



skinforum
international skin science network



SKIN & FORMULATION,
5th SYMPOSIUM & 17th SKIN FORUM

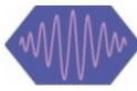
Reims Convention Centre – France



23-24 september 2019

PROGRAMME & ABSTRACTS





Dear Colleagues,

It is our pleasure to welcome you to Reims and to the “*Skin and Formulation 5th Symposium – Skin Forum 17th Annual meeting*” co-organized by the APGI and Skin Forum.

The “*Skin and Formulation 1st Symposium*” was organised in Paris in 2003 with more than 170 delegates. Three years later the “*Skin and Formulation 2nd Symposium*” was held in Versailles in October, 2006. This second symposium attracted even more participants (235) and more than 100 posters were displayed. The first joint “*Skin and Formulation 3rd Symposium – Skin Forum 10th Annual meeting*” registered a total of 300 delegates and displayed 160 posters.

The aim of our second joint meeting here in Reims is to bring together academics, industrialists and young researchers in a unique forum which will focus on the skin and the formulations applied to it.

We hope you will enjoy the programme which has sessions dedicated to:

- Skin biology
- Skin product development - from concept to final product
- Biophysical tools in formulation development
- Big Data for advanced formulation design
- Rheology of skin products - industrial and regulatory perspectives

In addition, 8 young researchers have been selected to present their work to the meeting and we particularly hope you will enjoy their presentations as our young scientists are the future for our field.

We wish you a pleasant conference which will afford you many opportunities to meet new friends and colleagues. We also hope you will visit the exhibitor and sponsor stands that have helped so much to make our meeting a success. Of course, the Champagne Evening should be a pleasant moment to extend fruitful exchanges.

Vincent Faivre & Majella Lane
Chairs



Monday, September 23th, 2019

08:00 - 08:45

REGISTRATION (Centre des Congrès)

08:45 - 09:00 Dr. Majella Lane (Skin Forum) and Dr. Vincent Faivre (APGI)

Welcome

09:00 - 09:35 Dr. Anne-Marie Pensé-Lhéritier, EBI, France

OPENING

Sensory and sustainable challenges

LECTURE

SKIN BIOLOGY

09:35 - 10:10 Dr. Cécile Clavaud, L'Oréal, France

Skin microbiome signatures in healthy skin across daily life : perspectives for cosmetics

10:10 - 10:45 Pr Guoping Lian, University of Surrey, UK

Multi-scale modelling of solute partition in complex topical products and impact on transdermal permeation

10:45 - 11:15 COFFEE BREAK AND POSTER SESSION

11:15 - 11:50 Dr. Dominik Imfeld, DSM Ltd, Switzerland

Novel anti-aging approaches with bioactive lipids

11:50 - 12:10 Dr. Emilie Munnier, Université de Tours, France

Shielding the skin from biofilm: Spirulina platensis sustainable lipid extracts and their formulation

12:10 - 12:30 Marcella Sessa-Rivera, University of Barcelona, Spain

Release and permeation study of meglumine antimoniate semisolid dosage form for the treatment of cutaneous leishmaniasis

12:30 - 14:00

LUNCH BREAK

SKIN PRODUCT DEVELOPMENT: FROM CONCEPT TO FINAL PRODUCT

14:00 - 14:35 Pr. Jonathan Hadgraft, University of London, UK

Formulation science - a half century of progress?

14:35 - 15:10 Dr. Milica Lukic, University of Belgrade, Serbia

From emulsions' stability concerns to sensory properties: tools that we use

15:10 - 15:30 Mohamed Beladjine, Paris-Sud University, France

Co-encapsulation of immunosuppressant and anti-inflammatory agents in a Pickering emulsion for the treatment of skin disease

15:30 - 16:00 COFFEE BREAK AND POSTER SESSION

16:00 - 16:35 Pr Vincent Boly and Dr. Javier Arrieta Escobar, University of Lorraine, France

Integration of Heuristic Knowledge in the Design of a Skin Moisturizer

16:35 - 16:55 Julie Quartier, University of Geneva, Switzerland

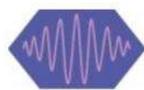
Polymeric micelle formulations for the cutaneous delivery of sirolimus: a new approach for the treatment of facial angiofibromas in tuberous sclerosis

16:55 - 17:15 Dr. Cristina Padula, University of Parma, Italy

New strategies for improving budesonide skin retention

17:30 - 20:30

CHAMPAGNE EVENING



Tuesday, September 24th, 2019

08:15 - 08:45

RE-OPENNING

BIOPHYSICAL TOOLS

08:45 - 09:20 Dr. Christoph Riethmüller, Centre for Nanotechnology, Germany
AFM of the skin barrier

09:20 - 09:55 Pr. Malcom R. Clench, Sheffield Hallam University, UK
MALDI-MSI for skin investigations

09:55 - 10:15 Claudia Vater, University of Vienna, Austria
Cytotoxicity of lecithin-based nanoemulsions on primary skin cell types and penetration monitoring by ATR-FTIR spectroscopy

10:15 - 10:45 COFFEE BREAK AND POSTER SESSION

10:45 - 11:20 Dr. Jean Doucet, Novitom, France
X-ray based methods for skin investigations

BIG BATA

11:20 - 11:55 Pr. Nicola Zamboni, ETH Zurich, Switzerland
Metabolomics and transcriptomics for aged skin

11:55-12:30 D Gewin Puppels
Quantitative analysis of in vivo skin penetration of topically applied materials

12:00 - 14:00

LUNCH BREAK

RHEOLOGY OF SKIN PRODUCTS

14:00 - 14:35 Pr. Florence Agnely, Paris-Sud University, France
Contribution of interfacial rheology for the study of topical emulsions

14:35 - 15:10 Dr. Pascal Brochette, Atellane, France
Rheology of skin products: industrial point of view

15:10 - 15:30 Sylvia Imbart, EBI, France
Relationship between sensory assessment and rheological properties of a sensorial referential

15:30 - 16:00 COFFEE BREAK AND POSTER SESSION

16:00 - 16:35 Dr. Valentine Ibekwe, MHRA, UK
Regulation point of view

16:35 - 16:55 Coralie Bellefroid, University of Liège, Belgium
Nucleic acids skin penetration enhancement: a combined approach of deformable liposomes and microneedles

16:55 - 17:15

CONCLUSION



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**We like to thank our sponsors and exhibitors
for their financial contributions helping us
to realise this congress.**



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Exhibitors





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BASF
We create chemistry

BASF offers comprehensive solutions to the pharmaceutical industry, ranging from a broad, high-quality excipients portfolio to chemical raw materials. With its expertise in polymer chemistry, its worldwide R&D-capabilities and the company's commitment to developing value-adding excipients, BASF continuously creates solutions to challenges related to Instant-and-Modified-Release, Solubilization, Softgels, Skin-Delivery and Biologics.



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Charles River is committed to helping our partners expedite their nonclinical development with exceptional safety assessment services, state-of-the-art facilities and expert regulatory guidance. Sharing our partners' goals to make a positive impact on the lives of people around the globe, we proudly support the development of human and veterinary pharmaceuticals, as well as industrial and agrochemicals, cosmetics and household products. From individual *in vitro* and *in vivo* toxicology studies to tailored packages, including full analytical support, our deeply experienced team will design and execute programs that anticipate challenges and avoid roadblocks for a smooth, efficient journey to your chosen market.



DSM

BRIGHT SCIENCE. BRIGHTER LIVING.

DSM in Personal Care & Aroma Ingredients

DSM Personal Care & Aroma Ingredients provides innovative personal care ingredients for some of the world's best-selling beauty products. Our strong scientific footing, combined with consumer and market insights from all over the world, results in transformational products that help to build brands and benefit consumers.

Our extensive portfolio includes key skin care ingredients such as lipids, natural bioactives and peptides, as well as vitamins, UV filters, hair care polymers and performance ingredients like emulsifiers and sensory modifiers. We complement this portfolio with our unique range of services in the areas of formulation expertise, sensory competence, technical support, quality assurance, and regulatory approval. Our business is driven by global mega trends, local consumer beauty insights, and growth opportunities that we uncover in emerging markets.

With our Aroma Ingredients portfolio we create solutions that enhance the senses. Our advanced chemical and biochemical expertise, in combination with a unique technology and production infrastructure, are the cornerstones of DSM AROMA Ingredients Innovation capability.



Lipoid

We Invest in Quality.

Welcome to the Lipoid Group

Lipoid is the world's leading producer of lecithins and phospholipids for the pharmaceutical industry. With an expertise of over 40 years, Lipoid offers the full range from vegetable (soybean, sunflower and alternative sources) or animal (egg) origin, as well as synthetic phospholipids and special lipid products.

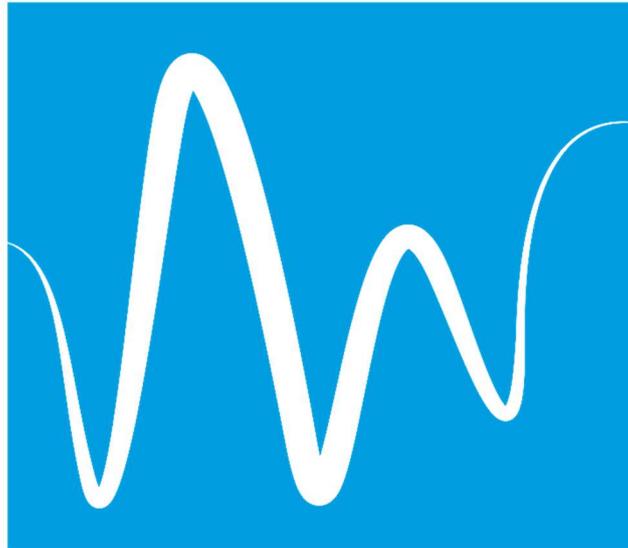
The products are well-known under the trade names LIPOID, PHOSPHOLIPON, PHOSAL, and PhytoSolve and are all manufactured under cGMP conditions in three independently operating plants in Germany.

Specialized in research and development of innovative botanical actives, Lipoid Kosmetik offers a broad sophisticated portfolio of high-quality cosmetic functionals, extracts and delivery systems based on lecithin and phosphatidylcholine. Pioneering the field of phospholipids and botanical extracts, Lipoid Kosmetik has gained an outstanding reputation in the global cosmetic industry. This long-standing expertise, in combination with the exceptional quality of our portfolio, enables our customers to develop and market innovative, functional and natural cosmetic products meeting the highest standards.



TRANSDERMAL. MARKETLEADER.

LTS is a leading pharmaceutical technology company that develops and manufactures innovative drug delivery systems such as Transdermal Patches ("TTS") and Oral Thin Films ("OTF") for the pharmaceutical industry. LTS' commercial offering encompasses more than 20 marketed products and a diverse pipeline of more than 30 development projects targeting multiple disease indications. LTS maintains its leading position through the continuous refinement of its core TTS and OTF technologies and by advancing emerging drug delivery technologies, including Micro Array Patches for the transdermal delivery of large molecule, biological actives.



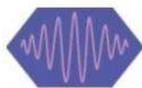
RIVERD
INTERNATIONAL BV

RiverD International pioneered the technology for rapid non-invasive molecular skin analysis by in vivo confocal Raman spectroscopy. The gen2-SCA family of skin composition analyzers provides detailed information about the molecular composition of the skin, with high spatial resolution.

This capability is widely used to study the intrinsic skin composition as well as the penetration of substances into the skin. Testimony to this are the close to 100 papers of RiverD-customers, that have appeared in the peer-reviewed scientific literature.

Since its start in 2002 RiverD has continued to devote much effort to the continuous further development of its technology and data analysis software.

Quantitative determination of the in vivo skin penetration of topically applied products (in micrograms/cm²) is a unique and important capability that has recently been added to RiverD's new SkinTools 3 data analysis software.



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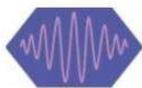
Unilever

SAFETY SCIENCE IN THE 21ST CENTURY

Developing and applying non-animal tools and approaches, through global scientific partnerships, to allow robust safety decisions using Next Generation Risk Assessment: Assuring the future safety of our consumers, workers and the environment



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INVITED SPEAKERS



Pr Anne-Marie PENSE -LHERITIER

Ecole de Biologie Industrielle, Cergy, France

Mail: am.lheritier@hubebi-edu.com **web:** <http://www.ebi-edu.com/en/>

Educational Qualifications & Achievements

Faculty of Pharmacy , Châtenay-Mâlabry. URA CNRS 1218 , University Paris -Sud

1981-1986: Pharmacist, Best Student Award

1986-1987: Master's degree in Galenic Pharmacy sponsored by the company Ceraver

1988-1991: PhD in Pharmaceutical Sciences at Hutchinson Worldwide

University of Grenoble

2013: Post-doctoral degree (Habilitation à Diriger les Recherches, HDR)

Current position : Ecole de Biologie Industrielle (Engineering School), Cergy, France

Since 1993: Full Time Professor of Formulation and Sensory Evaluation. These courses are designed to train engineering students in the design and development of products for life science industry.

Since 2005: Head of the Formulation and Sensory Evaluation Department. Numerous Partnerships of research, supervision of master and doctoral students.

Involvement

Board member: Sensory group of AFNOR (ISO), Société Française d'Analyse Sensorielle (SFAS), Association de Pharmacie Galénique Industrielle (APGI) European Paediatric Formulation Initiative (EuPFI).

Leader: Non-Food Sensory Evaluation Working Group at the European Sensory Scientist Society (E3S)

Sensory and sustainable challenges

Anne-Marie PENSE-LHERITIER

Cosmetics Consumers' demand for more natural ingredients and sustainable products continue to increase. In parallel, their wish of safe products and personalized formulation with the insurance of wellbeing is growing. Regarding consumer acceptance, target sensory characteristics are essentials and have to be defined and achieved (fragrance, color and texture). In a worldwide competition, if cosmetics companies want to keep or to increase their market they must develop more natural products, stable, safe and with the sensory qualities expected by consumers; That's the big challenge.

The cosmetic product is composed of a mixture of different ingredients each of them will participate to the sensory image of the formulation. For instance, emollients are known to influence the spreadability and residue attributes, gelifying agents impact the firmness, powders modulate the softness. When it is planned to design a new product with natural raw materials or to substitute some of them, the knowledge of these sensory features is essential. The standard sensory method used is the quantitative descriptive profiling, which allows to obtain accurate sensory images of ingredient and products. The results obtained with this method can be compared to those obtained with a product preference study: a preference mapping that links the sensory attributes to the consumers' hedonic expectations can be drawn up.

Improving the product development process required managing at the, same time economical, technical, regulatory... constraints. But the major challenge in the development of new products is the formulation and consequently the product performance, microbiological or physico-chemical stability. Methods using experimental design strategy are useful to reach all the specifications including sensory characteristics. Screening or mixture designs have proved their effectiveness in identifying the critical formulation attributes and/or the critical parameters of process. With their assistance, it could be established how the nature and the ratio of the ingredients determining significantly the final product characteristics. Consequently, the process of the development of products that target directedly the consumers' acceptability is more efficient.

In conclusion thanks to the dynamism of suppliers, more and more, qualitative raw material of vegetable or mineral origin are on the market. With the help of sensory methodology and experimental design, the change of ingredients in the formulas is easier as well as the setting up of eco-friendly process thereby achieving by the same the desires of consumers.



Dr Cécile CLAVAUD

**Project leader in skin microbiome,
L'Oréal Research and Innovation, France**

My initial expertise is chemistry and biochemistry thanks to a PhD thesis in medical imaging (CEA Saclay, 2002-2006). Then I learned medical mycology during 5 years post-doc at Institut Pasteur (Paris) where I studied the biochemistry of a *Aspergillus fumigatus*, a pathogenic mold responsible for nosocomial infections (2006-2011). During six years (2011-2017), at L'Oréal, my main area of expertise was skin and scalp microbiome profiling in any type of skin and skin area in order to identify key determinant associated to skin and scalp disorders. This work was possible thanks to my position at Open Research Europe, where I had the chance to establish partnerships with the leading academic researchers of the field.

Since 2018, I am in charge of laboratory activities dedicated to better understanding how commensal microbiota can impact positively the skin barrier function in reconstructed skin models.

Based on my expertise, I am also contributing to support the development of new microbiome based concepts for L'oreal brands, such as Vichy Dercos and La Roche Posay.

Most relevant Publications

[Complete Genome Sequence of *Malassezia restricta* CBS 7877, an Opportunist Pathogen Involved in Dandruff and Seborrheic Dermatitis.](#) Morand SC, Bertignac M, Iltis A, Kolder ICRM, Pirovano W, Jourdain R, **Clavaud C**. Microbiol Resour Announc. 2019 Feb 7;8(6). pii: e01543-18. doi: 10.1128/MRA.01543-18. eCollection 2019 Feb.

[Comparison of Healthy and Dandruff Scalp Microbiome Reveals the Role of Commensals in Scalp Health.](#) Saxena R, Mittal P, **Clavaud C**, Dhakan DB, Hegde P, Veeranagaiah MM, Saha S, Souverain L, Roy N, Breton L, Misra N, Sharma VK.

Front Cell Infect Microbiol. 2018 Oct 4;8:346. doi: 10.3389/fcimb.2018.00346. eCollection 2018.

[Novel phages of healthy skin metaviromes from South Africa.](#) van Zyl LJ, Abrahams Y, Stander EA, Kirby-McCollough B, Jourdain R, **Clavaud C**, Breton L, Trindade M. Sci Rep. 2018 Aug 16;8(1):12265. doi: 10.1038/s41598-018-30705-1.

[Aging-related changes in the diversity of women's skin microbiomes associated with oral bacteria.](#) Shibagaki N, Suda W, **Clavaud C**, Bastien P, Takayasu L, Iioka E, Kurokawa R, Yamashita N, Hattori Y, Shindo C, Breton L, Hattori M. Sci Rep. 2017 Sep 5;7(1):10567. doi: 10.1038/s41598-017-10834-9.

[Dysbiotic Bacterial and Fungal Communities Not Restricted to Clinically Affected Skin Sites in Dandruff.](#) Soares RC, Camargo-Penna PH, de Moraes VC, De Vecchi R, **Clavaud C**, Breton L, Braz AS, Paulino LC. Front Cell Infect Microbiol. 2016 Nov 17;6:157. eCollection 2016.

[Body Site Is a More Determinant Factor than Human Population Diversity in the Healthy Skin Microbiome.](#) Perez GI, Gao Z, Jourdain R, Ramirez J, Gany F, **Clavaud C**, Demaude J, Breton L, Blaser MJ. PLoS One. 2016 Apr 18;11(4):e0151990. doi: 10.1371/journal.pone.0151990. eCollection 2016.

[Characterization of the major bacterial-fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium.](#) Wang L, **Clavaud C**, Bar-Hen A, Cui M, Gao J, Liu Y, Liu C, Shibagaki N, Guéniche A, Jourdain R, Lan K, Zhang C, Altmeyer R, Breton L. Exp Dermatol. 2015 May;24(5):398-400. doi: 10.1111/exd.12684.

[Analysis of the response of human keratinocytes to *Malassezia globosa* and *restricta* strains.](#) Donnarumma G, Perfetto B, Paoletti I, Oliviero G, **Clavaud C**, Del Bufalo A, Guéniche A, Jourdain R, Tufano MA, Breton L. Arch Dermatol Res. 2014 Oct;306(8):763-8. doi: 10.1007/s00403-014-1479-1. Epub 2014 Jul 20.

