvements in sensorial and optical properties of formulations. A contribution from the hair follicles to nanoparticle delivery also suggests a role for the technology in specialised products to target these appendages.

It would be very advantageous to optimise topical formulations for a number of reasons. Where the active is expensive there are obvious cost of goods implications but for some compounds there are also safety issues. For example retinoids need to be delivered in sufficient amounts to have an effect but delivery of too much may cause an irritant response. The perception that more of an active in a product is necessarily better will not hold unless the thermodynamic activity of the active in that formulation is already optimal. This optimisation may be achieved using conventional strategies such as penetration enhancers and/or super saturation and will be particularly suitable for conditions where the skin barrier has not been compromised.

Finally, advances in the range and sensitivity of analytical techniques available to pharmaceutical scientists are providing better insights into vehicle effects on skin delivery of actives. Building on these findings we should be optimistic about our ability to engineer and design better formulations for the benefits of our consumers and for our patients.

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Swollen Alpha Gel: A new formulation with high humectant concentration for a unique sensorial performance

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Keywords: Swollen alpha gel, humectant, X-Ray Scattering, rheology

Long lasting hydration and anti-aging cares remain key consumers' needs for skincare products all over the world. With aging or when exposed to external aggressions such as dryness, UV light or cold temperatures, the upper skin layer, the Stratum corneum, gets stiffer. When it stiffens, it induces tightening at the skin surface. At a deeper level, numerical simulation shows that it increases also compression in the folding zones, with potential dermis degradation [1].

Recent clinical studies have shown that daily application of a concentrated solution of humectant, such as 20% glycerol, allows to get a clear leap of performance on immediate and long-term hydration scores (corneometry), skin radiance, as well as skin suppleness and crow's feet wrinkles (4 months). However, formulating a humectant at a high concentration remains a real challenge to offer consumers with a pleasurable and unique sensorial experience prone at conveying high skincare benefits, without stickiness and greasiness.

A unique sensorial performance was achieved with a new emulsion technology based on a Swollen Alpha Gel (SWAG) and high humectant concentration. The product is structured by a network of soft crystallized platelets, based on fatty alcohols and a mild anionic surfactant, and highly swollen by water and humectant. The rheology of the texture is characteristic of a weak gel with a shear-thinning behavior, consistent with an easier application and a higher slippery effect determined by sensorial tests, as compared to classical emulsions. As observed in Small Angle X-Ray Scattering experiments, SWAG can still be present in product coating after drying, which also provides a great potential for slow **r**elease of active ingredients for long lasting benefits.

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Nanotoxicology, a crucial step in order to bring nanomedicines to the market

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Keywords. Nanomedicine, Distribution, Toxicity, Regulation

Nanotechnology has strong potential for targeted delivery of active drugs or contrast agents. In most cases, these systems consist of self-assembled biodegradable polymers or lipids considering that their degradation products must be well tolerated and easily removed from the body. More recently, small diameter inorganic particles have proved to be of interest, particularly in imaging, which has led to major development of research in this field. For a nanomedicine, the regulation is *a priori* the same as for all other drugs. However, there are several recommendations issued by the ANSM (French National Agency for the Safety of Medicines and Health Products) or EMEA (European Medicines Agency) or FDA that have been submitted. These recommendations are based on the knowledge gained over the last twenty years about the fate of nanoparticulate drugs after administration to animals or humans. Many parameters have been identified that modulate pharmacokinetics and fate of nanomedicines. It's basically their size, shape, chemistry and surface properties. Most of nanomedicine applications concern the delivery of molecules active in cancer or inflammation, two pathologies in which the vessels are permeable to small diameter particles. However, all other organs targeted by nanomedicines, among which the liver need to be considered for toxicity at cellular and tissular level. Finally, even local delivery needs deep investigation into toxicological aspects. For instance, lung delivery of nanomedicines for the treatment of infectious or inflammatory diseases needs to integrate a wide exploration of nanoparticles deleterious effect over this tissue. The presentation, in its overall, will try to establish relations between, physico-chemical properties of nanomedicines, their biodistribution and toxicity in liver targeting and lung delivery.

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Potential and pitfalls of ocular mRNA delivery for protein expression in the retina

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Keywords. Müller cells, lipid nanoparticles, mRNA, modified mRNA, pDNA, ocular, intravitreal, subretinal, drug delivery, retina

Purpose: Ocular delivery of messenger RNA can result in the local expression of proteins in the retina The local expression of neurotrophic factors by the delivery of messenger RNA (mRNA), for example, could be beneficial to halt retinal cell degeneration. Also for gene editing (e.g. CRISPR/cas9) the ocular delivery of mRNA encoding for Cas9 can be of interest. mRNA therapeutics have recently experienced a new wave of interest, mainly due to the discovery that chemical modifications to its molecular structure could drastically reduce its inherent immunogenicity and perceived instability (1,2). Nevertheless, the efficient delivery of mRNA remains a major bottleneck and has not even been evaluated for ocular applications. Here, we evaluated the potential of mRNA delivery to the retina after intravitreal and subretinal injection. Therefore, mRNA modifications and nanoparticles were first optimized in vitro to transfect retinal cell types such as Müller cells and RPE cells. Then, selected formulations were injected *ex vivo* and *in vivo* to assess the amount and extent of protein expression.

Methods : MIO-M1 cells and RPE cells were seeded in 24-well plates. For transfections in the presence of vitreous, MIO-MA cells were seeded in a transwell system. Transfections were performed in OptiMEM® or in the presence of vitreous isolated from fresh bovine eyes, using eGFP encoding mRNA formulated in the commercial lipid-based carrier MessengerMAX®. The duration and level of protein expression was followed by flow cytometry for different mRNA concentrations and modifications. Also, the carriers' mobility in the vitreous was determined by single particle tracking (SPT) (3). For *in vivo* experiments, an intravitreal or subretinal injection was performed in mice with naked or complexed mRNA and GFP expression was assessed after 1 or 7 days. Also the potential to transfect *ex vivo* bovine retinal explants was evaluated using luciferase expressing mRNA. Bovine retinal explants were prepared in the conventional way (after removing the vitreous) or, alternatively, bovine retinal explants were prepared with an intact vitreoretinal interface, according to a protocol recently developed in our lab (4).

Results : MessengerMAX did not fully complex the mRNA, but resulted in nanoparticles that were able to transfect >80% of Müller cells and RPE cells in culture. The m1ψU(1.0) mRNA modification outperformed other mRNA modifications, resulting in a 25-fold higher protein expression. Subretinal injection of MessengerMAX/mRNA complexes *in vivo* revealed some GFP expression close to the injection site, especially 7 days after injection. Also in an *ex vivo* bovine retinal explant with the photoreceptor layer facing up, complexed mRNA resulted in the expression of the encoded protein. Naked mRNA, however, in both cases failed to lead to detectable protein expression, although the penetration of the mRNA into retinal cells could be visualized. After intravitreal (IVT) injection, very little amount of protein expression was found *in vivo.* In *ex vivo* retinal explants with an intact vitreoretinal interface, it became clear that the inner limiting membrane (ILM) is a large barrier for penetration of complexes into the retinal cell types. When this ILM was removed, however, more penetration of complexes into the *ex vivo* bovine retinal explants was observed, accompanied with protein expression. SPT analysis revealed that the negatively charged messengerMAX lipoplexes showed a high intravitreal mobility, leading us to conclude that mainly the ILM and not the vitreous itself is responsible for the low protein expression levels *in vitro* and *ex vivo* after IVT injection.

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Conclusions : Overall, we conclude that messengerMAX lipoplexes complex mRNA, are stable in vitreous, and have the ability to deliver mRNA to the retina after subretinal injection. After intravitreal injection, however, the ILM limits penetration of complexes into the retina, thereby preventing the transfection of retinal cells and thus the production of proteins. As the expression levels *ex vivo* and *in vivo* are much lower when compared to *in vitro* cell culture, we will investigate further how to overcome the ILM as delivery bottleneck for mRNA after intravitreal injection.

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New Adjuvants for Vaccines

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Keywords. adjuvants, vaccines, emulsions, liposomes, DAMPs..

Adjuvants are substances from diverse biochemical or chemical origins, defined by what they do and not by what they are. They enhance the specific immune response against an antigen and hence are essential components of vaccines.

Aluminum salts are historical adjuvants that have been used in the vaccine industry for nearly one century. However, new adjuvants systems have been recently developed such as virosomes that are built of phospholipids and proteins from influenza virus particles, or liposomes and emulsions that contain immunomodulators and polymers. Vaccines are now entering a new era of nano systems and colloidal formulations.

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Nanoparticles for Suncare Application

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Keywords: Nanoparticles, Suncare, Cosmetic regulation, Organic nano UV filters

Nanotechnology has always been present in nature; for example, gecko feet or butterfly wings are nanostructured. This is a source of inspiration for innovation. Since the 1990's, nanomaterials are intentionally manufactured in different market sectors to design materials with outstanding performance. In cosmetic application, these nanomaterials are mostly present in suncare & make-up applications. According to the Cosmetics Regulation, nanomaterials need a specific safety assessment prior to placing them on the EU market. In case of nano UV-filters, they must be registered on Annex VI of the Cosmetic Regulation which include a positive SCCS (Scientific Committee on Consumer Safety) opinion.

The last approved UV filters for cosmetics are nanomaterials: Methylene Bis-Benzotriazol Tetramethylbutylphenol (MBBT) & Tris-BiPhenyl Triazine (TBPT) Zinc Oxide and Titanium Dioxide. Specifically, the new organic filters improve the protection against UV rays in both UVB & UVA range. As an advantage compared to other UV filters, the organic nano UV filters have high molecular weight & stays on the skin surface to scatter & absorb the light. These filters can be easily dispersed in water & do not require solubility data. Beside an easy use, these filters allow a different sensory of the sunscreen formulation.

Despite the very good performance of these innovative solutions, we observe emerging scepticism from stakeholders. Although the Cosmetics Regulation does address and manage the potential risks of nanoproducts, some doubts remain on the definition and the suitability of the testing methods for these products. In this context, it is specifically important to improve dialog on nanomaterials in the value chain and with consumers to understand the concerns, share information and discuss benefits and potential risks.

The future will tell us if the society is open to nano innovations in the cosmetic field.

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Therapeutic Peptide Delivery: How Can Nanosystems Help Address Present and Future Challenges

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Keywords. Peptide delivery, sustained-release injectable formulations, transport through membranes, nanosystems, nanotubes

Peptides have become very attractive drugs in the last decades, due to their selectivity, their high bioactivity and low toxicity. These drugs have been successfully developed for the treatment of major diseases like type-2 diabetes and cardiovascular disorders, various types of cancer and multiple sclerosis. Due to their poor stability in extreme pH conditions, their enzymatic degradation and poor absorption across epithelial membranes, as well as their short plasma half-life, peptides remain difficult-to-administer drugs. At the present time, they are predominantly administered via injection, using sustained-release (SR) injectable formulations mainly based on polymer matrices slowly releasing the peptide over months [1]. These formulations have become the most successful peptide formulations on the market and standards of care for many peptide drugs. The use of alternative routes of administration, like the oral route or the transmucosal route, might increase in the future, due to the invasiveness perception of injections, as well as disposal issues associated with used needles and complicated, multi-step injection protocols [2]. However, very low bioavailability due to limited permeability through the membranes remains a key challenge for these alternative delivery routes [3]. In addition, new challenges have emerged recently, related to the need for intracellular delivery of peptides to new targets in cancer treatment, as well as the crossing of the blood-brain barrier (BBB) for peptide delivery to the brain. Then, in this context, nanodelivery systems (e.g. nanotubes, nanoparticles or nanocapsules) can provide appropriate solutions to address present and future challenges of peptide delivery. This paper will more particularly focus on functional nanosystems for SR peptide formulations, peptide delivery across cellular membranes (either intestinal epithelium or BBB) or into cells to target intracellular receptors. It will present various successful nanosystems for peptide delivery that have entered the clinic or even progressed to the market. Finally, it will discuss more prospective approaches mainly intended to help peptides cross membranes or enter cells.

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3D printing and formulation

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Keywords. 3D printing; additive manufacturing; bioprinting; health applications; silicone

Additive manufacturing and particularly 3D printing is foreseen for the next 10 years to have major impacts on economy and innovation developments. These innovations were and will be sustained by technological developments but strong efforts shall also been placed in formulation and re-formulation in order i) to make industrially useful materials “printable” and ii) to discover new material functionalities. Typical situation is represented by viscoelastic materials such as silicone with poor rheological properties, impossible to print in atmospheric conditions since a significant yield stress character of the material is required to maintain the shape of the 3D object after deposition. This character was achieved through liquid silicone rubber re-formulation [1] and impact of this re-formulation on feasibility of 3D printing will be presented with examples in bio and health systems.
Formulation will also be presented as an innovation leverage enabling the discovery of potential smart materials with predefined properties. Our group have been working on this approach for the last 3 years, digging deeply into the 3D printing technologies and ink formulation to achieve complex 3D objects with new capabilities. These objects, called 4D printed objects can have several properties such as catalysis, biomolecular recognition and biological interactions abilities [2-3].

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Lipid-based drug delivery systems: Influence of excipient structural properties on their preparation processes

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Keywords. Lipid, self-assembly, thermotropic properties, lyotropic properties, dispersion.

More than 35% of commonly used active pharmaceutical ingredients (API) are classified as poorly water-soluble according to the Biopharmaceutics Classification System. In addition, leads obtained with high-throughput screening tend to have even higher molecular weights and lipophilicity than older molecules. In the case where such a lead is transformed into a conventional drug, classical formulation strategies are no longer adequate to achieve acceptable bioavailability. Although a number of alternative technologies are being developed, lipid-based formulations have shown a lot of significant results in enhancing the bioavailability of lipophilic drugs. Unfortunately, the thermotropic and/or lyotropic properties of lipids could be complex and have to be considered with care during product and process developments [1]. This will be illustrated here through the examples of microspheres and *Janus* nanoparticles obtained by prilling and high-pressure homogenisation respectively.

Prilling by ultrasonic jet break-up is an efficient process to produce homogeneous in size and perfectly spherical microparticles for controlled drug delivery [2], taste masking and/or administration to populations having difficulties to swallow. However, the material thermotropic properties could affect the process feasibility and the final product properties especially with lipid-based excipients. Here, six excipients were first characterised and then used to produce microspheres on a pilot scale prilling apparatus. The experimental results were compared to theoretical calculations, notably the droplet solidification time [3] which is a key-parameter of this process. It appeared that monotropic polymorphism and supercooling effect have to be considered with care during formulation development as these behaviours significantly interfere with the process control.

High-pressure homogenisation is widely spread in food and pharmaceutical industries in order to prepare nano-dispersions as nanoemulsions or solid lipid nanoparticles with narrow size distributions. We recently used this process to produce lipid-based *Janus* nanoparticles which contain an oily compartment “stuck” to an aqueous compartment bounded by a bilayer containing phospholipids and non-ionic surfactants [4]. The original design of these particles and their stability with time and temperature result from the lyotropic and thermotropic behaviours of some macrogolglycerides used in the formulation. These anisotropic particles are promising tools to incorporate API with opposite solubilities.

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Product engineering : a combined approach for the design and the processing of drug delivery particles

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Keywords.
Particle, Capsule, Process, Emulsion, Microfluidics,

The design of drug delivery systems has attracted high attention in the last decades because of their potential for drug delivery applications, especially for effective cancer therapies[1]. Among these products, polymer particles appears as very promising tools[2]. Depending on their administration, their size ranges from nanoscale to several hundred micrometers, making them called nanoparticle or microparticle. Due to their structure, particles can also be divided into two main groups: spheres[3] (matrix systems) and capsules[4]. Capsules are vesicular systems exhibiting a core-shell structure, the core acting as a liquid reservoir for drugs and the shell as a protective membrane. The versatile nature of the inner core (allowing the encapsulation of hydrophilic or hydrophobic substances depending on its polarity), their high encapsulation efficiency and the low amount of solid content make capsule extremely attractive. Moreover, the release kinetics of the drug can be controlled by adjusting the nature and thickness of the polymer shell, but also the polarity and the volume of the liquid core[5].
This talk will discuss on the fabrication processes of particle and capsules with appropriate end-use properties in the context of potential biomedical applications. There exist many different processes to prepare particles from mechanical to chemical ones. The choice of the process associated with the appropriate formulation can facilitate the production of particles with the appropriate end-use properties. We will illustrate this with particle characteristics such as their size/size distribution, capsule morphology[4,7], responsiveness, depending on the expected specifications (administration method, biocompatibility, drug release kinetics, furtivity/targeting…). Besides, we will show the new trends of process development on particle tailoring as well as toward continuous[8], low-energy or interfacially reactive processes[4,7].

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Mass transfer studies through biological membranes. A chemical engineering approach.

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Keywords. Diffusion, skin, drugs, Franz cell

This contribution describes the classical way that the process of drug diffusion through skin is studied experimentally. One use the Franz cell in association with a linear model based on Fick diffusion model, linear equilibrium relations and the assumption that the skin can be assumed to be a dense membrane. It appears that the way the Franz cell is used is related to the existence of an analytical solution of the model that is available in classical textbooks [1].

Due to numerical methods, improvements in the description of the process of drug transfer can be proposed.

Firstly, the highly complex multiphase/multilayer structure of the skin can be taken into account. Such models have been proposed in the literature devoted to mass transfer through skin [2]. They are more or less complex depending on the properties of the studied layer. In the Stratum Corneum, the pure diffusion model is always assumed due to its avascular nature. However, some papers propose to take into account its structural properties by 2D or 3D “biphasic brick-and-mortar” models with simple partition coefficients between the two phases but diffusion coefficients variable in space [3]. Some improvements were further made adding porosity and tortuosity in the model. In the viable epidermis (VE), some authors have added a convection term to model the transport into blood and lymphatic capillary partition. But the transport has also been modelled using a dispersion term, which includes diffusion and convection at the same time. As far as we know, all these models remain generally based on a linear description of thermodynamic equilibrium and diffusion in order to get, if possible, analytical solutions [4]. Compartment models are also commonly used within this framework [2].

Secondly, modern diffusion theory (Irreversible thermodynamics approach, Maxwell-Stefan approach [5,6]) as well as network model approach could be used provided that more sophisticated thermodynamic models of the skin and knowledge on skin structure to be available.

The way the Franz cell is used can also be improved. Only the first moments of the experiments are exploited since the above-mentioned analytical solution is correct only during this period. Such a constraint is no more necessary if a numerical technique is used to solve the model equations and the all duration of the experiment can be used.

Other experimental techniques can also be considered, such an open receptor compartment [7].

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Abstracts of oral communications

Development of hyaluronic acid-based nano-complexes for pentamidine encapsulation

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Keywords: hyaluronic acid, polyarginine, pentamidine, polymer-drug complexes, nanoparticles.

Introduction.
During the last decades, the development of pharmaceutical nanocarriers based on natural polysaccharides has received growing interest thanks to their biocompatibility, biodegradability and non-immunogenicity properties. Among the different natural polysaccharides, hyaluronic acid (HA) has been widely used in pharmaceutical field thanks to its interesting physicochemical and biological properties. HA is an important component of the extracellular matrix belonging to the class of anionic glycosaminoglycan (GAGs) and highly distributed throughout connective, epithelial, and neural tissues [1]. Moreover, HA is able to interact with different cell surface proteins like Stabilin-2, CD44 and other membrane-integrated glycoprotein overexpressed on various cancer cells leading to an increased uptake at subcellular level [2]. Thanks to these properties, HA has provided a promising platform to physically encapsulate or chemically conjugate various drugs for various drug delivery applications. This work aims at developing new polymeric nanocarriers made of HA and polyarginine (PArg) for the encapsulation of a hydrophilic model drug: pentamidine isethionate (PTM). PTM is an antiprotozoal agent commonly used for the treatment of different parasitic infections [3]. Recent works showed that PTM has also a strong antiproliferative effect both in *in-vitro* and *in-vivo* models on solid tumors [4]. An extensive physico-chemical characterization of nanocarriers was carried out using different techniques in order to validate these new types of nanosystems for the delivery of different hydrophilic drugs like PTM.

Materials & Methods.
Polyelectrolyte complexes made of HA and pentamidine (HA-PTM PEC), blank NP and loaded nanoparticles (PTM-loaded NP) were prepared by ionotropic gelation technique [5]. Seven different HA-PTM PEC were obtained by adding 500 µL of HA aqueous solution (2.5 mg*mL^{-1}) over 500 µL of PTM (concentration ranging from 0.6-9 mg*mL^{-1}). To obtain blank NP, different solutions containing HA (concentration ranging from 1.25-5 mg*mL^{-1}) were added to an equal volume of PArg (0.27 mg*mL^{-1}). Similarly, to prepare PTM-loaded NP, 500 µL of different amounts of PTM (ranging from 0.5 to 0.17 mg*mL^{-1}) were added to 500 µL of a cationic solution containing a fixed amount of PArg (0.18 mg*mL^{-1}). This pre-mix was left under agitation for 10 min and then, 500 µL of HA (concentration ranging from 0.83 to-3.3 mg*mL^{-1}) were added.
All the formulations were characterized in terms of size, polydispersity (PdI), zeta potential and morphology using transmission electron microscopy (TEM) and Cryogenic electron microscopy (cryoTEM). The encapsulation efficiency (E.E.%) was evaluated using UV spectra and ionic-exchange chromatography. *In vitro* release of PTM from PTM-loaded NP was studied upon incubation of NP suspension in PBS at 37 °C using vertical diffusion Franz cells®. Stability of NP (size, PdI, zeta potential and leakage of drug) was also evaluated under storage at 4 °C for 3 weeks before and after freeze-drying. Finally, *in vitro* cell viability on human lung carcinoma (A549) and human breast adenocarcinoma (MDA-MB-231) cell lines was estimated using 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay following 72 h of exposure to different concentrations of PTM, blank NP and PTM-loaded NP.

Results.
Polyelectrolyte complexes (PEC) are defined as complexes formed though the electrostatic interaction between oppositely charged structures. In this study PTM was selected as a cationic drug and HA as polyanionic polymer. Using ionic gelation technique, different HA-PTM PEC having a molar ratio HA/PTM ranging from 6.61 to 0.41 were prepared. During the formation of PEC two different population were identified; one having a hydrodynamic diameter of around 10 nm (soluble complex) and another one around 150 nm (insoluble complex). Moreover, the increase of PTM concentration was accompanied by a growth of the peak

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at 10 nm with a simultaneous decrease of the peak at 150 nm. The zeta potential of all complexes was negative and ranged from -21.7 to -3.1 mV. Regarding the electrophoretic mobility, a reduction of HA-PTM PEC mobility for an increasing concentration of positive charge (PTM) was observed.
To stabilize the insoluble HA-PTM PEC and to maximize the amount of associated drug, PArg was selected as cationic polymer able to reticulate HA. Different blank NP with a molar ratio HA/PArg between 0.82-8.25 were developed. The average size of the resulting NP ranged between 112 and 244 nm with a low polydispersity index (< 0.2). Zeta potential values ranged from +33 to -22mV and an inversion of the values was observed when the charge ratio HA/Parg was lower than 1.24.
After that, PTM-loaded NP with different concentration of PTM (from 0.5 to 0.17 mg*mL^{-1}) were prepared. All the NP shown an average size between 155 and 203 nm with a good polydispersity index (< 0.1) and a negative zeta potential ranging from -24 to -18 mV. PTM-loaded NP were able to encapsulate high amount of PTM (80%) ensuring a constant release over time (60 % of the drug released in PBS following 10 h of incubation). Besides, freeze-dried blank NP and PTM-loaded NP preserved their integrity in term of size and morphology following reconstitution in water. *In vitro* studies on lung (A549) and human breast (MDA-MB-231) cancer cell lines shown that PTM-loaded NP had a better antiproliferative activity than the free drug suggesting an enhanced internalization of encapsulated drug.

Conclusions.
The present study provides insight into a new HA based nanocomplex for positive hydrophilic drug encapsulation. In a first set of experiments, HA-PTM PEC were studied. The complex obtained were not stable and PArg was used in order to stabilize and to maximize the amount of the drug associated to the system. Blank NP and PTM-loaded NP and were developed using ionotropic gelation technique. The NP obtained were able to encapsulate PTM in high amount (80%) and retain the drug during the time. Following *in vitro* studies, PTM-loaded NP were more effective in reducing cell viability as compared to free drug. Overall, this study highlights the promising potential of HA-PArg nanoparticles as novel carrier systems for hydrophilic drug.

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Formulation of organosilicate particles complexed with bioactive molecules: adsorption and release studies.

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Keywords. Organosilicates, Laponite, Montmorillonite, Adsorption, Drug release.

Introduction.
Numerous studies have described the potential use of clays as interesting carriers of active pharmaceutical ingredients (APIs) due to their abundance, lamellar structure, high surface area [1]. However, clays represent limits such as contamination and variation of compositions. Several researchers tried to synthesize composites with the same structure of clays but with more homogeneity and affinity with the organic molecules. Thus, a new family of particles called organosilicates has emerged. we synthesized and fully characterized lipophilic aluminum/calcium organosilicate particles (OS_L particles) through one-step sol-gel reaction under mild conditions using a mixture of CaCl2, AlCl3 and three alkoxysilanes as starting materials able to stabilise w/o emulsions [2]. The aim of our study is to investigate the BZ3 adsorption capacity of OS_L particles. Regarding BZ3, it was selected as a hydrophobic molecule [3].

Materials & Methods.
Adsorption experiments: The BZ3 adsorption capacity of OS_L particles, LAP and MMT was evaluated by typical batch adsorption experiments. To study the effect of important parameters, batch experiments were conducted. For each experimental run, 0.5 g of each adsorbent was contacted with 25 mL of BZ3 in 50 mL solution, conical flask wrapped with aluminium foil to prevent BZ3 light-induced decomposition. This mixture was stirred in a temperature-controlled water bath (5, 20 and 38°C). Samples were withdrawn at different time intervals (0–24h), and then analysed by HPLC to evaluate the BZ3 concentration in the supernatant.
Determination of adsorbed amount of BZ3 by HPLC: Samples were analysed for BZ3 residual concentration by high-performance liquid chromatography (HPLC) using an alliance e2695 (Waters, Milford, MA, USA) equipped with a UV detector and a Supelco column nucleosil C18 (250 mm × 4.6 mm, 5 µm,). The mobile phase, which was degassed and filtered before analysis, consisted of acetonitrile (A) and methanol (B) and water with 0.1% acetic acid (C). The eluent flow rate was 1.0 mL.min^{-1}. The elution conditions applied included the following: 30% A, 30% B and 40% C. UV measurements were carried out at 288 nm.
Desorption studies: The *in vitro* BZ3 release profile from the three adsorbents was carried out in different medium using direct dispersion methods according to [4] with modifications.to fulfil the sink conditions, an amount of complexes equivalent to 1.2mg of BZ3 was added to 100mL of release medium and This mixture was stirred in a temperature-controlled water bath.at designed time interval, 1mL of the dispersion was removed immediately and replaced with fresh PBS solution. Physical mixture was obtained by simply grinding of a BZ3-adsorbent mixture.

Results.
Temperature effect: Batch experiments were carried out at pH 10 and the temperature effect was studied at different temperatures 5, 20 and 38°C in a thermostatic system, with an outer circulating-water bath to evaluate the adsorption capacity of BZ3 onto OS_L particles, LAP and MMT (Fig. 1) In the case of OS_L particles; the increase of % BZ3 adsorbed in each temperature values were significate which prove the positive effect of temperature on BZ3 adsorption onto OS_L particles.

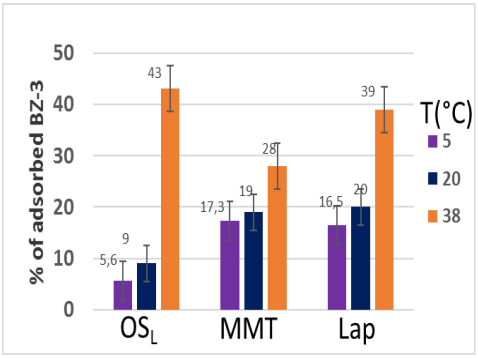

Fig.1. Effect of temperature on the adsorption amount of BZ3 onto OSL particles, LAP and MMT after 24h (pH=10, BZ3 concentration=2.5 g. L^{-1}, adsorbents at 2 g.L^{-1}. (n=6)

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Adsorption isotherm

The equilibrium adsorption isotherm could provide information about the homogeneity and heterogeneity of the adsorbent surface. The isotherms for the adsorption of BZ3 on OS_L particle, LAP and MMT showed in Fig. 2 exhibited a L2 type isotherm according to Giles classification [5].

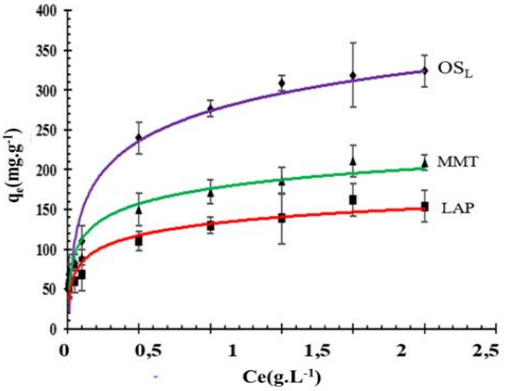

Fig.2: Adsorption isotherms of BZ3 on OS_L, LAP and MMT (pH 10, temperature=38°C, BZ3 concentration=2.5g. L^{-1}, adsorbents at 2 $g.L^{-1}$, contact time 24h), (n=6).

Desorption studies: As denoted in Fig.3, around 100% of BZ3 in the physical mixture quickly diffused onto the PBS solution within the first 1h of incubation's particles show a biphasic release profile; A burst effect with 40% being released in the first 2h followed by a sustained release with around 45% throughout the entire incubation period. This behaviour can be explained by the fact that BZ3 adsorbed in the surface of OSL particle is dissolved first then the BZ3 molecules intercalated inside the particle's matrix were dissolved witch takes more time.

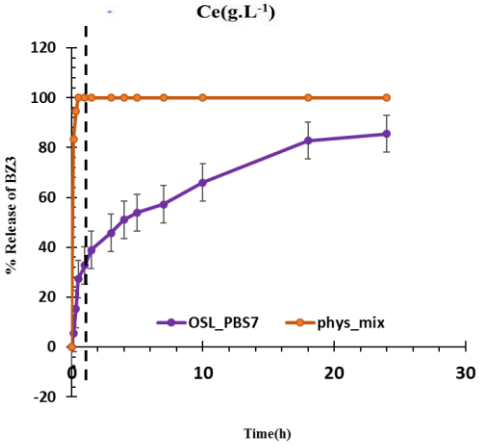

Fig.3: % release of BZ3 to PBS solution from OS_L.

Conclusions.

The ability of OS_L particles to stabilize W / O emulsions having been proven, and with a view to a synergistic approach of the two stabilizing and complexing effects of OS_L particles with bioactive molecules, would make it possible to combine its properties with a single formula applied topically. Desorption studies have shown a biphasic release profile.

The following experiments will be carried out to test the adsorption of hydrophilic organosilicates(OS_H) to complex active ingredients according to their degree of hydrophilicity.

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Pectin modification by esterification with Octenyl Succinic Anhydride (OSA) to obtain new drug delivery systems

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Keywords. Pectin, esterification, green process, bead, curcumin.

Introduction.
For the last decades, pectin has become one of the most studied biopolymer for drug targeting and biomedical applications [1]. Indeed, pectin has interesting properties such as low cost, high stability, good gelling property, biocompatibility and non-toxicity [2]. However, its use can be limited due to its rapid hydration, swelling and dissolution in water [1]. This study consists to overcome these limits by pectin chemical modification to develop a new drug delivery system. The chemical modification consists in grafting hydrophobic segments onto pectin backbone using mild conditions. After comparing two methods of grafting to obtain modified pectin [3], esterification route was selected to functionalize hydroxyl functions of pectin [4] using octenyl succinic anhydride (OSA). Various factors of formulations and process (pectin, octenyl succinic anhydride and potassium carbonate amounts and temperature process) were explored within an experimental design in order to understand and control the chemical modifications [5]. Then, the new materials are used to formulate drug delivery systems.