[Chemical organization of the cell wall polysaccharide core of *Malassezia restricta*.](#) Stalhberger T, Simenel C, **Clavaud C**, Eijsink VG, Jourdain R, Delepierre M, Latgé JP, Breton L, Fontaine T. J Biol Chem. 2014 May 2;289(18):12647-56. doi: 10.1074/jbc.M113.547034. Epub 2014 Mar 13.

[Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp.](#) **Clavaud C**, Jourdain R, Bar-Hen A, Tichit M, Bouchier C, Pouradier F, El Rawadi C, Guillot J, Ménard-Szczebara F, Breton L, Latgé JP, Mouyna I. PLoS One. 2013;8(3):e58203. doi: 10.1371/journal.pone.0058203. Epub 2013 Mar 6. Erratum in: PLoS One. 2013;8(10). doi:10.1371/annotation/bcff4a59-10b7-442a-8181-12fa69209e57.

Skin microbiome signatures in healthy skin across daily life : perspectives for cosmetics

Cécile CLAUD

Our body's largest organ, skin, is constantly exposed to external environment. It is covered with various microorganisms, including fungi, bacteria, archaea and viruses, among which bacteria outnumber others. The microbiome plays three main essential roles: protection against pathogens by secreting antimicrobial molecules; regulation of the innate immune system and reinforce the skin barrier function and wound healing. Thus, it is essential to preserve the equilibrium to prevent from skin disorders.

Several studies, among which our work has contributed, have revealed that the skin microbiome is determined by ecological zones of the skin including sebaceous, moist and dry environments. In addition to ecological zones, host-related factors such as age and exposome factors such as urbanization, pollution and topical treatments are also shown to affect the skin microbiome in association with an altered skin quality.

Furthermore, the skin microbiome is linked to skin disorders. Recent studies on Atopic dermatitis or Dandruff suffering subjects have shown that microbial shift in bacterial and fungal communities were observed in lesional and in non-lesional sites, suggesting that the disorder is related to a systemic process that is not restricted to the site exhibiting clinical symptoms. The findings from the past years indicate that we don't have to rely solely on antifungals/antibacterial agents for treating skin disorders. Indeed, by controlling the bacterial, fungal species equilibrium and skin barrier is a good opportunity to imagine new holistic treatments to get better long-lasting effects. Thus recent studies proposed the direct modulation of the skin microbiome by new approaches such as topical application of prebiotic, transplantation and phage therapy.

Altogether, these recent advances in understanding our microbial counterpart, supports the fact that the skin microbiome will be key in the near future for a more personalized dermo-cosmetics.



Pr Guoping Lian

Guoping Lian is Professor of Chemical and Process Engineering at the University of Surrey and also Science Leader of Unilever R&D Colworth UK.

He received his BSc in Mechanical Engineering from Hunan Agricultural University in 1982, MSc in Agricultural Engineering from China Agricultural University in 1984 and PhD in Chemical Engineering from Aston University in 1993.

He joined Unilever R&D Colworth UK in 1994 as a Research Scientist. In 2013, He was appointed Professor of Chemical and Process Engineering at the University of Surrey while still serving as Science Leader at Unilever R&D Colworth. His research interest covers Agriculture Engineering, Chemical Engineering, Food Engineering, Pharmaceuticals, Pharmacokinetics and Toxicology. He specialises in multi-scale and multi-physics modelling of complex nano/micro structures of foods, pharmaceuticals and personal care products. He also specialises in multi-scale and multiphysics modelling of the pharmacokinetics of transdermal delivery and bioavailability of dermatological drugs and cosmetic care actives. At Unilever, Professor Guoping Lian has led the development of a number of leading-edge in-silico models to support bigger, better and faster innovation in foods, beauty care and home care products. His early work includes the development of tea infusion model for the design, optimization and marketing of tea-based beverages (pyramid tea bags) and flavour model for flavour enhancement of low fat foods. Since being appointed as Professor of Chemical and Process Engineering at the University of Surrey in 2013, he has set up a number of international and intersectoral collaborations and obtained significant research funding. His recent research focuses on the development of multiscale and multiphysics models for in-silico prediction of the pharmacokinetics of transdermal permeation and bioavailability of dermatological drugs and cosmetic actives. The models he developed with co-authors are becoming important non-animal alternatives to support innovation in cosmetics products and safety assurance of dermal exposure.

Multi-scale modelling of solute partition in complex topical products and impact on transdermal permeation

Guoping Lian*¹, Mattia Turchi², Qiong Cai², and Tao Chen¹

1

University of Surrey – United Kingdom

2

University of Surrey – United Kingdom

Abstract

Solute partition in multiphase topical products of dermatological drugs and cosmetic care is an important thermodynamic parameter for transdermal permeation. Understanding how solute partition in multiphase topical products affects transdermal permeation is critically important for the design and optimisation of transdermal bioavailability and bioequivalence. We present progress in developing a multi-scale modelling approach for predicting solute partition in multiphase microstructures of topical product and its impact on transdermal permeation. Initially, the thermodynamic equilibrium of solute partition in multi-phase complex fluids of micelles and emulsions has been predicted using molecular dynamics simulation combined with COSMOmic, an extended COSMO-RS (conductor-like screening model for real solvent) method for quantum chemistry calculation of chemical potential of solute in heterogeneous structures of micelles, droplets and membranes [1]. Molecular dynamics simulation has been used to predict the self-assembly of SDS and SLES surfactant micelles and oil-in-water microemulsion droplets. The self-assembled surfactant micelles and oil-in-water droplets are then entered into COSMOmic for predicting the chemical potential and partition of a wide range of solutes in the self-assembled microstructure. Chemical potential and partition of the solutes in self-assembled micelles and oil-in-water droplets have been also predicted using Steered Molecular Dynamics and Umbrella Sampling. Predicted solute partition in micelles and oil-in-water emulsions has been compared with published experimental data and there is a good agreement [2]. The effect of solute partition in micelles and oil droplets on transdermal permeation has been predicted using our latest microscopic PBPK model [3]. Integrating molecular dynamics and COSMO modelling with microscopic PBPK modelling allows for the impact of both the molecular features of actives and the formulation features of surfactant micelles and emulsions to be systematically analysed.

Klamt, A.; Huniar, U.; Spycher, S.; Keldenich, J. COSMOmic: A Mechanistic Approach to the Calculation of Membrane–Water Partition Coefficients and Internal Distributions within Membranes and Micelles. *J. Phys. Chem. B* **2008**, *112* (38), 12148–12157.

Turchi, M., Cai, Q., Lian, G. *In silico* prediction of the thermodynamic equilibrium of solute partition in multiphase complex fluids: A case study of oil-water microemulsion. Accepted by Langmuir

Chen, T., Lian, G., Kattou, P. In Silico Modelling of Transdermal and Systemic Kinetics of Topically Applied Solutes: Model Development and Initial Validation for Transdermal Nicotine. *Pharmaceutical Research* 2016, *33*(7), 1602-1614



Dr Dominik IMFELD

**Swiss Nationality,
born in Zurich**

- 1991: MSc in Biochemistry at Swiss Federal Institute of Technology, the ETH in Zurich
- 1991-1994: Ph. D. at ETH Zurich studying protein structure, folding and stability
- 1994-1997: Postdoc at Novartis in Basel. Working on projects for blood coagulation using peptide engineering for factor VII inhibitors then investigation of the role of apoptosis in cancer and introducing proteomics
- 1997 -2009: at Pentapharm Ltd, final position head of R&D Biotechnology
- since 2010 to current position at DSM: Research group leader at DSM Personal care and now Head of Claim Substantiation for skin actives.
- 22 years of experience in skin biology research and development of a variety of skin care active ingredients with key contribution to the development of peptide based active ingredients now widely used in skin care products. He is inventor on several patents and author or co-author of many publications

Novel anti-aging approaches with bioactive lipids

Dominik IMFELD

Skin aging is a global concern that is becoming even more important with a growing population living in higher prosperity and the aging population in well-developed countries. The perception and manifestation of aging differs however for different ethnicities based on varying behavioral habits, exposure to different environments and depending skin type. However, the signs of aging such as wrinkling, skin pores and inhomogeneous skin tone are to some extent in common and the main accelerator for skin aging is UV light.

Peroxisome Proliferator-Activated Receptors (PPARs) are members of the nuclear hormone receptor family, they regulate gene expression by forming heterodimers with retinoid X receptors. PPARs are generally considered to be key targets in skin care applications. Especially PPAR alpha activators improve epidermal differentiation, reduce inflammation, increase extracellular matrix components and modulate pigmentation.

The ability of saturated and unsaturated long chain fatty acids to bind and activate all three PPAR sub-types has been well documented. We widened our search on fatty acid derivatives such as hydroxylated free fatty acids. After an *in vitro* gene reporter assay screening we focused on further evaluating 10-hydroxy stearic acid (10HSA) as a promising candidate thus it became our "bioactive lipid". 10HSA was identified as a primary PPAR α agonist with $EC_{50} = 5.5 \times 10^{-6}$ M and this was further supported by *in silico* docking studies.

10HSA increased *in vitro* on fibroblasts the collagen type I and type III levels by up to 96% and 244%; $P < 0.01$) and *ex vivo* on human skin explants the collagen type III by +57% ($P < 0.01$). Also, *ex vivo* on UVB irradiated skin 10HSA inhibited MMP-1 gene expression (83%; $P < 0.01$) and mitigated sunburn cell induction (34% vs. vehicle control) and p53 up-regulation (46% vs. vehicle control; $P < 0.01$). Mass-spectrometry-based proteomics on 10HSA treated fibroblast secretome showed significant increases in proteins associated with the WNT pathway that could reduce melanogenesis and proteins that could modify dermal fibroblast activity and keratinocyte differentiation. Clinically in a full-face, double blind, vehicle-controlled trial, 10HSA showed a statistically significant decrease of size of conspicuous skin pores ($P < 0.05$) after 8 weeks of application and age spots became significantly less pigmented than the surrounding skin ($P < 0.05$) after 4 weeks. Finally, 10HSA showed synergistic effects on stimulation of collagen3 synthesis when applied in combination with retinol on *ex vivo* human skin.



Pr Jonathan HADGRAFT

Jonathan Hadgraft was educated at the University of Oxford where he obtained his M.A. and D.Phil. in Chemistry. Subsequently the Faculty of Medicine awarded him a D.Sc. In May 2004 he joined The School of Pharmacy, University of London where he is Emeritus Professor of Biophysical chemistry. He has held academic posts at the Universities of London, Strathclyde, Nottingham, Cardiff and Greenwich. During his academic career he has been involved in the supervision of over 70 Ph.D. students. He has been scientific adviser to Laboratório Estudos Farmacêuticos (Lisbon) and has been a visiting professor at the North West University, Potchefstroom, South Africa and Monash University, Melbourne, Australia. He is an adviser to the EMA.

His major research interests are in the application of physical chemistry to drug delivery, with special reference to the skin. The research group he is associated with uses a range of biophysical techniques to probe the mechanisms of skin penetration and its modulation.

He has been elected to Fellowships of the Royal Society of Chemistry, the Academy of Pharmaceutical Scientists, the American Association of Pharmaceutical Scientists and the Controlled Release Society. He is on the scientific advisory boards of a number of pharmaceutical companies. Over the years has contributed to over 500 publications and serves on several of editorial boards of pharmaceutical science journals. He has given numerous plenary lectures in Europe, the US, South America, Japan and Australasia. He has been involved in a number of litigation cases in the UK, Europe, US and South Africa.

He is a VP of Southwold Rugby Club and a member of The British Alpaca Society.

Formulation Science – a half century of progress?

Jonathan HADGRAFT

One of the major drivers for investigating the formulation strategies for topical delivery was the synthesis of potent fluorinated corticosteroids. There was a recognition that only small concentrations of the active were required for topical effect and that it was important to ensure that systemic delivery was not too high such that to produce unwanted side effects. The significance of concentration and the interplay between solubility and partition coefficient was appreciated. One of the advantages of investigating the steroids was that they could be studied *in vivo*, as they were vasoconstrictors. The degree of pallor on the skin surface was a crude indicator of the degree of absorption and was also a function of the potency of the steroid. It was also known that penetration enhancers could be added to formulations to improve delivery, a lot of the preliminary studies involved dimethyl sulphoxide: even today we do not know its precise mechanism of action. Other penetration enhancers were evaluated, and companies formed explicitly to produce novel enhancers which could be incorporated into transdermal products that were extensively developed for systemic delivery in the 1990s.

The effects of thermodynamic activity on skin penetration were documented and in formulation science a number of researchers made use of supersaturation to improve absorption. There was a general recognition that synergistic effects could be obtained by combining different types of enhancement strategies including supersaturation. A low bioavailability of topical applications had been known for some time and the use of supersaturation highlighted the problem of crystallisation on and in the stratum corneum.

In some designs the production of supersaturation resulted from the evaporation of a volatile solvent and there was a general recognition that topical applications changed significantly after application to the skin. This is a particular problem because the application dose is only $2 \text{ mg}\cdot\text{cm}^{-2}$; this equates to an application thickness of only $20 \mu\text{m}$. Many formulation studies, *in vitro*, were conducted with an infinite dose and questions have to be asked about their relevance. Formulation components will evaporate, penetrate into the skin and actives will crystallise. There has been a hope that this can be predicted using physicochemical parameters such as solubility, partition behaviour, molecular size and solubility parameter. Little is understood about the permeation characteristics of excipients and until this is determined it is difficult to see how a predictive model can be built. Modern developments in instrumental techniques are now allowing study of these and in the next decade we will learn a great deal more.



Dr Milica LUKIC

Milica Lukic studied Pharmacy at University of Belgrade where she got her PhD in Cosmetology in 2014. She is an Assistant Professor at Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy-University of Belgrade, engaged in teaching at courses of Cosmetology and Pharmaceutical technology at integrated academic studies and post graduated – specialist and PhD studies.

Her research interest encompasses pre-formulation and formulation of emulsion systems for cosmetic and pharmaceutical application, physicochemical characterization of colloidal systems with the use of different techniques and development and optimisation of in vivo methods for safety and efficacy evaluation. Until now, dr Lukic has authored/co-authored more than 100 papers, presentations (oral or poster) and technical solutions.

From emulsions' stability concerns to sensory properties: tools that we use

Milika LUKIC

Emulsions have found an extensive range of applications within different industries and due to their unique physicochemical and functional properties they are used in everyday life in a variety of consumer products. Regardless of the significant technological progress, to develop conventional emulsion is, to their very nature, still a challenging task with multiple endeavours. Cosmetic emulsions have to meet legal requirements, regarding quality (long-term physical, chemical and microbiological stability), safety and efficacy, together with pleasing consumers' preferences based on sensory characteristics – considered as crucial for products' sale potential. Therefore, formulation of cosmetic emulsions greatly reflects all the complexity of this task, requiring a multidisciplinary approach and the knowledge from different scientific fields like chemistry, physics, technology, physiology, psychology etc. Although, the fulfilling of consumers expectation was considered to be an imperative for cosmetic, a modern patient has corresponding demands for topical drugs as well.

Since formulation and often necessary reformulation of emulsion are time and money consuming, in the past decade great scientific efforts have been made to simplify these processes and they are presented in this presentation. Correlations between instrumental parameters and consumer percept attributes have been studied and modifications of existing methods and implementation of techniques not usually employed in emulsions' development and/or characterization have been made. Tools like rheological measurements, textural and sensory analysis, thermal techniques, skin biophysical methods and factorial design have been used and different combined approaches are demonstrated in considerable number of studies concerning emulsions development.

Obtained results have opened different perspectives in emulsion investigation and development. They enabled achieving satisfying results in shorter time with less expense in (re)formulation processes. Additionally, these results were valuable for advancement in existing and progress of new methodologies and strategies used in different fields.



Pr Vincent BOLY

His research focuses on innovation processes. One of the major aspects concerns the metrology of innovation both in its theoretical and technological dimension (development of evaluation software). Thus, these developed approaches allow companies to measure their capacity to innovate and are in line with new international standards. The second part concerns the management of the upstream stages of innovative projects. His work focuses on the development of theoretical models and methodologies in the areas of needs analysis, value chain approaches, customer/supplier co-innovation, and the digital chain for formulated products.



Dr Javier ARRIETA-ESCOBAR

Currently works as a Postdoctoral Researcher in the field of product design at the ERPI (*Equipe de Recherche sur les Processus Innovatifs*). He holds a double Doctorate in Industrial Systems Engineering from the University of Lorraine and in Chemical Engineering (with honours) from the National University of Colombia. He worked during 5 years in the formulation of personal care products in Colombia and during that time he followed a distance learning program of Cosmetic Science from the Society of Cosmetic Scientists in the UK. He also has a MSc. degree in Chemical Engineering from the University of Applied Sciences of Mannheim in Germany and a BSc. degree (with honours) in the same area from the National University of Colombia.

Integration of Heuristic Knowledge in the Design of a Skin Moisturizer

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The design of optimal mixtures is considered an important challenge in many industrial sectors, especially for formulated products like cosmetics. Besides the physicochemical properties of the final product, in the domain of cosmetic formulations, the perceived performance of the product is of paramount importance, so greater consumer involvement needs to be integrated into the modelling problems. This work focused on developing a systematic design approach of a structured product incorporating consumer expectations. As a case study, the design of a moisturizing cosmetic emulsion was carried out.

Starting with a list of product attributes, the importance level of each attribute and their interinteractions were determined by exploiting data from usability tests of a commercial product (survey on 32 women) and computing them with fuzzy measures, in particular the Choquet integral [1]. These consumer parameters along with some heuristics regarding the formulation were next incorporated into a systematic CAMD methodology, as shown in previous studies [2,3]. Then, a set of 5 body lotions were generated under multiple objectives (cost, greasiness, and toxicological profile). In the end, an experimental validation at lab scale was carried out, starting from 36 possible main components (6 non-ionic emulsifiers, 24 emollients and 6 rheology modifiers). The results show that the incorporation of the consumer preference significantly affects the choice of the ingredients and can help to reduce the time and resources spent in cosmetics design.

The paper proposes an-easy-to apply methodology, which allows to select the most important consumer attributes taken from usability tests and integrate them along with heuristics to obtain a set of feasible alternatives that are optimised to meet different specified requirements. This *in-silico* methodology could be used as a preliminary design tool for unexperienced cosmetic scientists and designers.

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Dr Christop RIETHMULLER

Dr. Christoph Riethmüller studied chemistry at the universities of Konstanz and Berlin. After studying arginine-metabolism in Archebacteria at the Max-Planck-Institute in Munich, he obtained a PhD from University of Gnanz for the enzymology of the signalling molecule nitric oxide, which emanates from arginine precursor. As a lecturer in human physiology at University of Muenster, he started performing atomic force microscopy (AFM) on living and fixed biological cells. The research focused on nanoscale correlates of barrier function in epithelia and endothelia. Around 2007, topography quantitation came into play, since biologically relevant forces leave tiny traces at the cell surface. The patented quantitation method is termed nAnostic (nanoscale topography for diagnostics). In 2009, Dr. Riethmüller founded the company Serend-ip GmbH, which delivers nAnostic service for research groups in science and industry - including the DERMATACT skin assay .