Materials & Methods
Materials
Low methoxyl (LM) pectin (Cargill) (Unipectin® 300C, DE = 27-33%) was dried at 80°C, under vacuum, for 4h. Octenyl succinic anhydride (OSA), (Aldrich) and potassium carbonate (K_2CO_3), (Aldrich) were used as received.

Synthesis of modified pectin
Briefly, pectin was manually milled with OSA and K2CO3. The heterogeneous mixture was heated in an oil bath for 15 min. After cooling at room temperature, the mixture was washed in acetone by successive centrifugations until take off of all the ungrafted OSA and dried one night at 30°C under vacuum.

Experimental design
A two-level factorial design (using 4 factors and resulting in a total of 16 runs) was built in order to study the influence of formulation and process parameters. It was used to screen various factors including: pectin amount (X1), octenyl succinic anhydride amount (X2), potassium carbonate amount (X3) and temperature (X4). Each factor was evaluated at low (−1) and high (+1) level. The choice of the low and high values was based on the preliminary study results. The experimental response evaluated was the percentage of substitution determined by 1H NMR.

^{1}H NMR
^{1}H NMR analyses were carried out on a Brüker 500 UltrashieldTM apparatus. Pectin and modified pectin were dissolved in D2O and 1H NMR spectra were recorded at 500 MHz and 25°C. The proton NMR spectra of modified pectins were used to determine the percentage of substitution by comparing the integration of the peak accounting for the proton in position 3 in the galacturonic acid residue and the one accounting for the methyl of OSA.

Beads formulation
500 mg of pectin and modified pectin at 19% of substitution was solubilized in 10 mL of water. 100 mg of curcumin was added to pectin solution under stirring. The mixture was dropped in a calcium chloride solution at 10% (m/m) using a peristaltic pump at 2ml/min. After 5 min of complexation with Ca^{2+}, the beads were washed with water to take off the free Ca^{2+} and dried over night at 30°C. The aspect and the surface area of the beads was determined by optical microscopy.

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***In vitro* release study**
25 mg of each kind of beads were weighed and added to 100 mL of intestinal medium (phosphate buffer, pH = 6.4) containing Solutol® 5 g/L. The experiment was performed at 37° C with a paddle speed of 50 rpm in a dissolution apparatus SOTAX AT7 Smart (type II apparatus). The amount of released curcumin was assayed by UV spectrophotometry at 426 nm using a calibration curve. Then the percentage of released curcumin was determined and plotted over time. All experiments were performed in triplicate.

Results
Synthesis of modified pectin
The reaction involves OSA as reactant and potassium carbonate as initiator to activate hydroxyl functions on pectin. The aliphatic chains bring hydrophobic segments on pectin and anhydride functions react with pectin hydroxyl function by esterification.

^{1}H NMR
The proton NMR spectra of modified pectins were used to confirm the grafting and evaluated the experimental response. A broad modified pectin range is determined by 1H NMR analyses. These results are used to evaluate the main effects of factors previously cited.

Experimental design
Four high risk factors were identified in a risk analysis study to have potential impact on percentage of substitution. The percentage of substitution varied from 1.7% to 90% for the various factor combinations. The most significant factors were pectin amount, K2CO3 amount and temperature. A positive effect on percentage of substitution is observed with amount of K2CO3 and temperature of reaction. A negative effect is observed with amount of pectin. The amount of OSA has no effect in this condition. The results of experimental design are used to scale-up the synthesis process to obtain a greater amount of modified pectin for formulation.

Beads formulation
After scale-up, modified pectin at 19% of substitution is used to formulate beads. During drying, the decrease of the surface area is reduced by 37% for modified pectin and 79% for LM pectin. This result is directly related to the OSA content and confirmed the reduced affinity of the modified material towards water.

In vitro release study
The kinetic of curcumin release show a slowest release with a higher lag time for the beads based on modified pectin at 19% (113 ± 5 min) against beads based on native pectin (98 ± 1 min). Moreover, encapsulation efficiency shows a better result with modified pectin for a similar encapsulation yield.

Conclusions
To conclude, a free solvent process is used to obtain modified pectin ranging from 2 to 90% of substitution. The results of experimental design are used to scale-up the chemical modification of pectin. These modified pectins hold their gelation ability with calcium cations and allow the development of new drug delivery systems. A slower release of a hydrophobic model drug is observed from a 19% OSA grafted pectin compare to a native pectin.

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Core-shell encapsulation systems as a help to formulate active cosmetic ingredients and enhance their skin penetration.

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Keywords.
Nano-encapsulation, active cosmetic ingredient, semi-solid forms, skin penetration

Introduction.
Active cosmetic ingredients (ACI) encapsulation in nanocarriers is a strategy widely explored by researchers to enhance skin penetration. This improvement is directly linked to the size (critical threshold is usually thought to be at 100 nm) and the chemical composition of the encapsulation systems. For their successful use in cosmetology, several challenges have to be overcome, concerning ACI loading/release as well as the system stability when dispersed in an end product. Based on the results of a public-private research project, we demonstrate that nanosystems with a lipid core and a hydrophilic shell, of a size superior to 100 nm, developed in our lab present the essential qualities to be considered as efficient encapsulation systems useable in the cosmetic field.

Materials & Methods.
Two core-shell nanosystems were studied: Lipid nanocapsules (LNC), obtained by the phase inversion temperature method as a reference, and alginate-based nanocarriers (ANC) obtained by emulsion-surface gelation assisted by ultrasounds. Two commercially available ACI were encapsulated: an ester of linoleic acid and piperonyl alcohol (Omegalight®, skin lightener) and an ester of punicic acid and tyrosol (Delipidol®, lipolytic agent). The nanosystems were characterized by dynamic light scaterring and zetametry. HPLC was used to determine the concentration and the integrity of the ACI after encapsulation. Fluorescence-based analytical approaches were used to study the stability of the nanosystems when dispersed in an end product. A cost-effective method based on infrared spectroscopy and multivariate analysis was developed for the rapid quality control of lipid-based encapsulation systems in viscous media. Sensory analysis was performed by trained experts by means of a triangle test. Fluorescence confocal spectral imaging and HPLC measurements were used to evaluate the interest of the nanosystems to deliver ACI to the skin.

Results.
Both esters were successfully encapsulated. Nanosystems showed a size of ≈120 and ≈230 nm for LNC [1,2] and ANC [3,4,5] respectively. High loadings were achieved, especially for Omegalight® (up to 80% m/m). HPLC studies showed that the fatty ester is protected from hydrolysis in aqueous media by the shell. The nanosystems are stable 6 months when stored at 4°C. Semi-pilot batches of the systems were prepared and showed properties similar to those of the laboratory samples.

The nanosystems were dispersed in several semi-solid formula and no leakage of the capsules was measurable after one month (normal or accelerated aging conditions). These systems made of an oily core and a hydrophilic shell both permitted to include high concentrations of lipid-derived ACI in a hydrophilic phase or in a water-based cosmetic product without modifying its stability or its sensory properties (up to 20 % m/m).

At last, besides their size (> 100 nm) and negative zeta potential, the core-shell capsules provided an accumulation of the ACI in both reconstructed and human skin of the same order of magnitude. This accumulation is higher when the nanosystems are dispersed in a semi-solid form.

Conclusions.
All these data show that LNC and ANC are nanosystems of interest in cosmetic formulation. During this work, several original analytical approaches were developed that could be adapted to other nanosystems or end products.

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Development of a nasal drug delivery composite nanosystem for Alzheimer's desease treatment

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Keywords. nanomedicine, nasal administration, thermosensitive gel, Alzheimer's desease

Introduction.
The treatment of central nervous system (CNS)-related disorders is the greatest challenge because of a variety of formidable obstacles in effective drug delivery [1]. Drug access to the CNS is hindered by the presence of the blood–brain barrier (BBB), which limits the free entrance and diffusion of most drugs and other external contents from the bloodstream to the brain [2]. In the last years, the intranasal route has risen as a route to transport drugs directly from nose-to-brain avoiding the BBB [3]. Indeed, as the brain and nose compartments are connected to each other *via* the olfactory/trigeminal neural pathways and *via* peripheral circulation [4], the Intranasal (IN) drug delivery is one of the important drug delivery options for brain targeting. It also offers minimal invasiveness, painlessness, self-administration, and high patient compliance [5]. Alzheimer's disease is a neurological disorder that results in cognitive and behavioral impairment. Conventional treatment strategies, such as acetylcholinesterase inhibitor drugs, often fail due to their poor solubility, lower bioavailability, and ineffective ability to cross the blood–brain barrier [6]. IN route could be interesting in this pathology. Nevertheless, it is also important to ensure sufficient drug residence time and drug absorption across the nasal epithelium although the mucociliary clearance [7]. The use of nanocarrier based formulations enhancing nasal absorption of drugs can be indicated as a promising approach for the enhancement of the efficacy of Nose-to-Brain delivery [8,9].
In consequence, the present study aimes at developing and characterizing an innovative composite formulation based on a thermosensitive gel enabling sustained delivery *via* liposomes of an active pharmaceutical ingredient (API) of potential interest for Alzheimer's disease after nasal administration. This API was shown to be a selective inhibitor of the butyrylcholinesterase but presents a low permeability through brain blood barrier model. The present formulation aimes at targeting the brain by countering the BBB.

Materials & Methods.
Thermosensitive gel was prepared by mixing Poloxamer 407 and Poloxamer 188. API-loaded liposomes were prepared by the thin film hydration method. Osmolarity and gelation temperature ($T_{sol\text{-}gel}$) of formulations, defined in a ternary diagram, were investigated by rheometry and visual determination. Various parameters such as lipid ratio, concentration and lipid to drug ratio were investigated to obtain acceptable entrapment efficiency (EE). Properties of formulations were evaluated in terms of granulometry, zeta potential, and EE after purification step on a Sephadex™ column. Mucoadhesion were evaluated by detachment and work of adhesion method by TA.XT plus Texture Analyser and using mucin disc. Kinetic release studies were performed in nasal simulated fluid.

Results.
At the issue of assays, a mixture composed of Poloxamer 407/Poloxamer 188 (15/1%, w/w) was selected, being compatible with intranasal administration in terms of Tsol-gel and with the olfactory mucosa (280 ± 20 mOsmol, pH 6). Mucoadhesion studies showed that *in situ* gel formulations present good natural mucoadhesive characteristics that could be increased in presence of liposomes within the gel. From kinetics release studies, it appears that the composite formulation enables a delayed and complete release of the API, different from those obtained with API loaded gel or API solution.

Conclusions.
From these results, it appears that a successful formulation of a promising API-loaded liposomes in a thermosensitive hydrogel for nasal delivery was realized. It will be soon the object of further biological evaluation.

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Skin absorption of ions from thermal spring waters

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Keywords. skin penetration, thermal spring waters, ions, Franz cell

Introduction.
Skin absorption of highly hydrophilic species including simple inorganic ions has long been believed limited because of the hydrophobic character of *stratum corneum* [1]. However, the regard to current knowledge, this seems to be an oversimplistic approach. The use of thermal spring waters (TSW) in treatment of various skin (atopic dermatitis, psoriasis, eczema etc.) and systemic diseases (ex. osteoarthritis, mental stress, sleep disorders) is well documented [2,3] and cosmetic formulations made with TSW as an active cosmetic ingredient exhibit a definite activity, contradicting this view [4,5]. Even though TSW have been applied in treatment since centuries, often with measurable positive outcome, little was known about the mechanisms underlying their biological activity. Nowadays, natural treatment methods along with modern balneology and balneotherapy as well as related therapeutic approaches such as thalassotherapy and hydrotherapy undergo their "renaissance" and, therefore, scientists have been searching for the explanations of TSW activity [6]. Most of the beneficial effects of TSW are due to mineral composition, here we are particularly interested in the fluxes of calcium and magnesium as the main TSW components playing an important role in skin barrier recovery [7]. Therefore, in this work we investigate skin penetration of these ions from a commercialised thermal spring water.

Materials & Methods.
Commercialised TSW was analysed in terms of pH (pHenomenal® pH, VWR, Fontenay-sous-Bois, France), osmolality (Osmomat 030 Cryoscopic osmometer, Gantec, Berlin, Germany), conductivity (CDM210 Conductivity Meter, MeterLab™, Vaulx En Velin, France) and the ionic content using ion-exchange chromatography (930 Compact IC Flex, Metrohm, Villebon Courtaboeuf, France).
Cutaneous absorption experiments were performed using viable skin samples. Full-thickness pig skin explants were mounted in Franz cells and provided with a glucose-rich, isotonic medium containing minimal amount of ions which was developed in order to ensure skin viability and to prevent the interference with the analytical method applied. The permeation of cations was assessed after 24 h exposure to marketed TSW. Skin layers: *stratum corneum*, viable epidermis and dermis were separated and ion concentrations were quantified in the receptor fluid and in each skin layer using ion chromatography.

Results.
Investigated TSW has a neutral pH and is characterised high ionic content manifested by measured osmolality value of 180 $mOsm \cdot kg^{-1}$ and conductivity of 9 $mS \cdot cm^{-1}$. The ratio of measured Ca^{2+}: Mg^{2+} concentrations is 5.5. Both magnesium and calcium cations TSW can diffuse from TSW through healthy skin *in vitro* however, penetration rates for these two cations differ. The values of total absorbed quantities (values of absorbed amount recovered from receptor fluid, and skin compartments) were 5% for Ca^{2+} and 12% for Mg^{2+}. Moreover, cation distribution in skin layers after 24h showed important differences: Ca^{2+} was significantly accumulated in *stratum corneum*, viable epidermis and dermis while with lower concentrations recovered from receptor fluid while Mg^{2+} penetrated through the skin without accumulation, therefore most of the penetrated amount was recovered from acceptor fluid. Nevertheless, the distribution of studied cations in skin layers after 24 h exposure indicates that their diffusion occurs according to the concentration gradient.

Conclusions.
Our results demonstrate that the beneficial effects observed during treatment with TSW is not only due to their action on surface of the skin but also is associated to penetration of the minerals into and through the skin. Our further work will focus on the impact of formulation type, in which TSW are used as an active cosmetic ingredient, on the skin penetration of minerals.

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Synthesis, Characterization and Pre-clinical Evaluation of Iodinated Polymer Nanoparticles as a Contrast Agent for Computed Tomography using a Spectral Photon counting CT

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Statement of Purpose.

Contrary to conventional Computed Tomography (CT) that provides a single attenuation measurement, Spectral Photon Counting CT (SPCCT), can discriminate between different elements by recording the energy spectrum of the tissues attenuation at each voxel of the image. This allows material decomposition that provides quantitative determination of the concentration of a specific element. Iodine-based materials are widely used and efficient contrast agents for X-ray imaging. Contrast agents in the form of nanoparticles that specifically bind to the tissue to be imaged, provide contrast enhancement in respect to small molecules. The purpose of this study is to combine the SPCCT technology and contrast agents as nanoparticles. In order to do so, iodinated polymer nanoparticles were designed.

Methods.

The iodinated polymer material was prepared by binding an iodinated molecule to poly(vinyl alcohol) (Fig 1) [1]. Radiopaque moieties were successfully incorporated to the polymer. The very high iodine content determined by ^{1}H NMR and ICP was over 70 wt%.

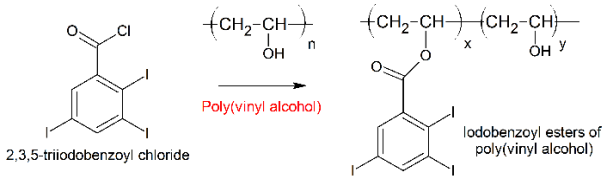

Figure 1. Iodinated polymer synthesis: covalent linkage of 2,3,5-triiodobenzoic chloride onto poly(vinyl alcohol).

Nanoparticles of iodinated polymers were prepared using the Nanoprecipitation method followed by a concentration step by means of centrifugation/redispersion. A suitable concentration for *in vivo* studies could be reached. Dynamic Light Scattering and Electron Microscopy allowed measurements of particle sizes and stability assessments. Radio-opacity of aqueous suspensions was assessed *in vitro* on phantoms of different concentrations. Discrimination of attenuation profiles from mixed elements and their quantification within a single scan were done. Biodistribution was studied *in vivo* by experiments on small animals (intravenous injections to rats), performed on a conventional CT and on the SPCCT for the sake of comparison.

Results.

Iodinated nanoparticles. Nanoparticles were stable and remained well-separated (Fig 2) for over 1 month in H_2O and human serum at 4, 20 and 37°C. Their mean diameter was in the 80-140 nm range, depending on the preparation method.

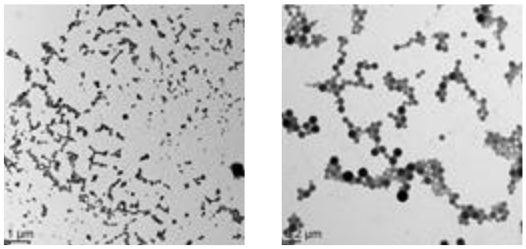

Figure 2. TEM images of aqueous dispersions of iodinated polymer before (left) and after centrifugation/redispersion.

SPCCT evaluation/phantom imaging.

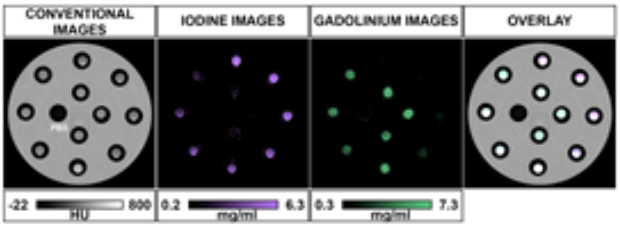

Figure 3. Conventional, Iodine, Gadolinium and overlay images are depicted.

Mixed contrast agents could not be differentiated with conventional CT. Contrast agents were accurately differentiated in the iodine and gadolinium specific images of SPCCT (Fig 3). There was a linear relationship between SPCCT measurements and known concentrations of iodine (measured by ICP-OES) [2].

***In vivo* imaging**. Nanoparticles accumulate in the liver and spleen up to 5 days post-injection. This is not the case for a standard solution of iodinated contrast agent that clears out after 30 min to 1 h.

Conclusions: The use of nanoparticles allows imaging the liver and the spleen for long. The SPCCT technology opens the door to quantitative determination of concentrations *in vivo* and multicolor imaging, in case of specific labelling of different parts of the body.

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Nanoemulsions as a useful tool for drug development

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Keywords: Nanoemulsion, Self-emulsification, Preclinical studies, Hemolysis assay

Introduction

Many drug candidates under development pipelines exhibit a low aqueous solubility and can be considered as Biopharmaceutical Classification System (BCS) classes II or IV molecules. "Develop-ability" of such molecules must integrate formulation strategies as soon as possible [1]. Indeed, these new chemical entities will never become drug candidates if innovative formulations are not considered. To enhance the solubility, and *in fine* the bioavailability of compounds, lipid based drug delivery systems (LBDDS) are the new trend in formulation and drug development [2]. However, considering the poor available quantity of lead compounds, to develop one innovative formulation for one compound is a heavy task. The LBDDS that we want to develop must be transposable to many drugs with various physico-chemical properties and be administrable by different routes (oral or parenteral routes). Among LBDDS, nanoemulsions (NE) which refer to nanosized droplets of oil dispersed in water, can be considered as a perfect delivery system easy to produce. They permit high drug loading, drug's protection, decreased drug's intrinsic toxicity and promote a prolonged drug release and an increased bioavailability [3]. Before their use in preclinical tests, NE have to undergo a variety of assays to identify potential safety concerns [4]. In particular, *in vitro* blood compatibility assays are helpful to identify acute toxicity due to the hemolysis caused by excipients or the formulation.

Materials & Methods

In order to define appropriate excipients, an *in vitro* test was set up in the laboratory to define hemolytic properties on human blood cells of excipients used alone or formulated. Through a design of experiments and a ternary diagram, the feasibility of NE with appropriate properties was determined by varying the relative proportion of excipients. Then, several process parameters (heating temperature, stirring time, stirring speed) have been modified to optimize the method. NE were prepared with generally recognized as safe (GRAS) excipients by spontaneous emulsification method, a low-energy process requiring no sophisticated equipment, and without using any organic solvent. The average hydrodynamic diameter associated with the polidispersity index (PDI) were checked and determined by dynamic light scattering. To be compatible with intravenous administration, osmolarity and pH were controlled thanks to a suitable phosphate buffer and the formulation was sterilized by sterilizing filtration. The drug recovered in NE, the NE stability in biomimetic media and the release kinetics studies were assayed by high performance liquid chromatography (HPLC).

Results

Among the most commoly used excipients for NE formulation, twenty excipients were tested and a medium chain triglyceride and two surfactants were selected for formulation assays. Based on a ternary phase diagram, the best formulation with optimal granulometric properties was defined. The nanodroplets are monodisperse in size with a diameter of 52.1 ± 2.1 nm and a PDI of 0.172 ± 0.015. Excipient having an osmotic effect, osmolarity of the formulation was adjusted by optimization of the phosphate buffer. The blank formulation, ready to use, presents an osmolarity of 277.3 ± 5.8 mOsm/L and a pH of 7.1 ± 0.2. Encapsulation assays of BCS classes II and IV were performed, and the stability of the formulation was shown in time, and also after dilution in various complex biomimetic media (Phosphate Buffered Saline (PBS), gastric medium).

Conclusion

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Drug development is a highly complicated and tedious process. To make it easier, LBDDS may be useful. The developed NE is safe, easy to produce and ready to use with granulometric and physicochemical properties optimized. Thus, it appears that the developed NE is appealing from the early drug discovery stages to allow *in vitro* and *in vivo* experiments.

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A new 5-FU derivative encapsulated in lipid nanocapsules : *in vitro* evaluation on 2D and 3D models.

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Keywords. 5-fluorouracil, Lipid Nanocapsules, Spheroids, HCT-116, 5-FUC12

Introduction.
5-Fluorouracil (5-FU) is an antineoplasic agent used against a wide range of solid tumors (such as breast, head and neck, colon, pancreas and stomach tumors) [1]. A reason for the lack in 5-FU efficiency is caused by a non-favorable pharmacokinetic profile and important toxicity [2]. In this respect, to improve toxicity/efficacy balance [3], numerous modifications of the 5-FU structure have been performed and novel derivatives of 5-FU have been reported [4, 5]. However, the results obtained using these derivatives are still marginal and 5-FU biodistribution and toxicity remain a challenging issue in oncology. Strategies based on nano and micro medicines appear as novel therapeutic approaches to optimize drug biodistribution and antitumor effect [6-8]. The final aim is to design systems with a high drug loading and an optimal release of chemotherapeutic agents into the tumor tissue, which reduce drug accumulation and toxicity in healthy tissue [9-10]. Even if the encapsulation in nanosystems results in an increased therapeutic efficacy of the drug, its high hydrophilicity represents a limiting step for the loading of such nanosystems as well as for the premature release of 5-FU from the nanocarriers when injected in blood.

Materials & Methods.
In the present work, using a simple synthesis process, 5-FU was modified with lauric acid to give a lipophilic mono-lauroyl-derivative, namely 5-FUC12. Then 5-FUC12 loaded lipid nanocapsules (LNC) have been developed. The rationale behind this strategy is that, increasing the lipophilicity of the drug, it will be possible to obtain a higher drug loading and a better controlled release of the drug once encapsulated into the lipid nanocarriers. These LNC have been characterized in terms of size dispersion, zeta potential, entrapment efficiency, stability in complex media. The feasibility and transposability of the system were evaluated and the batch formulation was scaled up 20-fold. The final product sterility and endotoxin content was assessed according to the European Pharmacopoeia recommendations. *In vitro* studies on colon (HCT-116) cancer cell lines to assess the efficacy of the derivative both in its free form or encapsulated into LNC were performed. Besides, three-dimensional (3D) spheroids made of HCT-116 cells were generated and the effect of 5-FU derivative alone and loaded into LNC was studied.

Results.
5-FU was modified with lauric acid to give a lipophilic mono-lauroyl-derivative namely 5-FUC12 with a yield of reaction of 70%. 5-FUC12 obtained was efficiently encapsulated into LNC (encapsulation efficiency above 90%) without altering the physico-chemical characteristics of LNC. Blank and 5-FUC12-loaded LNC displayed an average size of 65nm, a low polydispersity (<0.1) and neutral zeta potential. The encapsulation of 5-FUC12 led to an increased stability of the drug when in contact with plasma, being the drug detectable until 3h following incubation. Two-dimensional HTC-116 cell model viability reduction after 24h was assessed using a resazurin assay. 5-FUC12-loaded-LNC exerted a toxic effect at low doses (2.5 µM) in comparison with 5-FU or 5-FUC12 in solution (4µM and 20µM respectively). Blank LNC reduced cell viability at a concentration of 0.193 mg/ml. Multicellular tumor spheroids (MCTS) were prepared with HT116 cells and epifluorescence microscopy techniques were used to measure their volume as an assessment of toxicity. Contrary to 2D models, blank LNC in 3D models displayed high toxicity at concentrations starting from 0.875 mg/ml. While, loaded LNC, 5-FU or 5-FUC12 were able to reduce the volume of the spheroids at lower drug doses (2µM) showing a similar anticancer activity.

Conclusions.
A new lipophilic derivative of 5-FU was successfully synthesized with a high yield of reaction. This 5-FUC12 derivative was encapsulated in LNC which characterization showed that those particles remained stable in terms of size distribution, potential zeta, entrapment efficiency content over time. The batch was scaled up 20-fold, and the final product was stable, sterile and endotoxin-free. 5-FUC12-loaded-LNC had an enhanced cytotoxic effect on human colorectal (HTC-116) cancer cell line in comparison with 5-FU or 5-FUC12 in 2D

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and 3D model, while blank LNC displayed a reduction in cell viability only at high concentration. Globally, our data suggest that the encapsulation increased the activity of the 5-FUC12. However, in depth evaluations concerning the permeability of spheroids to LNC need to be perfomed to disclose the potential of these nanosystems for cancer treatment.

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High shear microfluidic rheology rheometer
Rheology optimization of ophthalmic eye drops

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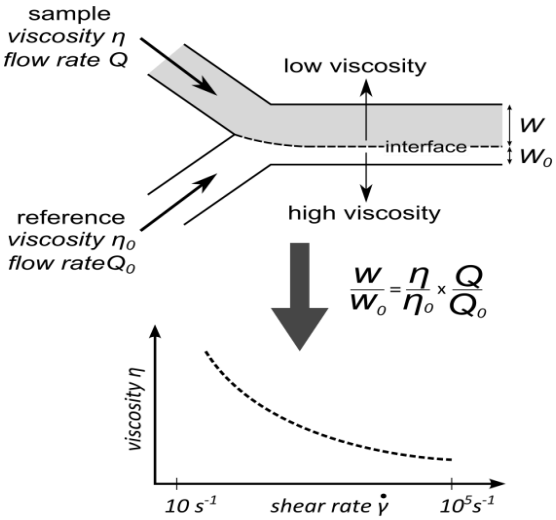
Keywords. High-shear viscosity, microfluidic, eye drops

Introduction.

Topical application represents the main route for administration of drugs to treat eye disorders. It is widely recognized that efficiency of ophthalmic formulations highly relies on their rheology. As blinking phenomena submits eyedrop to a wide range of shear rates, the rheology of formulations must be carefully optimized, and its full knowledge allows for residence time to be increased while keeping a maximal patient compliance. According to literature the shear rates during blinking are estimated between 4,000 s^{-1} and 30,000 s^{-1}. Viscosity measurement using conventional rheometers remains challenging regarding the low viscosity of the eye drops solutions and cannot perform measurement at such shear rate values.

Materials & Methods.

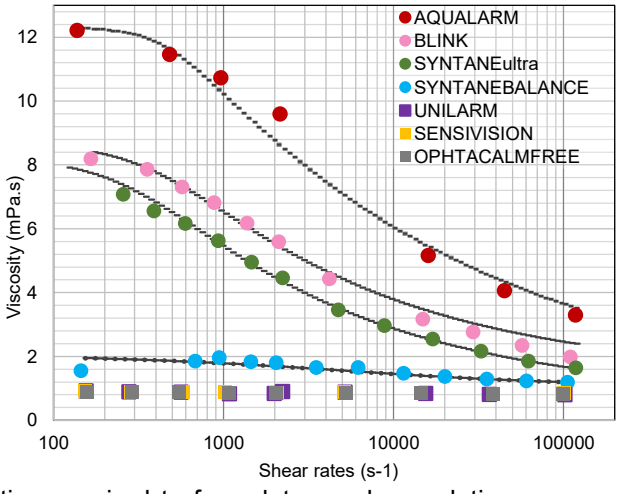
Fluidicam^{RHEO}, a microfluidic based instrument, allows to measure viscosity as a function of shear rate and temperature in a single experiment set-up. Using a small sample volume, the technology allows flow viscosity measurements of low viscosity samples in a wide range of shear rates (up to 10^5 s^{-1}). During the measurement, a sample and a viscosity standard are pushed together through a microfluidic chip (Y-junction) at controlled flow rates. Images of the resulting laminar co-flow are acquired with a digital camera and the position of the interface is measured. The interface position is related to the ratio of flow rates between the sample and the reference allowing to determine the viscosity. In this work different formulations of eyedrops have been analyzed with Fluidicam^{RHEO} over a range of shear rate from 150 – 180 000 s^{-1} at 34 °C (corneal surface temperature) in order to identify the rheological behavior of the formulations.



Results.

As shown in the graph, depending of the use of eyedrops formulations, the physical properties and the rheological behaviour vary. Newtonian formulations (purple, grey and yellow squares) are usually used for cleaning reasons with viscosity around tears viscosity values, providing a short retention time allowing to clean the corneal surface with less therapeutic effects.

Whereas non-Newtonian formulations (red, pink, green and blue dots) can reach high viscosity values at low shear rates providing a higher retention time, they are usually used for therapeutic reasons and topical treatments.



Conclusions.

FLUIDICAMRHEO allows accurate viscosity measurement at a range of shear rate representative of the stress applied during blinking. Thus, the instrument provides relevant information required to formulate eyedrop solutions with optimum physical properties. The use of microfluidic device allows to perform precise viscosity characterization with minimum sample requirements. The viscosity measurement is made efficient, as analyzing 7 samples, studied in this note, requires less than 30 minutes.

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Translation of Nanoparticle Formulations from Lab to Industrial Scale Synthesis: The Case of Squalene-Adenosine Nanoparticles

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Keywords. Drug-delivery systems, impurities quantification, clinical translation, Squalene Adenosine nanoparticles

Introduction.
A large variety of nanoparticle based delivery systems have become increasingly important for diagnostic and/or therapeutic biomedical applications [1]. Physical and chemical drivers that lead to multiple types of particle instabilities complicate both the ability to produce, appropriately characterize, and consistently deliver well-defined particles. This frequently leads to inconsistencies, conflicts in the published literature, and ultimately, failure in the clinics [2]. Part of the problem lies in the fact that developing drug-delivery systems with complex features has prevailed over the desire to treat a disease effectively with a simple, robust, and safe formulation [3]. Another issue lies in the challenges of scaling up nanomaterial synthesis from the lab to industrial scale.

Recently, Squalenoylation has emerged as a simple, safe and robust method to produce nanoparticles encapsulating a vast range of therapeutic agents [4]. Conjugating squalene, an endogenous, biocompatible and biodegradable lipid, to drug candidates yields nanocarriers with high drug loading efficiency, low toxicity, and high blood residence time. These nanoparticles have showed promising results in the treatment of diverse diseases such as cancer, ischemic stroke or in the management of pain [5] [6]. Efforts now need to focus on the translation from lab-scale experiments to semi-industrial batches allowing larger pre-clinical trials and eventually aimed at obtaining regulatory approval.

Through the example of squalene-based nanoparticles, we report here on the challenges encountered in the process of scaling-up nanomedicines synthesis. Notably, we demonstrate the influence of squalene analogs resulting from industrial scale up development. These impurities, even when present in low quantities, can have tremendous impact on the reproducibility of nanoparticle properties from batch to batch. Characterizing and solving these complications is a crucial step in the translation towards the clinical use of nanostructured drug carriers.