Nanoscale phenotyping reveals the impact of topical treatments

Christoph RIETHMULLER

Clinical definitions of skin health or disease are mainly based on visual appearance. For a quantitation of skin function below the clinical threshold, various physi(ologi)cal assays can be performed. Generally, a simultaneous presence of test person, instrumentation and trained staff is required. Here, we present a novel phenotyping assay based on tape strips, which can be sent by postal service. A single index number resembles the degree of biological stress of skin cells. The method principle extends the tactile sensation of a physician down to the nanoscale. Using atomic force microscopy (AFM) recordings, computer vision is employed to quantify topographical elements - resulting in a dermal texture index (DTI). The DTI is elevated in cases of filaggrin deficiency or upon exposure to external factors like detergents or sunlight. The high sensitivity enables to differentiate subclinical states. The results of early and current dermatological studies will be presented.



Pr Malcolm Ronald CLENCH

Malcolm Clench, originally a chemist worked as a Research Assistant for Dereck Gordon of Manchester Metropolitan University and Prof Michael Barber at UMIST between 1982 and 1985 being awarded a PhD for this work in 1988. After a period in the scientific instrument industry he moved back to to an academic appointment in 1990. He is Head of Research for the Biomolecular Sciences Research Centre Sheffield Hallam University.

Professor Clench pioneered the application of MALDI-MS imaging in the UK. He is Leader of the Imaging and MALDI Special Interest Group of the British Mass Spectrometry Society, a member of the Board of the Mass Spectrometry Imaging Society. He has published 120 papers and supervised 34 PhD students. His research work over the last ten years has been concerned with the study of toxico- and pharmacodynamic responses in tissue with a particular interest in skin and 3D skin models.

MALDI-MSI for Skin Investigations

Malcolm R CLENCH

Matrix assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) is a label free imaging technology that has been extensively employed in the pharmaceutical industry. In MALDI-MSI images are created by irradiating the surface of a section of biological tissue, which has been previously coated with an energy absorbing matrix, with a UV laser. Ions ejected from the surface are subjected to mass spectrometric analysis and images generated by plotting the intensity of specific m/z values with positional information as a grey or colour scale. [1] One of the key features of MALDI-MSI that makes its use appealing is the ability to detect and study the distribution of multiple compounds simultaneously in a label-free manner. Additionally by using, accurate mass measurement and tandem MS analysis, molecules can be identified directly on the tissue sections. The initial proof of concept study demonstrated the MALDI imaging of proteins in biological tissue [2], since then however it has been applied to the analysis of a wide range of pharmaceutical compounds in situations ranging from whole animal sections to drug eluting stents and 3D tissue models [3-6].

Here applications of MALDI-MSI to skin and skin models are presented. We have been examining the use of MALDI-MSI to study drug penetration in skin, drug metabolism in skin and wound healing. Data are presented from studies carried out using ex-vivo human skin and a commercial living skin equivalent model (Labskin) including data from a detailed study into quantitative imaging of Terbinafine along with some preliminary work on the chemical induction of metabolising enzymes.

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Dr Jean DOUCET

Jean Doucet is a French physicist, specialized in the structure of soft-condensed matter and biological tissues. His activity focuses on the analysis of molecular organizations in materials and human tissue for biomedical, pharmaceutical and cosmetic purposes. The use of synchrotron-based techniques has allowed him to go beyond the performance of conventional laboratory characterization techniques, and to develop new experimental protocols.

After a university career, Jean Doucet is now Managing Director of Novitom, the first full-service provider to specialize in 3D micro-imaging and micro-analysis based on synchrotron technologies. In the field of cosmetics, Novitom offers unique *in vitro* methods to test the efficacy of active ingredients, to improve the formulation and to substantiate the claims, that meet the needs of the R&D and marketing.

Jean Doucet is the author of over a hundred scientific publications as well as many articles for engineers.

X-ray based methods for skin investigation

Jean DOUCET, Emilie LECCIA

X-ray techniques have recently undergone spectacular technological developments, thanks to the use of synchrotron light sources, which has resulted in technological breakthroughs in the characterization and analysis of materials. The quality of the synchrotron X-ray beam is such that it leads to unique performances in terms of spatial resolution, sensitivity and kinetic tracking, offering in particular new approaches for the evaluation of cosmetic products.

There are three families of X-ray techniques: diffraction techniques, which provide information on molecular and supramolecular arrangements, spectroscopic techniques that provide information on the nature of the elements and chemical bonds, and radiographic techniques to visualize the internal morphology of the samples.

The ability to work with micrometric-sized X-ray beams has opened the field of microanalysis. A major example concerns diffraction carried out on stratum corneum (SC) after the application of a product. It is now possible to evaluate the changes in the SC lipids as a function of depth, to discriminate between mixing with endogenous lipids or insertion of globules, or to know if the treatment fluidifies or crystallizes the lipids. These data are supposed to be related to permeation and mechanical response.

Micro-fluorescence imaging, a spectroscopic technique for observing and quantifying the distribution of chemical elements, is another new opportunity offered by micro-beams. Operated in 2D scanning mode, this technique becomes a powerful tool to analyze the penetration of non-purely organic actives or ingredients into the skin after *ex vivo* treatment on explants.

The breakthrough of X-ray microtomography is due to the outstanding characteristics of synchrotron X-ray beams in terms of flux, monochromaticity and parallelism. The 3D images of the reconstructed volumes combine a submicron spatial resolution, a high contrast sensitivity, all of which are compatible with temporal tracking in a controlled environment. The interest of this 3D microscopy is easily realized for the analysis of explants, to visualize and measure the thickness of the layers, to evaluate their density or to quantify the orientations of collagen fibers.

Synchrotron light sources have thus considerably boosted the performance of X-ray based methods. It is interesting to note that these new opportunities are very much in line with the requirements for further evaluation of the effect of cosmetics by offering new tools complementary to commonly used tools. In addition, the results are provided in a form suitable for both R&D and marketing services!



Dr Nicola ZAMBONI

Biosketch

2003 PhD, Institute of Biotechnology, ETH Zürich

2004 –2005 Postdoctoral fellow, Stanford Genome Technology Center, Stanford University, USA

Since 2005 – Principal Investigator and later Adjunct Professor, Institute of Molecular Systems Biology, ETH Zürich

Nicola Zamboni graduated in 2003 in the group of Jay Bailey at ETH Zurich in the field of metabolic engineering and ^{13}C metabolic flux analysis. As a PostDoc in Stanford, he developed and applied metabolomics-based approaches for unraveling metabolic changes in eukaryotic cells. Since 2005, he is a PI at the Institute of Molecular Systems Biology in Zurich. His lab focuses on the development of mass spectrometry and computational methods to characterize metabolic dynamics in complex systems and reverse engineer cellular regulation.

Metabolic drivers of skin function

Nicola ZAMBONI

Over the past years, we investigated the metabolic underpinnings that characterize fundamental epidermal processes in humans, including keratinocytes differentiation, acute and chronic responses to oxidative stress, aging, and wound healing. In all of these studies, we adopted a systems biology approach that integrates metabolomics, transcriptomics, genomics, and a large portion of computational biology. Our primary tool to investigate metabolic networks is mass spectrometry. We established world-wide unique methods for non-targeted metabolomics that allows us to profile thousands of metabolites and lipids at virtually any throughput. Such measurements provide an information on what pathways are changing upon external or internal perturbations.

The key challenge in this approach is the interpretation of the results. This is a necessary step to move beyond merely descriptive studies and deliver mechanistic insights. To identify causal and relevant changes, we resort to computational methods. They are built on biochemical principles and aim at integrating all available information to isolate the most likely reactions (genes) that drive a phenotype. At this stage, transcriptomics data from arrays or RNA sequencing can be merged to distinguish between transcriptional and other forms of regulation.

I illustrate pros and cons of our approach with two vignettes derived from published studies on the acute response of skin cells to UV and oxidative stress [1] and skin aging [2]. In the second and main part, I present an unpublished analysis of metabolic changes in differentiating keratinocytes in the in vitro high calcium model. Through integrated analysis of metabolites and transcripts, we obtained a comprehensive landscape of the metabolic changes that keratinocytes undergo to support morphological changes in the epidermis fulfill their barrier function. By modulating directly metabolite levels and enzyme activity, we can both stimulate and impair proliferation and differentiation of keratinocytes in vitro.

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Pr Florence AGNELY

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Florence AGNELY is Professor of Pharmaceutical Physics at University Paris-Sud/ University Paris-Saclay. Graduated from the Ecole Supérieure de Physique et de Chimie Industrielles de la ville de Paris (ESPCI), she obtained a PhD on physical chemistry of hydrophobically modified polymers from Pierre and Marie Curie University under the supervision of Dr I. Iliopoulos and Dr R. Audebert. She worked as a post-doc researcher at the joint CNRS-Elf Atochem research unit headed by Dr L. Leibler (Levallois Perret, France). She currently supervises the “Physical Pharmacy” team at the Institut Galien Paris-Sud. Her research activities are devoted to the design and characterization of polymer-based systems and of emulsions for pharmaceutical or biomedical applications. She is in charge of the master program « Pharmaceutical Technology and Biopharmacy » of the University Paris-Saclay and is board member of the Formulation Group of the French Chemical Society.

Contribution of interfacial rheology for the study of topical emulsions

Florence AGNELY

Emulsions are widely used in the formulation of pharmaceutical and cosmetic topical products. They are found in creams, but also in some ointments, gels, pastes, ... Emulsions offer the possibility to encapsulate an active molecule inside the droplets of the dispersed phase, ensuring its protection against environmental stress and degradation (oxygen, light, enzymes, acidity, etc.), and allowing its controlled delivery. By topical route, emulsions often improve the permeability of the active compound. However, emulsions are thermodynamically unstable systems (except microemulsions) and are prone to phase separation. The use of stabilizers is thus required for their formulation and for ensuring their long-term stability. Until now, emulsions have mostly been stabilized by synthetic surfactants that adsorb at the oil/water interface. Unfortunately, even with authorized synthetic surfactants, irritations or allergic responses can be observed [1]. Alternative stabilization approaches have been developed such as the use of biopolymers [2] or solid particles [3].

In this context, interfacial rheology appears as a valuable tool to assess and compare the stress/strain relationships occurring at interfaces stabilized by surfactants, biopolymers or particles. The drop method can be used for dilational rheological measurements. The volume of a pendant (or a rising) drop is submitted to sinusoidal variations, thus resulting in expanding or compressing the surface of the drop. Other techniques are based on shear solicitations of the interface and some of them consist in using a rheometer equipped with a biconical disk or a ring. This geometry is placed at the interface between the two liquids and rotates to determine the rheological parameters of the studied interface. Both types of experiments (dilational and shear experiments) allow the measurement of interfacial elastic and viscous moduli and a better understanding of the mechanisms involved in the formation and the stability of the emulsions [4, 5]. The potentiality and the limitations of interfacial rheology for the study of topical emulsions will be discussed and illustrated from results obtained in our group and others.

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Dr Pascal BROCHETTE

PhD in Physical Chemistry.

Specialised in interfacial physical- chemistry and in dispersed systems.

Founding director of the company Atellane. 20 years of experience in continuing education with a technical and scientific focus

Diplômes

Habilitation à Diriger des Recherches (HDR), Rennes I Univ. [1998]

Doctorat de chimie-physique, Pierre & Marie Curie Univ., Paris VI [1984]

Cursus

1987–1999 - Head for a R&D team specialised in emulsions, microemulsions and surfactants at the Lacq Research Group (GRL, Elf Group).

1999-2019 – Founding member of *Atellane*, a company devoted to training and advising. Technical and scientific training for the cosmetic, pharmaceutical, chemical, food industries. Scientific consultancy, troubleshooting.

Skills

- Training engineering;
- Training projects management;
- Scientific outreach & scientific illustration;
- Interfacial physical-chemistry, surfactants, dispersed systems (emulsions, suspensions, microemulsions), rheology, solubility parameters;
- Physical chemistry of formulation, knowledge of raw materials.
- Problem solving techniques;
- Mixing and industrial transposition;

Rheology of skin products > Industrial point of view.

Pascal BROCHETTE

Formulated products intended to be applied to the skin must meet the consumers expectations. Among these, the flow properties are particularly important: they dominate the sensory effect during skin application. For certain skin conditions, a shear thinning product is preferable to avoid damaging the consumer epidermis or cause pain.

The rheological profile is one of the characteristics conferred to the product during the formulation step. For most products to be applied on skin, the ideal profile is a shear thinning behaviour with a yield stress (i.e. casson body).

When this product goes through the production process, the expected properties must be checked on each batch. Usually, the rheological profile is reduced to a single viscosity measurement, most often using the Brookfield method.

We will discuss the following points:

The ideal rheological profile for a topical product.

The types of networks that give this profile, with examples of corresponding thickening raw materials.

The industrial implementation of some of these thickening raw materials.

The question of quality control with only one point on the rheological profile.

The consumer "windows" on the rheological profile and the relevance of the speed-mobile choice.

More industrial issues such as the influence of the rheological profile on the product mixing or transfer, and on priming of pumps.



Dr Valentine IBEKWE

Education

2005 : PhD – The School of Pharmacy, University of London

1999 : B Pharm – The School of Pharmacy, University of London

Employment

Since June 2005

Pharmaceutical Assessor, Licensing Division, Medicines and Healthcare products Regulatory Agency (MHRA), UK

Roles:

- Assessment of marketing authorisation applications for new and generic products and applications for variations in National and European procedures; primarily for ophthalmic and dermatological products.
- Providing advice to companies on development and submission/regulatory strategies at MHRA scientific / regulatory advice meetings and CHMP scientific advice.
- Contributing to the development of European guidelines on quality

Rheology of skin products: a regulatory perspective

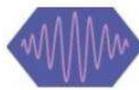
Valentine IBEKWE

An overview of current regulatory requirements regarding characterisation and control of rheological properties of skin products will be presented.

The rheological characteristics of a product can affect the transformation during application, kinetics of the active substance and, potentially, efficacy of the product. From a patient usability perspective, rheological properties affect the ease of application and feel which can influence overall patient acceptability and compliance. Therefore, adequate characterisation and specifying meaningful controls for rheological properties throughout shelf life should be an important aspect of development of new and generic topical products. For generic products, the current draft EMA guideline for quality and equivalence outlines the criteria for demonstration of extended pharmaceutical equivalence in lieu of clinical equivalence data, which includes demonstrating equivalence of microstructural and rheological properties of the generic and reference products.

Although the importance of rheology is widely understood and despite availability of appropriate methods for full rheological profiling, data on rheological properties in many marketing authorisation applications for skin products are found deficient. For instance, submissions for generic products usually include only single-point viscosity tests for comparison of rheology of the generic and reference medicinal product and for QC/lifecycle management of the product.

Data requirements for characterisation and control of rheology of topical semisolid and structured liquid products to support initial marketing authorisation applications and lifecycle management will be discussed. The most common deficiencies relating to rheological data identified in initial marketing authorisation and post-approval variation applications will also be discussed.



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Reims, France
23-24 September, 2019



ORAL COMMUNICATION SELECTED ON ABSTRACT

Shielding the skin from biofilm: *Spirulina platensis* sustainable lipid extracts and their formulation

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Abstract

Biofilms, bacterial or fungal, are involved in numerous skin, hair and dental inconveniences or pathologies. Dislodging the biofilm is very difficult; thereby developing antiadhesion active ingredients that can shield the skin and prevent the installation of biofilm is of high concern.

Our consortium is most of all interested in the development of natural ingredients that could be easily introduced in pharmaceutical or cosmetic products intended for skin care [1]. Biomimetic and sustainable approaches appeared an obvious direction to follow to develop ingredients with no impact on the quality and innate regulation of the skin.

Free fatty acids (FFA) are antimicrobial agents that are naturally occurring on skin. They are in charge of the microbiota regulation and exhibit wide antimicrobial spectrum depending on carbon chain length and number of double bonds. Two major factors limit the development of FFA as antimicrobial ingredients: the toxicity and low selectivity of solvents used for their extraction; and the FFA sensitivity to oxidation, especially considering polyunsaturated fatty acids (PUFA).

Microalgal biomass represent a natural renewable source of FFA especially of PUFA [2]. Among all microalgae, *Spirulina platensis*, a blue cyanobacteria, is a good model, thanks to its richness in FFA and its easy cultivation. Lipophilic pigments, such as chlorophylls and carotenoids, were also found in microalgae and were usually co-extracted with lipids. In order to perform a green and selective extraction of microalgal FFA, various biosourced solvents were screened. Lipid and pigment amounts, combined with FFA profile were used to select the optimal conditions.

Anti-biofilm formation activity of those extracts was investigated against fungal, bacterial and mixed biofilms and gave promising results. An encapsulation of the lipid extracts was performed using different lipid-based sub-micrometric encapsulation systems to ensure their protection against oxidation and to facilitate their incorporation in water-based end products [3]. Data did not show any degradation of lipid extracts during encapsulation process. The encapsulation did not affect the anti-adhesion properties of the extract. Moreover, it conferred an activity against certain types of pre-formed biofilms. The ingredients showed a low toxicity on keratinocytes *in vitro*. The results indicate the FFA and encapsulated FFA are promising ingredients that could participate to shielding the skin from biofilm formation.

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Release and permeation study of meglumine antimoniate semisolid dosage form for the treatment of cutaneous leishmaniasis.

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Abstract

Background: *Leishmania infantum* is the protozoan responsible of cutaneous leishmaniasis (CL) in Spain, causing usually self-limited ulcerative lesions, although they may sometimes take up to two years to heal. The treatment consists in intralesional administration of antimony salts that require multiple injections, which cause acute pain and make patients unable to tolerate the treatment. Due to the difficulty and discomfort of this administration route, topical treatments would be an interesting alternative.

Purpose: The aim of this study was to characterize and evaluate in human skin a formulation containing 30% of meglumine antimoniate (MA) in a gel base, in order to achieve a new dosage system of the drug as well as reduce side effects.

Methods: The release study of the MA formulation was performed in Franz Diffusion Cells (n=5) with hydrophilic polypropylene membrane (0.45 m) using water as receptor medium for 54.8 h at 32° C. *Ex vivo* permeation assay was performed in damaged human skin in Franz Diffusion Cells (n=5). As receptor phase was used water kept at 32°C for 27.7 h and stirred continuously. A sample of 300 µL was withdrawn from the receptor compartment at the end of the study. Finally, skin was removed from Franz Cells, cleaned with gauze, soaked with sodium lauryl sulfate and rinsed with distilled water. The permeation area was cut and weighed. MA contained in skin was extracted with water for 20 minutes under sonication. Samples containing antimony were analysed by ICP-OES (Inductively coupled plasma optical emission spectrometry). In both studies percentages of the antimony released, permeated or retained was evaluated. Release study was described in terms of percentage of drug (%Rel). Skin permeation was described in terms of percentage permeated of drug (% Per) and amount (mg) retained of drug by cm²/g of skin (Aret).

Results: The % Rel of antimony was 21.5 ± 3.4. The % Per of antimony was 28.7 ± 2.7, and the Aret of antimony (mg/cm²/g) was 32.2 ± 3.6.

Conclusions: According to the antileishmania activity for the MA gel evaluated *in vitro* on amastigotes (1) the amount retained in human skin will probably be enough to heal comfortable and safety.