Materials & Methods.
Nanoparticle Formulation: Squalene-Adenosine (SQAd) was obtained either by *in-lab* synthesis or by industrial process from a private partner. SQAd nanoparticles were prepared using the nanoprecipitation technique. SQAd was dissolved in absolute ethanol and added dropwise under strong stirring to a 5% (w/v) dextrose solution. Ethanol was then completely evaporated to obtain an aqueous suspension of pure SQAd nanoassemblies. Nanoparticle size and surface charge were systematically measured after preparation using a Malvern Nano ZS and morphology observed by cryogenic transmission electron microscopy.

Prep-HPLC: The HPLC UV-VIS detector was set at 270 nm. The HPLC was coupled to a Mass Spectrometer with ionization ESI Plus. Separation and quantitation were performed on a 5 um column inverse phase C18 column. Using the following gradient (H2O / ACN:MeOH (50:50) 80-100% in 10 min) different batches of SQ Ad bioconjugates were introduced into the system and pure samples were reconstituted from collected fractions.

Cytotoxicity assay: An MTT test was performed on four cell lines (MCEC, HepG2, Miapaca, HUVEC) in 96 well plates.
Cells were plated and grown for 24 hours. Subsequently, cells were treated with different concentrations of nanoparticles ranging 1 ug/mL to 200 ug/mL in 100 uL of complete cell medium. Cells were incubated for 24, 48, or 72 hours before an MTT assay was performed. For the MTT assay, cells were washed with PBS, and incubated for 2 hours with medium containing 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-

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diphenyltetrazolium bromide. Medium was removed after 2 hours and 100 uL of DMSO was added to each well before absorbance reading at 570 nm.

Results.
An HPLC-MS study of different batches (industrial *vs in-lab*) of SQAd showed varying impurity profiles that were not easily detectable by NMR study. UV-VIS integration of the HPLC chromatograms indicated that industrial batches contained ~5% impurities. Impurities were isolated, characterized and proven to be derived from squalene analogs. DLS and TEM studies showed that NPs that were formulated with impure industrial SQAd had structures, surface charge and sizes that were radically different from lab synthesized SQAd NPs. These differences in physico-chemical properties were confirmed by small-angle X-ray scattering studies of the crystal lattice of different batches of SQAd NPs. Isolation of the main impurities showed that their LC_{50} was more than 10-times higher than what could be found with pure SQAd. This factor alone could greatly influence the reproducibility of pre-clinical results and ultimately the efficiency of the drug development process.

Conclusions.
We show that industrial scale synthesis of bioconjugates using lab scale based synthetic methods can induce the presence of impurities that are not always easy to detect. As such an especially careful approach in terms of toxicity and pharmaceutical development should be adopted in the maturation process of these innovative materials. Indeed, nanoparticle based delivery systems are often complex structures that aim at accumulating bioactive molecules inside of tissues and cells. This makes them a class of materials that is especially sensitive to variations in purity from batch to batch. Understanding and solving these issues is a crucial part of the pharmaceutical development of nanomedicines, and one which cannot be left for the industry to deal with alone.

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Development of Gold nanoparticles for the characterization of different bacteria by Surface Enhanced Raman Scattering

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Keywords: Surface-Enhanced Raman Spectroscopy, Bacteria characterization, Rhodamine 6G (R6G), Gold nanoparticles

Introduction

Identification and quantification of microorganisms has become an essential issue in many areas like human health care and diagnostics, food and water safety and biodefense [1]. The conventional counting method, using plating and culturing, is the most straightforward and widely used method for this purpose but it appears tedious and time consuming. Since its discovery, Surface Enhanced Raman Spectroscopy (SERS) technique is increasingly used as faster and more convenient alternative. This technique allows to provide a high enhancement of Raman scattering from molecules adsorbed on a rough noble metal surface (silver, gold or copper). This phenomenon is explained by the combination of two well documented mechanisms: Electromagnetic effect (EE) and chemical effect (CE) [2]. The objective of our work was the development of gold nanoparticles (AuNps) as SERS substrate for the characterization of bacteria in order to avoid the use of the tiresome conventional counting method.

The present study was divided in two parts. The first part consisted in developing the AuNPs synthesis method. Physico-chemical features of the synthetized AuNPs and their SERS efficiency using R6G model analyte have been investigated as a function of the synthesis experimental conditions. In the second part, the optimal operating conditions were used for bacteria characterization. The developed method was first tested for the quantification of *Lactobacillus rhamnosus GG* (LGG), a Gram-positive bacterium indicated for the treatment or the prevention of antibiotic-associated diarrhea. This strain being widely used in encapsulation systems, its characterization is required to determine encapsulation efficiency and bacteria release kinetics [3]. Then the potential of the developed method was investigated by characterizing other bacterial strains such as gram-negative bacterium *Escherichia coli* and gram-positive bacterium *Bacillus subtilis*.

Materials & Methods

SERS active AuNps were produced by an acidic treatment of Au nanostars precursors which were synthesized in a one-step process by reduction of $HAuCl_4$ by hydroxylamine.

RamanRxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, USA), equipped with a thermoelectrically cooled CCD detector, was used in combination with a non-contact probe. Raman spectra were acquired under 785 nm irradiation (400 mW). To record SERS signal, 50 µL of R6G aqueous solution or bacteria suspension were added to 5 mL of nanoparticles suspension and spectra were measured in 1 cm Quartz-cell with 15 sec data acquisition and two spectral accumulations. Data collection was operated using the Holoreact™ software and spectra were processed using Matlab® R2009b software (Mathworks Inc., Natick MA, USA).

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Results

Because of the weakness of inelastic light scattering phenomena, conventional Raman spectroscopy requires highly concentrated samples. In the case of R6G, no Raman signal is detected at a concentration lower than 0.1 mM (Fig.1). Our developed SERS active uncoated spherical AuNPs provided a great enhancement of R6G Raman signal with the possibility of detection at a concentration lower than 5 nM (Fig.1). Moreover, the signal provided by these stable AuNPs in acidic environment appeared to be 10^2-10^3-fold greater compared to star-like Au precursors or classical citrate-reduced AuNPs of same dimensions. This surprising result could be explained by the significant contribution of the chemical enhancement in the overall increase of Raman signal as well as the absence of stabilisation agent that could hinder the proper analyte adsorption at nanoparticle surfaces. The magnitude of the signal enhancement was further improved by taking the advantage of the electromagnetic effect through partial aggregation of the nanoparticles suspension but with some disadvantages (low signal stability, rise of signal background…). Optimal operating conditions were thus identified and then used for bacteria characterization.

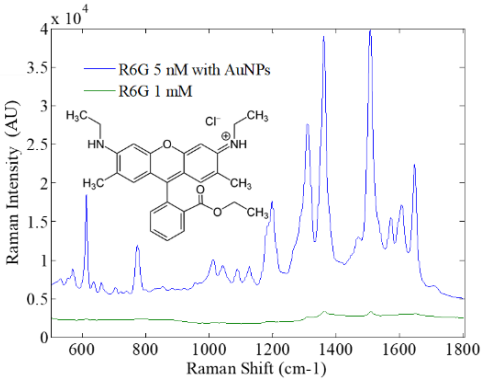

Fig. 1. Rhodamine 6G Raman signal enhancement by AuNPs

In the case of LGG Raman signal, the peak at ≈ 735 cm^{-1} (Fig. 2a) was the most important feature and might be attributed to polysaccharide structures (peptidoglycan) of the bacteria cell wall. The corresponding Raman intensity measured at this peak was observed to linearly evolve with LGG concentration with a variability lower than 20% (Fig. 2b). This satisfying result showed that the developed analytical method was suitable for LGG quantification purpose. The use of this method for the characterization of two other strains, namely *E.Coli* and *B.Subtilis*, showed also its potential in bacteria discrimination assay. Indeed, Gram-negative *E.Coli* and Gram-positive LGG and *B.Subtilis* displayed some differences in their overall spectra (Fig. 2a) allowing to discriminate them especially through Principal Component Analysis (PCA).

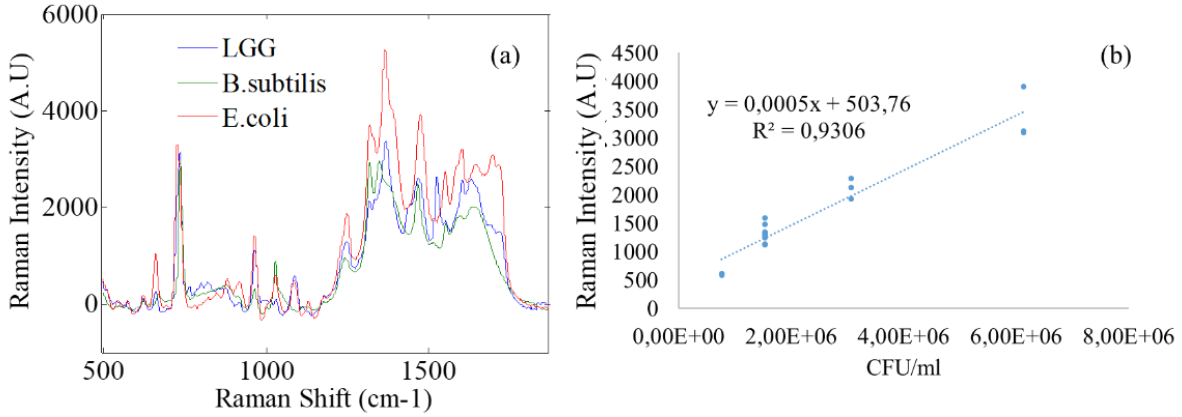

Fig. 2. (a) SERS spectra of LGG, *E.Coli*, *B.Subtilis* in presence of uncoated spherical nanoparticles, (b) SERS signal (735 cm^{-1}) as a function of LGG concentration.

Conclusions

In this study efficient, convenient and fast SERS method employing uncoated spherical gold nanoparticles was developed allowing the detection and quantification of small chemical molecule and LGG microorganism. This method has also been successfully employed for the detection of gram-negative (*E.Coli*) and gram-positive (*B.Subtilis*) bacteria. Further experiments will be performed to investigate the influence on bacteria SERS signal of formulation excipients classically used for bacteria encapsulation purpose.

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Posters sessions

Posters 01 - 20

P. 01	Virginie	CAPRASSE	Silicone Solutions for Acne Management
P. 02	Laurie	MAES	Impact of silicone polymers on pain management drug delivery
P. 03	Yi	ZHOU	Nano- versus Micro-particles for S-nitrosoglutathione formulation
P. 04	Enrica	CAPPELLOZZA	In vivo and ex vivo biodistribution of solid lipid nanoparticles
P. 05	Rama	ARAB	36% of oral administrations of liquid forms of 10 drugs are substitutable with suitable solid forms
P. 06	Alice	DENIS	Innovative Rheology Modifiers for Mucosal Formulations
P. 07	Aurelie	MALZERT-FREON	Lipid-based nanoformulations strategies integrated in drug discovery
P. 08	Mohamad	TARHINI	Protein based nanoparticles: Synthesis, optimisation, and biomedical application
P. 09	Mariana	MACIEL DE OLIVEIRA	Encapsulation of dihydrocaffeic acid in a phenylboronic acid-modified hyaluronic acid hydrogel
P. 10	Awa Nakognon	TUO EPSE KOUASSI	Physicochemistry and antioxidant properties of ivorian plants, encapsulated for treatment of alopecia in black women
P. 11	Armelle Adjoua Sandrine	AKA APSE ANY-GRAH	Optimization and safety of a gel based on sacoglottis gabonensis (humiriaceae) extract
P. 12	Sabrina	BARBOSA DE SOUZA FERREIRA	Mechanical properties of emulgels containing curcumin: Texture profile analysis, syringueability and softness determination
P. 13	Armelle Adjoua Sandrine	AKA APSE ANY-GRAH	Development of a vaginal innovative gel with ceftriaxone and with cefixime for the treatment of the gonorrhea
P. 14	Armelle Adjoua Sandrine	AKA APSE ANY-GRAH	Development of dermo pharmaceutical forms based on the fruit of Alchornea Cordifolia for the treatment of dermatophytes
P. 15	Mariana	VOLPATO JUNQUEIRA	Preparation and characterization of microsponges containing metronidazole using different organic phases
P. 16	Armelle Adjoua Sandrine	AKA APSE ANY-GRAH	Moringa oleifera (moringaceae): from oil of grain to antioxidant cream
P. 17	Annalisa	ROSSO	Rationally designed tacrolimus-loaded nanoemulsion: a novel stable oral drug delivery system
P. 18	Roland	RAMSCH	Characterization of Aluminum salts suspension in vaccines using Static Multiple Light Scattering
P. 19	Claudia	COGNE	Influence of impurity on ice crystal nucleation
P. 20	Lorine	LE PRIOL	Microencapsulation of Edible Oil by Using Bio-based Complexes as Wall Materials against Lipid Oxidation

P. 22	Greta Camilla	MAGNANO	Nanoceria, a promising strategy for decontamination of chemical warfare agents
P. 23	Roland	RAMSCH	Dispersibility and stability characterization of TiO2 nanoparticles in cell culture media with static multiple light scattering
P. 24	Roland	RAMSCH	Analysis of gelation capability of human platelet lysate
P. 25	Lynda	LAMOUDI	Simulation numérique du coefficient de diffusion d'un Principe actif à partir d'un système à géométrie variable
P. 26	Dally	LABA ISMAEL	Study of some rheological properties of a poloxamer 407 thermogelifying gel / starch of ipomoea batatas from cote d'ivoire
P. 27	Camille	DUMONT	Solid lipid nanocarriers: high potential formulations for oral delivery of peptides
P. 28	Emilie	MUNNIER	PEO-PPO-PEO nanomicelles prepared by a green emulsion-evaporation process: characteristics and thermoresponsive properties.
P. 29	Catherine	CHARCOSSET	Optimization of liposomes formulations for improving the depigmenting effect of cysteamine
P. 30	Paula	MURESAN	Generating Injectable Intrathecal Formulations for Poorly Soluble Drugs
P. 32	Emilie	MUNNIER	Encapsulation of Spirulina platensis green extracts for anti-biofilm use
P. 33	Daniele	MASSELLA	Scalable production of polymeric nanoparticles for transdermal delivery and bio-functional garments production.
P. 34	Fahima	TAMANI	How differently do single PLGA microparticles behave?
P. 35	Baptiste	ROBIN	Emulsions stabilized with biodegradable PLGA nanoparticles: influence of nanoparticle physicochemical properties
P. 36	Fabrice	OFRIDAM	Elaboration of Eudragit L100 based particles via precipitation process: effect of added secondary polymer
P. 37	Bouameur	SARAH	Contact angle: A screening test for formulation of Solid Lipid Nanoparticle
P. 38	Sylvie	BEGU	PEG free formulations for antioxydant topical delivery
P. 39	Narimane	LAMMARI	Encapsulation of vegetale oil via nanoprecipitation process using Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammoniethyl methacrylate chloride) (Eudragit RS 100)
P. 40	Florian	VANNESTE	Influence of lipid formulation on the lipid adsorption onto polymer particles
P. 41	Nathalie	SINTES-ZYDOWICZ	A design of experiment approach to non-isocyanate polyurethane nanoprecipitation: Toward an optimized preparation of polymer nanoparticles
P. 42	Jean-Marie	AUBRY	A promising tool to accurately predict the surface tension of liquids: application to cosmetic oils for spreading properties improvement

Silicone Solutions for Acne Management

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Keywords. Acne, Silicone, topical formulation

Introduction.
Acne is a common skin disease that occurs when hair follicles become clogged with dead skin cells and oil from the skin. Acne has been estimated to affect 660 million people globally, making it the 8th most common disease worldwide[1].
We will go through recent studies where silicone technologies have been successfully used as topical excipients in dermatological formulations. Innovative formulation concepts dedicated to acne demonstrating drug release and skin benefits including, non-occlusivity, long-lasting and desired aesthetics are highlighted in this poster. These formulations have been developed utilizing only salicylic acid as the chosen active ingredient

Materials & Methods.
The release experiment with salicylic acid was carried out using Franz diffusion cell console at 32°C for a period of 20 hours. Polyether sulfone membrane (PES, 0.22μ pore size, Millipore Corporation, Billerica, MA) was used as the membrane. All samples were analyzed using UPLC system to determine the salicylic acid.

The in vitro permeability of salicylic acid was assessed through dermatome piglet skin tissues using a receptor medium made of acetate buffer pH 5.5. Experiments were done over a 20 hour period with a Logan 912 auto-sampler system. Temperature was set to 32°C. Permeated samples were analyzed by UPLC. At the end of the diffusion period, the different skin layers, which included stratum corneum, epidermis and dermis, were separated for salicylic acid recovery analysis.

The sensory evaluation for skin care products is designed to provide a sensory profile of selected formulations assessed individually and rated versus one another, evaluated by an experienced panel. All sensory data are analyzed using critical response tables with significance for $\alpha<0.05$.

Formulation concept:

- Concept 1- Invisible Acne Films (2488 & 2514) - is based on the development of a formulation containing salicylic acid and tea tree essential oil that forms an invisible and conformable film on the skin. Two Invisible Acne Film formulations are developed.
- Concept 2 – Acne Lotions (Clear & White) - The aim of this concept is to develop lotions with salicylic acid which are white or clear according to the ratio between the different ingredients.
- Concept 3 – Protective Acne Patch - The aim of this development is really to develop a small patch loaded with salicylic acid and designed for acne treatment and spot camouflage.

Results.
Concept 1, Invisible Acne Films, the composition of the formulation has an impact on the release of active. The presence of the silicone fluid and medium chain triglycerides in the formulation 2514 allows the release of salicylic acid compared to a formulation 2488 without these excipients. Invisible Acne Film 2514 with silicone fluid and medium chain triglycerides shows a smoother, greasier and tackier feel on the skin versus the Invisible Acne Film 2488.

Concept 2 – Clear Acne Lotion or White Acne Lotion – a faster penetration rate is observed when salicylic acid is formulated in White Acne Lotion. The salicylic acid distribution profile in each skin layer is different between the White and the Clear formulations. Salicylic acid concentrations in stratum corneum and dermis are higher for the Clear Acne Lotion. In terms of sensory profile, Clear Acne Lotion compared to White Acne Lotion, shows a higher film presence and smoother feel, it is greasier and tackier with a less mattifying effect.

Concept 3: The aim of this development is really to develop a small patch loaded with salicylic acid and design to treat acne and mask spot. The release of salicylic acid from this patch is comparable to a commercial benchmark.

Conclusions.

The versatility of the silicone chemistry in terms of functionalities and characteristics, translated into an easy to use excipient tool box for topical applications. A wide range of formulation options to load, stabilize and release various drugs for dermatological and local treatments are offered as demonstrated by the different silicone concepts loaded with salicylic acid in this poster. Optimizing the efficiency of drug delivery and behavior of the formulations on skin would certainly lead to better perceived efficacy by the user associated with a more pleasant sensory feel required for this specific skin disease. Such performance benefits may potentially increase the patient compliance to the treatment requirements.

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Impact of silicone polymers on pain management drug delivery

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Keywords. silicone, lidocaine, pain management, topical formulation, substantivity

Introduction.
Silicones are widely used in various medical and pharmaceutical applications such as wound and scar management devices, topical and transdermal therapeutic systems. They have been successfully formulated as pharmaceutical excipients in dermatological and topical drug delivery forms to improve medication efficacy and acceptance by the patients, which are critical requirements in pain management drug.
The aim of this study was to assess the benefits of selected silicones on key parameters of topical forms e.g. substantivity and skin permeation, using lidocaine as model drug.

Materials & Methods.
To conduct the comparison study, two different silicone materials were considered according to their polymeric structure and consequently their resulting performance attributes as detailed hereafter:

- DOW CORNING™ Q7-9120 Silicone Fluid, 20 cSt: a non-occlusive and non-volatile silicone fluid
- DOW CORNING™ Dimethiconol Blend 20: a silicone gum blend enhancing substantivity and film forming properties on skin.

Each silicone material was formulated in two different types of topical formulations: water-in-oil and anhydrous gel.

The *in-vitro* permeability experiments were conducted for 20 hours at 32°C in Franz cell apparatus using dermatomed piglet skin as membrane and phosphate buffered saline (PBS) as the receptor fluid. The amount of drug that permeated through the skin was determined using UPLC.

The skin substantivity versus time was evaluated in order to quantify the durability and long lasting effect of the formulation. The test was performed by applying the formulations onto panelist forearms; the silicone staying on skin was detected and analyzed by infrared spectroscopy using an attenuated total reflectance Fourier transform infrared spectrophotometer (ATR-FTIR) equipped with a skin analyzer device.

Results.
There was no significant difference in lidocaine delivery between the two W/O emulsions containing either silicone fluid or silicone gum blend. After 20 hours, the whole lidocaine content was recovered in both the skin and receptor chamber and about 15% of lidocaine was dosed in the skin. A similar repartition of lidocaine in each skin compartment was also observed after 20 hours.
The nature of the silicone polymer in the emulsion impacted the substantivity. The W/O emulsion with silicone fluid showed poor substantivity on skin with less than 10% of silicone remaining on skin after three and five hours. The W/O emulsion with silicone gum blend showed a better substantivity, around 35% of silicone remained on skin after three hours and more than 20% after five hours.

A higher diffusion rate of lidocaine was obtained with the Anhydrous Gel containing the silicone fluid. 25.1% of lidocaine were recovered in the receptor medium and skin layers for the Anhydrous Gel with silicone fluid versus 17.3% for the Anhydrous Gel with silicone gum blend.
The total amounts of lidocaine in the skin were similar but with different distribution profiles in the skin layers: the lidocaine concentration in the stratum corneum was higher for the Anhydrous Gel with silicone gum blend.
The nature of the silicone polymer also influenced the substantivity of the in the Anhydrous Gel. With silicone gum blend a good substantivity was observed with 60% of silicone remaining on skin after five hours. The Anhydrous Gel with silicone fluid showed a lower substantivity with only 30% of silicone remaining on skin after five hours.

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Conclusions.
The unique and versatile characteristics of silicone chemistry in terms of functionalities and characteristics offers a wide range of formulation options to load, stabilize and release various drugs for dermatological and local treatments as demonstrated by the lidocaine formulations either in emulsion or anhydrous gel forms. Silicone polymers can allow for a sustained delivery of lidocaine by enhancing the substantivity of the formulation on the skin surface. As result, formulators can design more efficient drug delivery systems with enhanced skin performances that increase patient compliance and efficacy.

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Nano- *versus* Micro-particles for *S*-nitrosoglutathione formulation

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S-nitrosoglutathione (GSNO) is a physiological nitric oxide donor and a promising drug for several acute or chronic diseases (e.g. stroke, atherosclerosis, …). However, this nitrosated tripeptide is highly hydrophilic and easily degraded while exposed to oxygen, light, metallic cations or enzymes. The formulation of GSNO, especially for oral delivery, is therefore very challenging.

In this work, three formulations based on the same raw materials (GSNO and Eudragit RLPO) were prepared with emulsion/solvent evaporation methods: nanoparticles (W/O/W, NP-W), microparticles (W/O/W, MP-W) and microparticles (S/O/W, MP-S). These particles were characterized as regards their size (dynamic light scattering/laser diffraction and scanning electron microscopy), zeta potential, GSNO encapsulation efficiency (EE) (fluorimetry), *in vitro* release (USP IV apparatus), cytocompatibility and intestinal permeability (Caco-2 cells monolayer). Moreover, the three formulations were obtained as dried powders, after optimization of their freeze-drying. Characterization was also performed on these stabilized formulations, including their residual water content (evaluated with a coulometric Karl-Fisher determination).

The three particles were able to encapsulate GSNO with similar EE (NP-W = 28.7 % ±3.3 % (n=11), MP-W = 24.4 % ± 3.7 % (n=7), MP-S = 25.1 % ± 3.6 % (n=5), direct determination). Freeze-drying using sucrose as protectant led to elegant cakes, easy to re-suspend, with similar EE as fresh formulations. On the other hand, particles sizes were not significantly modified by drying (NP-W: 290.7 nm ± 14.5 nm *vs* 299.7 nm ± 12.7 nm, n=6; MP-W: 70.8 µm ± 7.5 µm *vs* 68.5 µm ± 5.5 µm, n=3; MP-S: 147.5 µm ± 38.5 µm *vs* 105.6 µm ± 22.6 µm, n=3). Zeta potential of NP-W remained unchanged and highly positive (around + 55 mV), as expected according to the polymer used. Freeze-dried particles had a residual water content around 5 % and all their characteristics were maintained for at least 6 months after storage at 4°C under inert atmosphere. GSNO release from NP-W was very fast, almost similar to the dissolution profile of the free drug, while MP formulations delayed the release during the 2 first hours of the experiment, with a more progressive profile for MP-S compared to MP-W. All formulations were compatible with Caco-2 cells for up to 8.5 mg/mL of freeze-dried powder (corresponding to 2.8 mg/mL of polymer and 0.017 mg/mL of GSNO, i.e. 50 µM of GSNO). Intestinal permeability of GSNO released from these formulations is currently under evaluation in an *in vitro* intestinal barrier model.

At the end, three different particles loaded with GSNO were obtained as dried and stable formulations, thus facilitating their preclinical use. Among them, microparticles obtained with a S/O/W process could represent the most interesting lead to explore for GSNO oral delivery. Better results in terms of EE and sustained release might be obtained by reducing the size of GSNO powder (currently 40 µm). Due to GSNO fragility, this step is challenging: experiments are ongoing using supercritical fluid technology.

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In vivo and ex vivo biodistribution of solid lipid nanoparticles

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Keywords. Solid Lipid Nanoparticles, Fluorophores, *In vivo* and *ex vivo* biodistribution, Fluorescence microscopy, Transmission electron microscopy

Introduction.
Solid Lipid Nanoparticles (SLN) are well known drug delivery systems due to their versatility and advantageous properties as molecule protection from enzymatic and chemical degradation upon administration, thus allowing increased drug bioavailability and modulation of drug release. Moreover, they are composed by a biocompatible and biodegradable matrix that ensures a safety profile [1].
The aim of this study is to investigate the biodistribution of SLN produced by a protocol based on emulsification and ultra-sonication method. These nanoparticles are functionalized with polysorbate 80 (P80), a non-toxic surfactant that increases the nanoparticles half-life, avoiding opsonisation with the complement activation and uptake by the reticulo-endothelial system (RES) [2]. Accordingly, preliminary data obtained by comparing SLN produced by the same protocol, with and without P80, demonstrated lower RES uptake values of SLN functionalized with P80 [3].
In this study, to evaluate both *in vivo* biodistribution and *ex vivo* tissue accumulation of nanoparticles, SLN were labelled with two different fluorophores, i.e., Indocyanine green (detectable by the optical imager for the *in vivo* study) and Rhodamine (detectable by fluorescence microscopy for the *ex vivo* study).

Materials & Methods.
SLN are composed by tristearin as lipid phase and an aqueous poloxamer 188 solution, functionalized with P80 and labelled with Indocyanine green (Cardiogreen) and Rhodamine (SLN-CaRo). SLN were prepared by emulsification and ultra-sonication method reported in [4], with minor modifications. They were characterized for their physico-chemical properties, and their stability was evaluated from the dimensional point of view and lipid composition up to 6 months. SLN-CaRo were i.p. administered to male athymic mice and fluorescent emission kinetic was acquired with an IVIS Spectrum (Perkin Elmer) at predefined times within 4 hours. Target organs (liver, kidney and spleen) were excised after 4 hours from administration, and immediately acquired with the same parameters: excitation filter 740 nm and emission filter 800 nm for Indocyanine green, excitation filter 570 nm and emission filter 620 nm for Rhodamine. Some treated mice were perfused with an aldehyde fixative solution after optical imaging acquisition, and the target organs were excised and processed to be stored at -80°C until use. Seven µm-thick cryostat sections obtained from these samples were stained with Hoechst 33342 and analyzed with conventional fluorescence microscopy (Olympus BX51) while, for brightfield microscopy analysis, cryostat sections were stained with Oil Red O and Mayer's hematoxylin solution. For transmission electron microscopy, the samples were fixed with a paraformaldehyde/glutaraldehyde solution, post-fixed with osmium tetroxide, dehydrated and embedded in Epon-Araldite. Seventy nm-thick sections were observed with a Philips Morgagni transmission electron microscope (FEI).

Results.
The production of SLN labelled with two different fluorophores made it possible the combined *in vivo* and *ex vivo* studies on biodistribution and tissue accumulation of nanoparticles following systemic administration.
The *in vivo* studies proved a preferential uptake of SLN in the anatomical area of liver following i.p. administration. The data are confirmed by the *ex vivo* tissue accumulation observed in the whole excised livers and in sections of these, whereas no evident fluorescent signal was detected in kidney and spleen.
Brightfield microscopy images of livers revealed no histological alteration after treatment, but a marked increase in lipid content, especially in the centrilobular vein area, was found. Fluorescence microscopy images demonstrated that SLN are able to enter the hepatocytes, especially those around the centrilobular vein, thus localizing in the cytoplasm. The absence of evident structural damage confirms previous data on biocompatibility and biodegradability of SLN.

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A relevant increase in lipid content in hepatocytes treated with SLN was observed also at transmission electron microscopy. Despite SLN biocompatibility, a cellular stress can be noticed: the nuclei were almost without heterochromatin clumps, there was a rarefaction of glycogen and the rough endoplasmatic reticulum lost its classical arrangement. However, no relevant organelle damage was found.

Conclusions.

The *in vivo* and *ex vivo* images indicate that SLN functionalized with P80 accumulate in the liver, as the main metabolising organ. SLN are able to enter the hepatocytes, localizing in the cytoplasm, and inducing a limited damage. In particular, an accumulation of SLN was observed in the cells around the centrilobular vein, an area of hepatic lobule with a particular physiological role, where also lipid droplets where found to accumulate.
These results provide a solid methodological background for combined *in vivo* and *ex vivo* studies on the biodistribution of SLN, and demonstrate how the SLN administered systemically accumulate in the liver. This should be taken into consideration in planning the utilization of these nanoparticles for therapeutic purposes.

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36% of oral administrations of liquid forms of 10 drugs are substitutable with suitable solid forms

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Oral liquid forms are commonly prescribed for children. Solid oral forms provide important advantages compared with liquid forms in terms of cost, stability, transportability, excipients' safety and dosing frequency. The gap between the prescriptions amounts of liquid forms and equivalent solid forms is attributed to the low swallowability and the dosage unacceptability of solid dosage forms. Based on data of 312152 oral drug administrations, we aimed to determine the best opportunities of liquid formulations substitution with suitable solid dosage forms in paediatric practice without any regulatory limitations and the potential economic impact associated with. The 10 drugs have the best opportunities of substitution are identified, and the possibility of substitution in each of them is determined, according to the swallowability and dosage acceptability of available solid forms, conformity with the instructions of the summaries of product characteristics. the 10 identified drugs are paracetamol, cyamemazine, clonazepam, valproic acid, hydroxyzine, furosemide, prazepam, amitriptyline, levetiracetam and prednisolone. The solid dosage forms could be used in 36% of the total administrations of liquid forms of the 10 drugs. Almost all substitution opportunities are associated with cost savings.

Innovative Rheology Modifiers for Mucosal Formulations

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Keywords. Polymer, Mucoadhesion, Tolerance, Dermopharmacy

Introduction.

Formulation development for mucosal applications is progressing nowadays with the raise of topical forms. From the oral cavity to the vaginal and perianal regions, several active ingredients can be delivered. To overcome washout from biological liquids such as saliva, mucoadhesive excipients are selected to allow prolonged contact with the targeted site, thus improving drug availability and treatment efficiency.

Mucoadhesion is the ability of a material to adhere to a mucous membrane, resulting from interactions with the mucins. In this context, conventional polyelectrolytes [1] are known to provide mucoadhesive hydrogels. However, their ionic interactions with mucin are affected by physiological pH variations, especially in acidic condition such as in vaginal area, as well as in low pH formulations driven by API efficacy concerns.

Innovative polyelectrolytes rheology modifiers were developed to achieve more robust structures, largely ionized on a wide range of pH in order to optimize API stabilization and mucoadhesion.