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Co-encapsulation of immunosuppressant and anti-inflammatory agents in a Pickering emulsion for the treatment of skin disease

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Abstract

Currently, pharmaceutical emulsions are mostly stabilized by synthetic surfactants, which raises direct or indirect toxicity and environmental issues (Cserhati et al., 2002). In long-term topical treatment, skin irritation is often observed (L'emyer et al., 2015). Alternative stabilization approaches have been developed, such as the use of solid particles. Such emulsions are called Pickering emulsions. Within this context, we formulated Pickering emulsions stabilized with biodegradable and biocompatible poly (lactic-co-glycolic) acid (PLGA) nanoparticles (NP) for a topical use. Such emulsions allow the incorporation of two active pharmaceutical ingredients (API) in the same system, the first one in the NP and the second one in the oil phase. In fact, this novel co-encapsulated system could improve patient compliance by reducing the doses and application frequencies. Furthermore, the API could be delivered with different kinetics and potentially act locally at different levels. We intend to take advantage of this novel co-encapsulated system to improve the treatment of chronic inflammatory skin diseases such as psoriasis or atopic dermatitis. Currently, immunosuppressant or anti-inflammatory agents are used to treat these diseases (Gottlieb, 2005). In this work, innovative Pickering emulsions co-encapsulating two API were successfully prepared, with either cyclosporin A (CysA) or tacrolimus (TAC) encapsulated in NP, and calcitriol (CAL) solubilized in Miglyol, at high drug loading. The influence of the API on the stability, rheological properties, structure and droplets size of the emulsions was assessed. The safety and biological effects of emulsions on keratinocyte cell lines (HaCaT) were also investigated after 5 days of exposure using MTT assay. Viability and proliferation of immune T cells from blood of healthy volunteers were also examined after T cell receptor (TCR) activation by flow cytometer. Results showed that whatever the API encapsulated, emulsions had a similar macroscopic and microscopic structure, identical rheological behavior and exhibited a good stability for at least 55 days. No toxicity on HaCaT cell line was noticed for the emulsions, with or without API as revealed by MTT assay. A significant decrease of TCR-induced Tcell proliferation was observed with emulsions co-encapsulating API, due to an important immunosuppressive activity. Thus, these Pickering emulsions might be promising for the treatment of chronic inflammatory skin diseases.

Polymeric micelle formulations for the cutaneous delivery of sirolimus: a new approach for the treatment of facial angiofibromas in tuberous sclerosis.

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1

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Abstract

Facial angiofibromas (FA) are benign, but often highly unaesthetic, tumors present as pink papules that are characteristic of tuberous sclerosis. Current treatments (e.g. surgery, electrocoagulation or laser treatment) are invasive and are associated with poor patient compliance. However, as the disease involves the mTOR pathway, sirolimus (SIR; rapamycin) has been used off-label for the topical treatment of FA [1]. Unfortunately, there are no approved SIR topical products and the formulations that have been employed are far from optimal for this indication. Considering the physicochemical properties of SIR, the preparation of a patient-friendly and efficient dermatologic formulation is challenging. The aim of this study was (i) to formulate SIR loaded polymeric micelles using D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS), which is approved by the FDA and EMA, (ii) to investigate their ability to target delivery of SIR into the epidermis and upper dermis and (iii) to determine the cutaneous biodistribution of SIR, i.e. the amounts present as a function of depth (resolution at 40 μ m).

An optimized 0.2% formulation (50 mg of SIR per g of polymer) was developed and was shown to be stable for at least 6 months at 4 ° C; micelle diameters were \sim 10 nm. From the micelle solution, a 0.2% micellar hydrogel with a skin-friendly pH of 5.2 was formulated and was stable over a period of 3 months at 4 ° C. Skin penetration experiments were performed using porcine skin and vertical Franz diffusion cells over a period of 12 hours under both infinite and finite dose conditions (500 mg/cm² of micelle formulation and 10 mg/cm² of micellar hydrogel, respectively). These studies enabled the skin deposition of SIR to be quantified and the cutaneous biodistribution to be determined. The 0.2% micelle formulation significantly increased SIR deposition in each skin layer and the total deposition of SIR was a > 3.3-fold greater than the control (0.2% w/w ointment) (142.1 \pm 42.3 and 43.6 \pm 27.8 ng/cm², respectively). Studies with the 0.2% micellar hydrogel demonstrated that SIR deposition in the viable epidermis, which is the target skin layer, was significantly higher than for the control (98.8 \pm 44.0 ng/cm² and 48.6 \pm 24.1 ng/cm², respectively); there was no transdermal permeation.

In conclusion, polymeric micelles enabled SIR bioavailability in the viable epidermis to be improved and a micellar hydrogel may offer a new more patient-friendly option for the topical treatment of FA.

Salido-Vallejo et al.: Actas Dermo-Sifiliográficas, 2014, 105: 558-568

New strategies for improving budesonide skin retention

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Abstract

Corticosteroids are still considered as first-line treatment for many dermatological conditions. Budesonide is a highly potent topical steroid, presently used mainly for the treatment of respiratory diseases and ulcerative colitis. In the early '80s, different studies reported its equivalent or higher anti-inflammatory activity, compared to other steroids, in the topical treatment of psoriasis or atopic dermatitis. Despite this, the development of new formulations for the dermal delivery of budesonide has been poorly investigated. The goal would be to have a formulation able to reduce the penetration of the drug across the skin improving its retention into the skin.

The aim of the present work was the *in vitro* evaluation of poloxamer hydrogels as potential vehicle for the dermal delivery of budesonide. In particular, we focused on the effect of binary poloxamer mixtures on the retention of the drug in the different skin layers. The effect of inclusion of budesonide in hydroxyl-propyl-beta-cyclodextrin was also investigated.

Two hydrogels containing 20% of Pluronic 407, alone or in combination with Pluronic 403, were prepared. Each formulation was loaded with 0.5% of Budesonide, using either budesonide or its inclusion complex with hydroxypropyl-beta-cyclodextrin. All formulations were characterized in terms of drug release and *in vitro* skin retention. Differences in drug release from hydrogel formulations were evaluated according the SUPAC-SS guidelines in vertical diffusion cells across cellulose acetate membrane. *In vitro* skin retention experiments were performed using vertical diffusion cells with pig ear skin as model for human skin. Formulations were applied in finite dose conditions (10 mg/cm²) for 6 h. At the end of the experiments, the unabsorbed formulation was removed, the skin was cleaned and the epidermis was heat separated from the dermis. Budesonide was then extracted from the skin layers with a validated method and all samples were analyzed by HPLC. As reference, two commercial formulations (ointment and cream) were used.

When a binary mixture of Pluronic was used, budesonide skin retention was reduced, due to the lower release from the formulation (as observed in the *in vitro* release test). The use of cyclodextrin inclusion complex did not modify budesonide release and skin retention from both hydrogels. Interestingly, no budesonide was found in the skin from the commercial formulations, probably in line with the lower release. Budesonide found in the receptor compartment was in all cases lower than the LOQ of the analytical method (0.05 µg/ml), suggesting the absence of possible systemic absorption.

Cytotoxicity of lecithin-based nanoemulsions on primary skin cell types and penetration monitoring by ATR-FTIR spectroscopy

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Abstract

As constituents of cellular membranes, lecithins feature high biocompatibility and great emulsifying properties due to their amphiphilicity. Accordingly, naturally occurring emulsifying agents could replace other skin damaging emulsifiers like sodium dodecyl sulfate or sodium laureth sulfate.

We developed lecithin-based nanoemulsions and evaluated their cytotoxicity potential on primary human keratinocytes and fibroblasts using two different cell proliferation assays (MTT assay and BrdU assay). In former studies we examined the effect of nanoemulsions using the MTT assay after an incubation time of 24 h and found that lecithin-based formulations had a less cytotoxic effect on human skin cells than formulations based on conventional emulsifiers like sodium dodecyl sulfate. In this study we examined a shorter incubation time (2 h) and additionally assessed cell proliferation using the BrdU assay which measures the synthesis of new DNA in cells. Also these studies showed that the lecithin-based formulations were superior to conventional surfactants, suggesting higher skin friendliness. To investigate the *in vitro* penetration depth of the deployed lecithins and oil components into the stratum corneum, we employed ATR-FTIR spectroscopy in combination with tape stripping. We identified the components in the superficial layers of the stratum corneum, suggesting that the nanoemulsions may have a low skin irritation potential.

In summary, we developed nanoemulsions using naturally occurring lecithin-based emulsifiers and confirmed their low cytotoxic potential on human skin cells employing two different cell proliferation assays and successfully monitored the skin penetration of the nanoemulsion components.

RELATIONSHIP BETWEEN SENSORY ASSESSMENT AND RHEOLOGICAL PROPERTIES OF A SENSORIAL REFERENTIAL

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1

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Abstract

Standardizing panelists and protocols in nonfood sensory evaluations yields reproducibility and accuracy. To realize this aim, lexicon and referential are required. Indeed, lexicons provide a tool for communication within the panel, while referential exemplifies the attributes. Despite their usefulness, few lexicons are published in the literature. Therefore, our team developed a specific referential to evaluate the visio-tactile features of the skin care products (Lh eritier., 2015). This tool kit (EBITouch R) is composed of 14 formulated or selected raw material covering wide-range of sensorial properties. These later are categorized into 5 categories (appearance, pick up, application, afterfeel 1 minute and 2 minutes).

Recently, a growth of research projects on the correlation of sensory characteristics and rheological properties of cosmetics has been observed (Elezovic et al., 2017). Studies focus either on the characterization of raw material or formulations, but no studies on referential. This work presents the first stage of a research project aimed at characterizing all the references.

Regarding this purpose, rheological measurements were performed on the sticky, non-sticky references. All analyses were performed using a rotational rheometer (Anton Paar, Austria) equipped with parallel 50 mm plate geometry. Flow curves were obtained by varying the shear rate from 0.01 s⁻¹ to 10 s⁻¹ at 20 °C and the power-law model was used for the determination of the consistency parameter and the flow behavior index (Moravkova and Filip, 2013). Furthermore, references have undergone double compression cycle using a texture analyser (Lloyd instrument, Ametek, England) equipped with a 10 mm diameter cylinder probe with a trigger force of 0.3 N.

Interestingly, references showed shear-thinning behaviour which are demonstrated by the decrease of viscosity under the increase of shear rate and a non-Newtonian flow behavior that is traduced by a flow index (n) in the range of 0 - 1. As expected, the consistency value has almost doubled for the non-sticky (251.81) reference in comparison with the sticky one

(96.39).

Additionally, the textural profile analysis showed a significant difference ($p < 0.05$) between references. For instance, the instrumental values of the stickiness, and penetration work of the probe for the sticky reference were found to be equal to 0.49 N and 0.02 J, respectively, higher than values measured for the non-sticky reference (0.03 N and 0.001 J for both stickiness and penetration work, respectively). These relevant results suggest that the formulation of these references was successful for the discrimination of the stickiness perception.

Nucleic acids skin penetration enhancement: a combined approach of deformable liposomes and microneedles

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Abstract

There is more and more interest in the use of nucleic acids carried by non-viral vectors to cure topically several skin diseases. Indeed, topical administration displays a lot of advantages compared to systemic administration [1]. However, cutaneous administration of large macromolecules such as nucleic acids is impeded by the stratum corneum (SC) acting as an efficient barrier. To overcome this drawback, deformable lipid-based nanovectors have been developed by adding ethanol (ethosomes) and/or edge activators (Transfersomes R) in the formulation [2]. Previous work of our team showed that deformable liposomes (containing both ethanol and edge activator) were not able to pass through the intact skin. Nevertheless, deformable liposomes were able to go deeper in the dermis once the SC was removed. Thenceforth, there is a need to bypass this barrier to achieve *in vivo* feasibility. For this purpose, the application of deformable liposomes combined with the use of microneedles (MNs) is appropriate to reach different skin layers.

The aim of this study is to evaluate the interest of associating deformable liposomes or ethosomes with MNs allowing to circumvent the SC barrier. Since the SC will be bypassed, the remaining question is the consistency of the use of deformable liposomes containing ethanol and edge activator supposed to be useful to improve the penetration through the SC.

The *ex vivo* effectiveness of MNs to perforate the skin was assessed on skins placed on Franz diffusion cells. Different lengths of MNs were tested to determine which design allows the deeper penetration. In addition, we compared, on a perforated skin, the diffusion of liposomes, ethosomes (containing 25% v/v ethanol) and deformable liposomes (containing ethanol and 15% w/w sodium cholate as edge activator) into the dermis.

We highlighted that the use of ethanol and sodium cholate seems necessary to enhance the penetration and diffusion into the dermis even if SC is bypassed by means of 1400 μm length microneedles. Hence, this study provides a promising approach to carry nucleic acids into the dermis as an innovative treatment.

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POSTERS

QbD approach in cosmetic formulation - development of oral care products

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1

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Abstract

INTRODUCTION

The development of a cosmetic product using an experimental design represents a systematic approach that allows to set the best ranges for the formulation factors with a minimal number of experimental runs. This new approach in the field of cosmetic formulation provides novel inputs to formulators in order to choose the appropriate ingredients in an accurate range to obtain the product that satisfies consumer wish.

The aim of the present study was to prepare a optimal toothpaste formulation using an experimental plan to select the appropriate range of the quantitative and qualitative parameters.

MATERIAL AND METHODS:

The experimental work was conducted according to a factorial experimental design with three factors and three levels (Modde 12.1 software, Umetrics, Umea, Sweden). The studied factors were the percentage of silica (15-25%), the percentage of xanthan gum (0,5-1,5%) and the percentage of sodium carboxymethylcellulose (0-1%). The software generated 14 experimental runs and 3 centered points and the experimental trials were performed in triplicate. The dependent variables have been: firmness, consistency, rigidity, adhesiveness, stringiness evaluated by texture analysis (Brookfield CT3 Texture Analyzer) and viscosity determined with a Brookfield cone-plate CAP2000 Viscometer. Quality-of-fit assessment, coefficient calculation and statistic parameters evaluation were performed with Modde 12.1 software (Umetrics, Sweden). Based on the experimental results, an optimal formulation was generated and analyzed.

RESULTS:

Silica and sodium carboxymethylcellulose revealed a positive influence on the consistency, firmness, adhesiveness and viscosity values. In addition, an interaction between silica and sodium carboxymethylcellulose and also between silica and xanthan gum was observed. Therefore, combining high ratios of hydrophilic polymer with high silica amounts leads to a decrease in consistency, firmness, rigidity, stringiness and viscosity values. Finally, the validation of the model was carried out by preparing an optimal toothpaste formulation containing 15% silica, 0,5% xanthan gum and 1 % sodium carboxymethylcellulose; a good correlation between the model predicted and the experimental response was obtained.

RESULTS:

The present study showed the advantage of using QbD to set the variables influencing the preparation process and to determine the optimal level of formulation parameters for developing oral care products.

ACKNOWLEDGEMENTS: This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI2017-0251/4PCCDI/2018, within PNCD III.

Development of a mouthwash containing polyphenolic extracts from *Vitis vinifera* by-products

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1

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Abstract

INTRODUCTION

Vitis vinifera by-products represent a valuable source of bioactive compounds with potential applications in the pharmaceutical and cosmetic field. Recently, wine-making by-products are subjected to intensive research, due to its bioactive potential, knowing that over 70% of the polyphenols remain in the pomace.

The aim of the present study was to determine the optimal conditions for the preparation of a mouthwash containing polyphenolic extracts from *Vitis vinifera* by-products based on an experimental design. In a previous study, the polyphenolic extract from *Vitis vinifera* by-products, pomace and canes, was obtained under optimal extraction conditions and phytochemical profile and antioxidant properties have been determined.

MATERIAL AND METHODS:

The preparation process was carried out based on a factorial experimental design developed by Modde 12.1 software (Umetrics, Umea, Sweden) using three independent variables: percentage of ethanol and xanthan gum and the stirring rate during the preparation process. The experimental trials were performed in triplicate for all 11 combinations and the results have been evaluated by means of statistical analysis using ANOVA test. The instrumental characterization of the mouthwashes was performed using Brookfield CT3 Texture Analyzer (consistency, stringiness, adhesiveness) and rheological measurements were determined with a rotational rheometer Brookfield DV-III Ultra. Organoleptic characteristics have been visually evaluated on a VAS scale and the stability of the samples was also determined.

RESULTS:

According to the experimental data, the percentage of xanthan gum influenced the physical properties of the mouthwashes, increasing the consistency, adhesiveness, stringiness and viscosity values and reducing the organoleptic characteristics of the products. The stability was also influenced by the xanthan gum concentration and by the stirring rate. Further, the optimal characteristics of a commercially available product were set as restriction criteria for the optimization and the optimal formulation was prepared and analyzed; a good correlation between the model predicted and the experimental response was obtained.

CONCLUSION:

The study achieved the optimum experimental conditions for the development of a mouthwash containing polyphenolic extracts from *Vitis vinifera* by-products.

ACKNOWLEDGEMENTS: This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI2017-0251/4PCCDI/2018, within PNCD III.

Synergising excipients to boost skin delivery.

A case study with lidocaine hydrochloride.

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1

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Abstract

Skin delivery remains the most difficult pathway for drug absorption since the *Stratum Corneum* forms an effective barrier against the environment. To improve the skin penetration of drugs, different strategies can be adopted, such as the use of chemical penetration enhancers (CPEs)*. The exact mechanism of CPEs is not yet fully elucidated, however they could be divided into 2 groups: solubility enhancers, by increasing the gradient of drug diffusion, or penetration facilitators, by disturbing the lipid domain of the *Stratum Corneum**. The challenge is to find the right match between the drug and the CPEs to achieve the target solubility and diffusion/permeation into the skin.

Diethylene glycol monoethyl ether (Transcutol R P, TC), is used for solubilization and propylene glycol monolaurate (Lauroglycol™ FCC; PGML), isopropyl myristate (IPM) are the non-polar excipients used in this study as diffusion enhancers. They were tested alone or in mixtures with lidocaine hydrochloride (LID HCl), a local anesthetic.

High Performance Liquid Chromatography (HPLC) was used for the evaluation of drug concentration. The drug solubility of LID HCl was found to be higher in hydrophilic vehicles and the rank order was: Water > TC > PGML > IPM.

An *in-vitro* permeation study was conducted using vertical diffusion cells (Franz cells, StratM membrane, 6 Franz-type diffusion cells diffusion area of 1.76 cm²). LID HCl was added in each vehicle or mixtures at 25% of its saturated concentration to compare the diffusion performance with the same thermodynamic activity. The results highlight the efficiency of a binary mixture compared to a single vehicle system, providing higher diffusion LID HCl after 8 hours, with 31.1 ± 9.5% of the applied dose for the mixture TC/PGML, 22.0 ± 4.0% for TC and 5.0 ± 1.1% for PGML. Very fast drug diffusion was observed in all mixtures with uptake less than 1 hour, whereas a lag time around 3 hours was observed for single vehicles. The binary system TC/PGML (7/3, w/w) showed the highest skin permeation enhancement after 24 hours with 48.3% of the applied dose. TC increased drug solubility in the formulation, whereas PGML pushed the drug to partition into the membrane. PGML provided better diffusion result compared to IPM when associated to TC at the same ratio.

This study highlights the synergic effect of combining TC and PGML to increase diffusion of LID HCl through a membrane.