The objective of this study was to challenge the mucoadhesive properties and mucosal tolerance of new ingredients, at realistic use levels in semi-solid and liquid formulations. The three rheology modifiers chosen were synthetized by two polymerization routes providing liquid or powder final appearance. Robust structures versus pH variations were achieved by introduction of a monomer containing a strong acid group. A comparative method was developed to assess mucoadhesion with a texture analyzer [2]. In addition, tolerance of the selected polymers was evaluated at a pre-clinical step on reconstructed mucosa.

Materials & Methods.

1. Mucoadhesion

The measurement is based on detachment force principle using TA-XTplus texture analyzer (Stable Micro Systems, Surrey, UK). Mucin disks from porcine stomach (type II) were prepared by compression and fixed to the lower end of the probe. The samples were placed inside the Pelletier cabinet at 25°C and the probe was lowered until it reached the hydrogel surface. The probe was then withdrawn until complete detachment to record the work of adhesion (three replicates). The results were plotted against polymer concentration in aqueous gels.

2. Tolerance

Tolerance is scored with *in vitro* reconstructed 3D models (Skinethic™ mucosa, supplied by Episkin).

- Gingival epithelium composed of normal human gingival cells cultivated in a defined medium.
- Vaginal epithelium from human vulva epidermoid carcinoma cells line cultivated in a defined medium.

30µl of each hydrogel are applied on the epithelium surface and cell viability is measured through a MTT test after different times of contact. Negative and positive controls were selected to validate the methodology and results.

Results.

1. Mucoadhesion measurement

The mucoadhesion against the concentration of three polymers is presented in figure 1 below. Results depend on polymer structure. Polymer 3, including hydrophobic groups provided a positive response at low doses only.

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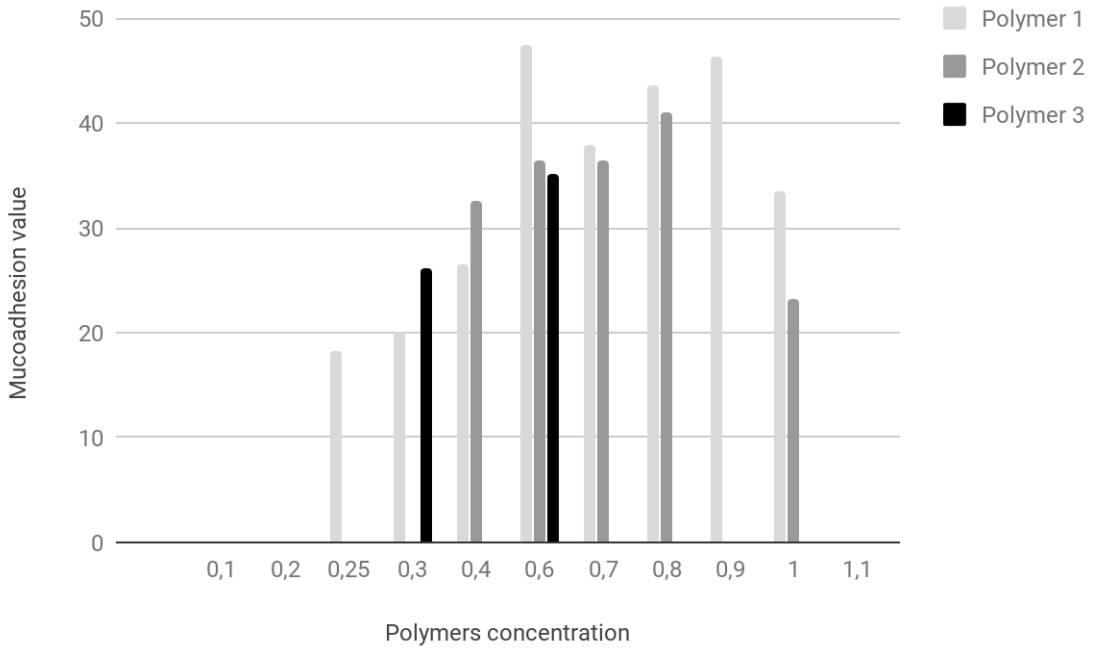

Figure 1 - Comparison of mucoadhesion according to polymers concentration

2. Tolerance assessment

In the validation step, controls' response demonstrated a selective model sensitivity, according to the gingival or vaginal target, indicating relevance of the models to screen mucosa tolerance of ingredients. The three selected polymers resulted in high cellular viability after 24H of contact and appeared well tolerated at efficient use levels.

Conclusions.

The dermopharmacy rheology modifiers proved to be highly tolerated on mucosal epithelia. The mucoadhesive performance of the three polymers have been confirmed in a wide range of pH, in accordance with their chemical structure. Moreover, the pH robustness of the polymers improves APIs' stabilization.

The innovative rheology modifiers are promising versatile materials for all mucosal applications

The next perspective is to assess mucoadhesion of finished products to evaluate the influence of other excipients on the adhesive properties.

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Lipid-based nanoformulations strategies integrated in drug discovery

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Keywords. Drug discovery; early formulation; drugability: polyvalent nanoemulsions

Introduction. An increasing number of promising lead molecules discovered in recent years present high molecular weight, high lipophilicity, and in some cases, poor permeability. To achieve an acceptable exposure of these leads in pharmacokinetics, pharmacology and/or toxicology studies, formulation strategies can be proposed as early as preclinical studies to permit efficient early animal experiments. Such a formulation work can be made by considering intrinsic properties of molecules, and its developability character [1]. It can also be proposed to be as polyvalent as possible, taking into account that, at the drug discovery stage, molecules are only partially characterized, and available in low quantities. In particular, lipid-based formulations appear as really appealing in drug discovery [2].
The Screening and Drugability platform of the CERMN has developed assays to experimentally determine the physico-chemical properties of new molecules that will influence their ADME-tox profile. Various formulations strategies based on drug delivery lipidic nanosystems have been also developed to overcome solubility issues of some promising drugs of therapeutic interest in treatment of cancers or of neurodegenerative pathologies as Alzheimer's disease [3]. This communication proposes to focus on results obtained for a promising formulation strategy based on nanoemulsions.

Materials & Methods. Concerning the drugability evaluation of molecules, solubility is determined by the shake-flask method, permeability is defined by the GIT and/or BBB-PAMPA methods, logP or CHI logD are measured by UHPLC, toxicity is evaluated using a liposomes leakage assay, haemolytic properties are determined on human blood, and the cardiac toxicity is evaluated from hERG inhibition by a fluorescence polarization assay.
Nanoemulsions have been formulated by a spontaneous emulsification method [4]. They were systematically characterized in terms of granulometric properties, surface potential, drug recovery efficiency, pH, osmolarity, *in vitro* drug release, kinetic stability, and biopharmaceutical properties (pharmacokinetics, stability in biomimetic media) (Figure 1).

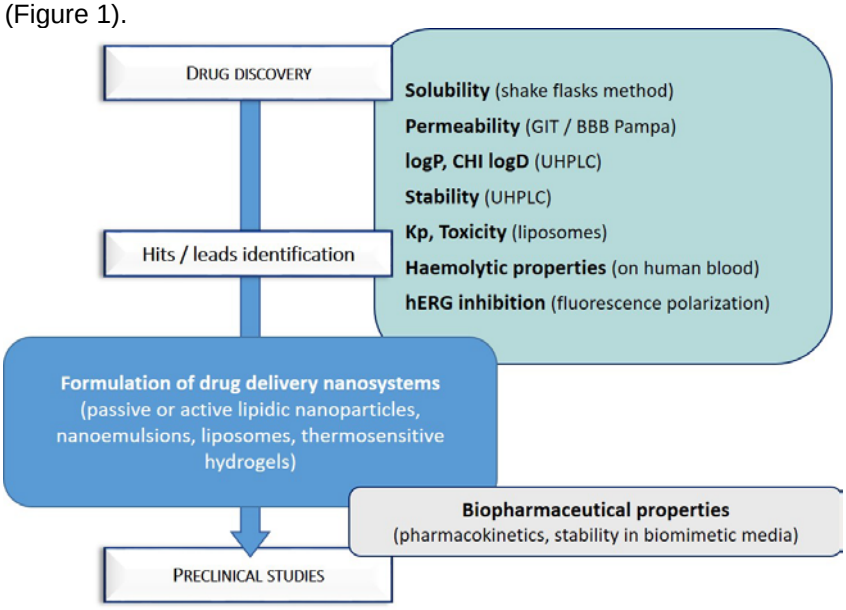

Figure 1. Drugability evaluation and formulation strategies integrated in the drug discovery.

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Results. Assays that we have developed to characterize drugability of new drugs are designed to be precise, miniaturized to be poorly molecules consuming, and well adapted to High-Throughput Screening (HTS). They were applied to various hits and leads. They have permitted to establish structure-property relationships of compounds, and to assess relative differences in terms of physico-chemical properties which could be determinant for their biological properties

The formulate-ability of various pharmaceutical ingredients (API), with different physico-chemical profiles, in a nanoemulsion designed to be orally or parentally administrable was explored. It was shown that these nanoemulsions efficiently increased apparent solubility of pyridoclax, an original poorly water soluble lead, very promising in treatment of chemoresistant cancers. Such nanoemulsions can be extemporaneously reconstituted before use. They also maintain the solubility of the drug in various biomimetic media. Thus, they provide a valuable option as formulation strategy for improvement of drug properties, and permit to perform preclinical studies on the most promising leads.

Conclusions. By experimentally determining physico-chemical properties of leads, and by proposing efficient and polyvalent formulation strategies, we provide valuable options to easily permit preclinical studies of leads.

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Protein based nanoparticles: Synthesis, optimisation, and biomedical application

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Keywords. Nanoparticles, Proteins, colloids, Antibacterial, Elastase

Introduction.
Nanoparticles are nowadays largely investigated in the field of drug delivery. Among nanoparticles, protein-based particles are of paramount importance since they are natural, biodegradable, biocompatible and nontoxic [1]. From the wide variety of proteins generally used to prepare nanoparticles, albumin has been largely used because of its availability, innocuous degradation metabolites, water soluble, well characterized and available in pure form which makes it perfect for nanoparticles dispersions preparation [2]. The majority of reported studies has been focused on BSA since it has good acceptance in the pharmaceutical industry and used as carrier system or just as protein model in numerous fundamental studies [3]. The presence of a large number of parameters affecting the nanoprecipitation process is observable in the literature [4].

Materials & Methods.
BSA nanoparticles were prepared by nanoprecipitation process and the effect of various experimental parameters such as BSA concentration, solvent-non-solvent volume ratio, ionic strength pH, crosslinker, etc. was investigated. HSA nanoparticles were also prepared and optimized to be used as carriers for Neutrophil Elastase (NE) and Human Secretory Protease inhibitor (SLPI). The activity of both enzymes was evaluated after encapsulation and the antibacterial potency of the formulation was investigated.

Results.
BSA nanoparticles were successfully prepared by nanoprecipitation method and optimized to achieve a size of about 150 nm and a zeta potential of -25 mV. In addition, a successful reproduction of the method was achieved using HSA. These particles prove to have a decent stability. The encapsulation of NE and SLPI within the HSA nanoparticles showed that the activity of both enzymes was not affected by the particles or by the encapsulation process. Finally, the antibacterial activity testing showed that both formulations have an excellent activity against *Pseudomonas aeruginosa* colonies.
Conclusions. Albumin based nanoparticles can be successfully prepared without the use of any toxic compounds which leads to a clean formulation that can be safely used for *in-vivo* application. In addition, NE and SLPI loaded HSA nanoparticles represent a good candidate for the development of an anti-bacterial agent. However, further testing is required to completely optimize these formulations.

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Encapsulation of dihydrocaffeic acid in a phenylboronic acid-modified hyaluronic acid hydrogel

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Keywords. Dihydrocaffeic acid; UVB; Phenylboronic acid; Hyaluronic acid; Hydrogel.

Introduction.
UVB irradiation (280-320 nm) induces overproduction of reactive oxygen species, which promotes directly molecular damages, and increases matrix metalloproteinases (MMPs) expression and apoptosis through mitogen-activated protein kinases (MAPKs) pathway, leading to skin aging [1]. Dihydrocaffeic acid (DHCA) is a phenolic compound with potential antioxidant capacity described in the literature [2]. In addition, our initial studies demonstrated that DHCA decreased oxidative stress and attenuated MMPs expression and apoptosis by inhibiting MAPK pathway on L929 fibroblasts irradiated with UVB. In this way, the incorporation of DHCA in topical delivery systems is a promising strategy to prevent skin photoaging. Polymeric hydrogels have gained increasing attention in topical drug delivery due their biocompatibility, capacity to protect drugs from the influence of the environment and to modify or control their release [3]. Hyaluronic acid (HA) is a biocompatible biopolymer with beneficial properties for the skin, such as hydration, skin repair and wound healing. Moreover, HA presents interesting physico-chemical properties, including viscoelasticity, high water retention capacity and lubrication. Thus, HA is widely used in the pharmaceutical and cosmetic fields [4]. It is well known that catechols react with boronic acids, including phenylboronic acid (PBA), through boronate ester bond formation [5]. Thus, the encapsulation of drugs with catechol group through complexation with PBA in desired delivery systems could be an interesting approach. We therefore sought to incorporate DHCA in a hydrogel based on HA modified with APBA moieties (HA-PBA).

Materials & Methods.
The association constant (*Ka*) between DHCA and PBA was determined by ^{1}H NMR titration in 0.01 M deuterated phosphate buffered saline (PBS, pD 7.4). HA-PBA was synthetized by an amide coupling reaction using 3-aminophenyl boronic acid (APBA), HA (Mw 100 kg/mol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as a coupling agent. Then, DHCA was complexed to HA-PBA (in a [DHCA]/[PBA] ratio of 1:1) in 0.01 M PBS pH 7.4 by stirring overnight at room temperature. The percentage of DHCA complexed was evaluated by reversed-phase high performance liquid chromatography (HPLC). The HPLC methodology was validated according to International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Q2(R1). The hydrogel based on HA-PBA was prepared by boronate ester crosslinking using a HA modified with a glucose derivative (HA-glucose). The HA-glucose conjugate was also synthetized by an amide coupling reaction between a glucose derivative possessing an amine function, HA (Mw 100 Kg/mol) and DMTMM. Thereafter, the hydrogel was obtained by simply mixing the HA-PBA-DHCA complex and HA-glucose (Figure 1). The rheological properties of the freshly prepared hydrogel was then evaluated, as well as after 7 days upon storage at 4 °C. The preliminary DHCA release from 782 mg of the hydrogel was carried out using cell culture insert (0,4 µM pore size) coupled to a 24 well plate. The receiving phase was composed of 1 mL of 0.01 M PBS pH 7.4 or pH 6.0, in sink conditions. The system was maintained under stirring at 32 ºC, and an aliquot of 150 µL was taken at the times 0.5, 1, 2, 4, 6, 8 and 24 h to quantify the amount of DHCA released by HPLC. A blank was performed with DHCA diluted in distilled water.

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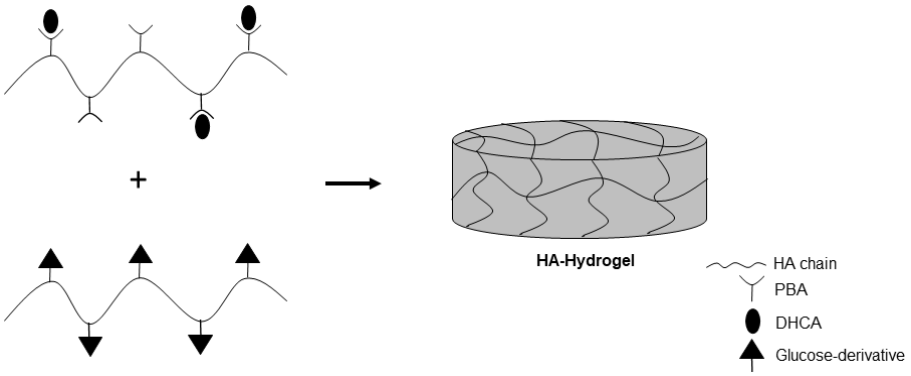

Figure 1: Synthesis of HA-based hydrogel containing DHCA via reversible boronate ester bond formation.

Results.

We found that DHCA has a great affinity for PBA (*Ka* 1726.71 ± 28.4 L/mol). The degree of substitution (DS, average number of substituting groups per repeating unit of HA) of the HA-PBA and HA- glucose was found to be 0.50 and 0.12, respectively, from ^{1}H NMR analysis. Regarding the validation of HPLC assay, it was specific, linear using seven concentrations in the range of 2.5–100 µg/mL of DHCA, accurate and precise. The limit of detection and quantification of DHCA was found to be 0.011 µg/mL and 0.0335 µg/mL, respectively. Upon incubation of DHCA with HA-PBA in PBS, pH 7.4 (in a [DHCA]/[PBA] ratio of 1:1), and removal of non-complexed DHCA by centrifugation using centrifugal filter devices, a total of 57.1 ± 1.6% of DHCA was complexed with HA-PBA. The remaining free PBA moieties on the HA backbone were then used for boronate ester crosslinking by mixing the HA-PBA-DHCA complex and HA-glucose. Gel formation was observed almost instantaneously. Dynamic rheological experiments confirmed the gel-like behavior of the mixture, with an elastic modulus (G') higher than the viscous modulus (G'') within the whole range of frequencies covered (0.01-10 Hz). After 7 days upon storage at 4 °C, the rheological properties of the hydrogel did not change, indicating good stability. This study thus established the feasibility of using HA-PBA to selectively complex the water-soluble active molecule DHCA and to embed it in a boronate ester-crosslinked network obtained by mixing the HA-PBA-DHCA complex and a HA-glucose. In the next step, we investigated the release of DHCA from the hydrogel at 32 °C, at pH 7.4 or pH 6.0, in a period of 24 hours. Cumulative releases of 21.1% and 33.7% were obtained for the hydrogel system, and 105.5% and 133.5% for the blank (DHCA alone), at pH 7.4 and pH 6.0, respectively. Thus, the hydrogel prolonged DHCA release, and this release was greater at pH 6.0, which reproduces the cutaneous pH.

Conclusions.

DHCA was successfully encapsulated in a novel hydrogel through a simple but original method of production, which combines selective complexation of the active molecule with the HA backbone and self- crosslinking in mild condition of HA. Moreover, a prolonged and pH triggered release was obtained. In this way, we started the development of a promising product to prevent photoaging.

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Physicochemistry and antioxidant properties of ivorian plants, encapsulated for treatment of alopecia in black women

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Keywords: Physicochemistry, antioxidant, plant extracts, hair

Introduction

Alopecia is a dermatological disorder that has been recognized for more than 2000 years. It involves absence or loss of hair, especially the head [1]. It is common throughout the world and has been estimated to affect between 0.2% and 2% of the world population. More than 10% African women are affected by hair loss. Indeed, the study presented by the International Journal of Dermatology indicates that 11% of black women are affected by hair loss, against 5% of women of Caucasian origin. Hair loss is therefore a real problem for black women, affecting their image, their self-esteem and their daily lives [2]. Trauma especially traction alopecia with oxidative stress done on the hair follicle, is the most known cause of alopecia in black women. The search for treatment results into few drugs of synthetic origin, but side effects associated with them can not be neglected. Herbal drugs or their formulation are viable alternative to synthetic drugs. Natural remedies have been used for their antioxydant properties in the treatment of alopecia [3]. Our study focuses on 03 hydroalcoholics extracts plants of the Ivorian flora : *Arbrus precatorius L.* (Fabaceae), *Lawsonia inermis L.* (Lythraceae) *and Heliotropium indicum L.* (Boraginaceae), making phytochemical screening of these plant extracts and finally to evaluate their antioxidant activity**.**

Material and Methods

The extracts of the three plants are obtained after maceration in 70% ethanol-water 30% for 48 hours using a magnetic stirrer at room temperature (25 ° C), followed by spinning. in a square of fabric, then a double filtration on cotton, on wattman paper 3 mm and evaporated at the rotavapor at 70 ° C. The concentrates were evaporated at 50 ° C in an oven for 48 hours. Soft extracts obtained, allowed us to carry out our phytochemical and antioxidant tests. The different groups of compounds and their contents in the extracts were highlighted according to the methods described by Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Bekro et al. (2007) (for sterols and polyterpenes: using liberman's reagent; for alkaloids: using Dragendorff's and Bouchardat reagent; for tannins, catechic tannin: using stiasny's reagent and gallic tannin: using reaction with ferric chloride; for polyphenols: using reaction with ferric chloride, for flavonoids: using cyanidine reaction; quinones: using Borntraegen's reagent; for saponins: shake during 15 seconds the test tubes which contain plant extracts and then, standing for 15 minutes. A persistent foam greater than 1 cm indicates the presence of saponosides. and for polyphenols: using Folin-ciocalteu method). The method of the antioxidant activity of these three hydroalcoholics extracts was performed by the test with 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Parejo et al [4]. A range of concentrations (0-200 µg / mL) of plant extract or acid ascorbic (antioxidant reference) was prepared in methanol. A volume of 2.5 mL of this solution, was mixed with 2.5 mL of prepared DPPH (100 µM) also in methanol. After homogenization, the mixture was incubated at ambient temperature (25° C) away from light. After 15 minutes of incubation, the absorbance was read at 517 nm against a blank containing only methanol. The inhibition percentage of the DPPH radical was calculated according to the following equation : Inhibition of DPPH (%) = (1- (OD test / OD white)) x 100. The IC50 which was the concentration of plant extract or acid ascorbic responsible for 50% of inhibition of the DPPH radicals, was determined on the graph representing the percentage of DPPH inhibition as a function of concentrations of extracts and acid ascorbic.

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Results

The phytochemical screening revealed that *Arbrus precatorius L.* (Fabaceae) and *Lawsonia inermis L.* (Lythraceae) contain sterols, polyterpenes, polyphenols, flavonoids [1] and alkaloids. The antioxidant activity is : (IC50 = 0.124 ± 0.090 µg / mL) for *Abrus precatorius*, (IC50 = 0.113 ± 0.090 µg / mL) for *Lawsonia inermis*, and (IC50 = 0.118 ± 0.090 µg / ml) for *Heliotropium indicum*. These amounts are close to IC of vitamin C (IC50 = 0.082 ± 0.065 µg / mL), antioxydant reference, suggesting of real antioxidant activities for these extracts.

Conclusion

These plants extracts contain sterols, flavonoids and polyphenols which are very good antioxidative compounds. Thus, they are, according to our study, considered as antioxidant and antiradical plants. It would be interesting to proceed by encapsulation of our extracts to find a valuable delivery systems in order to protect the activity extracts, to facilitate their penetration in the scalp for a better oxygenation, fighting against hair loss and to promote hair growth.

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Optimization and safety of a gel based on *sacoglottis gabonensis (humiriaceae)* extract

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Keywords: hydrogel, *Sacoglottis gabonensis*, opportunistic germs, optimization, Buruli ulcer.

Introduction:
Buruli ulcer (BU) is a debilitating infection of the skin and soft tissues. It is caused by *Mycobacterium ulcerans* [7]. It can lead to deformities and permanent disabilities. It is the third mycobacteriosis in the world [5, 9]. Treatment recommended by WHO is based on antibiotic therapy and surgery. There are many limiting factors to this treatment, so traditional treatment is the best choice for patients [1]. Unfortunately the methods of use of these plants are non-reproducible and non-aseptic. *Sacoglottis gabonensis* (SG) is a plant species used in traditional medicine for the treatment of this disease [4]. Its activity on *Mycobacterium ulcerans* has been demonstrated with a MIC = 0.78 mg / ml and a CMB = 12.5 mg / ml on the Ivory Coast strain [6]. It has also shown its activity on five strains of *Mycobacterium ulcerans* with resistance levels lower than 1 from the concentration of 20 mg / ml. [9]. A thermosensitive and mucoadhesive hydrogel based on this plant developed in the pharmaceutical galenic laboratory of the UFR SPB has presented interesting properties for the cutaneous route [2, 3]. Our study aimed to optimize and characterize it.

Methods:
After harvesting and identification of the plant, we proceeded to its physicochemical characterization and to the study of its antioxidant activity by the method of inhibition of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and by the FRAP method (reducing power with respect to the ferric ion). The choice of solvent for optimizing the solubility of the extract was carried out according to the modified OECD method. Formulations based on poloxamer 407, carboxymethylcellulose and *Sacoglottis gabonensis* were made according to the SCHMOLKA cold method [8] with the two most suitable solvents. The evaluation of the *in vitro* activity on germs that superinfect Buruli ulcer guided the choice of optimized gel. This one has been the subject of a physicochemical characterization. HAAKE viscometer viscosity and TA1 texturometer adhesion studies were performed. The maintenance of the *in vitro* antioxidant activity of the extract was verified. A chamber passage study of USSING at 37 ° C in rat jejunum was conducted as well as an *in vivo* safety test in rabbits according to OECD methods 404 and 405.

Results:
The sieving technique improved the solubility of the aqueous extract by 30%. The particle size chosen was less than 0.05 mm. The extract showed good antioxidant activity (DPPH: IC 50 = 2.5 µg / mL, FRAP: 0.141 mmol Eq Trolox / g ES). Its pH was 4.89 at 25 ° C and 4.79 at 37 ° C. The solubility of SG in 50/50 water-ethanol (45.5 g / L) was the best. The pH of the hydroalcoholic gel was 5.88 at 25 ° C and 5.74 at 37 ° C while that of the aqueous gel was 6.01 at 25 ° C and 5.99 at 37 ° C. Our formulations were homogeneous and effective on staphylococcal strain 23UB17. However, the most effective gel was the hydroalcoholic gel with an inhibition diameter of 21 mm. This optimized gel was viscoelastic and thermally sensitive with a Tgel of 22 ± 2 ° C. It has good adhesion properties (Wad = 2.75 mJ). The antioxidant properties of the extract have been preserved in the formulation. This gel showed good safety, unlike the extract, which was found to be a primary eye irritant. Finally, the membrane passage of the compounds gave encouraging results.

Conclusion: The optimization of the gel based on *Sacoglottis gabonensis* has resulted in an improvement of its *in vitro* efficacy, its safety and its adhesion properties while retaining its thermosensitive character.

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Mechanical properties of emulgels containing curcumin: Texture profile analysis, syringueability and softness determination

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Keywords. Curcumin, emulgels, textural properties, seringueability, softness.

Introduction.
Curcumin (CUR) is well known due to its antioxidant, antimicrobial, anti-inflammatory, antirheumatic and anti-cancer properties. Nevertheless, CUR exhibits high hydrophobicity and low chemical stability, which can impair its pharmacological properties [1]. In this sense, the incorporation of CUR in drug delivery systems, such as emulgel, can increase its solubility, stability and consequently, bioactivity. Emulgels are oil-water systems, where the water phase is gelled by polymers, such as acrylic acid derivatives. These formulations can provide increased availability and modified release of the drug. The development of emulgel systems composed of poloxamer 407 (P407), Carbopol 974P® (C974P), sesame oil (SO) can provide a new nanostructured system for CUR delivery. Thus, the aim of this study was to prepare and evaluate the mechanical properties (texture profile analysis, syringueability and softness) of emulgels systems containing CUR.

Materials & Methods.
Emulgel systems were prepared containing 15% (w/w) P407, 0.25% (w/w) C974P, 0.75% (w/w) SO with and in the absence of 0.1% (w/w) CUR. The mechanical properties were evaluated using a TA-XT_2plus Texture Analyzer. For texture profile analysis, the formulations were evaluated in TPA mode of the equipment and at 5, 25 and 37 °C, using a cylindrical polycarbonate probe. Seryngueability and softness characteristics were evaluated in compression mode at 25 °C with syringe and 37 °C with a conical perspex probe, respectively. Textural parameters (hardness, compressibility, adhesiveness, elasticity and cohesiveness), softness and seringueability were derived from force-time plots. The effects of presence of CUR and temperature on textural parameters were statistically evaluated by two-way ANOVA. On the other hand, the effect of presence of CUR was evaluated by one-way ANOVA on softness and syringueability results. A level of $p < 0.05$ was accepted to denote significance.

Results.
The CUR presence significantly decreased the compressilibity ($p < 0.05$), but it did not influence significantly the other textural parameters. Otherwise, the increase of temperature significantly increased hardness, compressibility and adhesiveness, due to the structure and organization of thermoresponsive polymer, P407. The syringueabiliy and softness results were not influenced by the incorporation of CUR in these preparations.

Conclusions.
The systems containing CUR demonstrated suitable mechanical properties for the development of new CUR drug delivery systems.

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Development of a vaginal innovative gel with ceftriaxone and with cefixime for the treatment of the gonorrhea

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Keywords: vaginal gel, gonorrhea, cefixime, ceftriaxone

Introduction.
The gonorrhea, due to Neisseria gonorrhoeae NG (or gonococcus), is one of the Sexually transmitted infections the most spread in the world. It constitutes a real problem of public health, with 106,1 million new infections per year (1). In the African region, we considered at 92,6 millions the total number of people having contracted a curable sexually transmitted infection in 2008 among which 21,1 million cases of gonorrhée (2). In Ivory Coast, the report of the project IMPACT - IC concerning the IST and the HIV showed 8.7 % prevalence to the HSH and 11.6 % at the workers of the sex (woman).
In the face of the importance of this infection and the emergence of antibiotic resistance, and due to limitations of oral treatments as well as an insufficient bioavailability of certain antibiotics, our objective was to develop gels dedicated to vaginal administration with the antibiotics Céfixime and Ceftriaxone that present an acceptable efficiency against the gonoccoque located in the vaginal cavity.

Methods.
After a preliminary experiments which allowed us to retain the poloxamer 407 (P407), the hydroxylpropylméthylcellulose (HPMC), the buffers citrate and bicarbonate of sodium as solvent, we formulated two definitive gels constituted by ceftriaxone and by cefixime according to the cold method of Schmolka described in 1972 (3). The rheological and organoleptic properties of the obtained gels deposited onto polyropylene surface and porcine vaginal mucous membrane were characterized by using the rheometer Kinexus More (MALVERN) and a texturometer TA1 (AMETEK). Gel stability was investigated by centrifugation at various speeds (1000, 3000 and 5000 rpm during 5 mn) and by applying several cycles of temperature (during 16 hours in-18°C, then during 8 hours in 25°C±2°C and finally in the temperature of the laboratory (21±2°C) during 5 mn). This allowed to determine the conditions of preservation of these gels. To mimick the dilution of the formulations which could arise after application, we mixed 3g of each formulation with 0.75 ml of model vaginal mucosa before to determine their textural and rheological properties. The ceftriaxone and the cefixime were quantified in the formulations by UV-visible spectroscopy. In vitro release assays were performed using Franz cell and the gel efficiency against the gonococcus was characterized.

Results.
Two gels were formulated. The first one was made of P 407 (20 %), HPMC 4KM (1 %), NaHCO3, Cefixime and buffer citrate and the second one included P 407 (20 %), HPMC 4KM (1 %), NaHCO3, Ceftriaxone and citrate buffer of pH 4 - 5. The sodium bicarbonate improved the solubility of the cefixime, whereas the citrate buffer allowed to maintain the pH of the gels in a vaginal pH (4.5). 3 g of these gels contained 250mg of Ceftriaxone (dose required in the treatment of the gonorrhea) or 100mg of Cefixime. The percentage of recovery of antibiotics in the gels was upper to 95 ± 2 % in the presence of bicarbonate, whereas it was 33 % in the absence of bicarbonate. They presented a viscoelastic and shear-thinning behavior. The elastic properties were more pronounced than the viscous properties (G ' > G "), except for low frequencies where the properties of the liquid dominated. No thixotropic behavior was observed at 37°C and 25°C. The sodium bicarbonate increased significantly the viscosity of our systems. This phenomenon was observed at low temperature (15°C). For temperatures above the P407 Tgel, this influence was not significant. The gelation temperature was 23°C for the gel of Cefixime and 20°C for the gel of Ceftriaxone. The gels remained stable in all the tested centrifugation conditions and in the various cycles of temperature. The dilution of the formulations with 0.75 ml of model vaginal fluid pulled a progressive increase about 6 °C of the gelation temperature. The adhesive work of the gel without PA was 506.7 ± 20. 10^{-3} mJ on pork's vaginal mucous membrane and of 139.5 ± 15.10^{-3} mJ on membrane of polypropylene. The adhesive work on pork's vaginal mucous membrane of the gel of Cefixime was 456.4 ± 20.10^{-3} mJ and that of the gel of Ceftriaxone of 421.7 ± 22.10^{-3} mJ. On the

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membrane of polypropylene, they were 347.3 ± 22.10^{-3} mJ and 415.7 ± 19.10^{-3} mJ, respectively, indicating no significant evolution from one type of membrane to the other. For all the gels, the adhesive work was higher on pork's vaginal mucous membrane than on polypropylene one even if this difference was not significant for the gels with active ingredients. At 25 °C and 37°C, the adhesive work of the gels without active ingredients was more important than those of the gels with active ingredients. The adhesive work was better at 37°C for our systems. These results are promising to obtain a good adhesion of the gels after vaginal administration. For both gels, at least 80 % of active ingredients were released after 45 minutes. At the concentrations of 83 mg/g of ceftriaxone and 33mg/g of céfixime in the gels, an in vitro total inhibition of the gonococcus was observed.

Conclusion.

The gels with cefixime and with ceftriaxone presented an acceptable pH of 4.5 for the vaginal mucosal membrane, a gelation temperature lower than 30°C, viscoelastic and shear-thinning properties as well as a good adhesion onto the vaginal mucosal membrane which make them capable of being administered by the vaginal route. The high percentage (> 80 %) of antibiotic released after 45 mn, as well as the in vitro efficiency of our gels on the gonococcus are promising results which should allow to envisage studies of membrane permeation across pig vaginal membrane and in vivo assays for the efficiency evaluation using animal models.

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Development of dermo pharmaceutical forms based on the fruit of *Alchornea Cordifolia* (Euphorbiacees) for the treatment of dermatophytes

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Keywords: *Alchornea cordifolia*, lyophilisate, ointments, lotions, Trichophyton

Introduction.

Dermatophytes are conditions caused by microscopic filamentous fungi that have an affinity for keratin (epidermis, nails, hair). They cause in humans and animals superficial lesions called dermatophytes: epidermophytes, intertrigo, onyxis, ringworm, folliculitis. Fungal infections are common reasons for consultation in Dermatology **(1)**. As symptoms, they present polycyclic lesions, pruritus, inflammatory lesions, superinfections, cracking at the bottom of interdigital folds and broken hair and nails lose their glow. These symptoms cause a permanent gene, a bad smell and sometimes deep scars. Although the global prevalence is 20 to 25% **(2),** there is very little research and development for the development of new antifungal drugs. However, there are many plants with excellent antifungal activities; but their traditional use in the form of decoctions, juices, extracts and other primary forms does not allow efficient activity and daily use. The objective of this work is to produce dermopharmaceutical forms from an extract from the fruit juice of *Alchornea cordifolia* and to demonstrate that the antifungal activity is preserved.

Methods.

The fresh pulp separated from the seeds of *Alchornelia cordifolia (* Euphorbiaceae) was used to prepare the juice. They were ground in a porcelain mortar until it became a paste. The paste was then pressed with a mechanical press to get the juice. The juice distributed in flasks was frozen and freeze-dried in a "Christ Alpha" lyophilizer. The lyophilizate obtained was the subject of organoleptic, physicochemical and textural characterizations.

Anhydrous ointments and hydrophilic ointments were made; they differed in nature as far as the base excipient and the lyophilizate concentration (20%, 33% and 50%) are concerned. For the anhydrous ointments, the lyophilizate was dispersed in the wetting agent and then introduced with stirring into the base excipient. For hydrophilic ointments, the lyophilizate was dissolved in the osmosis water and then mixed in the hydrophilic base excipient. All the stable formulations were stored for one month in the laboratory at a temperature of 25°C to ensure the preservation of their stability. Stable formulations after one month of storage have been *in vitro* and *in vivo* evaluated on different Trichophyton species (*T. rubrum, interdigital T., T. soudanense, T. mentagrophytes*) according to two techniques: incorporation and direct connection. *In vivo* tests on white mice were performed after T. mentagrophytes infestation on scarified skin parts. The reference substance was griseofulvin.

Results.

The 125 mg / ml lyophilizate solution of the fruit pulp showed an activity identical to that of the 125 mg / ml solution of griseofulvin. The lotions were made with ethanol at different degrees and at concentrations of 1/50 and 1/100. Ointments at different concentrations of the lyophilizate (20, 33, 50%) were made with different types of excipients. All lotions and ointments showed in vitro antifungal activity identical to that of griseofulvin up to 16 and 30 days of incubation. The ointments showed better in vivo activity compared to lotions, and the 50% vaseline-based ointment revealed an activity identical to that of griseofulvin, with a healing time of 8 days.

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Conclusion.

The lyophilizate of A. cordifolia fruit juice formulated as an ointment and lotion, maintained a good antifungal in vitro and in vivo activity against Trichophyton species, compared to griseofluvine.

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Preparation and characterization of microsponges containing metronidazole using different organic phases

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Introduction.
Drug delivery systems offers several advantages when compared to conventional dosage forms. Among the benefits, it can be mentioned the modulation of the release process, reduction of toxicity, improvement of drug availability into a specific site, which leads to better adherence to treatment by patients [1]. Polymeric microsponges (MS) are rigid and porous structures able to incorporate a relatively large amounts of drug into their interconnect channels. MS constitute a relatively new strategy, thus has a few studies using them[2]. Therefore, the aim of this work was to obtain and characterize MS developed with different organic solvent proportions and containing metronidazole (MTZ) as a model drug.

Materials & Methods.
MS was prepared by *quasi*-emulsion technique. A dispersion of ethylcellulose (0.5%, w/w) and HPMCphtalate (0.03%, w/w) was prepared in dichloromethane (DCM) (100, 80, 70 and 50%, v/v) and ethanol (0, 20, 30 and 50%, V/V) (organic phase), being denominated M10, M8, M7 and M5, respectively. Aqueous solution (1%, w/v) of porogenic agent and MTZ (2.5%, w/w) was dispersed in polymeric solution[2]. This dispersion was dripped into an aqueous poloxamer 188 dispersion (aqueous phase) and remained in magnetically stirring for 24 h. MS were dried at 60 °C in the hot air oven and they were evaluated as morphology by SEM, product yield (PY), drug content (DC), entrapment efficiency (EE) and particle size (PS) by dynamic light scattering (DLS).

Results.
All MS displayed spherical morphology, smooth surface and uniformly porous structures. MS prepared at concentrations higher than 80% of DCM showed more spherical morphology and more homogenous size. Moreover, the increase of DCM from 50 to 100% reduced the size from 5.31 to 0.91 µm. PY values were 68.54, 38.89, 49.49 and 56.62%, EE was 8.28, 10.90, 18.67 and 11.45%, and DC was 1.63, 2.14, 3.67 and 2.24% to M5, M7, M8 and M10, respectively.

Conclusions.
Therefore, it was possible to prepare MS with different organic solvent proportions containing a metronidazole, but the better results were obtained using high amount of dichloromethane, being possible the using of a maximum of 20% of ethanol.

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Moringa oleifera (moringaceae): from oil of grain to antioxidant cream

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Keywords: *Moringa oleifera,* antioxidant cream, stability, safety

Introduction:
The oil from *Moringa oleifera (Moringaceae)* thanks to its "bio" origin and its antioxidant properties is very popular with researchers. It is therefore a first-rate cosmetic raw material [2, 4]. Among the most used cosmetic forms, creams occupy an important place thanks to their particular structure conducive to skin penetration. Our research team was interested in this type of formulation for the valorization of this plant from the local pharmacopoeia [1, 3]. The goal of our work is to develop an antioxidant cream based on *Moringa oleifera* seed oil (Moringaceae).

Methods:
After extraction of *Moringa* oil with Soxhlet, its physicochemical properties were determined. The critical HLB method made it possible to formulate an emulsion from this oil. A cream was then made. After being prepared with stirring in a water bath at 70 ° C. and then in a micro-vortex, the cream obtained was characterized from a physicochemical point of view. During its characterization of rheological tests with the HAAKE viscometer and a textural analysis with texturometer TA1 were carried out. The maintenance of antioxidant activity after formulation was verified by FRAP and DPPH methods. Stability under normal conditions (27 ± 2 ° C) and accelerated (40 ° ± 2 ° C) was performed. The sensory analysis conducted on a sample of ten people was correlated with the rheological and textural parameters. Finally, an acute toxicity study after dermal and ocular administration in albino rabbits was performed according to OECD methods 404 and 405.

Results:
The extraction of the oil gave a yield of 33.58%. The density was 0.88 ± 0.01g / cm^3 at 27 ° C; the viscosity of 3355 ± 0.68 mPa.s; the refractive index of 1.46 ± 00; the pH of 5.7; the acidity at 1,18 ± 0,20% oleic acid; the peroxide value of 4.60 ± 0.56 meq O_2 / kg; the iodine number of 62.18 ± 0.88 g I_2 / 100g; the saponification number of 209.44 ± 8.57 mg KOH / g; the unsaponifiable matter content of 0.93 ± 0.42% and the carotenoid content of 4.57 ± 0.01 mg / g. His critical HLB was 9.97.
The cream formulated had a whitish, homogeneous appearance, and was odorless and unctuous. It was hydrophilic, stable to temperature variations and centrifugation, shear thinning and thixotropic. Its pH was 5.47. The particle size ranged from 0 to 5 microns. The cream was stable at room temperature, cold and heat for three weeks. It exhibited an antioxidant effect, good textural properties and showed no acute toxicity.

Conclusion:
The results obtained are promising. Indeed, our cream based on Moringa seed oil has satisfactory organoleptic and galenic characteristics. Its antioxidant property, adhesion and absence of acute toxicity allow us to consider a study of the effect perceived by an expert dermatologist and the effect felt by users.

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Rationally designed tacrolimus-loaded nanoemulsion: a novel stable oral drug delivery system

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Keywords. PEGylated nanoemulsions, oral delivery, tacrolimus, freeze-drying

Introduction.
In the last decade, nanoemulsions (NE) have provided innovative solutions in oral delivery of poorly water-soluble drugs. NE are nanosystems having a characteristic vesicle structure made of an oily core surrounded by a surfactant shell. They offer major advantages due to the simplicity of production, high stability and capability to increase the oral bioavailability of encapsulated compounds. In fact, following oral administration, they are able to protect the associated drug from gastrointestinal degradation, while prolonging intestinal residence time and promoting interactions with the intestinal epithelium [1]. In this work, an optimized design of a novel oil in water NE using the emulsion phase inversion (EPI) technique is presented. The chemical composition (influence of surfactant mixture and surfactant-to-oil ratio), temperature of formulation and morphological structure [2] of NE was assessed. Stability studies in simulated gastrointestinal fluids (GI) (Ph. Eur. 9th Edition) and in colloidal suspension over time were performed. Finally, the NE was converted into a solid dosage form using the freeze-drying technique with a view to further improving the stability of the system.

Materials & Methods.
NE were prepared using the EPI method. Briefly, the organic phase, made of oil (Miglyol® 812) and a combination of hydrophilic and hydrophobic surfactants (Myrj®52 and Labrafil® M1944CS), was heated at 80°C under magnetic stirring to solubilize the excipients. The aqueous phase (PBS 5mM solution), heated at 80°C too, was then titrated into the organic phase. To shape NE, the system was subject to constant stirring by way of an Ultra-Turrax® at 80°C. Optimization of NE was done using pseudo-ternary phase diagrams plotting surfactant mixture, oil and water phases. In the attempt to identify the NE feasibility domain, different Km values (Km= mass Myrj® 52/ mass Labrafil® M1944CS) ranging from 1 to 5 were evaluated. Moreover, the surfactant to oil ratio (SOR) and the influence of temperature were also studied. Particle size and polydispersity index (PDI) were determined by Dynamic Light Scattering (DLS). The zeta potential values were measured using the Laser Doppler Electrophoresis (LDE) technique. The morphology of the particles was investigated by transmission electron microscopy (TEM), differential scanning calorimetry (DSC) and X-ray powder diffraction analysis (XRPD). Then, the hydrophobic model drug, tacrolimus (TAC), was encapsulated into NE. TAC was firstly solubilized in the organic phase and then the same process describe for the blank NE was followed. The encapsulation efficiency (EE) of TAC into NE was determined by size exclusion chromatography on a Sephadex™ G-25 resin, followed by HPLC analysis. NE stability in simulated GI (Ph. Eur. 9th Edition) up to 8 hours at 37°C and in colloidal suspension upon storage during one month at both 20°C and 37°C was assessed [3]. Finally, NE was converted into a dried solid powder using the freeze-drying technique. The NE was dried without cryoprotectant and in presence of cryoprotectant trehalose at different concentrations. Then, NE powders were re-suspended in water and physico-chemical characterized.

Results.
The aim of this work was the optimization of a novel stable NE formulation obtained by EPI method. The final goal was to maximize the oil content in order to encapsulate hydrophobic active drugs. The NE feasibility domain was identified by mean of pseudo-ternary diagrams. Following the optimization step, at Km of 2.5 and SOR of 2.86 we obtained stable NE showing a hydrodynamic size of around 100 nm and polydispersity index (PDI) lower than 0.2. From DSC and XRPD morphological analysis we demonstrated that NE had a PEGylated rigid shell, which was crystalline following the evaporation of the water phase and amorphous when in colloidal suspension. The formation of a PEG-water complex in the hydrated PEG corona might be the cause of the abovementioned amorphous state [4]. These NE were able to encapsulate TAC, the hydrophobic model drug, in a high amount (EE 99.5%), without alteration of the physico-chemical properties of the system. Then, TAC-loaded NE stability in simulated gastrointestinal fluids (GI) and in colloidal suspension was assessed. In both studies, no alteration in terms of size and PDI was observed. Moreover, no leakage of the drug from NE was observed upon storage during 1 month in colloidal suspension at 20°C. Finally, NE were successfully converted into dried powders. Blank NE could be freeze-dried in absence of cryoprotectant (NE 27% w/v),

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however the addition of low amounts of trehalose (2.5% w/v), at NE concentration of 13.5% w/v, improved the water re-dispersion. Following powder reconstitution in water, the results of size analysis showed that NE recover their initial physico-chemical characteristics, as confirmed by DLS and TEM measurement.

Conclusions.

In the present work NE stabilized by a rigid PEGylated shell were successfully obtained by EPI method. NE were able to encapsulate the hydrophobic drug tacrolimus in high amount. TAC-loaded NE were proved to be stable both in GI fluids and in colloidal suspension. Moreover, upon storage during 1 month in colloidal suspension at 20°C no leakage of the drug was detected. Finally, dried NE powders were obtained using the freeze-drying technique without alteration of the nanocarrier physico-chemical properties. All the results here presented demonstrate the feasibility of producing highly stable solid NE by EPI method and unveiled new possibilities for the exploitation of this novel system for the oral delivery of lipophilic drugs.

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Characterization of Aluminum salts suspension in vaccines using Static Multiple Light Scattering

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Keywords. Stability, vaccines, aluminium salts, redispersibility

Introduction.
Adjuvants such as aluminum salt particles (Alum) are commonly added in vaccines to enhance their immune responses. However, these adjuvants usually aggregate and then settle over time due to their electrical charges. The resulting sediment can be loose or compact. In latter case, it is difficult to disperse the sediment again. Vaccines efficiency depends on the forces of intermolecular bonds. The stronger the forces, the compacter is the sediment, the less easy is the redispersion and the vaccine's efficiency decreases. To avoid uncontrolled flocculation and decrease of vaccine efficiency, the solutions are usually flocculated in a controlled way. The effectiveness of such flocculation is usually measured by the sediment height after 24 hours. The higher this height is, the looser the intermolecular bonds, the better is the redispersibility. It is clear, that this test takes too much time for routine or quality control.

Materials & Methods.
Turbiscan is based on static multiple light scattering. A detection head moves up and down along a flat-bottomed cylindrical glass cell. The detection head is composed of a pulsed near infrared light source (wavelength = 880 nm) and two synchronous detectors. The transmission detector (at 180°) receives the light, which goes through the sample, while the backscattering detector (at 45°) receives the light scattered backward by the sample. The detection head scans the entire height of the sample, acquiring transmission and backscattering data every 40 µm.

Results.
A typical scanning profile is shown in Figure 1. In the top graph we can see the intensity of the transmitted light over the sample height (0mm is bottom of the cell). Dark blue curves represent the beginning of the experiment, red curves the end. It can clearly be seen that there is a clarification on the top of the sample. We calculated the peak thickness of the clarification zone, which gives us a kinetic (middle) from which we can obtain a "settling onset time", which correspond to a threshold of the peak thickness. Finally, we compared the settling onset time (less than 1 h) with the height of the final sediment after 24h.

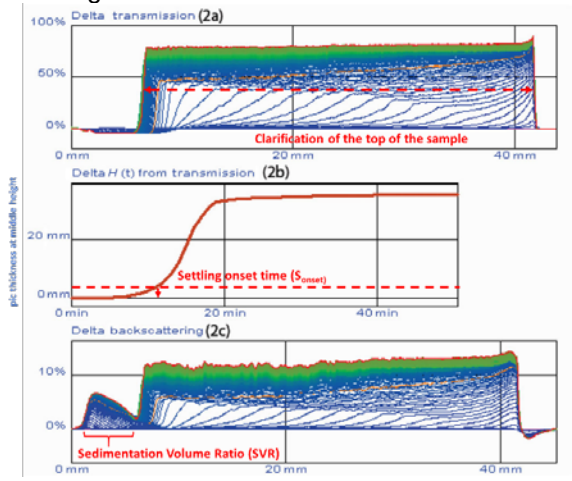

Figure 1: Typical transmission profile (top), backscattering (bottom), peak thickness (middle).

Conclusions:
This work presents a new approach to study the redispersibility of aluminium adjuvants, that usually tend to precipitate and decrease vaccine efficiency. The obtained settling onset time, which is less than an hour, is in good correlation with the results obtained after 24h. This method allows therefor to reduce significantly the measurement of vaccine samples in both fields, development and in quality control.

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Influence of impurity on ice crystal nucleation

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Keywords. Freezing, nucleation, Crystal growth, modelling

Introduction.
Freeze drying is a useful technology in the pharmaceutical industry. It has become the preferred method for sensitive, high-value pharmaceuticals because it enables moisture removal at relatively low temperatures under easily maintained sterile conditions. But one of the fundamental problems during the process is that the freezing often creates heterogeneous ice-crystal structures among the vials within a batch as well as among different batches. During freezing, the aqueous solution in vial is cooled below its thermodynamic crystallisation temperature and remains in a subcooled, metastable liquid state until ice nucleation occurs. The primary homogenous nucleation is spontaneous nucleation where the formation of the solid phase particle is not brought by the presence of any solid phase. Usually, large subcooling is required to initiate this kind of nucleation. The primary heterogeneous nucleation is the most primary nucleation. The formation of new solid phase particle is catalysed by the presence of foreign solid particles which has lower surface energy than that of a new solute particle. Therefore, it requires lower supersaturation than homogeneous nucleation.

The objective of this work is to study the freezing behaviour of aqueous solution, without as well as with foreign particles. We used two techniques to investigate the phase transitions, a differential scanning calorimeter and a cryo-microscope. These techniques enable the freezing temperatures measurement, but also in situ visualisation that reveals ice morphology.

Materials & Methods.
Aqueous solutions of citric acid were prepared at different concentrations [5-60% wt]. The choice of citric acid was motivated by its use as pharmaceutics excipients for freeze-drying. 1% wt of desert sand has been added to the aqueous solution to simulate the presence of foreign particles. Two Arizona Test Dust (ATD) has been used mainly composed of silica: (i) the ultra ATD (mean particle size: 5 µm), (ii) the normal ATD (mean particle size: 10 µm).

The calorimeter (DSC) used in this study is from Thermal Analysis Instruments (TA Q200) including a refrigerated cooling system allowing operation over the temperature range from 193 to 823 K. To determine the phase change temperature, the experiments were performed according to the following program: (a) isotherm at 278 K, (b) cooling rate at 10 $K \cdot min^{-1}$, (c) isotherm at 203 K during 5 min, (d) heating rate at 10 $K \cdot min^{-1}$. The results reported in this work correspond to data during the heating phase (steps d). All DSC curves were normalized with respect to the sample mass.

The observations of the freezing process in situ were carried out with an optical cryomicroscope OLYMPUS equipped with a Linkam cold stage and a video capture software at 10 images per second. We performed optical cryo-microscope measurements at 10 $K \cdot min^{-1}$, in the temperature region between 275 and 163 K. It allows an estimation of ice nuclei numbers, ice growth speed and ice morphology.

Results.
In this work, we present the results of cryo-microscope and DSC of different aqueous solutions, including or not ATD. We observe different tendencies:

(i) The surfusion temperature (difference between the freeze temperature and the melting temperature) varies proportionally with acid fraction. Moreover, we note a constant effect of ATD on surfusion temperature (+6°C) (Figure 1).

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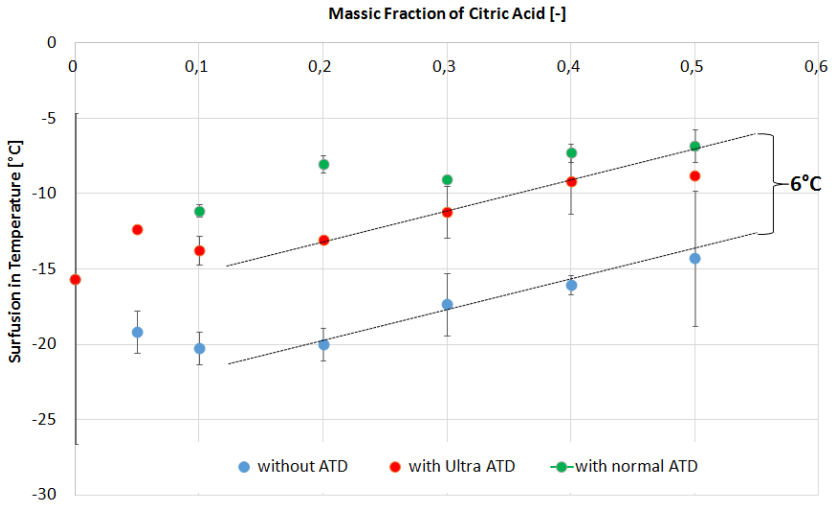

Figure 1: Influence of acid citric fraction on surfusion temperature. Blue point: without ATD; red point: with Ultra ATD; green point: normal ATD.

(ii) Ice nucleus centers and ice growth are also dependent on acid citric fraction. For a sample with a weak fraction (lower than 0.4), we observe a radial growth from a unique point (Figure 2a). For higher acid fraction, we observe multiple ice centers, with a dentrical growth (Figure 2b).

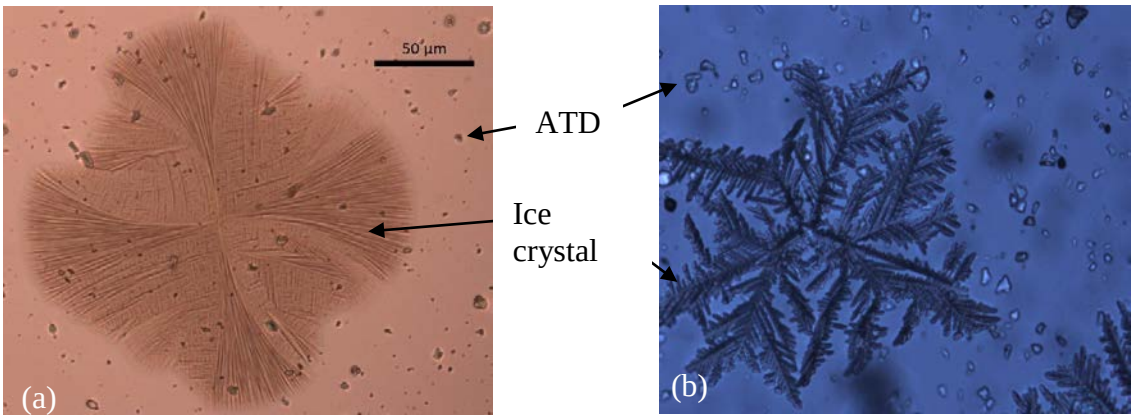

Figure 2: Influence of acid citric fraction on crystal morphology. (a) acid citric fraction: 0.4wt (without polarizer); (b) acid citric fraction: 0.6wt (with polarizer).

(iii) Ice propagation speed is a function of the acid fraction, but is not really affected by the presence of ATD. The growth rate could be well predicted by the Classical Nucleation Theory.

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Microencapsulation of Edible Oil by Using Bio-based Complexes as Wall Materials against Lipid Oxidation

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Keywords. Oxidative stability · Microencapsulation · Sunflower oil · Bio-based wall materials · Spray drying

Introduction.
Polyunsaturated fatty acids (PUFAs) are essential to enable normal growth and maintain good health of all higher organisms. They have proven positive biological effects on blood pressure regulation, modulation of inflammatory or even immune responses [1]. Unfortunately, they cannot be synthesized in the body and need to be provided by the diet [2]. Due to the highly unsaturated nature of PUFAs, they are sensitive to oxidation and thermic degradations leading to the production of hydroperoxides and unpleasant flavors and smells. The encapsulation of these compounds appears to be an efficient solution to protect them from this oxidation. The spray drying process was selected for this purpose, as it is a relatively inexpensive, fast and easy to use process. The choice of encapsulating agents is a vital step in spray drying as it influences the properties of produced microparticles. The main objective of this study was to evaluate the influence of the addition of different polysaccharides (alginate, inulin, maltodextrin and pectin), in association with pea protein isolates, as wall materials for the encapsulation of PUFAs-rich vegetable oil by spray drying process.

Materials & Methods.
Sunflower oil was kindly donated by the SAS PIVERT (Compiègne, France) and stored at room temperature. The different wall materials, pea protein isolates (PPI) (MyProtein, United Kingdom), alginic sodium salt (A) (Sigma-Aldrich, United Kingdom), inulin (I) (Louis François, France), maltodextrin (MD) (Sigma-Aldrich, USA) and pectin (P) (Sosa Ingredients, Spain) were also procured and stored at room temperature. All others chemical were of analytical grade.
Briefly, 10g of polysaccharides (A, I, MD or P) and 50 g of PPI were dispersed in 500 mL of distilled water using a high-speed disperser to form 12% w/v final wall material solutions. The solutions pH were adjusted to 7.8 with a 0.1 M NaOH solution. Then, the emulsions were prepared by adding 10% w/v of sunflower oil (core/wall ratio 6:5) and mixed again at 10,000 rpm for 5 min. The premixed emulsions were then stabilized by passing through a high pressure homogenization device operated at 400 bars for two passes. The sizes of the emulsion droplets were evaluated using a laser particle size analyzer. Emulsions viscosities were measured at imposed shear rates.
The freshly homogenized emulsions were then spray dried using a lab scale spray dryer. The applied air inlet temperature was 160°C, the liquid flow rate was 9 mL/min and the aspirator rate was set at 100%. The recovered solid yield was calculated as the ratio of the powder weight collected after drying experiment and the initial amount of dry materials in the prepared emulsions. Accelerated oxidation tests were carried out on the pure and microencapsulated sunflower oil. The samples were exposed to high temperature (100°C) to undergo oxidation. The results were expressed in hour, named the induction period (IP), and defined by the time corresponding to the inflection point of the conductivity versus time curve. The higher the induction time, the more stable the sample. The morphology of the spray dried microparticles was observed with an environmental scanning electron microscope.

Results.
The results showed a direct correlation between the viscosity of the emulsions and the yields of spray drying and morphologies of the microparticles. Emulsions stabilized with PPI-A and PPI-P showed higher viscosities, correlated to the hydrocolloidal nature of alginate and pectin (Table 1). The transformation of these emulsions into dry powders gave low solid yields (19 and 23%, respectively) and larger microparticles (Table 2 and Figure 1). Actually, high viscosity resulted in microparticles agglomeration and accumulation inside the drying chamber. Moreover, a higher solid content in sprayed drops will increase the size of the dry microparticles [3]. IP of microparticles containing PPI-I, PPI-MD and PPI-P wall materials were significantly higher than that found for microparticles formulated with PPI. On the contrary, IP of microparticles made of PPI-A was significantly lower than the one made of PPI (Table 2). These results were consistent with the observations of the external

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structures of the microparticles (Figure 1). Formulations made of PPI-I, PPI-MD and PPI-P gave spherical shapes and individual microparticles whereas the image of microparticles formulated with PPI-A showed inter-particle bridges, characteristic parameter of poor evaporation of water during the process.

Table 1
Volume weighted mean diameters ($d_{4,3}$) and viscosities of sunflower oil-in-water emulsions stabilized by pea protein isolates and alginate (PPI-A), pea protein isolates and inulin (PPI-I), pea protein isolates and maltodextrin (PPI-MD) and pea protein isolates and pectin (PPI-P).

Samples	$d_{4,3}$ (µm)	Viscosity (mPa.s)
PPI-A	10.34 ± 0.01^{a}	181.0 ± 0.1^{a}
PPI-I	13.68 ± 0.04^{b}	21.2 ± 0.1^{b}
PPI-MD	1.08 ± 0.01^{c}	26.1 ± 0.1^{c}
PPI-P	3.28 ± 0.01^{d}	256.9 ± 0.1^{d}

[a-d] Means in each column followed by different letters were significantly different ($p < 0.05$)

Table 2
Induction periods (IP) and solid yields of microparticles produced from emulsion stabilized by pea protein isolates (PPI), pea protein isolates and alginate (PPI-A), pea protein isolates and inulin (PPI-I), pea protein isolates and maltodextrin (PPI-MD), pea protein isolates and pectin (PPI-P) and pure sunflower oil as control.

Samples	IP (h)	Solid yield (%)
Control	12.27 ± 0.67^{a}	-
PPI	21.26 ± 0.44^{b}	50
PPI-A	19.97 ± 0.12^{c}	19
PPI-I	23.47 ± 0.20^{d}	40
PPI-MD	24.45 ± 0.10^{e}	35
PPI-P	27.18 ± 0.28^{f}	23

[a-f] Means in each column followed by different letters were significantly different ($p < 0.05$)

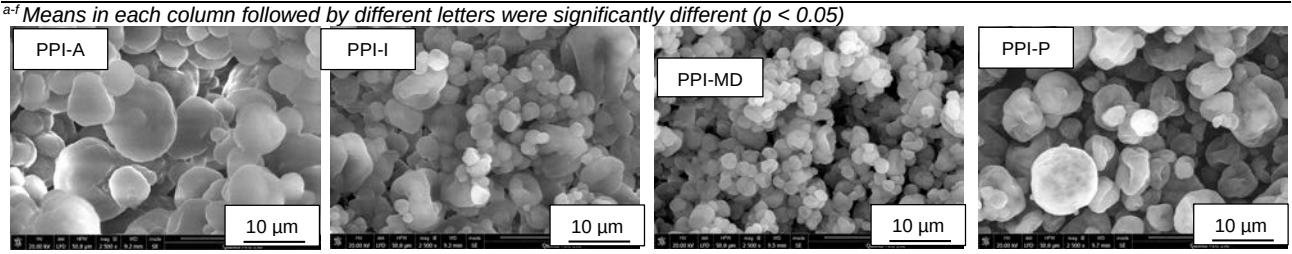

Figure 1. Environmental scanning electron micrographs of microencapsulated sunflower oil in pea protein isolates and alginate (PPI-A), pea protein isolates and inulin (PPI-I), pea protein isolates and maltodextrin (PPI-MD) and pea protein isolates and pectin (PPI-P). The magnification was set at 2500×.

Conclusion. This study intended to do an analysis of the variation of the composition of wall materials on the oxidative stability of microencapsulated sunflower oil. The obtained results demonstrated that the nature of the bio-based carrier agents strongly affected the oxidative stability of the active material. Inulin, maltodextrin and pectin were suitable additives to pea protein isolates for this purpose. The addition of 2% w/v of these polysaccharides allowed to respectively increasing to 2.21, 3.19 and 5.9 2h the IP of microparticles exclusively made of PPI. These findings are of importance for providing a solution to develop PUFA-enriched formulations for food and feed industries.