Development and evaluation of novel monolayered transungual delivery system for itraconazole

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Abstract

Introduction: Onychomycosis accounts for about 50% of all nail diseases and affects 2–13% of population worldwide. It is fungal infection affecting nails, both fingernails and toenails. The treatment options available for this diseases are very limited and are associated with one or more issues. The major hurdle in the treatment of this diseases is the delivery of drugs into thick and hard nail plate. Thus, there is an acute need of developing new strategies, improved delivery systems and formulations. The current work is an attempt to address aforementioned issue and deals with the development of a monolayered lacquer for nail employing itraconazole as a model drug.

Methods: The formulation trials were initiated by screening various polymers and plasticizers. Further a diverse variety of penetration enhancers alone or in combination were screened to obtain improved drug delivery across the nail. To prepare nail lacquers, the selected ingredients were mixed with drug in blend of organic solvents. The prepared lacquer were further characterized and evaluated for different parameters. The permeation profiles of drug was evaluated using human nails. The developed lacquers were further tested for anti-fungal activity using *Candida albican*.

Results: Eudragit and triacetin were selected as polymer and plasticizer, respectively for forming a film/layer on nail. Urea in combination with salicylic acid, in 1:1 weight ratio, were found to be appropriate penetration enhancer. Confocal microscopy, revealed extensive distribution of the fluorescent dye across the human nail plate through the developed lacquer in comparison to control that was restricted in the top layer. The developed lacquer has shown higher drug permeation (@ 3.5 times) vis-`a-vis drug solution. When compared with marketed nail lacquer the developed matrix nail lacquer was found have significantly higher anti-fungal activity.

Conclusion: Conclusively, an efficient and stable nail lacquer was developed for potential transungual delivery of itraconazole to the nail bed which can ensure better efficiency against onychomycosis.

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Acknowledgments: This work is financially supported by Science and Engineering Research Board, New Delhi, India under Start Up Research Grant (Young Scientist).

Characterization and topical delivery of terbinafine

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Abstract

Skin fungal infections are one of the most common disease forms worldwide. Various topical formulations of terbinafine (either as the base or hydrochloride salt) are currently available for management of such conditions. As there is limited data in the literature relating to the physicochemical properties of the base, the initial goal of this work was to conduct a comprehensive characterisation of the molecule and subsequently to develop novel formulations with improved skin delivery compared with a commercial terbinafine base preparation. Terbinafine base was synthesised in house followed by nuclear magnetic resonance (NMR) analysis. The base form was characterised using Differential Scanning Calorimetry (DSC). In order to develop novel topical formulations for terbinafine base, a number of solvents, namely isopropyl myristate (IPM), propylene glycol monolaurate (PGML), Transcutol R (TC), propylene glycol (PG), polyethylene glycol 200 (PEG 200), oleic acid (OL) ethanol (EtOH) and isopropyl alcohol (IPA) were selected. Solubility and stability studies were also conducted. Subsequently, ternary and quaternary formulations (1% w/w) were prepared and evaluated with Franz type diffusion cells and mass balance studies in porcine skin. NMR spectroscopy confirmed the complete conversion of the hydrochloride salt to terbinafine base. DSC analysis showed one endothermic event for the base indicating that the melting point is 41.3 ° C. Solubility studies confirmed that terbinafine is soluble at a concentration of 1% (w/w) in the selected solvents. The molecule exhibited excellent stability in all solvents over 5 days with no degradation issues. The permeation and mass balance studies indicated that ternary (IPM:PG:IPA, IPM:PEG 200:IPA) and quaternary formulations (PG:TC:IPM:IPA and PG:PEG 200:IPM:IPA) outperformed the commercial terbinafine gel (one-way ANOVA, *p < 0.05). The next stages of the work will focus on further development of these formulations with evaluation of their performance *in vitro* using human skin. Optimised formulations will ultimately be investigated *in vivo* with human subjects.

Topical spironolactone in a natural non-ionic emulsifier-based vehicle – a safety assessment

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Abstract

Spironolactone is a synthetic steroidal diuretic, which is being used in dermatology as a topical therapy for acne and hirsutism (1). Currently, spironolactone is not approved by the FDA/EMA for dermatological indications; *alternatively, compounded topicals with spironolactone are offered*. There are no data on cutaneous safety of topical spironolactone. The appropriate selection of emulsifying excipient is important in compounding practice. *Cetearylglucoside and cetearyl alcohol* is FDA certified as "*Alkyl glucoside*" surfactant intended for topical formulations. Emulsions with this mixed emulsifier provide additional hydration which is essential in maintaining healthy skin (2). Patch test is useful in predicting the safety of topical drugs; this tool can even detect irritation without being affected by anti-inflammatory effects of investigated drugs (3).

Following the preformulation tests, we prepared P (placebo emulsion) and P-S (emulsion with 5% spironolactone) using "*Alkyl glucoside*" surfactant. The safety of the samples was assessed in a 24h patch test on human volunteers under the supervision of a dermatologist, employing objective *in vivo* biophysical measurements which could be used in patch test. The parameters measured baseline, as well as after removal of 24h occlusion were: stratum corneum hydration (SCH), transepidermal water loss (TEWL) and erythema index (EI). Untreated skin was used as control- (UC) and skin under occlusion, without the treatment (UCO).

Both samples showed a satisfactory safety profiles. After 24h, there was no statistically significant increase of TEWL and EI vs. baseline, as basic biophysical parameters that could indicate the skin irritation. Certain increase in TEWL was registered at all test sites, with the exception of UC, which indicates the connection of TEWL increase to skin occlusion. A significant increase in the degree of hydration after the treatment for both samples was observed; it was not significant on UCO.

Our results indicated that 5% spironolactone in emulsion based on alkyl polyglucosides could be preliminary considered as safe for topical use. Further investigations are needed. The investigated emulsions also show the ability to improve skin hydration state, which can be very useful in the acne treatment.

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Understanding the synergistic action of propylene glycol and Transcutol R P on dermal delivery of methadone

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Abstract

Introduction:

The use of methadone for the management of pain has received great interest in recent years. Currently, oral and intravenous formulations are available for clinical use. Emerging studies revealing peripheral actions of methadone indicate that patients may benefit from dermal delivery which can contribute to improved safety profiles. The objective of the present work was therefore to develop novel dermal formulations of methadone that would target pain in a safe and efficacious manner.

Methods:

A range of solvents including d-limonene (LIM), ethyl oleate (EO), octyl salicylate (OSAL), Transcutol R P (TC) and propylene glycol (PG) were used to prepared binary systems for dermal delivery of methadone. *In vitro* permeation and mass balance studies were conducted under finite dose conditions (5 $\mu\text{L}/\text{cm}^2$) in porcine skin to evaluate the efficacy of these binary formulations. New high performance liquid chromatographic (HPLC) method and gas chromatography (GC) methods were developed and validated for the analysis of methadone and solvents.

Results

Promising penetration enhancement was observed for all binary formulations compared with neat solvents. PG:TC (50:50) was the most effective formulation of the binary formulations tested ($p < 0.01$). Compared with neat TC, a 40-fold increase in the cumulative amount of methadone permeated was observed for PG:TC (50:50) after 12h. To investigate the effect of PG and its synergistic action with TC on permeation, the skin uptake of PG and TC were analyzed using GC. The results showed that the inclusion of PG in the binary formulations clearly facilitated the permeation of TC. TC has excellent solubilizing properties and methadone appeared to "track" the permeation of TC. It is likely that the presence of PG increased the permeation of TC which further enhanced the permeation of methadone.

Conclusion:

Understanding the fate of excipients provides insight into the mechanisms of permeation enhancement. Ongoing work will focus on further formulation optimization and preliminary studies *in vivo*.

Preparation, characterization and dermal delivery of methadone

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Abstract

The use of methadone for the management of pain has received great interest in recent years. Currently, oral and intravenous formulations are available for clinical use. Emerging studies revealing peripheral actions of methadone indicate that patients may benefit from dermal delivery which can contribute to improved safety profiles and high local concentration. We hypothesize that methadone free base should be more permeable than its hydrochloride salt and should be a suitable agent for transdermal administration.

The first stage of the work required the preparation of methadone free base, as confirmed by nuclear magnetic resonance (NMR). Methadone free base and its hydrochloride salt were then characterized using a series of studies including thermal analysis, distribution coefficient determination and solubility measurement. A new high performance liquid chromatographic (HPLC) method was developed and validated for methadone. *In vitro* permeation and mass balance studies were conducted under finite dose conditions ($5 \mu\text{L}/\text{cm}^2$) in porcine skin and human skin to identify potential candidate components for dermal formulations. The melting points for methadone free base and its hydrochloride salt were observed to be 76.38°C and 236.92°C respectively. Compared with the hydrochloride salt, the free base generally had good solubility in a wide range of solvents. The highest cumulative amount of methadone permeated from d-limonene (LIM) at 24 h, in both porcine and human skin, 41.32 ± 4.66 and $13.32 \pm 3.42 \mu\text{g}/\text{cm}^2$ respectively. Ethyl oleate (EO), Transcutol R P (TC) and octyl salicylate (OSAL) also appear to be promising candidate components of dermal formulations for methadone base. To our knowledge, this is the first *in vitro* permeation study to evaluate the permeation of methadone through porcine and human skin under finite dose conditions. Ongoing work will focus on formulation optimization and delineating mechanisms of permeation enhancement for specific vehicles.

FMX101 4% topical minocycline foam: drug delivery into the pilosebaceous follicles, sebum softening effect and bacterial susceptibility

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Abstract

Purpose: Characterize skin penetration profile of minocycline from FMX101 4%, test the impact of FMX101 4% on the physical properties of model human sebum and investigate susceptibility and acquired resistance of *Cutibacterium acnes* to FMX101 4% topical minocycline foam.

Methods: Skin penetration studies were performed with excised human skin, utilizing MedFlux-HTTM flow-through diffusion cell system. Following exposure to FMX101 4%, skin samples were extracted and the amount of minocycline was determined by LC-MS/MS. Mixtures of FMX101 4%, FMX101 vehicle or an oil-in-water emulsion with model sebum were analyzed by differential scanning calorimetry (DSC), rheometry, and light microscopy. Susceptibility of *C.acnes* to FMX101 4% was tested by broth and agar dilution. Spontaneous resistance in clinical isolates of *C.acnes* by direct selection, and after serial passages was measured.

Results: skin application of FMX101 4% resulted in less than 1% of the applied dose of minocycline permeating through the skin barrier, while the concentrations in the dermis and epidermis reached 1680ng to 4818ng of minocycline per cm² of the skin surface after 24 hours of exposure. Importantly, the sebaceous appendages constituted approximately 50% of total delivery into the skin layers (dermis and epidermis combined). Model sebum displayed a peak melting temperature at ~ 37 ° C. Mixing sebum with FMX101 (4% or vehicle) lowered the peak melting temperature to below 35 ° C and decreased sebum viscosity. Conversely, mixing sebum with oil-in-water emulsion increased the peak melting temperature to > 37 ° C. Rheological test results supported observations obtained by DSC. Light microscopy evaluation demonstrated that sebum and FMX101 were easily intermixed, whereas sebum and oil-in-water emulsion were immiscible. FMX101 4% and minocycline had MIC₉₀ values of 0.25/0.5µg/mL. Spontaneous resistance to FMX101 4% in 7 strains of *C.acnes* occurred at a frequency of < 1×10⁻⁸. Minocycline retained potent antibacterial activity against *C.acnes* over 15 serial passages; thus, no selective growth advantage for minocycline-resistant mutants occurred under these conditions.

Conclusions: FMX101 4% demonstrated significant skin penetration of minocycline in epidermis and dermis, with a focus delivery to the sebaceous appendages. FMX101 (4% and vehicle) demonstrated miscibility with sebum. Such sebum softening effect may reduce clogging of the pilosebaceous unit and explain the improved delivery of minocycline. FMX101

4% has a favorable microbiologic profile against *C.acnes*. The MIC₉₀ of FMX101 4% was 4-fold lower than bacitracin and tetracycline, and 8-fold lower than clindamycin. Spontaneous resistance or development of second-step mutations due to exposure to FMX101 4% occurred at a low frequency.

In vitro crisaborole skin distribution after topical application

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Abstract

Atopic dermatitis (AD), known also as atopic eczema, is a chronic inflammatory condition of the skin, which affects up to 5 % of adult population, and 15-20% of children. The main treatment for AD for many years has been topical corticosteroids.

Crisaborole is non-steroidal phosphodiesterase 4 inhibitor and represents the first non-steroidal medication approved for the treatment of atopic dermatitis (AD) in over a decade. Clinical trials demonstrated its efficacy and safety, improving the quality of life of patients. Crisaborole was approved in 2016 by the FDA for the treatment of AD in adult patients and launched in 2017 as 2% ointment (Eucrisa R); in Europe it is under evaluation by the EMA.

The aim of this work was to study the delivery of crisaborole to the skin in vitro, from a 2% ointment. Objectives of the study included the determination of crisaborole distribution in the various skin compartments (stratum corneum, epidermis, dermis) and the evaluation of penetration across the skin, to estimate the risk of systemic effects. An additional objective was to set up and validate a simple method for quantitation of crisaborole in skin layers.

The experiments were carried out in vitro, using pig ear skin as a well-accepted model of human skin. At the end of the permeation experiments, the skin was tape-stripped, the epidermis was removed from dermis and crisaborole was extracted and analyzed by HPLC. The developed analytical method was linear from 0.06 to 6 $\mu\text{g}/\text{ml}$ and resulted to be selective, precise and accurate. The extraction from all the skin layers was quantitative.

After 4h of contact with the formulation (2% ointment), crisaborole was found in all skin layers. The skin distribution of the drug included stratum corneum (4%), viable epidermis (17%) and dermis (77%); 2% of the total amount recovered was in the receptor compartment. In fact crisaborole permeated the skin in very small amounts (the flux being approx. $0.2 \mu\text{g cm}^{-2} \text{ h}^{-1}$, with a lag-time of approx. 4 h), as assessed in 24h permeation experiments. In conclusion, after topical application of a 2% ointment, crisaborole was present in all skin layers, with the highest percentage found in the dermis, followed by epidermis and stratum corneum.

Use of silicones in development of a highly persistent sprayable emulsion containing essential oils for treatment of skin common infection

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Abstract

Silicones are currently used in many different healthcare applications, including registered pharmaceutical products (as excipients for topical creams and drug loaded devices) and pharmaceutical process aids [1]. The simultaneous presence of "organic" groups attached to an "inorganic" backbone gives silicones a combination of physicochemical properties as low surface tension and low viscosity which allow good spreadability and unique sensory profile [1-3]. The aim of this study was to develop a sprayable silicone emulsion with high skin substantivity in order to promote long-lasting effect at skin superficial layers of hydrophobic (siliphilic) active ingredients including essential oils.

After a preliminary study using basic paraffin emulsions aiming at identifying the impact of physical and rheological properties on emulsions sprayability, the development of silicone emulsions was undertaken. The development step consisted in combining various silicone ingredients including linear Polydimethylsiloxane (PDMS) fluids, silicone resins (for filmforming properties), silicone acrylates, silicone emulsifiers and co-emulsifiers, silicone gum blends and silicone elastomers with various organic viscosifiers and emulsifiers in water. That led to the selection of four compositions on the basis of emulsion physical aspect and viscosity. Those emulsions were characterized and the emulsification technique was optimized in order to improve the physical and rheological stability. The high substantivity, the water resistance and the wash-off resistance were demonstrated *in vivo* for all optimized emulsions using a suitable IR spectroscopic technic. An *in vitro* water vapor permeability testing of the optimized emulsions revealed the absolute non-occlusive property of one of those emulsions. After approval by ethics committee, an *in vivo* tolerance study performed on healthy volunteers showed absence of skin irritation reactions after several applications of that emulsion. Different methods of incorporation of essential oil with demonstrated antimicrobial properties were then compared based on physicochemical stability of final emulsions; which led to the selection of the best one. The percutaneous diffusion assay of that emulsion using Franz cells demonstrated its suitability for retention of essential oil at superficial skin layers.

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Influence of NaCl concentration on the fabrication of alginate-albumin microparticles by transacylation in emulsion

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1

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Abstract

Our team developed a method for the fabrication of microcapsules using natural polymers that are biocompatible and biodegradable, and for which no use of toxic reagents is needed. This method uses a transacylation reaction to create amide bonds between a protein and an alginic acid ester [1]. The resulting capsules are stable (because covalently bound) and can be used as reservoirs for the delivery of drugs in various domains such as cosmetics or pharmacy [2].

This method uses an emulsification step, the aqueous phase containing both biopolymers being dispersed into an oil phase containing a surfactant. The state of the aqueous phase plays an important role in the fabrication of the microparticles, affecting the feasibility of the microparticles and their structure. It is, in fact, not homogeneous. In the range of polymer concentrations used, a phase separation is observed, surely because of electronic repulsion (both biopolymers carry the same charge) and thermodynamic incompatibility. The resulting mixture is a water-in-water emulsion, each water phase containing a higher concentration of its own polymer [3].

It has been shown that the addition of NaCl has an influence on the aqueous phase, modifying its phase separation and viscosity. NaCl concentrations from 0 to 3200mM have been tested in the aqueous phase in order to understand its mechanism of action. When rising concentrations in NaCl, the turbidity decreases, reflecting a lowering of the phase separation. Concerning the viscosity, the trend is opposite, being lowered at small concentrations of NaCl (lower than 400mM) and increasing above. A higher viscosity could indicate a better cohesion of the aqueous phase, which is consistent with the decrease of the phase separation that has been described earlier.

Adding NaCl to the aqueous phase enables the fabrication of microparticles in a larger range of polymer concentrations. Without NaCl it is impossible to obtain microparticles below specific concentrations in polymers (5% in protein and 2% in polysaccharide). In the presence of NaCl, particles are obtained for concentrations as low as 1.5% in protein and 1% in polysaccharide.

The presence of NaCl permits a better co-localization of the polymers, hence the fabrication of particles for low concentrations in polymer.

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3-O-ethyl-l-ascorbic acid: topical delivery from complex solvent systems

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Abstract

L-ascorbic acid (AA), commonly known as vitamin C, has been widely used in topical formulations for many years as an antioxidant and anti-aging ingredient. However, the physicochemical properties of AA are not optimal for skin uptake and the molecule is also unstable, readily undergoing oxidation on exposure to air. Recently, 3-o-ethyl-l-ascorbic acid (EA) has been developed as a stable vitamin C derivative. This structural modification protects the 3-OH group from ionization, thereby protecting the molecule from oxidation, but also results in significant changes in the physicochemical properties compared with the parent compound, AA. Although EA is widely used in cosmetic products skin permeation of the molecule has not been investigated to date. In this work, the physicochemical properties of EA were experimentally determined, and the stability and solubility of the molecule in selected solvents were examined. Additionally, finite dose porcine skin permeation studies were conducted with EA using a range of complex solvent systems. The vehicles studied were PG:PGML, PG:IPA, and 1,2-P:Lab at various ratios. The most effective vehicles were subsequently used to develop ternary solvent systems by the addition of a lipophilic solvent, either IPM or LabrafacTM. Permeation of EA was evident for all the solvent systems investigated. The formulation of PG:PGML (75:25) was the most effective in terms of percentage EA permeation compared with all other binary systems (41.93 %, $p < 0.05$). With regards to ternary solvent systems, the use of IPM proved to be more effective than LabrafacTM in promoting EA permeation. The optimum ternary vehicle was PG:PGML:IPM, resulting in significantly higher amounts of EA delivered through the skin compared with all other formulations ($p < 0.05$). Following mass balance studies, total recovery of EA was lower than 100 ± 15 % for all vehicles, reflecting partial breakdown of the molecule inside the skin. To our knowledge these are the first studies to report the physicochemical properties of EA as well as *in-vitro* skin permeation data of the compound. Studies are ongoing to identify complex vehicles for synergistic enhancement of EA skin delivery as well as to identify potential degradants of the molecule.

Tamoxifen loaded flexible lipid vesicles: Development, optimization and evaluation

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Abstract

Introduction: Tamoxifen (TAM) is a non-steroidal estrogen receptor modulator indicated in the management of breast cancer. It has also shown efficacy in some skin disorders i.e., psoriasis, keloid and cutaneous melanoma. The major goal of present investigation herein is to explore the alternative route (i.e., topical) for TAM along with its improved delivery. **Methods:** Screening and optimization of the various process and formulation variables was done by applying Taguchi design followed by face centered composite design (FCCD) and the same were validated. The optimized FVs were characterized for morphology, micromeritics, electric properties, degree of flexibility, thermal properties and X-ray diffraction analysis. These were evaluated for in-vitro cytotoxicity by MTT assay employing MCF-7 and HEK001 cell lines. Further, the anti-cancer effect was evaluated by employing DMBA induced skin cancer in mice, while anti-psoriatic effect was evaluated employing mice-tail model.

Results: Vesicles were prepared by the thin-film hydration technique, as this technique provided higher entrapment efficiency and ease of controlling variables. Ratio of Drug: Phospholipid (PL) and PL: Sorbitan monooleate (SM) were found to be most the influential variables. Optimization through FCCD, revealed that maximum entrapment and drug permeation along with smaller vesicle size were obtained at the intermediate levels of TAM:PL and PL:SM. Linear correlations and residual plots obtained between the predicted and observed values for all response variables validated the high prognostic ability of the polynomial models for optimizing TAM-FVs.

Characterization studies revealed the formation of positively charged nano unilamellar FVs having sufficient flexibility to cross the narrow pores of skin without losing their shape and drug contents. The intercalation of TAM within the lipid bilayers was confirmed through DSC and XRD studies.

In-vitro cytotoxicity studies revealed the superior efficacy of vesicular TAM (@ 2 times) vis-à-vis its solution. TAM-FVs showed significant increase (i.e., 37%) in tumor latency and significant decrease in the incidence of tumors (i.e., ~3 fold) vis-à-vis TAM in solution. Evaluation of anti-psoriatic effect on mice tail also revealed analogous results.

Conclusion: In clinical practice, the systemic side effects of oral TAM largely preclude the use of this drug. TAM administered topically, if effective and demonstrating an acceptable safety profile, might be a potential treatment for breast cancer and in complicated dermal

disorders like psoriasis. Accordingly, it can be concluded that the phospholipid-based vesicular systems hold great potential in ameliorating efficacy and safety of tamoxifen using topical route of administration.

Topical application of highly concentrated water-in-oil emulsions: physiological skin parameters and skin penetration *in vivo*

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Abstract

Water-in-oil (W/O) emulsions represent promising drug carrier systems for various dermatological and cosmetic applications. In a previous study, innovative W/O emulsions stabilised by the non-ionic silicone surfactant Dow Corning™ Emulsifier 10 were successfully developed and analysed *in vitro*. The use of the silicone surfactant enabled the preparation of highly stable emulsions with a water volume fraction of up to 80% (w/w).

The aim of this study was to further investigate the emulsions, focusing on the *in vivo* performance. Two formulations were selected; the emulsions were identical in composition except for the oil compound, in order to compare the influence of the oil type on various matters. The effect of repetitive application of the emulsions on physiological skin parameters, such as transepidermal water loss (TEWL) and skin hydration, was assessed. The formulations were applied daily on the volar forearm of ten participants. In regular intervals, the skin parameters were analysed non-invasively. In doing so, beneficial effects or a possible skin irritation potential of the tested emulsions on human skin should be elucidated. Further, *in vivo* penetration studies were performed to assess the drug delivery efficiency for the incorporated hydrophilic model drug fluorescein sodium. To this end, tape stripping studies were performed on the volar forearm of the participants.

The results of the *in vivo* studies showed that the developed emulsions were well tolerated. Overall, repeated application during 4 weeks led to an increase in skin hydration and slightly decreased TEWL values. Skin penetration studies revealed a trend towards higher skin penetration of fluorescein sodium from the emulsion prepared with isopropyl myristate compared to the formulation containing liquid paraffin.

Clotrimazole human skin penetration related with local anticandidal efficacy. Effect of formulation and kinetic skin clearance

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Abstract

Background: Clotrimazole (CLT) is an imidazolic broad-spectrum antifungal against skin or vaginal infections caused by different pathogenic dermatophytes or yeasts. The efficacy of imidazolic formulations is generally evaluated[1] in terms of maximizing skin retention and flux although, in fact, both terms are converse to enlarge drug residence in the specific skin biophase. As an example, *Candida spp.* are found in deep epidermis and an increased transdermal flux across 400 μm human skin is desirable[2]. Otherwise, dermatophytes are prone to accumulate in stratum corneum and, thus, requiring a maximized skin retention and a minimal permeation[3].

Purpose: An 1% CLT nanoemulsion (NE), a multiple W/O/W emulsion (ME) and a commercial O/W cream (CR) were evaluated in terms of *ex vivo* skin permeation (J) and retention (Qr) and antimicrobial susceptibility. Results were comparatively evaluated.

Methods: Skin permeation experiments were run with Keshary-Chien type vertical diffusion cells (400 μm thickness, 300mg donor formulation, ethanol:water:transcutol[®] P (50:40:10_{v:v:v}) receptor solution at 32°C) during 48h. Minimum inhibitory concentration (MIC) values were investigated against *Candida albicans* (ATCC10231), *Candida glabrata* (ATCC66032) and *Candida parapsilosis* (ATCC22019) following the Broth microdilution method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) applied to antifungal agents against glucose-fermenting yeasts. Affordable drug residence times were estimated as the time with drug retention levels above each corresponding MIC based on a stationary zero-order drug clearance.

Results: Similar skin permeations were observed for NE and CR (Qr: 16,92 and 22,72 $\mu\text{g}/\text{cm}^2/\text{g}$, Flux, 0,53 and 0,55 $\mu\text{g}/\text{h}\cdot\text{cm}^2$) while ME gave a higher permeation flux (1.40 $\mu\text{g}/\text{h}\cdot\text{cm}^2$). Skin retentions (n=3) were not statistically different but decreasing as: ME > CR > NE (33.26 \pm 3.56, 28.60 \pm 5.27, 16.92 \pm 3.92 $\mu\text{g}/\text{cm}^2/\text{g}$).

Microbiological testing demonstrated that ME and ME inhibit *Candida spp.* at lower concentrations than CR for strains of *C. glabrata* and *C. parapsilosis*. Similar activities were also observed for *C. albicans* susceptibility in all three formulations. Bearing in mind that ME achieved higher retained amounts of CLT in skin but also a higher drug flux, simulation of affordable CLT residence times showed the highest residence against *C. albicans* for CR (51 h vs. 24 h for ME).

Conclusion:

The highest permeation flux obtained with the ME assures high drug levels in dermis but also a rapid epidermic clearance. Delivery enhancement of clotrimazole depends not only on intrinsical permeation properties but also on specific location of drug in the skinbiophase.

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Variations on human skin permeation and cumulative skin retention of ethylhexyl-methoxycinnamate formulated in different disperse-systems

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Abstract

Background: Exposure to ultraviolet radiation (UV) may cause different skin diseases such as sunburn, photo aging or skin cancers. Ethylhexyl-methoxycinnamate (EHMC) is one of the most used UV filters in personal care products and especially useful in waterproof products due to its high lipophilicity (logP 6.1). Although, toxicity of these substances limits its use due to its unwanted transdermal absorption.

Purpose: Different formulation-types are comparatively evaluated in terms of heat-stability and influence on skin permeation and retention of EHMC.

Methods: Different W/O/W, W/O, O/W emulsion bases were used to vehiculize EHMC at the maximum level permitted by international regulations. Stable predefined emulsions containing the same proportions of organic UV- filter (5%) and non-ionic surfactant glycerol monostearate (ArP165) were compared in terms of skin permeation and retention. Franz-type diffusion cells filled with sink ethanol:water 60:40 were employed with 400 μm abdominal human skin to evaluate EHMC permeation from 15 mg containing formulations. Skin retention was evaluated with organic extraction as optimised previously. Permeation parameters are: drug flux (J), lag-time (Tl) and retained amount of drug (Qr).

Thermal stability was evaluated at ambient, 30, 40 and 50°C during three months storage

Results: W/O/W achieved an EHMC skin retention (Qr) higher than the conventional emulsions suggesting a potentially higher UV-filter efficacy. In terms of tolerability, lag time (Tl) for multiple emulsion resulted to be significantly larger than for the other formulations (8.5 h >> 5.6 h and 4.8 h), meaning a favourable slower penetration able to support an unique application for a day. Conversely, transepidermal flux (J) was also higher than for both conventional emulsions, suggesting a larger absorption risk. Concerning the differential effect of surfactants, it seems to be erratic. ArP165 is a surfactant specially indicated for oily or nonpolar creams. Comparatively, it does not influence in EHMC permeation profile.

Thermal stability in terms of drug content, at high temperatures (> 30°C) was slightly higher in multiple emulsions, although non-significant, than the conventional emulsions.

Conclusions: Multiple emulsions are shown to enhance the epidermal permeation of EHMC UV filter in comparison with W/O and O/W formulations. Its higher absorption-risk must be balanced with its lower penetration rate.

Human skin penetration of azolic antifungals formulated in triphasic multiple emulsions

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Abstract

Background: The habitual pharmacological treatment of skin fungal infections requires an optimal topical administration of azolic antifungals that is challenging due to their adverse physicochemical properties. It is desirable to achieve the highest epidermal penetration and the minimal transdermal permeation. In this sense, multiple emulsions (ME) are particularly useful to vehiculize lipophilic substances with a high content of surfactants able to modulate permeation.

Purpose: Characterize the relationship between the ME formulation of different azolic antifungals and its permeation performance compared with respective commercial O/W formulations.

Methods: Optimised multiple emulsions of similar compositions were obtained by twostep emulsification containing 1% Econazole nitrate, Bifonazole or Clotrimazole. Droplet homogeneity and size distribution were investigated by optical microscopy and also laser diffractometry reporting the standard percentile readings of the distribution, the volumeweighted mean diameter, $D[4,3]$ and volume-surface mean diameter, $D[3,2]$.

Skin permeation was investigated with 0.64 cm² vertical diffusion cells filled up with Ethanol:Transcutol R P:Water(50:20:30 v/v/v). Skin was obtained from leftovers of abdominal plastic surgery. 300 mg samples were applied and experiments lasted for a minimum of 24h. Permeated amounts per unit area (Q_t) were obtained by HPLC-UV. Permeation extent was described with 24h permeated amounts (Q_{24}) and permeation flux (J) calculated by linear regression between Q_t and time.

Drug penetration: At the end of each experiment, skin specimens were rinsed, blotted dry, weighted and minced. Drug extraction was run with fresh receptor solution under ultrasounds. Collected solutions were analyzed and drug retention levels ($\mu\text{g/g}$) were reported.

Results: In all three cases, azolic drugs were dissolved in the intermedial oily phase, whereas they appeared to be suspended in the commercial formulations. ME droplet size and its volume-weighted mean diameter were higher than with the commercial formulation, and resultant skin retention values too. Two variations in surfactants composition were included: larger droplet sizes were achieved in absence of cetyl palmitate and the presence of polysorbate didn't affected droplet size but modified dramatically its permeation performance.

Q_{24} and transdermal flux J of Econazole and Clotrimazole ME are clearly higher than the corresponding values with the commercial formulation, enhancing its deep epidermal penetration according with its anticandidal (yeast) indication. Conversely, polysorbate containing Bifonazole ME achieves a lower permeation and higher skin retention demonstrating an enhancement on its stratum corneum delivery, as required in dermatomycoses.

Conclusions: Multiple emulsions are shown as an efficient formulation option to individualize skin delivery of azolic antifungals in comparison with conventional emulsions.

Photostability of *trans*-resveratrol in topical microemulsions

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1

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Abstract

Resveratrol, a polyphenolic phytoalexin has scientifically proven ability to penetrate the skin barrier and exhibit antiaging activity. This poorly soluble natural antioxidant can mitigate the photoaging through the expression of AP-1 and NF-B factors; it aids in the production of collagen type I and II and decreases hyperpigmentation. Unsurprisingly, the use of resveratrol in cosmetology and dermatology is on the rise. However, the majority of its proven benefits are ascribed to the *trans* analogue, susceptible to photoisomerization, presenting a great challenge in the development of topical formulations. Microemulsions (ME) are recognized for their ability to improve solubility and skin penetration of active ingredients. Our aim was to evaluate different types of ME for the protection of *trans*resveratrol until its delivery to the skin tissue.

Five different ME were developed (water in oil (W/O), oil in water (O/W), W/O with white wax, W/O with polyacrylic acid and ME gel) and loaded with equal amount of *trans*resveratrol. Prepared ME were exposed to aging under stress conditions (irradiation with UVA-light (373 nm) or temperature of 40 ° C). During the process of aging, *trans*-resveratrol content in ME was assayed with HPLC, and antioxidant capacity was assessed with the reduction method of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results were compared with the samples, stored for 90 days at room temperature (21 ° C).

When ME were shielded from light, resveratrol maintained its antioxidant capacity (EC₅₀ 20 mg/mL) regardless of the temperature applied. Its degradation was far more likely in the presence of UVA light. Since the solubility of resveratrol is better in oil, lower EC₅₀ of O/W ME (87 mg/mL) in comparison to W/O ME (122 mg/mL) was observed after 28 days under UVA light. Nevertheless, viscosity of the formulations was proven to be even more important factor for the stability of resveratrol over the outer phase, as all three semi-solid formulations (W/O with white wax, W/O with polyacrylic acid and ME gel) retained 2- to 3-fold better antioxidant activity (EC₅₀ 38-58 mg/mL) over their liquid ME counterparts. Concurrent HPLC analysis of the *trans*-resveratrol content with the DPPH assay demonstrated that ME formulations with resveratrol retain much of their original antioxidant activity even after 90 % of *trans*-resveratrol is gone, implying that antioxidant activity can also be pertained to the *cis*-resveratrol. Our findings indicate great potential of semi-solid ME for the delivery of *trans*-resveratrol, improving its solubility and photostability.

Impact of silicone polymers on pain management drug delivery

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Abstract

Silicones are widely used in various medical and pharmaceutical applications such as wound and scar management devices, topical and transdermal therapeutic systems. Silicones have been successfully formulated as pharmaceutical excipients in dermatological and topical drug delivery forms to improve their efficacy and acceptance by the patients, which are critical requirements in pain management drug.

In our seminar, we will go through recent studies where silicone technologies have been successfully used as topical excipients in dermatological formulations to assess key parameters of topical forms e.g. substantivity and skin permeation, using lidocaine as model drug.

The presentation will review comparative studies which are conducted on a selection of silicone excipient formulated in emulsion. This will cover the impact of silicone chemistry, the impact of formulation ingredients and the impact of the lidocaine solubility on the skin delivery. Those evaluations are combined with various silicone characteristics on skin such as wash off resistance, substantivity and aesthetics benefits.

These studies also emphasize the versatility of silicone chemistry that provides to the formulator of pharmaceutical applications a wide range of technologies with various functionalities. Silicone technologies have been used as topical excipients in dermatological formulations to improve skin delivery and efficiency of drugs.

Ultradeformable cationic liposomes for Vitamin C delivery: development, characterization and drug release studies.

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Abstract

Vitamin C (Vit C) is a potent antioxidant with several applications in the cosmetic and pharmaceutical fields. Vit C can neutralize reactive oxygen species, treat hyperpigmentations and improve collagen biosynthesis. However, the biggest challenge in the utilization of Vit C is to maintain the stability and improve the drug delivery into the skin. In this sense, one strategy widely used to optimize the delivery of actives molecules to the skin is the use of encapsulation techniques. Liposomes are nanoparticles composed of phospholipids, able to penetrate the corneal layer and interact with their lipid matrix, releasing the active. Variations of these vesicles can be obtained by the addition of surfactants. Named elastic vesicles or ultradeformable liposomes, they have the ability to compress through the lipid channels of the *stratum corneum*, which could increase the penetration capacity.

The objective of the present work was to develop and characterize ultradeformable cationic liposomes for the encapsulation of Vit C. For this, liposomes composed of soy lecithin, cationic lipid DOTAP (1,2-dioleoyl-3-trimethylammonio propane) and cholesterol were obtained by mechanical method and homogenized by an extrusion apparatus. Polysorbate 80 was added to the formulation and incubated for 24 hours. The Dynamic Light Scattering experiments showed that the liposomes have a physical stability of at least 10 months and zeta potential of 46 mV. Characterization tests performed through Isothermal Titration Calorimetry and by edge tension measurements on Giant Unilamellar Vesicles (GUVs) indicated that the surfactant molecule is incorporated in the lipid membrane and can modify its properties. Cytotoxicity tests conducted on human keratinocyte cells (HaCaT) by the method of Alamar Blue suggested the almost non-existent toxicity of the liposomal compositions to keratinocytes. Drug release tests were carried out in vertical Franz diffusion cells, aiming to verify the influence of the surfactant molecule in the delivery of Vitamin C.

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The influence of innovative versatile polymers in skin delivery: formulation of diclofenac sodium and permeation study

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Abstract

The interest of topical applications of Active Pharmaceutical Ingredients (API) is well known to achieve direct local therapeutic action and avoid adverse systemic effects. However, it is always a challenge to reach a good compromise between perfect stability of API in its formulated vehicle and optimum bioavailability after application. Innovative rheology modifiers were developed to achieve more versatile structures, able to thicken and efficiently stabilize APIs in a wide pH range and withstand high concentrations of solvents and oils. These rheology modifiers are especially able to stabilize APIs and oils using a cold procedure, even without surfactant addition (cream gel vehicle). The aim of this study was to assess the influence of these rheology modifiers on the percutaneous penetration of diclofenac sodium in different types of vehicle (aqueous or oil continuous phase). As a model drug, widely used for its analgesic and anti-inflammatory properties [1], diclofenac sodium was formulated in emulsions, gels, cream gels and the patented technology Geltrap™. Skin penetration was carried out using Franz cells to compare formulation type and composition, benchmarks were also evaluated in the same conditions. Moreover, the influence of some penetration enhancers was considered, alone or in combination, at different amounts.

Drug permeation was studied using dermatomed human skin from plastic surgery according to the OECD guidance 428 "skin absorption: in vitro method". 30 mg of each formulation was applied on the skin surface without rubbing and replicated twice. Diclofenac sodium concentration in the receptor compartment was measured 24 hours after application.

Globally, formulation type appeared as a driving parameter to control skin penetration: gel < cream-gel Geltrap™ < emulsion. The innovative polymers did not affect the penetration of diclofenac sodium, irrespective of the formulation type neither of their use levels. For instance, in emulsion, results are similar with polymers at different doses (0,5 to 2%) or without polymers at all. This can be considered as a great advantage for the formulator who is able to choose the most suitable viscosity and texture without impact on the drug delivery.