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Nanoceria, a promising strategy for decontamination of chemical warfare agents

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Keywords. Cerium dioxide, chemical warfare agents, paraoxon, skin decontamination

Introduction

Organophosphorus compounds (OPs) are a class of chemical warfare agents (CWA) that have found a significant application during terrorist attacks and/or military conflicts to bring harmful impacts. Despite the ongoing calls for the complete destruction of CWA, they still represent an omnipresent threat for the military and are used as a mean to terrorize civilians[1]. These agents penetrate the body through inhalation, ingestion but mainly by skin penetration. Rapid skin decontamination is the most efficient way to reduce the risk of intoxication. The most efficient systems for skin decontamination are based on the application of absorbent powders or washing[2].

To remove toxic substances from contaminated sites, various strategies are developed and applied. Among these, recent studies showed the potentiality of metal oxide nanoparticles, and especially Cerium Dioxide Nanoparticles (CeO_2 NPs) to absorb and decompose CWA[3]. Due to their attractive surface properties, CeO_2 NPs act as reactive sorbents for fast degradation of OPs[4], and they provide a suitable method for skin decontamination[5]. Here, CeO_2 NPs of different physicochemical properties were synthesized by a chemical method, hydrothermal synthesis[6] and assisted by microwave treatment. Their potential to degrade paraoxon chosen as a model OP compound was evaluated in vitro in aqueous suspension. The influence of the size, morphology and surface state of NPs on the kinetics of degradation was studied.

Experimental Section

Synthesis and characterization of various CeO2 morphologies

Single crystalline nano-octahedra (NO) ,nanocubes (NC), nanorods (NR), a mixture of nanocubes and truncated nanooctahedra (NCO), Truncated octaedra (TO) and small agglomerates of sub-10 nm nanoparticles (SO) were selectively obtained by using a hydrothermal method under different conditions of temperature, concentration and precursor**.** X Ray Diffraction (XRD), Transmission Electronic Microscopy TEM, and Nitrogen adsorption were used to characterize nanoparticles Crystalline structure, size, and specific surface area. Results are presented in Table 1. The degradation kinetics of Paraoxon (POX) byCeO_2 NPs was quantified by measuring the concentration of both POX and p-nitrophenol, the product resulting from the cleavage of the P-O-aryl bound. (Fig. 1).

Sample	Crystal faces	d_{TEM} (nm)	$S_{BET}(m^2/g)$
NC	{100}	5-60	15
SO	{111}	150-260	6.5
NO	{111}	6-35	66.6
NCO	{100} + {111}	3-20	76.1
NR	Not determined	(7-9) X (50-200)	132.5
TO	{111}	7-10	20,8

Table 1. Characterization parameters of CeO_2 NPs

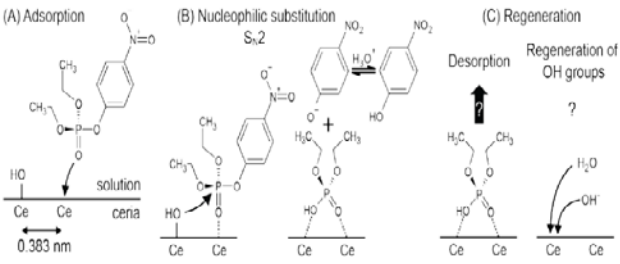

Fig.1. Degradation mechanism of Paraoxon

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Degradation efficiency of CeO_2 nanoparticles

The aim of this study was to correlate the physicochemical properties of the nanostructure with the efficiency of OP degradation. The results are expressed as degradation kinetic curves (POX conversion versus time figure 2). The influence of two parameters, the specific surface area and the crystal faces on degradation efficiency is clearly shown whatever the morphologies of CeO_2.

Without NPs, there was no degradation of POX in 5 hours (POX blank).

POX is totally or almost totally degraded for NO, NCO, NR, reaching 97%, 86%, 96% of degradation respectively after 5 hours of contact. After 30 minutes POX was completly degraded by truncated octahedra (TO). On the contrary, NC and SO are two morphologies less favorable for the degradation with only 14% and 24% of conversion at the end of the experience (Fig.2).

The {111} crystal faces of the nanopaticles present higher efficiency of degradation compare to the {100} faces. This can be explained from the adsorption step of the degradation process. There is a small number of adsorption sites for CeO_2 on cubic shape in comparison to other shapes. For the {111} crystal faces, the surface density of Ce atoms is 7.9 nm^{-2}, larger than the 6.8 nm^{-2} for the {100} crystal face, which favour the adsorption mechanism, first step of degradation mechanism, which would promote the nucleophilic substitution mechanism.

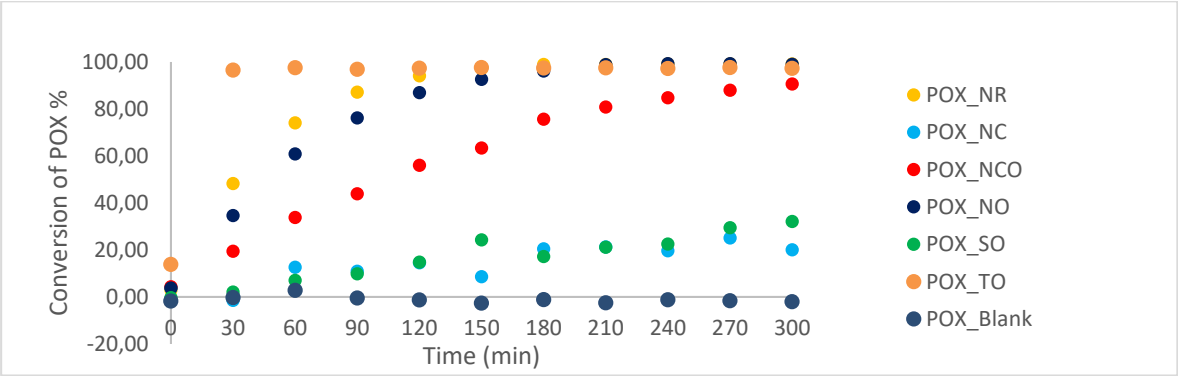

Fig. 2. Degradation efficiency of Paraoxon with different morphologies of CeO_2

Conclusions

The efficiency of degradation as a function of the crystallographic facets allow to better understand the kinetics of degradation. These results help to confirm that CeO_2 NPs elaborated with a certain shape, crystallography facets and specific surface area are promising materials for the degradation of POX. Therefore, CeO_2 NPs is a new strategy for developing a galenic formulation compatible with the constraints of the ground and that can be used for the defense of tomorrow.

Aknowledgment

The authors are thankful Direction Générale de l'Armement (DGA) for their financial support.

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Dispersibility and stability characterization of TiO_2 nanoparticles in cell culture media with static multiple light scattering

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Keywords. Stability, dispersibility, nanoparticles, toxicity, cell culture medium, SMLS

Introduction.

Since the last decades, nanoparticles (NPs) have been widely used in a broad range of industries such as cosmetic or food. In this context, concerns had been raised about the toxicity of such nanomaterials on human health. Thus, it is of paramount importance to understand the NPs behavior under conditions like those for in vitro or in vivo nanotoxicity studies.

The preparation protocol of the NPs dispersion greatly impacts their properties and their resulting toxicity when injected to cells [1]. Indeed, it has been proved that NPs agglomerate immediately when added to a cell culture medium and results on false dose estimation and in fine false toxicity interpretations [2]. Classically, proteins serums such as bovine serum albumin (BSA) or fetal bovine serum (FBS) are added to the dispersion to coat the NPs and thus avoid agglomeration. However, the dispersion method (whirling, sonication, …), both the BSA and NPs concentrations and the cell culture medium influence the BSA-NP interactions [3]. Then, the characterization of the stability and dispersibility of the NPs over time is very important to obtain a well-controlled dispersion for toxicological studies.

In this study, we propose to use SMLS to:

1. Evaluate the particle size related to the dispersion state and the stability of a TiO2 NPs stock dispersion in BSA-water solutions.
2. Estimate the dispersibility of the NPs stock dispersion diluted in presence or absence of cell culture media (e.g. DMEM) via the mean particle size.

Materials & Methods.

Stock dispersions, i.e. NPs in BSA-water mixture, as well as dilutions of the stock dispersion in cell culture medium were prepared following the NaNoREG protocol [1]. The TiO_2 NPs with primary size of 20nm were received as dry powder from Evonik. BSA and DMEM from Sigma-Aldrich were used as protein model and cell culture medium respectively.

We have used a Turbiscan Lab to characterize the dispersibility and stability in stock dispersions and dilutions in cell culture medium. This device uses the principle of SMLS that provides a non-intrusive optical characterization of a native sample without dilution. An infrared light source illuminates the sample and the backscattered (BS) and transmitted (T) light intensity signals are collected simultaneously by two sensors over the whole samples height and repeated over time. The resulting spatial and time dependent signals T and BS are directly linked to the fundamental properties of the dispersion (particles mean size, concentration, …) as well as physical instabilities (aggregation, sedimentation, …).

Results.

The influence of BSA mass fraction (ranging from 0% to 0.5%) on the dispersibility and stability has been quantified with Turbiscan in the NPs stock dispersion. *Figure 1* shows the results obtained for the mean agglomerate size. For BSA mass fraction lower than 0.2%, the mean agglomerate size is around 150nm which corresponds to the initial measured NPs mean size. In these cases, it can be concluded that the NPs are not agglomerating. For BSA mass fraction higher than 0.2%, the agglomerate size exceeds 1µm which highlights a strong agglomeration process. It is well established in literature [2], that for such high proteins concentration, a depletion attraction phenomenon occurs that causes the agglomeration of NPs. This agglomeration induces a strong decrease of the NPs concentration within 5 minutes. To avoid the depletion attraction, it is thus recommended to dilute this stock dispersion into a cell culture medium in less than 5 minutes.

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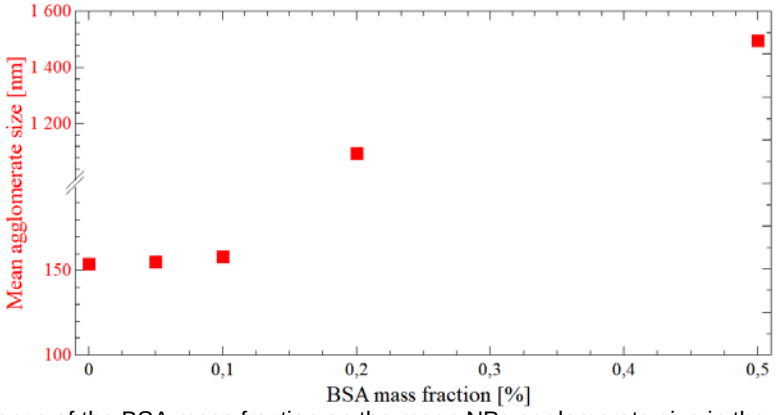

Figure 1 : Influence of the BSA mass fraction on the mean NPs agglomerate size in the stock dispersion

Finally, the dispersibility and stability in the case of the dilution of stock dispersions in cell culture medium (here DMEM) has been studied. These dilutions correspond to the final NPs dispersions to be injected to the cells for toxicity studies. The dispersibility and stability were quantified thanks to the initial mean NPs size, the mean agglomerate size and the NPs concentration kinetic at the bottom of the sample. This latter quantity is shown in *Figure 2*. In 10 hours, the effective delivered dose of NPs that would be in contact with adherent cells (at the bottom of the glass cell) is ten time higher than the initial administered dose for BSA mass fraction lower than 0.2%. This would result in false dose/cytotoxicity response when injected to the cells.

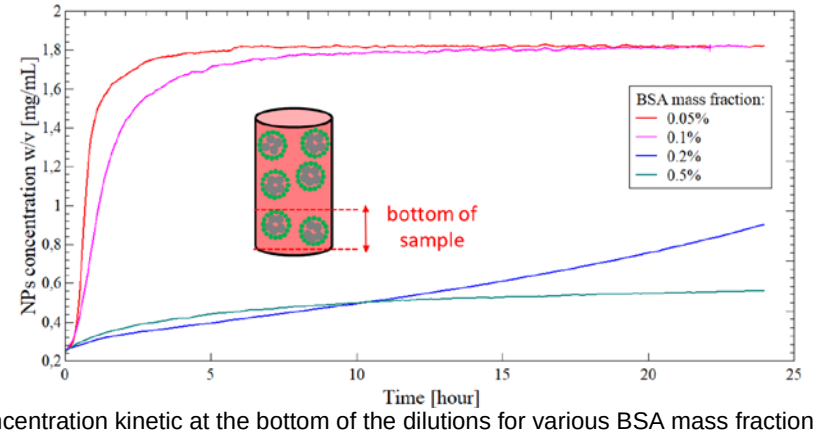

Figure 2 : NPs concentration kinetic at the bottom of the dilutions for various BSA mass fraction in stock dispersion

Conclusions.

The dispersibility and stability of stock dispersions as well as dilutions have been quantified thanks to the Turbiscan. It has been proved that best results in the cell culture medium are obtained for a BSA mass fraction of 0.5%. Paradoxically, at this same mass fraction, depletion attraction starts after 5 minutes in the stock dispersion. Thus, it is recommended to directly dilute the BSA into the cell culture media before adding the NPS to avoid this depletion phenomena and insure the best dispersibility and stability before injection to the cells.

These results are promising for screening the preparation of NPs dispersions in any in vitro nanotoxicological studies.

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Analysis of gelation capability of human platelet lysate

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Keywords. Platelet lysate, Gelation, Microrheology, gel properties

Introduction.
Human blood platelet lysate is an interesting alternative to Fetal Bovine Serum (FBS) in cell culture media: It is of human origin and contains therefore human growth factors that accelerate cell culture growth. It does not contain any animal components, such as FBS. The latter one has become questionable due to security (mad cow disease) and ethical (origin and source) reasons. Although the growth factors are much better, Human Platelet Lysate (HPL) forms an unstable gel in contact with calcium ions (Ca^{2+}), which is usually a compound in cell media, such as DMEM. Those gels are very weak and may undergo easily a syneresis under stress. In this work, we will show, how microrheology using Diffusing Wave Spectroscopy (Rheolaser Master) can help to analyze these delicate materials.

Materials & Methods.
Rheolaser Master uses Diffusing Wave Spectroscopy (DWS) [1,2], which consist of multiple backscattering of a coherent laser light source. The backscattered waves form interference patterns (Speckle image) which show an evolution in time depending to the motion of the scattering particles. The faster the particle move, the faster change the speckle image. By mathematical processing of a sequence of Speckle Images, one can correlate the evolution of the Speckle image to the motion of the particles. By further data treatment, the viscoelastic properties can be obtained indicating the elasticity and the viscosity of the samples. The use of an optical method allows the analysis of very weak gels in a non-invasive and non-destructive manner. 1µm polystyrene particles were added as tracers for the backscattering (0.1 wt%).
First study: The HPL was melted and diluted to 8 wt% in DMEM and then measured at 37°C.
Second study: The HPL was melted and diluted to 1, 5, 10, 15, 20 and 30 wt% in DMEM and then measured at 37°C.

Results.
The work will show two studies. The first part focuses on different HPL preparations, which have different gelling abilities. Figure 1 shows the evolution of the Elasticity Index (EI) as a function of time for 5 different preparations. The higher this Elasticity Index is, the higher is the sample's elasticity. The control sample does not show any gelation, which was expected. The Samples 2, 3 and 4 show an increase of elasticity, which indicates gelation. Sample 1 does not gel either, which is due to a special sample treatment.

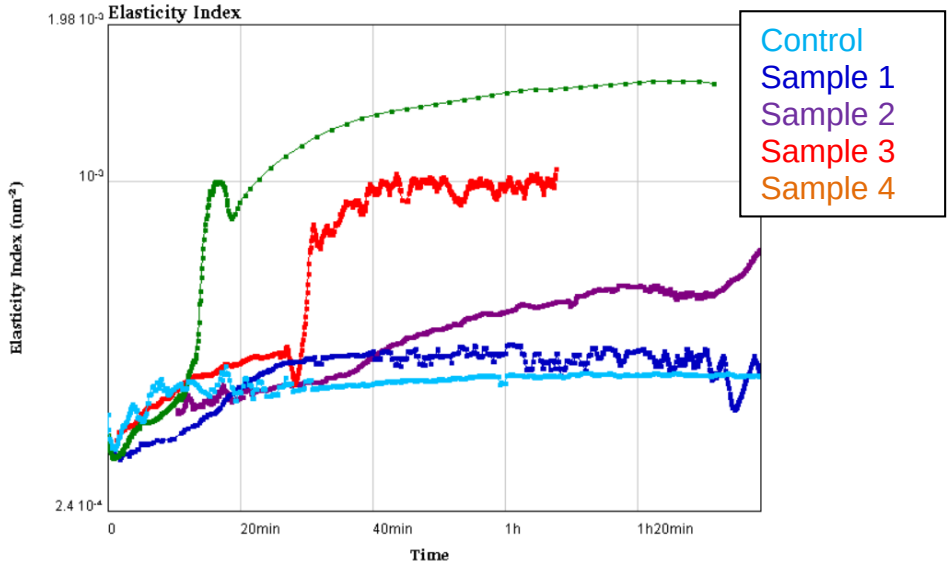

Figure 1: Evolution of Elasticity Index as function of time at 37°C.

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The second part of the study focused on the use of a gelling HPL systems and which is the best concentration for gelation. HPL was diluted to 1, 5, 10, 15, 20 and 30 wt% in DMEM and measured at 37°C. After 10 hours, the Elasticity Index value was compared between each sample. Only HPL samples diluted to 10 and to 15 wt% showed gel formation, whereas lower and higher concentrations show only flocculation, which make cell culture experiments impossible.
The low Elasticity Index (about 10^{-3} nm^{-2}) for the 10 and 15 wt% samples show the formation of a weak gel, which may be suitable for transfer with pipette. This is an advantage for cell culture handling [

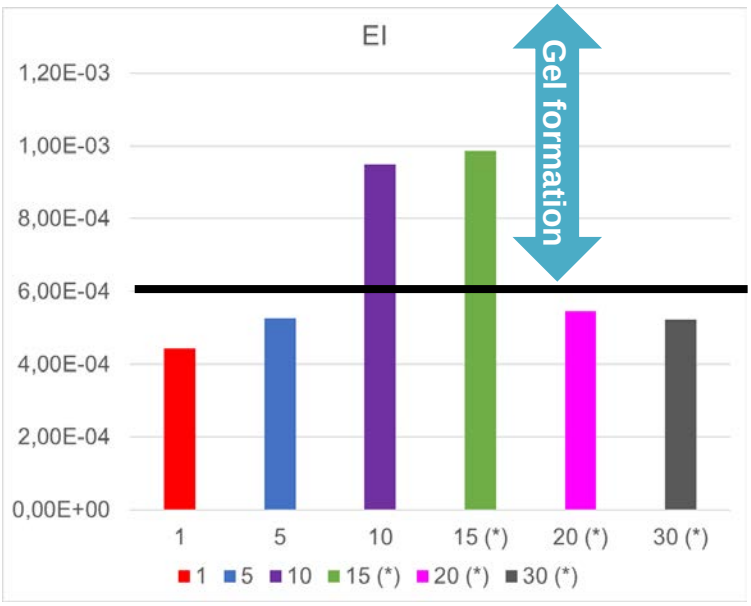

Figure 2: Elasticity Index after 10 hours at 37°C.

Conclusions:
This work presents a new approach to study the redispersibility of aluminium adjuvants, that usually tend to precipitate and decrease vaccine efficiency. The obtained settling onset time, which is less than an hour, is in good correlation with the results obtained after 24h. This method allows therefor to reduce significantly the measurement of vaccine samples in both fields, development and in quality control.

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Simulation numérique du coefficient de diffusion d'un Principe actif à partir d'un système à géométrie variable

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Mots clés: Modélisation, diffusion, principe actif, polymère.

Introduction:

Les modèles mathématiques qui décrivent la libération du principe actif des dispositifs pharmaceutiques hydroxypropylique méthylcellulose (HPMC), permettent l'élucidation des mécanismes fondamentaux de transport de masse. De nombreux modèles empiriques et réalistes ont été proposé.

Dans beaucoup de cas, l'utilisation d'un modèle empirique simple est entièrement suffisante. Cependant, des théories plus complexes et plus mécanistes doivent être appliquées si l'exactitude est exigée [1].

L'objectif de ce travail est de contribuer à la compréhension du problème complexe de la libération des principes actifs à partir des systèmes à base de matrice gonflable.

L'effet de quelques facteurs tels que la quantité de la matrice hydrophile et le pH du milieu, sur la libération du principe actif à partir des comprimés a été étudié. Le gonflement de ces matrices a été suivi dans deux milieux différents de dissolution. Les coefficients de diffusion de l'eau et de la substance active ainsi que les constantes de gonflement et d'érosion ont été estimés en employant un logiciel basé sur la méthode d'élément fini.

Matériels et Méthodes.

1. Travail expérimental :

Des comprimés cylindriques à base d'HPMC à différentes concentrations ont été immergés dans deux milieux de dissolution (milieu acide HCl 0.1N à pH=1.2, simulant le milieu gastrique et milieu tampon à pH=6.8, simulant le milieu intestinal), des prélèvements sont réalisés pour différent temps, la mesure de la taille (épaisseur et rayon) ainsi que la masse des comprimés sont effectués pour chaque temps. Un ensemble riche de données expérimentales a été obtenu pour les deux milieux de dissolution et exploité pour la réalisation de la partie simulation [2].

2. Modélisation de la diffusion dans un système à géométrie variable

Le transport de l'eau et du principe actif dans la matrice peut être regardé en tant que deux phénomènes de diffusion, qui peuvent être décrits par deux bilans de matière:

$$\rho \frac{\partial w_k}{\partial t} = \vec{\nabla}\left(\rho D_K \vec{\nabla} w_k\right) \qquad \text{Eq. 1}$$

Dans équation (1), la densité de matrice est ρ, w_k sont les fractions massiques de l'eau et du principe actif, D_K sont les coefficients de diffusion.

Pour résoudre l'équation (1), les coefficients de diffusion, D_K (pour k = 1, 2), doivent être évalués.

$$D_k(w_1) = D_k^*.\exp[-\beta_k.(1 - \frac{w_1}{w_{1,eq}})] \qquad \text{Eq.2}$$

Où Dk*/exp (β_k) sont les valeurs (pour k = 1, 2) des coefficients de diffusion de la matrice sèche ($w_1 = 0$), et les D_K* sont valeurs des coefficients de diffusion dans la matrice entièrement gonflée ($w_1 = w_{1eq}$).

Le mouvement d'un élément extérieur est dû au phénomène de gonflement et au phénomène d'érosion:

$$v = v_{swe} + v_{eros} \qquad \text{Eq.3}$$

Où v_{swe} est la vitesse dû au gonflement (une valeur positive) et v_{eros} est la vitesse dû à l'érosion (une valeur négative).

$$v_{swe} = \frac{d\delta}{dt} = -\frac{j_{1,swe}}{\rho} = -\frac{k_{swe} j_{1,diff}}{\rho} \qquad \text{Eq.4}$$

Où k_{swe} est la constant de gonflement et $J_{1,diff}$ est le flux de diffusion de l'eau.

$$v_{eros} = -k_{eros} A_{tot}(t) \qquad \text{Eq.5}$$

Où k_{eros} est la constant d'érosion et A_{tot} est la surface totale d'érosion du comprimé

Le logiciel utilisé pour réaliser la simulation est COMSOL Multiphysique, il est utilisé pour la résolution de tous types de problèmes scientifiques basés sur des équations aux dérivées partielles, il est basé sur la méthode des éléments finis.

Résultats.

1. Etude du gonflement et de l'érosion :

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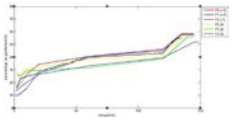

Fig.1 : Variation du taux de gonflement dans le milieu acide

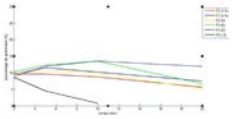

Fig.2 : Variation du taux de gonflement dans le milieu tampon

Nous remarquons pour le milieu acide HCl 0.1N, pH=1 .2, une croissance du taux de gonflement jusqu'à atteindre une valeur limite au environ de 140 min (saturation en eau de la matrice hydrophile). La pénétration de l'eau dans la matrice hydrophile augmente avec l'augmentation de la concentration en HPMC. Concernant le milieu tampon pH=6.8, nous constatons qu'il y a présence de deux phénomènes, le gonflement et l'érosion au même temps.

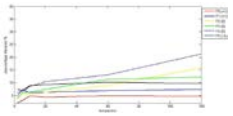

Fig.3 : Variation du taux d'érosion dans le milieu acide

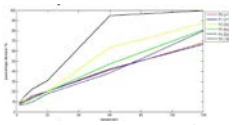

Fig.4 : Variation du taux d'érosion dans le milieu tampon

A partir de ces figures nous constatons que l'érosion (perte de masse) des comprimés ne dépasse pas 22% dans le milieu acide tandis que dans le milieu tampon phosphate le taux d'érosion atteint 100%.

2. Simulation des coefficients de diffusion de l'eau, du PA et les constants de gonflement et d'érosion.

COMSOL Multiphysics ® offre la possibilité d'utiliser un mode d'analyse spécifiquement créé pour résoudre les problèmes impliquant un changement de la géométrie dans le temps. Ce logiciel fournit comme résultat la déformation et la fraction massique de l'eau dans un quart du comprimé à différentes positions à un instant « t » (figure n°6).

Dans notre cas, nous pouvons déterminer expérimentalement dans le comprimé que la fraction massique de l'eau à l'instant « t » et non pas la fraction massique de l'eau à différentes positions pour un même instant.

A cet effet, Le volume total du comprimé sera utilisé pour réaliser la simulation. Ce dernier a pu être déterminé expérimentalement en mesurant l'épaisseur et les diamètres et de la même façon il sera déterminé à partir des résultats fournis par le logiciel

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Fig.5 : Comprimé après 120mn d'immersion

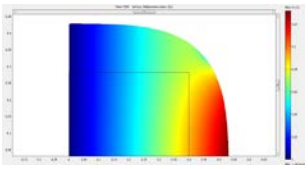

Fig. 6 : Quart du comprimé après 120mn par COMSOL

Les courbes du modéle ont été bien adaptées aux données expérimentales.

Pour le milieu acide, nous avons négligé l'érosion devant le gonflement, ce qui a facilité le travail de la simulation, et nous avons abouti à une estimation des paramètres suivants :

(k_{eros}=2.46*10^{-5}cm^{-1}/s, k_{swe}=4.3, D*$_1$=2*10-6 cm^2/s, D*$_2$=12*10^{-8} cm^2/s) avec une erreur relative de l'ordre de 2%.

Pour le milieu tampon phosphate pH=6.8, nous avons considéré les deux phénomènes en compétition l'érosion et le gonflement. Dans ce cas complexe, la simulation des résultats de l'expérience est plus difficile à réaliser et nous avons abouti à une estimation des paramètres suivants :

(k_{eros}=10^{4}cm^{-1}/s, k_{swe}=1.8, D*$_1$=1.2*10^{-7} cm^2/s, D*$_2$=2*10^{-4} cm^2/s) avec une erreur relative de l'ordre de 13%.

Conclusion

Dans le travail actuel, nous avons élaboré, en utilisant un logiciel de calcul basé sur la méthode des éléments finis, des modèles mathématiques qui peuvent décrire le processus de libération du principe actif des formulations pharmaceutiques solides destinées à l'administration par voie orale. Ces dernières sont immergés dans des solutions qui reproduisent les liquides physiologiques (milieu gastrique (HCl 0.1N) et intestinal (solution tampon phosphate pH=6.8).

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Study of some rheological properties of a poloxamer 407 thermogelifying gel / starch of *ipomoea batatas* from Côte d'Ivoire

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Keywords: Poloxamer P407, Gel, Rheology, Starch Glycerol

Introduction

The purpose of this work is to provide different formulations based on starch and poloxamer 407 (P407) in order to obtain a preparations having a higher contact time at the application site. The aim on this study is to develop thermogelling gels based on native starches and poloxamer 407 (F 127)

Materials and methods

Preformulation tests of gels of P407 at 20% and starches of sweet potato at different concentrations (1% to 10%, w/w) and P407 at 20% and glycerol starch (GA at 2.5g, 5g and 10g) were made. These gels were characterized by macroscopic test, microscopic test (OPTICA polarized optical microscopy) and physicochemical tests. The best formulation was selected for the rest of this work. Moreover, rheological studies were carried out, using a rotary viscometer (HAAKE), and stability studies during 28 days.

Result

The gel based on P407/2.5g GA showed good homogeneity, good shear thinning and thixotropic properties and heat sensitivity with a gelation temperature (Tgel) equal to 17.3 ° C. The pH was 6.7 at 37 ° C. In addition, the median particle size was 1.69 µm at 37 ° C. Which could presage a good cutaneous applicability.

Conclusion

The results obtained seem encouraging. However, the absence of active principle in the formulations, limited the interpretation of the results obtained. Also, more in-depth assessments in the presence of an API, should be made to better appreciate their impact on the physicochemical characteristics of these gels.

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Solid lipid nanocarriers: high potential formulations for oral delivery of peptides

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Keywords. Peptide, Lipid, SLN, NLC, *in vitro*

Introduction:
Peptides are a class of therapeutic molecules with a great potential to treat diseases in a selective and efficient manner. However, they are mainly administrated via parenteral route as their bioavailability through oral route is very low: peptides are easily degraded by the harsh gastro-intestinal environment and their absorption through the intestinal border is highly limited. Encapsulation of peptides in lipid-based nanocarriers could offer them external protection and facilitate their absorption through intestinal epithelium [1]. Efficiency of two systems, Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC), was evaluated using Leuprolide as model peptide.

Materials & Methods.
Hydrophobic Ion Pairs (HIP) were formed between leuprolide and sodium docusate [2]. Nanocarriers of Precirol®ATO 5 (glycerol distearate) loaded with HIP or Leuprolide were prepared with a High Pressure Homogenizer (HPH). The encapsulation efficiency (EE) was evaluated via HPLC. The size of the dispersions was measured via Dynamic Light Scattering (DLS) and Cryogenic Transmission Electron Microscopy (cryo-TEM).
The stability in size and polydispersity index of the blank nanoparticles was evaluated via DLS over 2h at 37°C in Simulated Gastric Fluid, and over 6 hours in water, Fasted State Simulated Intestinal Fluid (FaSSIF-V2) and Fed State Simulated Intestinal Fluid (FeSSIF-V2).
Peptide release from nanoparticles was performed over 6 hours in FaSSIF-V2.
Evaluation of particles ability to protect Leuprolide from proteases degradation was conducted in Tris buffer pH 6.8 containing either trypsin or α-chymotrypsin.

Results.
HIP of leuprolide-docusate were obtained with a complexation rate of 99%. Reproducible nanosuspensions of Precirol®ATO 5 were formulated via the HPH technique (size of 120 nm, PDI≤0.2). They were successfully loaded with HIP (EE=77% for SLN, EE=76% for NLC) and Leuprolide alone (EE=52% for SLN, EE=58% for NLC). Size and polydispersity index of the particles were stable under SGF conditions. Almost no release of peptide was observed under FaSSIF-V2 conditions. Nanocarriers demonstrated the ability to prolong Leuprolide resistance to proteases.

Conclusions
The HPH method is a reproducible technique to produce both SLN and NLC. Peptides can be successfully loaded in these nanocarriers and their reversible complexation via HIP formation enables increasing their payload. Dispersion of the nanosuspensions in simulated intestinal fluids did not affect size distribution or release of the peptide. Then, these systems have a high potential to protect and transport peptides to the systemic circulation. Evaluation of the impact of particles digestibility as well as their ability to cross the intestinal barrier are the next steps of this study.

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PEO-PPO-PEO nanomicelles prepared by a green emulsion-evaporation process: characteristics and thermoresponsive properties.

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Keywords.
Nanomicelles, stimuli-responsivity, emulsion- solvent evaporation

Introduction. The aim of this study was to prepare thermoresponsive nanosystems for a cosmetic use. Among polymers exhibiting thermoresponsivity, Pluronics® (or Poloxamers®) are of the most interest to prepare stimuli-responsive nanosystems as they are well-known and authorized for a cosmetic use [1, 2]. These triblock copolymers are formed by one hydrophobic poly(propylene oxide) (PPO) group surrounded by two hydrophilic poly(ethylene oxide) (PEO) groups. The hydrophilic-lipophilic balance (HLB) of Pluronic® varies as function of PEO/PPO ratio and modulates their affinity to active molecules. The biocompatibility makes them potential candidates for elaboration of encapsulation systems for protection then stimulated release of active cosmetic or pharmaceutical ingredients at temperatures higher than the body temperature. Unfortunately, those micelles, prepared most of the time by film hydration, show a low stability in time. The emulsion-solvent evaporation method was explored with three molecules of cosmetic interest.

Materials & Methods. Nanomicelles were prepared by an emulsion-solvent evaporation process assisted by ultrasounds. A design of experiment approach was applied to determine the ideal conditions of preparation. A green volatile solvent was tested to avoid the use of chlorinated solvent. For each nanosystem, the morphology (TEM and/or cryo-TEM), the size (DLS) and the zeta potential were measured. The thermoresponsivity of the systems was studied by DSC and size measurements as function of the temperature. The contents in active molecules were determined by HPLC.

Results. Three active ingredients have been encapsulated in Pluronics® F68 and F127: curcumin, for its antioxidant properties and its intrinsic fluorescence, panthenol for its calming and repairing activities and Punica granatum seed oil hydroxyphenethyl esters for their lipolytic activities. Emulsion-solvent evaporation was used to prepare Pluronics® nanomicelles. This technique usually used to prepare nanoparticles but barely used to prepare nanomicelles showed interesting results. The nanosystems have a high stability and a small polydispersity index (<0,2). The results show that the size (≈30 to ≈200nm), loading up to ≈ 80% for the ester) but also thermoresponsive and release properties strongly vary depending not only on the polymer tested, but also on the encapsulated molecule when the same conditions of preparation were used (concentrations, durations, and temperature).

Conclusions. Emulsion-evaporation technique can be used to prepare stable and reproducible nanosystems made of Pluronics®. The characteristics of the nanosystems obtained is strongly depending on the physico-chemical characteristics of the encapsulated molecule. Whereas panthenol thermoresponsive nanosystems car directly be used in cosmetics, the two other systems developed still have to be optimized.

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Optimization of liposomes formulations for improving the depigmenting effect of cysteamine

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Keywords. Cysteamine, encapsulation, liposomes, phospholipon 90H.

Introduction

Cysteamine is an aminothiol compound physiologically synthetized by human body cells. It presents several biological effects such as radioprotective, anti-cancer, anti-malaria, anti-cystinosis and depigmenting effect [1], [2]. However, cysteamine possesses unpleasant organoleptic properties (foul odor and bitter taste), hygroscopicity, instability in solutions and poor pharmacokinetic profile [3] thus its application is limited. The encapsulation of cysteamine in liposomes may overcome these drawbacks. Cysteamine has been encapsulated in liposomes to improve its delivery to cystinotic cells, its gastro-intestinal absorption and to prolong its radioprotective action. In this study, cysteamine was encapsulated in liposomes in various experimental conditions; the optimal formulation will be tested on hyperpigmented skin. The vesicles were characterized in terms of size, morphology, zeta potential and encapsulation efficiency.

Materials & Methods

Blank liposomes were prepared using ethanol injection method. Phospholipon 90H (Ph90H) (10 mg/mL) and cholesterol (chol) (5 mg/mL) were dissolved in absolute ethanol. The organic solution was later injected into the aqueous phase using a syringe pump at a temperature above the transition temperature of the lipid. The same procedure was applied to prepare liposomes loading cysteamine, except that in this case the aqueous phase contained cysteamine at various concentrations. The molar ratio Ph90H: chol: cysteamine was of 1:1:0.25; 1:1:0.4; 1:1:0.5; 1:1:1; 1:1:2; 1:1:5; 1:1:10; 1:1:15 and 1:1:20 in the different formulations. The quantification of cysteamine in liposomal suspensions was conducted using a modified Ellman method where several parameters such as dilution factor, dilution in 1% triton-X 100, and addition of an organic solvent were changed to enhance vesicles solubilization. The optimized method was validated in terms of linearity and repeatability. The size and zeta potential of the various liposome suspensions were evaluated by differential light scattering; the morphology of vesicles was examined by transmission electron microscopy.

Results

Ellman method was successfully applied in water but some difficulties were faced when applied in liposomal suspension; the supramolecular structures of lipids were not solubilized and interfered in dosage method. Among various organic solvents tested, methanol was the only organic solvent able to solubilize the liposomal suspension when added at the same volume ratio of phosphate buffer to cysteamine and Ellman reagent. The optimized method will be then considered to evaluate the encapsulation efficiency and loading rate of cysteamine in liposomes.

At the various Ph90H: chol: cysteamine molar ratios studied, two populations have appeared: the major one of nanometric size (96 %) and the second of micrometric size (4%). However, one nanometric population was obtained for blank liposomes. Also, the mean particle size of blank liposomes was 195 nm while a slight increase was observed after the addition of cysteamine in the different formulations. The presence of cysteamine at the different concentrations did not affect liposome lamellarity or morphology where the liposomes appeared oligolamellar and spherical in shape. The different liposomal formulations had a high negative zeta potential (-35 mV) meaning that the prepared liposomes were stable. A slight increase in the surface charge of the cysteamine loaded liposomes was observed compared to the blank liposomes.

Conclusions

The quantification of cysteamine in liposomal suspension using Ellman method was performed using some modifications. The addition of methanol as organic solvent was the best solution for the quantification of cysteamine in liposomal suspension. Cysteamine at low and high concentrations did not affect the size, zeta potential or the shape of liposomes. The optimized Ellman method will be applied in further studies to determine the encapsulation efficiency and the loading rate of cysteamine in liposomes.

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Generating Injectable Intrathecal Formulations for Poorly Soluble Drugs

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Keywords. Intrathecal, drug-delivery, lipid formulations

Introduction

Chronic pain bears a great societal and economic burden, being accompanied by a decline in normal functioning and quality of life. It has recently been highlighted as one of the leading causes of disability worldwide[1]. The need for novel analgesic treatments is expanding due to many patients suffering from intractable pain which is not currently treated by existing therapies. Numerous preclinical studies have suggested that cannabinoids acts as analgesics and have been shown to play a potential role in chronic pain management[2]. Cannabidiol (CBD) has been reported to have a therapeutic action on TRPV1 and TRPA1 in the spinal cord in association with pain management, however the main drawback associated with the use of cannabinoids in analgesia are the side-effects observed in most trials and extensive first-pass metabolism[3, 4]. CBD is an extremely lipophilic drug (logP 6.33) and its oral bioavailability is approximately 13% - 19%[4]. Therefore, these properties make it an excellent candidate for formulation in a lipid based nanoemulsion.

Recent developments in intrathecal drug delivery systems allow for this technique to administer drugs at therapeutic levels directly to the site of action in the subarachnoid space thus reducing adverse effects and by-passing first pass metabolism. To date there are very few formulations for intrathecal delivery in pain management and therefore the aim of this project is to expand on the formulations available for intrathecal delivery.

Materials & Methods

Density gradient ultracentrifugation using standard solutions of phosphate buffered saline of various densities was used to incorporate CBD in commercially available Intralipid nanoemulsion. Lipid particles loaded with CBD were separated by ultracentrifugation and the drug-containing upper lipid layer was collected. An alternative protocol involving CBD being vortexed, sonicated and incubated followed by slow-speed centrifugation with Intralipid to encapsulate drug in lipid particles was also carried out. Reverse-phase HPLC was performed to quantify the amount of CBD encapsulated within the lipid nanoparticles. Particle size analysis using dynamic light scattering (DLS) technology and Zeta-potential measurements were carried out to ensure stability of the lipid formulation.

An *in vivo* pilot study will be carried out in rodents in order to assess acute neurotoxicity of the Intralipid emulsion vehicle when injected intrathecally. Clinical signs of neurofunctionality will be observed alongside histological analysis (meningeal haemorrhage, necrosis, ischaemia) to ascertain the effects of the lipid vehicle on the CNS.

Results

Drug incorporation method optimisation was carried out. Vortexing then sonicating CBD in Intralipid followed by incubation was performed. This method led to quantifiable CBD concentrations based on HPLC, particle sizes ranging <300 nm and Zeta-potential ~ -42mV, however polarising microscopy could not discern whether drug was encapsulated or crystallised in the emulsion. Density gradient ultracentrifugation was successful in incorporating CBD within the oil phase of the emulsion as demonstrated by HPLC. The addition of CBD to Intralipid did not affect particle size (<300 nm) or Zeta-potential (-55mV) which remained stable for at least 7 days post incorporation.

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Conclusions

CBD exerts its therapeutic action on receptors at the spinal cord therefore a targeted formulation is required. Contemporary developments in intrathecal drug-delivery systems allow for this technique to be used to administer drugs directly at the site of action. Considering the lipophilicity of CBD, a lipid-based formulation is ideal for its delivery. It has been shown that CBD may be incorporated in the lipid phase of a commercially available nanoemulsion by the use of density gradient ultracentrifugation and remains stable for up to 7 days. The drug release from the formulation will be further characterised *in vitro* and then the acute neural toxicity of the formulation will be assessed in rodents.

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Encapsulation of *Spirulina platensis* green extracts for anti-biofilm use

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Keywords. Nanocarriers**,** alginate, biofilm, free fatty acids, sustainable extraction

Introduction.
Biofilm-related infections are currently a major health issue. Candida biofilms are especially involved in catheter-, mucosa- and skin- infections. Microorganisms organized as biofilms become poorly susceptible, even resistant, to conventional antimicrobials and therapeutic approaches [1]. Free fatty acid (FFA) have been recognized as a new class of antimicrobial compounds, naturally found on skin. Microalgae are a renewable source of FFA, especially of polyunsaturated ones [2]. Among all microalgae, *Spirulina platensis*, a cyanobacterium, is a good model, thanks to its richness in FFA and its easy cultivation. A vectorization using a nanocarrier was needed to allow those lipophilic extracts to enter biofilms.

Materials & Methods.
In order to perform a green extraction of microalgal FFA, four solvents and three extraction conditions were tested. The composition of the extracts was studied by HPTLC. The FFA-enriched extracts were encapsulated using two types of nanocarriers: lipid nanocapsules (LNC) [3] and alginate-based nanocarriers (ANC) [4]. The nanocarriers were characterized by dynamic light scaterring and zetametry. The integrity of the extract was checked by establishing the lipid profile and by the measurement of the antioxidant activity. Anti-adherence and anti-biofilm activity of those extracts alone or encapsulated in nanocarriers were determined on *Candida albicans* biofilms by the XTT test. The interesting formulations were tested for toxicity on keratinocytes and fibroblasts in culture (HaCaT cells and HS68 cells, DMEM, 10% FBS, 1% antibiotics).

Results.
Lipid and pigment amounts, combined with FFA profile were used to select the optimal conditions of extraction: 30 minutes of ultrasonic extraction using EtOAc or dimethylcarbonate (DMC) as extraction solvent.
The selected extracts showed anti-biofilm growth properties, but showed no significant efficiency to fight against settled biofilm. The hypothesis is that their lipophilicity prevent their penetration in the hydrophilic grown biofilm. To increase their penetration in the biofilms, the extracts were encapsulated in two types of nanocarriers with a lipid-core and a hydrophilic shell: in LNC by the phase inversion temperature method [3] and in ANC by emulsion-surface gelification assisted by ultrasounds [4]. Lipid profiles and antioxidant activity measurements did not show any degradation of lipid extracts during both encapsulation processes. For both types of extracts, LNC of ≈35, ≈55 and ≈ 100 nm were prepared. They showed very small polydispersity indexes and negative zeta potentials. The obtained ANC showed a hydrodynamic diameter of ≈ 230 nm and a zeta potential close to -20 mV. All the formulations were stable during 1 month when placed at 4°C and sheltered from light. Assays carried out on biofilms in formation showed that the encapsulation of the extracts had no impact on the anti-biofilm growth activity of the extracts. Assays carried out on settled biofilm showed that extract-loaded LNC did not have any antibiofilm activity, regardless of the size. In contrast, ANC showed a very interesting anti-biofilm activity when enriched with both extracts (up to 60% of inhibition). Those formulations did not show any toxicity for human skin cells in culture unless for very high concentrations (> 10%).

Conclusions.
The combination of the S. platensis lipid extracts and alginate-based nanocapsules allowed thus the development of an all in one active ingredient, exhibiting both anti-biofilm growth and anti-biofilm activity [5]. This combination could be a prime candidate to both prevent and cure biofilm-related infections such as chronic wounds.

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Scalable production of polymeric nanoparticles for transdermal delivery of different drugs and bio-functional garments production.

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Keywords. Bio-functional textiles, Polymeric Nanoparticles, Scalable production, Transdermal Release.

Introduction.
In the recent years, the use of polymeric nanoparticles (NP) as smart drug delivery systems has aroused a great interest in pharmaceutical research. Moreover, such nanomaterials can be employed in textile finishing processes in order to develop garments that can deliver active principles by exploiting the transdermal administration route [1]. Notwithstanding the great potential of integrating nanoformulations with textile materials for dermatological application the “market success” of such biomaterials faces several issues. The main limitation in the development of such technology mainly lies in the low productivity of NP manufacturing methods, which usually are not able to provide an adequate amount of nanoformulations for textiles finishing processes [2]. Flash Nanoprecipitation (FNP) technique is a good solution to overcome such issue thanks to its fastness simplicity and reproducibility of results. Generally speaking, FNP exploits the different solubility of a polymer in different solvents, which are miscible. The polymer dissolved in an organic solvent collides against a water jet in a micro reactor in which highly turbulent mixing is generated, the low affinity of the polymer for the aqueous phase leads to its precipitation in the form of nanoparticles [3].
In this research, the potential use of FNP to encapsulate three types of drugs having different chemical affinity to Polycaprolacone (PCL) has been evaluated, *i.e.* (i) a hydrophilic drug such as caffeine, (ii) a partially hydrophobic as melatonin, and (iii) a fully hydrophobic drug as Curcumin; moreover, the feasibility of finishing textile fabrics with such formulations was investigated.

Materials & Methods.
NPs were produced in a confined impinging jet mixer by dissolving PCL with the drug in acetone, with the exception of CAF, which was also solubilized in water. The obtained nanoparticles were characterized in terms of mean diameter, particle size distribution and Zeta Potential by dynamic light scattering (DLS). The surface morphology, chemical structure and thermal properties were investigated using scanning electron microscopy (SEM), Fourier-transform infrared (FTIR) and X-ray photoelectron (XPS) spectroscopies, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The Loading Capacity (LC) and Encapsulation Efficiency (EE) were determined from UV-visible spectroscopy. The NPs suspensions were attached to cotton fabrics by imbibition and a transdermal release test was conducted *in vitro* by Franz diffusion Cell experiments to determine the potential application of such materials as bio-functional garments.

Results.
Process parameters were adjusted to achieve the desired mean diameter, it was observed that NPs diameter tends to decrease with increasing inlet flow rate and to increase with increasing initial polymer concentration. EE was correlated to the drug water solubility, the higher water solubility the lower EE; moreover, no significant relationship between formulation parameters and EE was observed, suggesting that EE is controlled by the nature of the drug.
An increase in LC was observed by increasing the weight ratio of the drug to the polymer in the formulation, so that the drug particle content could be adjusted in all three cases. Drug distribution inside the NP depends on their chemical nature. Thus, it has been observed that curcumin and melatonin were completely trapped in the particle of the inner core, whereas caffeine was mainly present on the surface of nanoparticles, especially when dissolved in acetone. The NPs were successfully attached to cotton fabrics and the Franz cell release test proved a control of the release kinetics exerted by the functionalized textile system.

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Conclusions.
The findings suggested that the use of FNP process allows to obtain nanoparticles with tunable characteristics while keeping high the productivity and the simplicity of production. The nanoparticles structures and EE differed according to the chemical nature of the drugs. The produced formulations were proven to be suitable for textile functionalization and to impart to the fabrics drug eluting properties. The present research proposed and inquired a novel approach for bio-functional garments production. Such materials have shown the potential of acting as wearable drug delivery systems and therefore further studies will be conducted in order to better understand their interaction with living tissues.

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How differently do *single* PLGA microparticles behave?

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Keywords. Microparticles, PLGA, Drug release, *Single* microparticle, Mass transport phenomena, Swelling

Introduction. Different types of mass transport phenomena can be involved in the control of drug release from PLGA-microparticles, including for instance water diffusion, drug dissolution, drug diffusion, polymer degradation, autocatalysis and polymer swelling[1]. The relative importance of these phenomena can strongly depend on the composition and size of the systems, as well as on the preparation technique.
In order to better understand the mechanisms involved in the control of drug release from PLGA microparticles, monitoring the behaviour of *single* microparticles can be very helpful: For other multiunit dosage forms (e.g. pellets), it has been shown that the apparent drug release kinetics from *ensembles* of systems can be very different from the release kinetics of *single* dosage forms. For example, an apparent *about constant* drug release rate from an *ensemble* of pellets might be the sum of numerous very different *individual* drug release profiles[2]. The aim of this study was to get an idea of the potential differences in the drug release behaviour from *single* PLGA microparticles.

Materials & Methods.
Microparticle preparation
Microparticles were prepared using an oil-in-water emulsion-solvent evaporation technique. Ten % diprophylline and 90% PLGA 504H were dispersed in dichloromethane (4, 6 or 10 mL). This organic phase was emulsified into 2.5 L of an outer aqueous poly (vinyl alcohol) solution (0.25%, w/v) using a three-blade propeller under continuous stirring (1000, 1500 or 2000 rpm) for 30 min. This induced particle formation. The microparticles were hardened by adding 2.5 L of the same outer aqueous phase and further stirring at 700 rpm during 4 h. The microparticles were separated by filtration and subsequently freeze-dried.
Microparticle characterization
Drug release from ensembles of microparticles*:* Ten mg microparticle samples were introduced into Eppendorf tubes, filled with 2 mL phosphate buffer pH 7.4. The tubes were kept at: (i) 37°C under horizontal agitation (80 rpm), (ii) 20°C under horizontal agitation (80 rpm), (iii) 4°C without agitation. At predetermined time points, 1.5 mL samples were withdrawn (replaced with fresh medium) and analysed by HPLC-UV [mobile phase: acetate buffer (0.01M, pH 4.5): acetonitrile (65:35, v/v), λ: 274nm, 1 mL/min]. Each experiment was conducted in triplicate.
Drug release from single microparticles*:* Diprophylline release from *single* microparticles was monitored in 96- well standard microplates as follows: Briefly, a *single* microparticle was introduced into each well, which was filled with 100 µL phosphate buffer pH 7.4 (Figure 1) and closed with a cap (to minimize evaporation). The well microplates were kept at: (i) 37°C under horizontal agitation (80 rpm), (ii) 20°C under horizontal agitation (80 rpm), (iii) 4°C without agitation. At predetermined time points, 50 µL samples were withdrawn (replaced with fresh medium) using Hamilton syringe and analysed by HPLC-UV (as described above).
Swelling of single microparticles*:* The swelling of *single* microparticles was monitored using the same set—up as for drug release measurement and an Axiovision Zeiss Scope-A1 microscope.

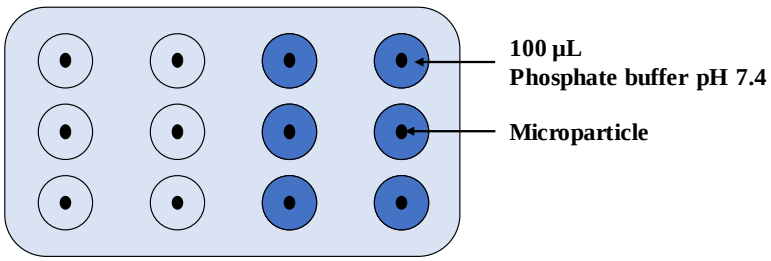

Figure 1. Schematic presentation of the experimental set-up used for drug release measurements from *single* microparticles.

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Results.

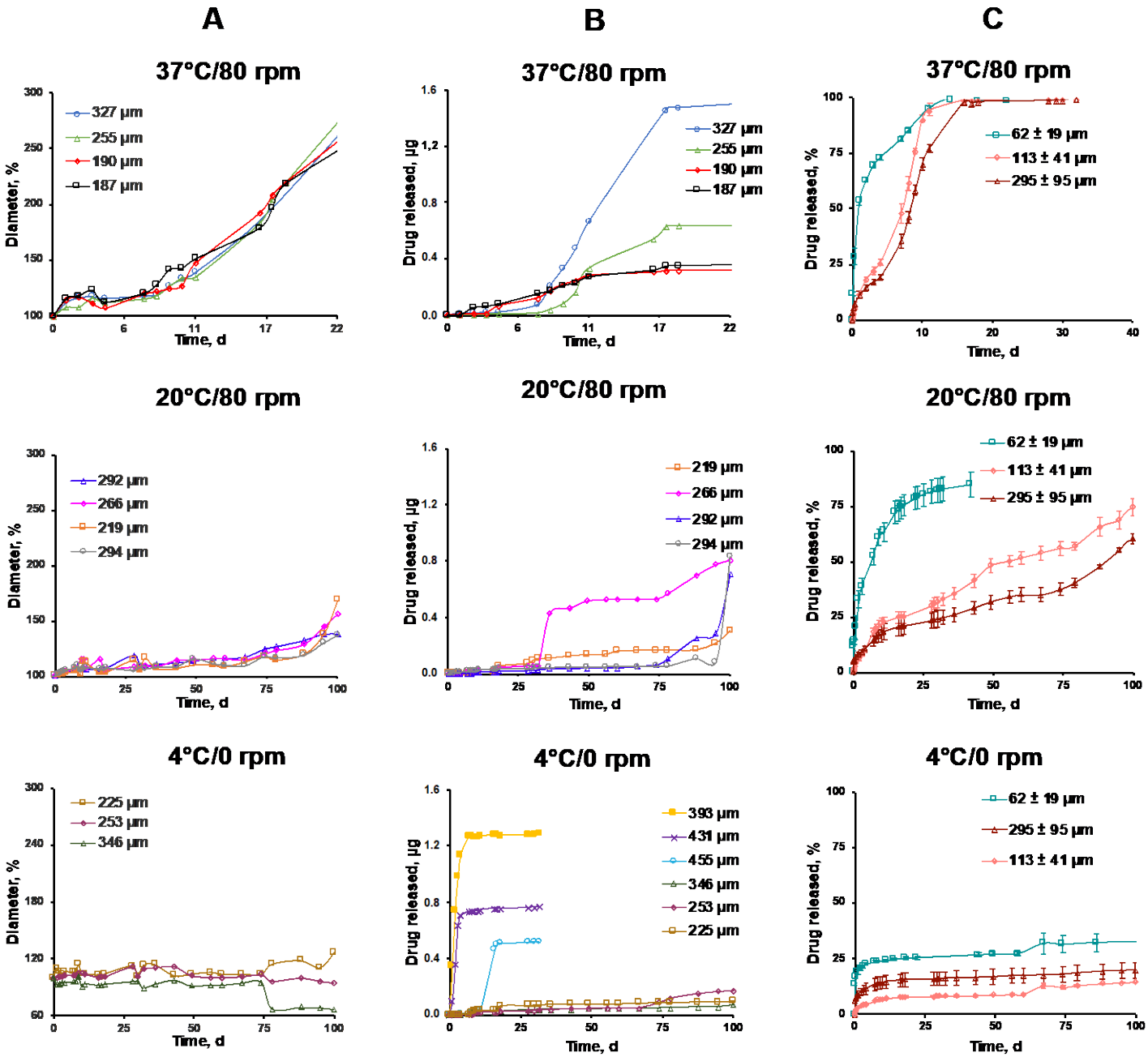

Figure 2: (A) Dynamic changes in the diameter of *single* microparticles of different size (as indicated) (B) absolute drug release rates from *single* microparticles, and (C) relative drug release rates from *ensembles* of microparticles upon exposure to phosphate buffer pH 7.4.

Conclusions. These first results seem to indicate that drug release from PLGA microparticles is mainly triggered by substantial system swelling, with “some accidents”: In certain cases, drug release sets on prior to particle fundamental polymer swelling, probably due to direct drug access to the surface. Hence, burst release from PLGA microparticles might be attributable to drug release from those microparticles, in which the drug is located at the surface or with direct access to the surface. In the other cases, “the drug has to wait” until substantial polymer swelling allows for sufficient drug mobility in the system.

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Emulsions stabilized with biodegradable PLGA nanoparticles: influence of nanoparticle physicochemical properties

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Keywords: Emulsion, Pickering, Nanoparticles, PLGA, Biocompatible

Introduction

Emulsions are widely used but thermodynamically unstable systems, mostly stabilized by surfactants. However, these amphiphilic molecules exhibit toxicity for the patient and for the environment [1]. Another way to stabilize interfaces is to use solid particles. This type of emulsion is called Pickering emulsions. For decades now, there has been a renewed interest for such emulsions considering their potential, for example in drug delivery, cosmetics or food industry. Nevertheless, most of the studied systems are not biocompatible, making them unsuitable for pharmaceutical aim [2]. In this context, we formulated biocompatible and biodegradable Pickering emulsions stabilized by poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NP) [3]. The goal of the present study is to understand the influence of PLGA nanoparticle characteristics on the properties of the emulsions. Three main parameters were studied: (i) the molar mass of the PLGA ($\bar{M}_w$ = 14 or 35 kDa), (ii) its ending moiety (ester or carboxylic acid), and (iii) the presence of poly(vinyl alcohol) (PVA), a biocompatible surfactant [4], on the NP surface.

Materials & Methods

NP preparation

Four kinds of PLGA were used: a 14-kDa PLGA, with an acid (PLGA 752H) or an ester ending (PLGA 752S), and a 35-kDa PLGA, also with an acid or an ester ending (PLGA 753H and S). PLGA NP were prepared by nanoprecipitation, as described previously [5]. Briefly, PLGA were dissolved in acetonitrile: the organic solution was added dropwise into water under stirring. The organic solvent was removed by evaporation under vacuum, with a rotary evaporator.

PLGA NP covered with PVA (PLGA-PVA NP) were prepared by emulsion-evaporation, following a previously described protocol [6]. PLGA 753H were dissolved in a mixture of dichloromethane and acetone. This solution was pre-emulsified by vortex with an aqueous solution of PVA and then emulsified with a sonication probe. Finally, the organic phase was eliminated by evaporation under stirring.

Contact angle measurement

NP hydrophobicity was determined by contact angle measurements, using the sessile drop method (Tracker, Teclis). A layer of NPs was formed by drying a drop of an aqueous NP suspension deposited on a mica surface. A drop of water was then deposited on the NP layer. The contact angle was measured by drop shape analysis over time.

Emulsion preparation

Emulsions with different water/oil ratios were prepared. NP concentration in aqueous solution was set at 25 mg/mL. The oil phase was composed of Miglyol 812N, a biocompatible fatty acid mixture [4]. Both phases were placed together in a vial and emulsified with a rotor (Ultraturrax, IKA T10 basic) at 20.000 rpm for 2 min.

Microstructure observation

Confocal microscopy: Fluorescent emulsions were observed at various water/oil ratios using a confocal scanning laser microscope (inverted Leica TCS SP8–gated STED, Germany), notably to determine the inversion phase ratios. NP were red-coloured with rhodamine, water was green-coloured with calcein.

Scanning electron cryomicroscopy (CryoSEM) observation: Each kind of NP were used to prepare an emulsion with 10% of oil. For each sample, an emulsion drop was deposited in a holder, and then quickly frozen by plunge in liquid ethane, without cryoprotectant. Samples were freeze-fractured at -150°C under high vacuum and then coated with iridium. Observations were made at -150°C in a Field Emission SEM Gemini 500 (Zeiss).

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Results

Contact angles allowed us to distinguish between hydrophobic NP (table 1, in yellow) and hydrophilic NP (table 1, in blue). Hydrophobicity could be explained by the presence of ester endings on the NP surface, whereas acid endings or PVA led to hydrophilic NP. Then, we studied the phase inversion oil percentage: below this value the NP stabilized O/W emulsions, and above this value, W/O emulsions. These values were consistent with contact angles: the oil amounts to obtain a phase inversion were lower for hydrophobic NP, following Finkle's rule. Thus, PLGA ending and PVA coverage had a great influence on the emulsion behaviour. PLGA molar mass also influenced it. Indeed, 752S PLGA NP were more hydrophobic than 753S PLGA NP, since they underwent phase inversion with very low oil quantities. Similarly, with hydrophilic acid ending, 752H PLGA NP were more hydrophilic than 753H PLGA NP and underwent phase inversion at a higher oil amount. In fact, low-molar-mass PLGA NP contained, for an equivalent mass, more polymer chains, so more ending moieties at their surface. Thus, the ending influence was increased by a low PLGA molar mass.

Table 1. NP characteristics and phase inversion oil percentage

NP type	Contact angle (°)	Phase inversion oil% (w/w)	$\bar{M}_w$ (Da)	Quantity of PLGA chains in 100 mg (mol)
PLGA-PVA 753S	85 ± 13	65	35,000	$2.9 \cdot 10^{-6}$
PLGA 752H	82 ± 2	32.5	14,000	$7.1 \cdot 10^{-6}$
PLGA 753H	80 ± 9	22.5	35,000	$2.9 \cdot 10^{-6}$
PLGA 753S	120 ± 7	17.5	35,000	$2.9 \cdot 10^{-6}$
PLGA 752S	120 ± 1	7.5	14,000	$7.1 \cdot 10^{-6}$

Then, we studied the microstructure of the emulsions by microscopy (with 10% oil w/w). We first noticed that, for PLGA 752H, 753H and 753S NP, we obtained multiple W/O/W emulsion (figure 1a). Indeed, because we were near to the inversion phase, both W/O and O/W interfaces co-existed. This latter seemed to be stabilized by a NP monolayer supported by aggregates, whereas the first one just comprised a NP monolayer. With PLGA-PVA NP (figure 1b), much more hydrophilic, we obtained a simple O/W emulsion, because we were far away from the phase inversion ratio. The interface was stabilized by a monolayer. Finally, PLGA 752S NP (figure 1c), the most hydrophobic NP, stabilized a W/O emulsion. This emulsion, much more viscous than the others, was stabilized by a 3D-NP network, connecting all the droplets with each other.

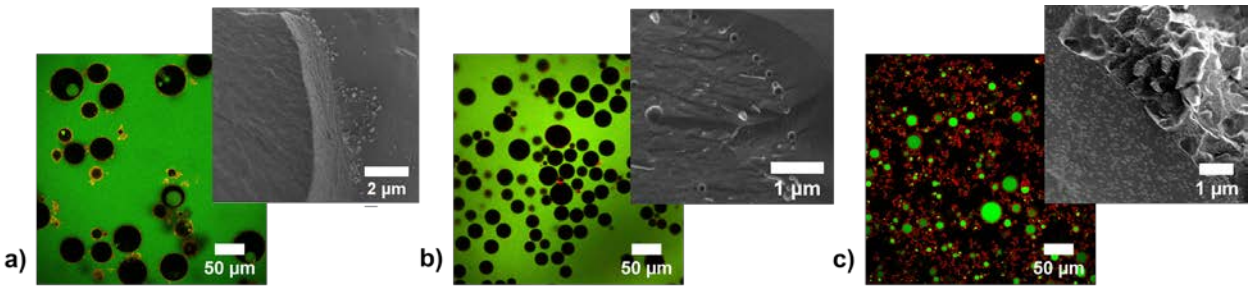

Figure 1. Confocal microscopy and cryoSEM pictures of emulsions with 10% of oil, stabilized by a) NP PLGA 753H NP, b) PLGA-PVA 753S NP, c) PLGA 752S NP

Conclusion

In this study, we showed the great influence of the PLGA ending, the PLGA molar mass, and the PVA coverage on the properties of emulsions stabilized by PLGA NP. Controlling these parameters can allow us to tune different kinds of emulsions, to finally propose encapsulation solutions for a wide variety of active substances.