E. Escribano et al., Assessment of diclofenac permeation with different formulations: antiinflammatory study of a selected formula, Eur J Pharm Sci 19 (2003) 203–210

Skin penetration of the anti-inflammatory frankincense extract using bacterial nanocellulose as drug delivery system

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Abstract

Due to inflammation-busting properties, it is claimed that frankincense is an effective treatment for a wide variety of inflammation-related conditions and discussed as a natural substitute for NSAID such as ibuprofen or naproxen. Its dermal application with effective penetration into the skin is of interest for the treatment of e.g. dermatitis, psoriasis and chronic wounds as well as in skin care for aging. Therefore, the biopolymer bacterial nanocellulose (BNC) was used as drug delivery matrix since it already proved to support the wound healing process [1] due to its unique three-dimensional network of nanosized fibers with excellent biocompatibility.

BNC, synthesized by the bacterial strain *Komagataeibacter xylinus*, was successfully formulated with frankincense extract using different techniques (adsorption, vortex and reswelling technique) and additives (like poly(ethylene glycol) and Poloxamers) that accomplished the homogenous and stable incorporation of the lipophilic extract into the hydrophilic BNC. Generating and following Ishikawa diagrams optimized the process of homogenous loading according to the concept of quality by design. Storage experiments showed constant values without loss of drug stability over more than 90 days. The loading procedure did not change the preferential characteristics of the BNC like high water absorption and retention, softness, and pressure stability. Depending on the type of additive, fast release systems for acute wound treatments (e.g. as masks) over up to 1-2 h as well as delivery systems for a prolonged frankincense release for chronic wounds over several days could be acquired. To investigate the amount, depth and distribution of penetrated frankincense in the skin, tape-stripping experiments were performed on porcine skin. Different application modes, application times and the utilization of additives and nanoemulsions were examined and showed correlations, e.g. between selected additive and depth of frankincense penetration.

The obtained results form the basis for the further development in vivo to achieve a tailor-made, enhanced, non-irritant, anti-inflammatory formulation that would serve as a better alternative for the dermal treatment thus providing better patient comfort and compliance.

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Acknowledgement: The authors gratefully acknowledge the Free State of Thuringia and the European Social Fund (2016 FGR 0045) for funding.

Fast inverted oil-in-water emulsions with natural antioxidants: assessment of stability and rheological properties

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1

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Abstract

The fast inverted oil-in-water emulsions, known as SWOP (*Switch-Oil-Phase*) emulsions invert into water-in-oil emulsions when applied to the skin and consequently form a waterresistance layer, favoring their use as cosmetic vehicles in sun protection products (1, 2). Use of a flavonoid dihydroquercetin (DHQ) in these products is interesting since it shows absorption in UV spectrum, besides its antioxidant ability (3). β -carotene (β C) is a well-known antioxidant and in a combination with DHQ could prolong its stability over time. The aim of this study was to incorporate 0.5% DHQ and combination of 0.5% DHQ and 0.2% β C into SWOP emulsions and to investigate the influence of β C on their stability and rheological properties during storage time. The samples of SWOP emulsion base (S) and emulsions with 0.5% DHQ, *i.e.* 0.5% DHQ and 0.2% β C (*SDHQ* and *SDHQ β C*, respectively) were prepared using hot/hot emulsification procedure. The emulsions were characterized by pH, conductivity, DSC, and rheological evaluation during the storage time (48 h, 30 days, 1 year) at room temperature. The pH values changed from \sim 5.3 (for all the emulsions, after 48 h) to 5.89, 5.87 and 5.74 for S, *SDHQ* and *SDHQ β C*, respectively (after 1 year). The emulsion S had the conductivity higher and less changeable ($1665 \mu\text{S}\cdot\text{cm}^{-1}$ /after 48 h, *i.e.* $1612 \mu\text{S}\cdot\text{cm}^{-1}$ /after 1 year, respectively) than *SDHQ* ($1675 \mu\text{S}\cdot\text{cm}^{-1}$ /after 48 h, *i.e.* $1060 \mu\text{S}\cdot\text{cm}^{-1}$ /after 1 year, respectively) and *SDHQ β C* ($1162 \mu\text{S}\cdot\text{cm}^{-1}$ /after 48 h, *i.e.* $372 \mu\text{S}\cdot\text{cm}^{-1}$ /after 1 year, respectively). In DSC curves endothermic peaks appeared in the same temperature range (100 – 120 °C) for all the emulsions. However, the differences in their enthalpies (-0.66×10^3 J/g, -1.01×10^3 J/g and -0.9×10^3 J/g for S, *SDHQ* and *SDHQ β C*, respectively) indicated slightly better stability of *SDHQ β C* in comparison with *SDHQ*. Rheological measurements revealed decrease in maximal apparent viscosities from 15.31 Pas, *i.e.* 15.23 Pas to 6.47 Pas, *i.e.* 5.36 Pas for *SDHQ* and *SDHQ β C*, respectively (after 1 year), and "shear-thinning" plastic flow behavior with a moderately pronounced thixotropy. The presented results revealed that stability and flow behavior of SWOP emulsions were influenced by DHQ during testing period, even in sample containing β C. Due to their specific structural characteristics, stability of SWOP emulsions as cosmetic vehicles probably would be improved by incorporation of additional antioxidants.

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”Modified bacterial cellulose as tailor-made delivery system for lipophilic agents”

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Abstract

Bacterial cellulose (BC) is a fascinating and sustainable hydropolymer with high potential in value added applications such as wound and skin care products or drug delivery systems (1, 2). It is characterized by a regular nanostructured cellulose fiber network, outstanding loading capacities for water and active ingredients, mechanical stability and excellent biocompatibility.

Many BC based skin products (e.g. for photodynamic therapy, laser or micro needling after treatment, modern wound care as well as for cosmetic applications) would benefit from a higher loading capacity of the hydrophilic material with natural, often lipophilic, substances. That is why, *post-* as well as *in situ*-modifications of BC during the bioprocess have been investigated in detail. At this, *post*-modifications selected should lead to a more lipophilic fiber network while *in situ*-modifications should provide increased pore sizes. Both modifications were tested regarding its effect on lipophilic model substance uptake and release behaviour, before the results were transferred to natural substances of interest.

BC was synthesized by the bacteria strain *Komagataeibacter xylinus* (DSM 14666) at 28 °C for seven days. For *in situ*-modification, different additives were tested. As a main result, the pore sizes of the cellulose network could be varied in the range of 2-10 µm. In case of *post*-modification, acetylation or oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and subsequent conjugation with more hydrophobic compounds was investigated. In case of both, *in situ* as well as *post*-modification, *in vitro* toxicity test MTT assay proved preserved biocompatibility of the modified biomaterial. Clear differences in loading capacities for model substances of varying lipophilicity and release profiles could be observed, underlining the potential of modified BC as controlled agent delivery system. These findings are now used in a collaborative project called InflammAging for the incorporation of lipophilic agents such as frankincense extract into BC for innovative and sustainable product design based on highly active natural compounds.

Acknowledgement

We gratefully acknowledge the Free State of Thuringia and the European Social Fund (2016 FGR 0045) for funding.

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New approach for the formulation of gels for topical administration: Study of the stability of a mixed thermogelling gel based on poloxamer 407 / native starch glycerol

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Université Félix Houphouët Boigny; BPV 34 Abidjan 01. Côte d'Ivoire Abstract – Côte d'Ivoire

Abstract

Justification. Native starches and their derivatives are among the most used excipients in pharmacy. However, their stability, that is to say the state of the product capable of remaining in a permanent equilibrium during its storage and its use over time must not be altered.

Purpose: This study aims to evaluate the stability of a mixed poloxamer 407 / glycerol mixed starch gel

Material and methods. Various formulations of poloxamer 407 gels and sweet potato starch glycerol were made. Preliminary stability tests were performed and selected an appropriate formulation containing 20% glycerol / poloxamer 407 (2.5% / 10%). The parameters studied were the macroscopic and physicochemical aspects and Haake viscometer rheology tests, followed by a 28-day stability study

Results. The pH of the gel was on average 6.75 ± 0.2 at 5°C and 6.72 ± 0.16 at 25°C . The mixed gel was homogeneous, with a particle size of 1.71 micron at 5°C , 1.69 micron at 25°C , thixotropic and rheofluidifier with a T_g of 17°C . The gel remained stable for 28 days of storage at 25°C

Conclusion. Glycerol starch did not modify the behavior of poloxamer 407. The mixture could serve as a reservoir of active ingredients for cutaneous applications

Keywords: gel, poloxamer 407, sweet potato, stability

Preformulation studies of hydrophylic cream bases with moisturizing properties formulated with polyglyceryl-3 methylglucose distearate

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Abstract

Oil-in-water emulsions play a dominant role in cosmetic skin care products. They are used in various skin care formulations such as facial creams, body lotions, sun care lotions, antiperspirant/deo roll-ons or even in some make-up foundations. The proposed formulations contain lipophilic phase between 20-30% and Polyglyceryl-3-methylglucose distearate (PGMGDs) as emulsifier in concentrations of 3-4%. PGMGDs has a very good compatibility with all types of oils including polar oils [1]. To ensure optimal consistency, stearic acid and cethyl palmitate were added in a concentration of 3-4%. Through the analysis of the rheological properties, was obtained a stable and easy-to-apply cream. Stearic acid and cethyl palmitate form liquid crystalline structures in the aqueous phase and the emulsion system will be stabilized [2]. The formulations proposed as creams bases were characterized in terms of homogeneity by visual examination, by touch textural analysis and appearance (color). Also, were determined the pH, spreadability, adhesiveness and viscosity of formulations. Optimum cream bases were tested on female human subjects with different Fitzpatrick phototype and with dehydrated skin on the inside of the forearms. The skin compatibility and hydration grade achieved after application of the cream were evaluated. Under the experimental conditions adopted in the screening test, the single application of the proposed products showed a very good skin compatibility. The evaluation of the hydration grade of the stratum corneum (Corneometer R CM 825 Courage + Khazaka) indicated that the products have an moisturizing effect four hours after application. The use of an experimental design to formulate a cosmetic product, allows to set the best ranges for the formulation and technological factors that influence the preparation of the O/W cosmetic emulsions. References 1. Mukherjee S, Yang L, Vincent C, Lei X, Ottaviani MF, Ananthapadmanabhan KP, A comparison between interactions of triglyceride oil and mineral oil with proteins and their ability to reduce cleanser surfactant-induced irritation, 2015, Int J Cosmet Sci, 37(4):371-82. Vasiljevic D, Dyekic L, Primorac M., Long-term stability investigation of o/w cosmetic creams stabilized by mixed emulsifier, 2012, Hemijska industrija 66(6):871-878

Development and evaluation of a vitamin loaded thermosensitive hydrogel for the treatment of skin burns

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Abstract

Background: Skin burns are severe injuries causing high rates of morbidity and mortality¹. Therefore, important efforts have been focused on the research of effective therapies for cutaneous wound healing. Chitosan is a polymer widely used in biomedical applications, it presents inherent analgesic, hemostatic and microbial effects²; and it is commonly used with hyaluronic acid

Purpose: The aim of this study was to develop, characterize and evaluate a thermosensitive poloxamer-chitosan-hyaluronic acid gel for the treatment of skin burns.

Methods: The thermosensitive gel was elaborated by adding Chitosan (CHT) and poloxamer (P407) to a solution of hyaluronic acid (HA), using the cold method under continuously stirring for 24 hours, vitamins A, D and E were dispersed in the polymers solution. Among the parameters evaluated in the physico-chemical characterization, authors report (i) time and temperature of gelation, (ii) rheological studies, and (iii) morphological studies. To assess the former, temperature of the gel was increased from 4 to 37°C, under stirring (400 r.p.m.). The temperature and time of gelation was established as the one that the magnetic bar stopped moving. Rheological measurements were conducted in hydrogels (HG) after 24 h of preparation, rotational and oscillatory determinations were carried out by a Haake Rheostress 1 rheometer. Morphological studies were performed by Scanning electronic microscopy (SEM). The wound healing efficacy of the HG was evaluated by burn inducing in mice. The formulation was applied topically once a day during 10 days. 4 groups were evaluated (control without treatment, blank HG, the developed HG and Silvederma R as reference formulation. A histological analysis of the burn wound was conducted, 5 µm sections were stained with hematoxylin-eosin and observed by microscope.

Results: HG showed a gelation temperature of 32°C and thermogelling time of 1.3 min. The rheological evaluation of the HG showed that the HG changed the viscoelasticity behaviour with temperature (thermosensitivity). SEM analysis showed that the HG formed a porous network with micellar disposition, which led to a homogeneous pore size around 500 nm and micellar sizes of about 60 nm. Observation of the histological sections showed the HG induced a re-epithelialization of the skin similar to Silvederma R

Conclusions: The thermosensitive hydrogel loading vitamins exhibited suitable physicochemical properties and important biological effects on wound healing. Thus, the HG developed is a great candidate to investigate in further clinical studies.

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SPRAY: Spectacular Polymer gels Release Ability

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Abstract

Users need some proof of efficacy about claims to choose goods of consumption, and this applies particularly to the field of cosmetics. The product consisting of the compound and its packaging contributes to the effectiveness and even the safety: whether in the dermatopharmaceutical or cosmetic field, the quality and quantity of compound delivered on the skin are essential, so the choice of formula delivery method, also the packaging, is part of its development. The spray is a mode of delivery commonly used in cosmetics.

Seppic, in its quest to provide functional ingredients as versatile as possible, was interested in the quality of sprays obtained from aqueous gels of two polymers. With the same chemical structure, the difference between the two polymers lies in the polymerization process[1].

In a first step, a simple and rapid characterization demonstrates the sprayability of the two gels. Secondly, the question was "Is it possible to discriminate sprayed gels by measuring specific parameters?". The shadowgraphy technique allows to obtain spectacular images and videos and speaking to all public; R&D Vision has developed algorithms for image processing in order to determine, in an objective and quantitative manner, certain characteristics of a spray (cone of the spray, length of penetration, wettability / impact)[2,3,4]. The varied and accurate measurements allowed distinguishing the gels' sprays with some discriminant parameters.

Whereas the viscosity at high shear brings results of the same magnitude, the third step was dedicated to assess the influence of interfacial and viscoelasticity phenomenon thanks to rheology measurements performed at URCOM laboratory[5].

At last, the sensorial evaluation of the spray has completed the study.

To conclude, the two gels, with a good sprayability, could find different applications.

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Imiquimod loaded liposomes for (trans)dermal delivery

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Abstract

Over the past years, (trans)dermal delivery of biologically active molecules has become very popular due to its advantages over other routes of administration.[1] Different methods and vehicles have been used to improve dermal delivery, among which, liposomes seem to be the most studied.[2] Imiquimod (IMQ) is a topically-applied imidazoquinolon, available in the market as a cream under the brand name Aldara R . This formulation is used for the treatment of several skin diseases, like actinic keratosis and basal cell carcinoma.[3] Poor aqueous solubility and low cutaneous permeability are disadvantages of IMQ that limit its efficiency for topical delivery by conventional formulations. [3] The aim of the present study is to prepare IMQ loaded liposomes and evaluate their ability to efficiently deliver the active substance to the desired skin layers. Thus, different IMQ containing liposomal formulations were prepared by using the thin film method. IMQ was either incorporated into the dry lipids, or was added to liposomes as a solution in acetate buffer (pH = 4). In order to obtain the desired particle size, all formulations underwent extrusion through polycarbonate membranes at 60 °C. The prepared IMQ-loaded vehicles were separated from the free drug by using mini-column centrifugation method and the amount of IMQ into liposomes was determined by HPLC. The prepared vesicles were characterized for their size, polydispersity index, zeta potential and entrapment efficiency. Liposomes with mean particle size between the range 130-140 nm and PDI of 0.18-0.19 were obtained. These results are within the desired values and indicate homogenous and monodisperse samples. The encapsulation efficiency reached values up to 64 %. A method for the IMQ determination into the different skin layers has been developed and skin permeation experiments are under evaluation.

The study was supported by SVV: 260401 and Czech Science Foundation (GACR 19-09600S) References

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Coated liposome: delivery system for better and faster efficacy

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Abstract

Active molecules with biological activities need to reach viable cells and may need to diffuse deeply into the skin. However, several molecules with high molecular weight like hyaluronic acid (HA), act by adding volume inside the upper layers of the skin through a physico-chemical mechanism, water absorption. In both cases, these molecules need a help to cross the *stratum corneum* (SC) to reach the targeted area. In this context, delivery systems are widely used in cosmetic and pharmaceutical industries to encapsulate active ingredients to cross the SC and improve product efficacy. As a vesicular delivery system, liposomes are commonly used in cosmetic formulations to increase the bioavailability of active ingredients and for their capacity to entrap both lipophilic and hydrophilic compounds. However, some cosmetic formulas and active ingredients are not compatible with liposomes and can induce their destabilization.

Coated liposomes (CL), a new generation of liposomes reinforced by polysaccharide coating, was developed to be more resistant in cosmetic formulas containing surfactants and/or electrolytes and to be able to encapsulate charged active ingredient.

The aim of this study was to show the interest of CL for improving the bioavailability of wide range of molecules (electrolyte, high molecular weight, low penetration profile molecule...) and for enhancing the product efficacy.

As a first step, we evaluated the delivery property of CL through human explant by using FranzTM diffusion cell. Two molecules with different penetration profile were used: caffeine (1.5%, MW=194.19g/mol) and an Hexapeptide (300ppm, MW= 870g/mol).

Secondly, we investigated the efficacy enhancement of a charged molecule with a high molecular weight, sodium hyaluronate (0.0025%, MW=1.6MDa), entrapped in CL. We evaluated the wrinkle appearance of the crow's feet area on volunteers by fringe projection.

Regarding the bioavailability study, after 24h, the global quantification of caffeine and hexapeptide showed that the active ingredients entrapped in CL improved the penetration in different skin layers compared to the molecule in water.

In vivo, immediately and until 2 hours after application, the CL of HA decreased the amplitude of the relief of wrinkles compared to the control.

In conclusion CL is a powerful carrier for enhancing the penetration of active ingredient regardless of the nature of the molecule. Moreover, it highlights its potential to boost the efficacy of low-penetration profile molecules by accelerating the penetration even in the upper layer of the skin. Thus, the entrapment in CL optimizes a short-term visible effect.

Influence of copolymer concentration and nature on tacrolimus-loaded micelles: evaluation of formulation characteristics and cutaneous drug delivery.

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Abstract

Polymeric micelles can solubilize and protect drugs and enable their targeted delivery. In dermatology, they have been shown to improve cutaneous delivery of poorly water-soluble drugs and to target the pilosebaceous unit [1-2]. The choice of polymer and its concentration are crucial to formulate stable micelles, but the influence of copolymer concentration and nature on cutaneous drug delivery is poorly understood. Here, micelles loaded with tacrolimus (TAC) and formulated with (i) D- α -tocopherol polyethylene glycol 1000 succinate (TPGS) polymer or (ii) methoxy-poly(ethylene glycol)-dihexyl substituted polylactide (MPEG-dihex-PLA) diblock copolymer were prepared and the formulation characteristics, TAC skin biodistribution and localization of the copolymers in the skin were compared. Stable TAC-loaded micelles (0.1%; 50 and 150 mg of TAC per g of TPGS) were prepared and characterized in terms of drug content, size and stability. Skin penetration studies using porcine tissue were performed for 12 hours to quantify skin deposition and to determine biodistribution of TAC and TPGS (by UHPLC-MS/MS). The results were compared to those obtained using TAC-loaded micelles (0.1%) prepared with MPEG-dihex-PLA copolymer [1].