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ELABORATION OF EUDRAGIT L100 BASED PARTICLES VIA PRECIPITATION PROCESS: EFFECT OF ADDED SECONDARY POLYMER

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Keywords: Eudragit L100, Precipitation, Particles, Polyelectrolytes, Morphology

Introduction

Polymers are largely used in numerous applications and especially in drug delivery in which they are mainly formulated to encapsulate active agents. Polymethylmethacrylate derivatives such as Eudragit figure among commonly used polymers. These polymers are mainly used to encapsulate active molecules for oral administration, then, the selected polymer should be pH sensitive such as Eudragit L100. This polymer is a copolymer of methacrylic acid and methyl methacrylate (in a ratio 1:1) and soluble above pH 6 [1]. Under acidic conditions, Eudragit L100 precipitates leading to particles encapsulating desired active molecules for oral administration [1], [2].

The aim of this study is to report on the effect of parameters affecting the colloidal properties of the obtained Eudragit objects. To target such objective, the effect of charged (polyacrylic acid, polyethylenimine) polyelectrolytes and non-charged (polyvinyl alcohol, dextran 40, Pluronic F68) polymers such as have been investigated. All obtained dispersions have been characterized in terms of size, size distribution, zeta potential and morphology.

Materials & Methods

Materials

Eudragit L100 was purchased from Evonik Röhm GmBH, Sodium hydroxide (pellets, Mw=40g/mol) and Polyethylenimine (Mw=25000 g/mol, Mn~10000) were purchased from Sigma Aldrich, hydrochloric acid from VWR Chemicals, Polyacrylic acid (solution of 40% wt, Mw=30000g/mol) from Aldrich Chemical Company, Sodium chloride (Mw=58,144 g/mol) from Laurylab, Polyvinyl alcohol (Mw=200000 g/mol) from Merck KGaA, Dextran 40 (Mw=40000 g/mol) from Applichem GmBH, and Pluronic F68 from Molekula Group.

Solubilization of Eudragit L100

A solution of Eudragit L100 at a concentration of 2.5 $g.L^{-1}$ was prepared by dispersing 0.25% in weight of solid in sodium hydroxide solution 10^{-2}M. The solution of Eudragit L100 was prepared under magnetic stirring at 500 rpm for 12 hours. The final pH of the Eudragit L100 solution was 6.9 with a complete solubilization.

Precipitation of the Eudragit L100 previously solubilized

Eudragit L100 previously solubilized was then precipitated by hydrochloric acid. For the precipitation without stabilizing agents, 40 ml of hydrochloric acid at different pH was added instantaneously to 40 ml of Eudragit L100 solution under magnetic stirring at 500 rpm. With stabilizing agents, 40 ml of hydrochloric acid 10^{-2}M was added under magnetic stirring at 500 rpm to a mixture of 30 ml of Eudragit L100solution and 10 ml of solution of one of the stabilizing agents at different concentrations. The resulting suspensions or solutions were maintained under stirring for 30 minutes and the resulting particles were characterized.

Characterization of particles

Particle size

Particles size was analyzed by laser diffraction using Mastersizer 3000 and by Dynamic Light Scattering using Zetasizer Nano ZS ((*Malvern Instruments).* Samples analyzed with Mastersizer were prepared with deionized water; samples analyzed with Zetasizer were prepared with NaCl solution at 10^{-3}M.

Zeta potential

2.5 ml of the final suspensions were diluted in 5 ml of NaCl10^{-3}M and Zeta potential measurements were performed on these samples using Zetasizer Nano ZS (*Malvern Instruments)*

Surface morphology

Particles morphology was observed by optical microscope, scanning electronic microscope and transmission electronic microscope.

Results

Without any stabilizing agent or added secondary polymer, objects of mean size in number was found to be around 10 µm. No significant change of the size distribution was observed when dextran, Pluronic F68 or

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polyacrylic acid were added, even with the highest amounts, corresponding to an addition mass equal to 1.3 times that of Eudragit. Besides, the measured zeta potential of the particles obtained either without any stabilizing agent or with these above additives was around -11mV and sedimentation of the particles was observed when the suspensions were stored. With high amount of polyvinyl alcohol added (additional mass equal to 1.3 times that of Eudragit), the size measurement showed a slightly increase in particle size. Results obtained with polyethylenimine were different. Indeed, this additive, allowed the production of narrowly size distribution of particles around 200 nm (size distribution shown on Figure 1). The zeta potential of these disperse particles was found to be +45mV and the produced suspension was of good colloidal stability since no sedimentation and no aggregation have been observed.

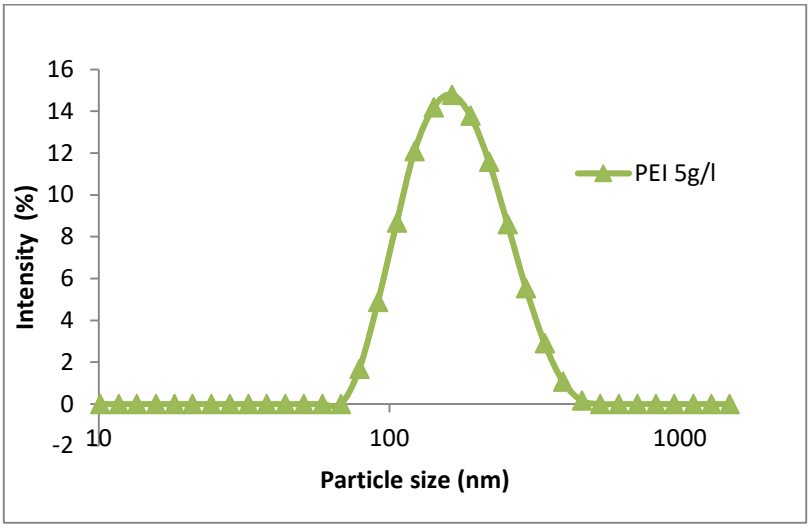

Figure 1: Size distribution by intensity of Eudragit L100 particles precipitated in the presence of polyethylenimine at a concentration of 5g. L^{-1} from Zetasizer Nano ZS

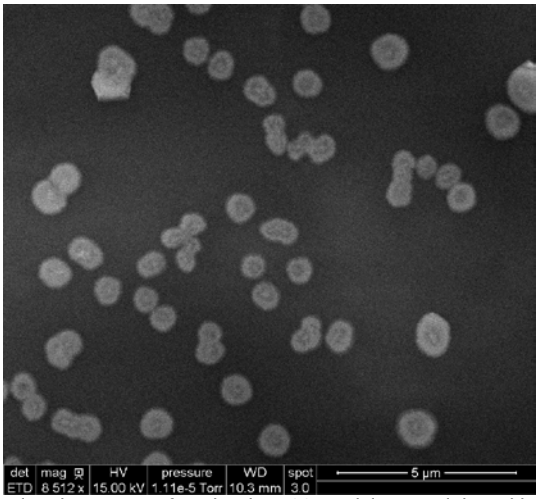

Figure 2: Image from scanning electronic microscopy of Eudragit L100 particles precipitated in presence of polyethylenimine 5g. L^{-1}

Conclusion

Among the stabilizing polymers investigated, polyethylenimine was found to be able to obtain a stable suspension of nanometric Eudragit L100 particles via direct acidic precipitation. Particles of 200 nm hydrodynamic size were obtained and confirmed by scanning electronic microscopy (Figure 2).

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Contact angle: A screening test for formulation of Solid Lipid Nanoparticle

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Keywords: SLN, nanoprecipitation, homogeneization, angle, contact.

Introduction

SLN are biocompatible nanovectors developed since the 90s to improve the bioavailability of active ingredients by [1] but also targeted release at a specific site[2, 3]. Unfortunately, their formulation is often very laborious and inconclusive. The use of screening tests for the preselection of excipients may be useful in this case. Among these tests, we assessed the contact angle of surfactant solution on candidate lipids for the preparation of SLN.

Materials & Methods

Placebo SLNs were prepared with three lipids: stearic acid (C18), palmitic acid (C16), and Compritol ATO 888 (C22) and stabilized with a 1% aqueous solution of surfactants (1: 1). .High Speed Homogenization method was used with Ultra turrax T 25. For each formula, two batches of SLN were evaluated on the size, polydispersity index and Zeta potential criteria with a Malvern Nano Zetasizer at a temperature of 25 ° C and triplicate.

The contact angle was assess directly by measuring the angle formed between lipid film (solidified after melting) and the tangent to the drop by using goniometer DIGIDROP Con-tact Angle Meter (GBX Scientific Instruments). The measurements were perform immediately after droplet positioning at 20°C in triplicate.

Results

The different formulations gave non-aggregated suspensions with particles size ranging from 1.11 ± 0.01 µm to 147 ± 2.5 nm and PDI less than or equal to 0.5. All Zeta potentials measured were favorable to satisfactory physical stability.

The surfactant solution gave with Compritol ATO 888 the lowest contact angle and the finest dispersion.

Conclusions.

Effective and rapid, measurement of contact angle can promote finely dispersed non-aggregating particles.

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PEG free formulations for antioxidant topical delivery

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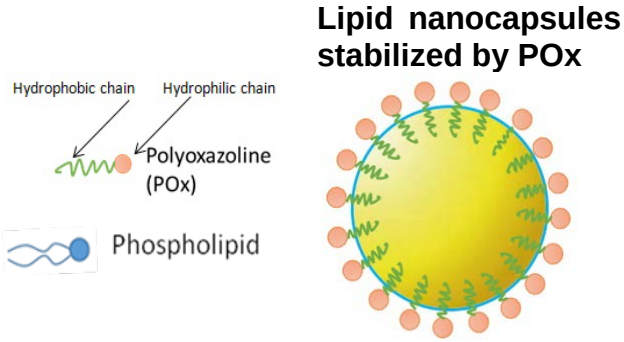
Keywords. Polyoxazolines, Lipid nanocapsules, Emulsion, Quercetin, Topical Delivery

Introduction.
The exposure of the skin to ultraviolet radiation and to certain environmental pollutants results in excess generation of oxidative free radicals, which can cause skin cells damage and potentially leading to skin cancer[1]. To address the specific challenge of enhanced oxidative stress in human skin, topical delivery of antioxidants must be improved in order to scavenge the excess reactive oxygen species in the epidermis. Thus, our research intends to prevent premature skin aging and skin cancer by focusing on the development of innovative phospholipid-based formulations loaded with natural lipophilic antioxidant such as quercetin.
Our formulations are designed for penetration enhancement through the *stratum corneum* and the epidermis by means of unsaturated phospholipids and amphiphilic polymers called polyoxazolines (POx) acting as chemical penetration enhancers.
POx are bioinspired polymers presenting similar properties to poly(ethylene glycol) (PEG)[2]. Considering the clinical awareness of PEG overuse leading to potential toxicity[3], POx also constitute a suitable candidate as a PEG alternative.

Materials & Methods.
The polyoxazolines are synthesized by a Cationic Ring Opening Polymerization (CROP) using different initiator of polymerization and degree of polymerization. The amphiphilic POx designed are constituted by a hydrophobic alkyl chain and a hydrophilic POx chain of various repeating units.
One of the main dermal delivery systems designed was the lipid nanocapsules (LNC) composed of an oily core surrounded by a stabilizing POx shell (Figure 1).



The LNC formulations were performed by a phase inversion method known to be a low energy process and solvent-free, leading to a high drug loading.

The POx and the LNC were evaluated for their penetration capacity using a preliminary evaluation method developed in our team. To do so, the affinity for lipid bilayer, membrane fluidization, interaction with the bilayer, and the depth capacity were analyzed by means of fluorescence spectroscopy, isothermal titration calorimetry and Raman microscopy. The antioxidant effect of the DDS loaded with quercetin were measured on NiH3T3 mice cells and with the DPPH assay[4]. Promising preliminary penetration results are currently confirmed with in vivo tests on mice ears.

Results.
Four POx of interest were synthesized: $C_{16}(POx)_{15}$, $C_{16}(POx)_{35}$, $C_{18:2}(POx)_{15}$ and $C_{18:2}(POx)_{35}$ enabling new DDS formulations for both architectures. The macromolecular design is completely controlled and the POx showed a narrow molar distribution.
The LNC were characterized by a size of 40-50 nm, demonstrating low dispersity (PDI<0.3) and good stability.

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Therefore, our research strives to prove POx value to topical formulations by its capacity to enhance the formulation stability and the skin penetration.

Conclusions

The preliminary penetration results were then confirmed with in vitro tests on human skin and in vivo tests on mice ears.

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Encapsulation of vegetale oil via nanoprecipitation process using Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammoniethyl methacrylate chloride) (Eudragit RS 100)

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The encapsulation of vegetable oils is considered a promising strategy to facilitate their applicability and to potentiate their effects in all the fields. For this context, Eudragit RS100 based nanoparticles were prepared by nanoprecipitation method to encapsulate two vegetable oils: sesame oil and date seed oil, extracted by supercritical fluid CO_2 process. The influence of parameters such as polymer amount, polymer-oil ratio, solvent type and oil amount was investigated. The particles were characterized in terms of their size, size distribution and zeta potential. The obtained nanoparticles showed a regular distribution and a high stability with values ranging between 200-500nm and 40-75mv for particle size and zeta potential, respectively.

Keywords: Vegetable oil, nanoparticle, Eudragit RS-100, nanoprecipitation, supercritical fluid.

Introduction

The various health benefits attended by the consumption of natural vegetable oils constitute an area of great research [1]. Nevertheless, their high sensibility to oxidation leads to the creation of off-flavors and cause major loss of oil quality, nutritional value and bioavailability. Additionally, the undesired taste of some oils limits their uses [2]. Thus, the nanoencapsulation serves as a promising approach by providing vegetable oils an extreme stability against all destructive environmental factors such as pH, oxygen, light, etc; enhancing their solubility in biological fluids, and masking their undesired flavor and aroma [3-4].

Among the several methods used for developing polymeric nanoparticles, the nanoprecipitation method is widely used due to its simplicity and reproducibility [5]. In this context, this method was for the first time used to design Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammoniethyl methacrylate chloride) (Eudragit RS 100) based nanocapsules as carrier for two kinds of vegetable oils: date seed oil and sesame oil, extracted by supercritical fluid CO_2 process.

Materials & Methods

Firstly, Eudragit-based nanoparticles were prepared by nanoprecipitation method. Then, the effect of the experimental parameters (polymer concentration, type of organic solvent and organic/aqueous phase ratio) on the colloidal properties of the particles was evaluated. Finally, the optimal conditions concluded were used to encapsulate sesame oil and date seed oil, separately.

Results

The addition of the oils affected the mean sizes of nanoparticles. For date cores oil, the particle size increased from 238.9 ± 3.6 to 366.2 ± 16.1 nm as the amount of oil increased from 50 to 400 mg.

However, for sesame oil, by increasing the sesame oil amount from 50 to 200, an increase in particle size was observed from 248.2 ± 1.6 to 411.3 ± 13.8 nm. However, at very high amount, a decrease in particle size was evaluated; the particle sizes were 292 ± 7.6 nm and 318 ± 5.6 nm for 300 mg and 500 mg of sesame oil, respectively.

All the formulations exhibit high zeta potential values (between 40 and 70mv).

Conclusion

The reliability of this work was to use a simple, a fast and reproducible technique to design vegetable oil based polymeric nanoparticles with a small particle size and high zeta potential.

These formulations could offer several advantages for topical and systemic delivery of cosmetic and pharmaceutical agents including controlled particle size, protection of the vegetable oil and ability to carry hydrophobic drugs.

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Influence of lipid formulation on the lipid adsorption onto polymer particles

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Keywords. Liposomes, Lipid / Polymer Particle Assemblies, LipoParticles, Drug delivery systems

Introduction.
Since the last century, the need of pharmaceutical industry has led to the emergence of new innovative galenic forms [1]. Several drug delivery systems (Figure 1) have emerged to circumvent the limitations of traditional medicine, particularly by ensuring a better bioavailability of drugs, by reducing their side effects, and by allowing pathological tissues or cells to be targeted. Among the systems developed, liposomes were the first drug delivery systems that have been successfully translated into real-time clinical applications [2], and the polymer particles have shown a significant therapeutic potential for controlled drug delivery [3]. With the aim of combining the advantages of both systems, this work proposes to cover the surface of polymer particles with lipid membranes, resulting in a biomimetic coating. The elaboration conditions of these colloidal assemblies (named LipoParticles) and their characterization were optimized [4-8].

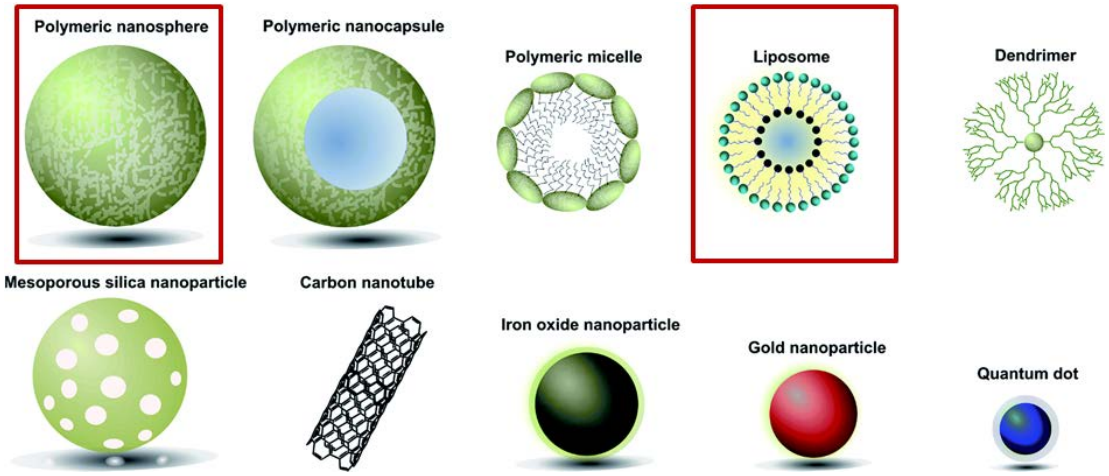

Figure 1: Representation of different types of nanocarriers used in biomedical applications [9]

Materials & Methods.
Vesicle Elaboration.
According to the lipid film hydration method [10], lipids (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC, and 1,2-dipalmitoyl-3-trimethylammonium-propane, DPTAP) were dissolved in chloroform, then the solvent was removed by rotary evaporation to get an homogeneous and thin lipid film. Multilamellar vesicles (MLV) were obtained by adding an aqueous solution to this lipid film, and by stirring this mixture. Finally, a dispersion of large unilamellar vesicles (LUV) was obtained by extrusion of the obtained MLV dispersion through calibrated polycarbonate membrane filters with pore diameter of 100 nm.
LipoParticle Elaboration.
LipoParticles were elaborated by adding an excess of vesicles to a dispersion of anionic polystyrene particles in water. The mixture was stirred for 1 h. Under such conditions, the adsorption and spreading of highly curved vesicles upon contact with the particles can lead to a lipid shell formation around the particle surface. Thereafter, dispersions were centrifuged twice to remove unbound lipids.
Quasi-elastic Light Scattering Analyses.
Mean hydrodynamic diameters (D_h) and mean size distributions (PDI value) were determined at 25 °C by quasi-elastic light scattering (QELS) (Zetasizer 3000 HS, Malvern Instrument, UK). The PDI value is a dimensionless measurement of the distribution broadness. The measurement angle was 90°, the laser was a Helium-Neon type operating at 633 nm, and the solvent refractive index and viscosity at 25 °C were 1.33 and 0.8872 cP, respectively. Samples were diluted in ultrapure water.
Electrophoretic Mobility Measurements.

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The electrophoretic mobility values were measured at 25 °C, using a Zetasizer 3000 HS apparatus (Malvern Instrument, UK). Electrophoretic mobility was converted to zeta potential according to Smoluchowski's equation. Samples were diluted in ultrapure water, and three independent measurements were recorded to obtain a mean zeta potential value.

Cryo-Transmission Electron Microscopy Observations.

For cryo-TEM observations, a small drop of sample was deposited onto an advanced holey carbon film (Quantifoil, EMS) coated copper grid. The excess of sample was removed by quick blotting with filter paper. The grid was immediately vitrified by plunging it into liquid ethane cooled by liquid nitrogen (Cryoplunge, Orsay University, Laboratory Physique des Solides). The sample was transferred into liquid nitrogen and inserted into the cold cryo-holder (Gatan 626). Subsequently, the cooled holder was quickly transferred into the vacuum column of TEM microscope (PHILIPS CM120, CTμ Lyon) maintained at liquid nitrogen temperature. The accelerating voltage used for the observation was 120 kV.

Results.

The reorganization of liposomes onto the polymer particle surface leads to LipoParticle assemblies. The driving force expected for this process is of electrostatic nature between cationic liposomes and anionic particles, with opposite surface charges. In order to study the effect of the lipid charge, two formulations of liposomes were prepared. The first one was slightly cationic with 10% molar of DPTAP (90/10 DPPC/DPTAP), and the second one was highly cationic with 90% molar of DPTAP (10/90 DPPC/DPTAP). Sizes of both liposome types were independent of lipid formulations since they were close to 135 ± 10 nm. Their size distributions were found to be relatively sharp (PDI = 0.082 ± 0.024). QELS characterization of anionic polystyrene particles disclosed a size value of 310 ± 30 nm (PDI = 0.047± 0.025). Zeta potential values of both lipid formulations and polymer particles were close to + 40 mV and – 35 mV, respectively. Assemblies resulting from these previous entities (*i.e.*, liposomes and polymer particles) were characterized by QELS, electrophoretic mobility measurements, and observed by cryo-TEM. This investigation has demonstrated that these assemblies were individualized, and non-aggregated, with a satisfactory size distribution (PDI = 0.083 ± 0.049). Interestingly, an inversion of zeta potential sign of final assemblies *versus* polymer particle one was detected with surface modification (from - 35 to *ca* + 20 mV). No significant difference in size and zeta potential was observed between both lipid formulations. However, cryo-TEM analyses have revealed two distinct morphologies for these assemblies, according to the initial lipid formulation. Indeed, slightly cationic liposomes were adsorbed onto polymer particles, without a reorganization of lipid membranes, while highly cationic liposomes seemed to show a reorganization of the lipid layer onto polymer particles.

Conclusions.

The valorisation of LipoParticles as nanocarriers in drug delivery area was already demonstrated. Herein, the morphology of these lipid/polymer particle assemblies was shown to be strongly dependent on the lipid formulation. With the aim of better controlling this assembly morphology, other parameters such as the nature of polymer particles or the ionic strength, are ongoing study.

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A design of experiment approach to non-isocyanate polyurethane nanoprecipitation: Toward an optimized preparation of polymer nanoparticles

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Keywords : Design of experiments, polyhydroxyurethane, nanoparticles, nanoprecipitation

Introduction.

Nanoprecipitation is a straightforward method to obtain nano-sized polymeric particles. Nanoparticles from different polymers have already been prepared with this technique[1], but very few studies have focused on polyurethane polymers, despite their excellent mechanical properties and biocompatibility. The present study describes the nanoprecipitation of an isocyanate-free polyurethane − (SB-HM)-PHU − synthesized from a diamine and a bio- based cyclic carbonate[2]. The purpose of the study is the improvement of the nanoprecipitation method applied to this novel polymer by the means of a full factorial design. Unlike the 'one-factor-at-a-time approach', this strategy allows to highlight possible interactions among the studied experimental factors. In the meantime, a better understanding of the physical-chemical phenomena involved during the process is provided.

Materials & Methods.

Monomer SB bis-CC and (SB-HM)-PHU syntheses

Monomer sebacic bis-(cyclic carbonate) (SB bis-CC) was synthesized according to work from Carre et al.[2] Polymer (SB-HM)-PHU was synthesized as follow: in a stoichiometric ratio, SB bis-CC and hexamethylene diamine (HMDA) were dissolved into THF and heated under reflux for 48h. After purification, (SB-HM)-PHU was obtained. Full characterization (^{1}H and ^{13}C NMR, FTIR-ATR, MALDI-ToF, TGA, DSC) was provided.

(SB-HM)-PHU nanoprecipitation

In a typical procedure, 25 mg of polymer were dissolved into 25 ml of DMSO (pre-solution). 360 mg of SDS were dissolved into 50 ml of water (non-solent). At 24°C, the pre-solution, filled up in a syringe, was added dropwise into the non-solvent at a rate of 35 ml/h. DLS analyses and zeta potential measurements were performed on the fresh samples, and repeated 17 and 27 days later for ageing study. The samples were also observed by cryo-TEM.

Experimental design for nanoprecipitation development

A two-level full factorial design – 8 runs and 4 center points – was used to investigate the method. Three independent factors with three coded values (-1, 0 and 1) were studied: polymer concentration in the pre-solution (X_1 ; 1, 3 and 5 mg/ml), water volume (X_2 ; 50, 100 and 150 ml) and SDS concentration in the non-solvent (X_3 ; 0, 1.25 and 2.50×10^{-2} mol/l). Two responses, obtained by DLS analyses, were determined: average particle size (Y_1) and polydispersity index PDI (Y_2). The responses were approximated by the following regression model: $Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{123}X_1X_2X_3$,
with b_i the main effects, b_{ij} and b_{123} the two-factor and three-factor interactions, respectively. Multiple linear regression analyses were performed using NEMRODW® software (LPRAI, Marseilles, France).

Results.

The particle mean size varied from 55 to 90 nm (Y_1) while the PDI ranged from 0.11 up to 0.52 (Y_2), depending on the operating conditions applied. These results indicated that the two responses were not correlated. After calculating the coefficients of each model and by considering the standard deviation determined for each response from the repeated center points, we highlighted strong main effects of both the polymer amount (X_1) and the water volume (X_2) on each response. Moreover, in the case of Y_1, the two-factor interactions X_1X_2 and X_1X_3 were also statistically significant while all the interaction effects seemed to influence Y_2. Finally, a low polymer concentration, a low water volume and a high SDS concentration were suggested to obtain the smallest particles. The smallest PDI was obtained with a high polymer concentration, a low water volume and a high SDS concentration. PDI appeared the parameter the most related to nanoparticle long term stability.

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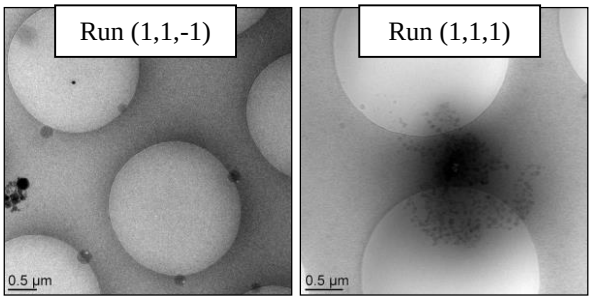

Figure 1. Cryo-TEM images of two different samples.

The cryo-TEM images accurately reflected the DLS measurements (Figure 1) despite the fragility of the particles under the electron beam.

Conclusions.

The process of PHU nanoparticle preparation by nanoprecipitation was optimized using a full-factorial design to obtain the lowest values of particle size and size distribution. The lowest PDI (0.10) was obtained with a PHU concentration of 5 g/l, a water volume of 50ml and an SDS concentration of 25.0 mmol/l while the lowest particle size (55 nm) was obtained with a PHU concentration of 1 g/l, a water volume of 50ml and an SDS concentration of 25.0 mmol/l. The presence of SDS was mandatory to prevent PHU nanoparticles from aggregation, provided that the concentration of SDS remaining in solution was inferior to the CMC. When this condition was not fulfilled, the presence of numerous micelles destabilized the nanosuspensions more or less rapidly. Cryo-TEM observations were consistent with DLS analysis and ageing study results.

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A promising tool to accurately predict the surface tension of liquids: application to cosmetic oils for spreading properties improvement

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Keywords
Cosmetic oils, surface tension, spreadability, oil/skin interface design, theoretical approaches

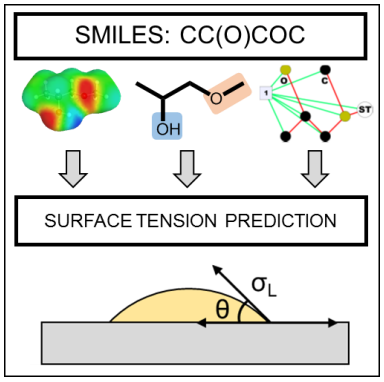
Introduction

Cosmetic oils are ubiquitous components of personal care formulations. The formulator has to design end-use products, which both meet the required functional specifications and are attractive for consumers. In personal care industries, consumers are increasingly responsive to sensorial qualities, such as excellent spreading properties on the skin and light, dry and silky after-feel. As it depends on consumer's feelings, sensorial profiles are difficult to anticipate. Understanding the origin of these sensorial characteristics requires to be aware of their relationships with physicochemistry. Spreadability of oils are closely related to both surface [1] and interfacial tensions, since the decrease of these two properties increases the wettability of the skin surface.

Surface tension is thus a key parameter to understand the interaction mechanisms involved at oil/skin surface interface. Several experimental methods exist to determine the surface tension. However, when virtual liquids are designed *in silico,* experimental determination turns out to be impossible to carry out. It becomes crucial to develop a reliable predictive model able to calculate accurately the surface tension. This is a major and ongoing challenge due to the complexity of the phenomena involved.

Materials & Methods

In this context, we compared three theoretical models able to estimate the surface tension of liquids in order to further predict the surface tension of cosmetic oils and to anticipate their spreading behavior on skin surface. Group contribution modelling allow the estimation of organic compounds surface tensions computing a molecule as a function of the contributions of the different groups included in its backbone [2]. Other theoretical calculations were carried out using the neural networks theory. Input variable parameters used are both thermodynamical descriptors from the COSMO-RS quantum chemistry theory [3] and simple topological molecular descriptors of the compounds. This is the specific case of graph machines [4].



Results

These three theoretical approaches were tested on a set of cosmetic oils and results were compared to experimentally measured surface tensions. Results prove firstly the efficiency of all the predictive models and highlight secondly the best predictions obtained with graph machine modelling.
In the meantime, contact angle measurements had been carried out on these cosmetic oils using two different substrates: Poly(methyl methacrylate) (PMMA), which is known to well mimic the surface properties of human skin and Vitro-Skin®, an artificial substrate, containing protein and lipid components, designing to have topological and surface properties similar to human skin. That exhibits a correlation between the

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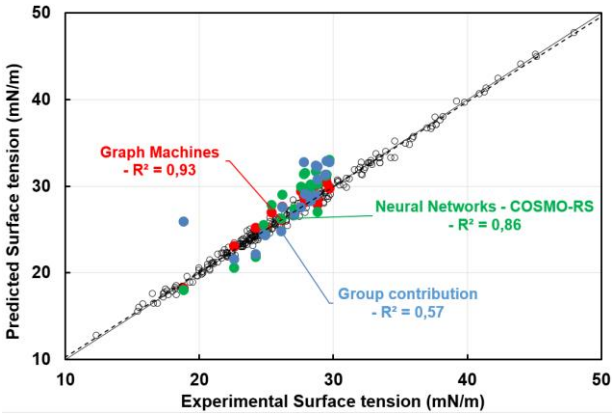
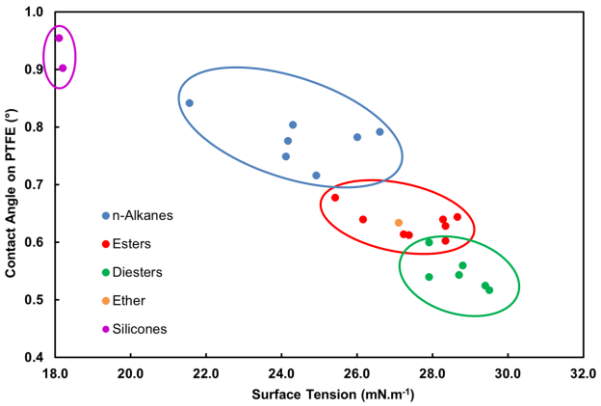
surface tension and the contact angle, and shows finally that, predicting the surface tension allows to anticipate the spreading behavior of the cosmetic oils on skin surface.





Conclusions

Finding an efficient theoretical model able to calculate *in silico* the surface tension of liquids such as cosmetic oils is an ongoing challenge of current times. Actually it allows to anticipate formulation issues such as spreading on the skin, since oil spreadability on skin surface is closely related to its surface tension. This study highlights the ability of graph machine to help designing surface such as human skin surface, understanding how components as cosmetic oils act on the human epidermis.

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