The variation of TAC loading (mgTAC/gcopo) in TPGS micelles enabled the impact of drug thermodynamic activity to be elucidated. While it had no influence on micelle characteristics, there was a statistically significant effect on TAC delivery (142.6 ± 25.3 and 373.0 ± 83.9 ng/cm² for 50 and 150 mgTAC/gcopo, respectively). The study of the nature of the copolymer highlighted the influence of their physicochemical properties on TAC micelle characteristics and skin delivery. Despite the same TAC loading (150 mgTAC/gcopo) and content (0.1%), micelle size was different (10 and 18 nm for TPGS and MPEG-dihex-PLA, respectively) and TAC aqueous solubility was also impacted. Moreover, MPEG-dihex-PLA micelle based formulations delivered 10 times more TAC than TPGS micelles (4.0 ± 1.3 and 0.4 ± 0.1 μ g/cm², respectively). Further studies also demonstrated that the molecular weight of the copolymers can influence their interaction with the skin: TPGS polymer (1.5 kDa) penetrated into the skin, whereas MPEG-dihex-PLA (5.1 kDa) remained at the skin surface and in hair follicles [1].

In conclusion, this work demonstrated that the nature and the concentration of copolymer must be carefully evaluated during the formulation of polymeric micelles: they not only influence drug solubilization but can also strongly affect the cutaneous bioavailability.

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Liposomes improve skin absorption of calcium and magnesium cations from thermal spring water

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Abstract

Thermal spring waters (TSW) have been used for medical and cosmetic purposes for many centuries. Their beneficial properties are well documented [1-2] and are mainly due to the cationic composition of the spring. Since cations contained in TSW penetrate skin fast, cosmetic formulations made with TSW as an active cosmetic ingredient are being developed to improve the penetration profiles. In this work, liposomes were used to slow down the absorption and to promote storage of active cations in dermis and viable layers of epidermis. We were particularly interested in the absorption of calcium and magnesium. These two cations are the main components of TSW, playing an important role in skin barrier recovery [3].

A marketed TSW was used to prepare liposomes (formed from saturated or unsaturated phospholipids) and emulsions: O/TSW, TSW/O and TSW/O/W. The composition of all emulsions included PEG-free emulsifiers based on vegetable raw material. A mixture of caprylic capric triglycerides was used as the oily phase.

Skin penetration experiments were performed using full thickness viable pig skin samples mounted onto Franz cells. Acceptor chamber was filled with a glucose-rich, isotonic survival medium with a reduced amount of ions to prevent interference in the analytical method applied. The permeation of Ca²⁺ and Mg²⁺ was assessed after 24 h exposure to TSW and tested formulations. Skin layers: *stratum corneum* (SC), viable epidermis (VE) and dermis (D) were separated, and ion concentrations were quantified in each of them as well as in the receptor fluid using ion chromatography.

The values of total absorbed quantities of cations and their distribution in skin layers after 24 h varied depending on the formulation type. Both cations were highly retained in the deeper skin layers (VE, D). The highest retention ratio (VE+D as compared to Qabs) was observed for liposomes formed from saturated phospholipids and TSW/O emulsion, however the latter exhibited the lowest Qabs values.

Our results demonstrate that the beneficial effects observed during treatment with TSW are associated with penetration of the minerals into and through the skin and are not only a surface action. Formulating TSW into liposomes or emulsions leads to modification of cation absorption profiles.

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Study of a new gel for dermal use based on a pollenic macrolide antibiotic with antifungal action

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Abstract

The pollenic macrolide antibiotics, as in the case of amphotericin B (AmB), have the capacity to generate the opening of the channels in the ergosterol membrane present in some fungi (*Candida* spp.) causing their death. These compounds are insoluble in most organic and inorganic solvents, that is we have proposed to formulate an AmB gel based on sepiigel³⁰⁵® (gel-AmB).

Propose. In this work we evaluate the impact of a gel-AmB on Transepidermal water Loss (TEWL) and on the Stratum Corneum Hydration (SCH) after topical application, predicting the rheological behavior and evaluating of short-term stability.

Methods and Materials. A total of 10 healthy volunteers, were recruited and the Cutometer R MPA 580 was used. Measurements were carried out on forearm. **TEWL:** the measurement was carried out with a Tewameter R TM 300 and **SCH:** the measurement was carried out were carried out by a Corneometer[®] 825, Courage and Khazaka, Electronic GmbH, Cologne, Germany).

Rheological behavior. The rheological measurements were performed using a rotational rheometer Thermo Scientific Haake Rheostress 1 (Thermo Fischer Scientific, Kalsruhe, Germany) equipped with cone plate geometry (60 mm diameter, 2° angle) with mobile upper cone Haake C60/2° Ti (0.105 mm gap) at 25 °C.

Finally, destabilization phenomena (coalescence, flocculation, sedimentation, creaming, etc.) were tested by multiple light scattering analysis of transmission (T) profiles during 24 h using the TurbiScanLab R (Formulation Co., L'Union, France) at 25 °C.

Results. The values of TEWL and SCH at 2 hours after the application of the gel-AmB did not show a statistically significant difference ($p \leq 0.05$) (TEWL basal: 8.47 ± 1.74 and TEWL after 2 h: 8.12 ± 1.72 g/m/cm², SCH basal: 33.58 ± 6.71 and SCH after 2 h: 36.42 ± 9.11 UC). Regarding the rheology, the AmB-gel presented trixotropy (value: 194.4 ± 47.09 Pa/s) and a pseudoplastic behavior (Cross equation= $r^2:0.9996$).

Conclusion. Our formulation will not cause any alteration in the biomechanical parameters of the skin. The gel-AmB could be applied to the skin, especially in mycosis processes since it was easily tolerated. None of the volunteers manifested burning, itching or redness. No instability was observed.

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Mesoporous silica particles intercalate at stratum corneum – targeting topical drug delivery

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Abstract

The potential application of micro- or nanosized particles in topical delivery is today an active and controversial topic (1). Particles beyond the size of a few nanometers are generally considered too large to penetrate the intrinsic barrier of the skin.

In this study, we explore mesoporous silica particles of sizes ranging from 400 nm to 7 μm when applied to skin *ex vivo* using a novel approach. Luminescent particles were created by controlled pyrolysis of a carbon precursor using the mesoporosity as nano-reactor (2).

Multiphoton microscopy demonstrated the particle interaction with the outermost skin layer stratum corneum and the accumulation in the skin microstructure. The nanosized particles (i.e., 400-600 nm), were found to intercalate down to a depths of around 25 μm despite washing, whilst the larger particles (2 and 7 μm) resided more superficially on the skin. Scanning electron microscopy confirmed the particle intercalation at stratum corneum. This imply the potential of targeting topical delivery facilitating controlled drug loading into skin microstructure by tuning of particle size. Proof of concept was demonstrated for particles loaded with metronidazole, a broad-spectrum antibiotic, using a transcutaneous drug delivery experiment.

The study demonstrates a novel concept for studying topical drug delivery using particle based systems, and shows how particles hold promise for targeting topical delivery.

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Evaluation of innovative PEG-free antioxidant nanoformulations for topical delivery

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Abstract

The skin exposure to ultraviolet radiation and to environmental pollutants results in excess generation of oxidative free radicals, inducing skin cells damage and potentially 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 (ROS) in the epidermis. Thus, our research intends to focus on innovative phospholipid-based formulations loaded with natural lipophilic antioxidant (quercetin). Formulations are designed for penetration enhancement through the *stratum corneum* 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 will also constitute suitable candidates as PEG alternative.

Therefore, our research also strives to prove POx value to topical formulations by its capacity to enhance the formulation stability and the skin penetration. Two main dermal delivery systems were designed : mixed micelles (MM)s and lipid nanocapsules (LNC)s. They are made of unsaturated phospholipids and POx constituted by a hydrophobic alkyl chain and a hydrophilic chain of various repeating units C16(POx)15, C16(POx)35, C18:2(POx)15 and C18:2(POx)35. Both formulations are defined by a nanosize : 30 and 60nm (PDI< 0.3) and quercetin loading of 90%. LNC loaded twice more quercetin than MM. IC50 determined using DPPH method were lower at 2µg/mL compared to the free quercetin (3.6µg/mL) reflecting a higher quercetin solubilization. The antioxidant effect was investigated on mice fibroblast cells for quercetin:5 µg/mL. ROS generated with TBHP at 10mM were scavenged but ROS essential for cell survey were preserved by the loaded MM[4] Promising results are expected for LNC antioxidant effect (ongoing). A complementary antioxidant test was performed on normal human keratinocytes with ROS generated by UVB radiation. The first results showed dramatic reduction the ROS over generated by the UV with LNC loaded with quercetin. To track down the penetration, Alexa fluorescent probe was grafted onto the POx. The penetration capacity and delivery of quercetin were compared for both formulations on mice ears (IPBS, Toulouse) to reach the targeted layer of the deepest layers of the epidermis. *Our lipid-polymer hybrid nanoformulations ensure promising features regarding size, stability, toxicity, encapsulation, antioxidant effect and quercetin delivery to deepest layers of the epidermis.*

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Study of Ex-vivo permeation of Chondroitin sulfate loaded to Solid Lipid Nanoparticles

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Abstract

Introduction: Our group has produced cationic solid lipid nanoparticles (cSLN) as a vehicle for pDNA and siRNA, in the transfection of cell lines [1]. SLN could be used as a vehicle for biomolecules. Chondroitin sulfate (CHON), is a component of the extracellular matrix of several connective tissues. Therefore, CHON is a potential therapeutic agent in Osteoarthritis (OA) [2]. Considering that topical route prevents the first-pass effect from occurring, research studies recommend topical nanotechnological systems as a new perspective for OA [2].

Purpose: The objective of this research was to evaluate the *ex vivo* permeation of CHON using SLN as a promoter.

Methods: We prepared SLN by hot microemulsion method [1], under optimized conditions. Different properties were tested, including entrapment efficiency of CHON (%EE), zeta potential and particle size. The transdermal absorption of 1.0 ml of SLN of CHON was estimated by an *ex vivo* permeation study, using porcine ear skin (n = 5) with a thickness of 0.6 mm, with Franz-type Diffusion Cells (0.64 cm² of permeation area), at 32 °C. Drug concentration in the receptor phase (water) was determined by a validated UV-Vis detection technique (520 nm). Transepidermal water loss values were used as proof of skin integrity. The permeated amount of CHON at 24 h, expressed as percentage of CHON encapsulated, was also showed by median and range.

Results: Nanoparticles showed spherical morphologies and particle size below 150 nm in average diameter. The initial concentration of CHON (0.4 mg/ml) produces % EE around 60 %. The median amount of CHON permeated through skin, was 78.09 % and the range (57.16 – 107.77) %

Conclusions: Although CHON has unfavorable characteristics for skin permeation, the study conducted with our SLNs confirms a promoter effect in the permeation of CHON across the skin.

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Study of transdermal human skin permeation of a chemical derivated from a natural flavanone formulated in a nano estructured system

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Abstract

Introduction: Nowadays, in the treatment of inflammation are used drugs steroidal (cortico steroids) and non steroidal drugs (NSAIDs); but unwanted side effects are present.¹ In order to found some alternatives; several kind of natural species has been studied.² In this way a natural flavanone (2*S*)-5,7-dihydroxy-6-(3-methyl-2-buten-1-yl)-2-phenyl-2,3-dihydro-4*H*-1-Benzopyran-4-one (**1**) was isolated from *Eysenhardtia platycarpa* and reported antiinflammatory, antioxidant and cytotoxic properties.^{3,4} Different structural modification were semi-synthesized from (**1**) represent a suitable strategy to obtain new compounds such as (8*S*)-5-hydroxy-2,2-dimethyl-8-prenyl-3,4,7,8-tetrahydro-2*H*-6*H*-Benzo[1,2-*b*:5,4-*b'*]dipyran-6-one (**1a**). Also, as an alternative treatment could be developed a nano system formulation applied directly onto the affected area.

Purpose: To investigate a nano system formulation as delivery system for flavanone (8*S*)-5hydroxy-2,2-dimethyl-8-prenyl-3,4,7,8-tetrahydro-2*H*-6*H*-Benzo[1,2-*b*:5,4-*b'*]dipyran-6-one intended for topical administration.

Methods: The Nano system formulation of flavanone (**1a**) (5% w/w) was prepared with, labrasol, labrafac, plurol oleique and propyleneglycol as excipients. The particle size was measured by Zeta-Sizer, Malvern Instruments. Permeation studies (n=6) were carried out with vertical Franz diffusion cell of 2.54cm² and dermatomed human skin (0.4mm) for a unique donor. The receptor phase was ethanol:water (70:30), under temperature of 32 ± 1°C. Samples were withdrawn at different time point scheme for 29h and quantified by means a validated HPLC method (water:ACN (20:80) as mobile phase; 1 ml/min flow rate; 208nm; Machery Nagel R C18 5mm, 25x4.6cm column). The Flavanone skin extraction was carried out with a mixture of Ethanol:water (70:30) under sonication for 20 minutes.

Results: Permeated amounts of flavanone (**1a**) after 29h were 15,8mg. The retained amount of flavanone (**1a**) in skin was 13,8mg/cm². The average flux was 5,5x10⁻¹mg/hr cm². The Tl was 16,9h; while the P1 and P2 were 1,7x10⁻²cm and 0,9x10⁻² 1/h, respectively.

Conclusions: The results demonstrate the possibility to use this flavanone formulated in a nano system formulation for topical purposes.

Acknowledge: Acknowledge to CONACyT, Mexico for the scholarship 709906.

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Pioglitazone nanoemulsion for the treatment of atopic dermatitis

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Abstract

Pioglitazone (PGZ) is a Peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonist that is used to treat type 2 diabetes mellitus. A regulatory role on inflammatory markers has been reported as an additional therapeutic activity for PGZ. The purpose of this study was to develop a Pioglitazone-loaded nanoemulsion (PGZ-NE) in order to evaluate its effectiveness for treating atopic dermatitis. Solubility studies and three pseudo-ternary phase diagrams were performed to establish the NE composition. The pharmacological efficacy of PGZ-NE was studied using hairless mice SKH-1. Animals were classified into three groups: Positive group (mice exposed only to oxazolone), treated group (mice treated with PGZ-NE) and negative group (mice not exposed to oxazolone nor PGZ-NE). The animals were sensitized with 5% oxazolone. One week later, the mice were treated with oxazolone 0.2% for 16 days. Topical application of PGZ-NE was carried out on the treated group one hour after oxazolone exposure. Biomechanical properties such as transepidermal water loss (TEWL) and stratum corneum hydration (SCH) were evaluated at predetermined time intervals (before treatment and at 8, 15, 18, 21, and 24 days). Finally, 24 hours after the final treatment, mice were euthanized and their skin was stored in order to evaluate gene expression and histology. A transparent and homogeneous PGZ-NE with spherical shape, nanometric size of lipid droplets and Newtonian behavior was obtained. The oxazolone treatment resulted in an increase of TEWL value and reduction of SCH which was evident in the positive control. The application of PGZ-NE in the treated group significantly reduced TEWL and improved SCH. This result can be associated with an enhancement of skin barrier function. Results from the RT-qPCR analysis revealed a significant increase in the expression of the pro-inflammatory cytokines IL-6 and TNF- α (positive control). Topical application of PGZ-NE on the skin of mice with induced dermatitis highly reduced the expression of both cytokines to levels close to the non-treated negative control. These results were consistent with histological analysis where signs of dermatitis including inflammatory cell infiltrate and loss of stratum corneum accompanied by an initial loss of dermal appendages were observed in

the positive control. The administration of PGZ-NE prevented the loss of stratum corneum, reduced the inflammatory cell infiltrated and also the alteration of the dermal appendages. Consequently, these results suggest that PGZ-NE may be used as a potential treatment for inflammatory skin diseases including atopic dermatitis.

Stabilities of lipid-based Janus nanoparticles for cutaneous applications

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Abstract

Our group recently developed lipid-based Janus nanoparticles [1,2] composed of a hydrophobic lipid compartment and a hydrophilic aqueous compartment bounded by a bilayer composed of phospholipids and nonionic polyoxyethylated surfactants. Due to the different compartments, the Janus particles should be used as an original nanocarrier for administration of molecules having opposite solubilities.

The aim of the present communication is to summarize the different stability studies made on these innovative formulations in the context of cutaneous applications. First of all, physical stability with temperature which is a critical parameter to consider in order to envisage the development of this formulation for pharmaceutical or cosmetic applications. Stable for more than 2 years at 21 ° C, these particles describes a two-step destabilization mechanism with increasing temperature: first, a slight size increase by Ostwald ripening and then a rapid coalescence. It appeared that the most efficient way to improve this physical stability should be to slow down the Ostwald ripening by modifying the composition and the concentration of the formulation. Then, the influence on preparation process of some conservatives and buffer has been consider in order to improve microbial stability. At last, these Janus particles were introduced into gels to facilitate their application on skin. The retention of gel rheological properties and morphology of the incorporated particles were notably investigated.

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Photoprotection potential and physicochemical characterization of *Bidens* spp. nanoemulsions

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Abstract

Exposure to ultraviolet radiation is the main cause of skin diseases and photoageing process, representing a big challenge for public health. The incidence and mortality from skin cancer are increasing in many countries and the use of different sunscreen products has been advocated by many health care practitioners. However, most products used against the harmful effects of Ultraviolet Radiation (UVR) are synthetic and, for this reason, several natural compounds with UVR absorption property have been studied and used to substitute or reduce the quantity of synthetic agents. *Bidens* spp., a traditional medicine plant wide used in Brazil, has a variety of compounds, which justifies its pharmacological properties, was used in this work to develop two different oil-in-water nanoemulsions. Nanoemulsion is an interesting option since smaller droplets promote uniform coating of the skin, which increase the potential of UVR protection. Therefore, the main objective of this work was to study the sun protect factor (SPF) properties against UVR radiation and physicochemical characteristics of *Bidens* spp. nanoemulsion (N1) and *Bidens* spp. Gel-nanoemulsion [with 5% (w/w) of Modified Starch to increase viscosity] (N2). The oil phase were composed by Hydrogenated PEG-40 Castor oil; *Bidens* spp. extract at 0.5% (w/w); Span 60 and Sunflower oil and the aqueous phase was ultrapure water. The pH was measured after nanoemulsions preparation and visual stability test of formulations were evaluated after 24h. Physicochemical characteristics as size, polydispersion index (PdI) and zeta potencial were analysed in Zetasizer and efficiency of UV protection was evaluated with *in vitro* spectrophotometric method in UV-Vis. Nanoemulsion N2 was analysed in Rheometer with the purpose to evaluate viscosity. Formulation N1 presented pH 5.62 and N2 pH 5.67, suggesting no changing after polymer addition. Visual stability test showed no phase separation and no changing of colour. The size of nanoemulsions N1 and N2 were $72.10 \pm 0,39$ and $73.34 \pm 0,27$ nm, respectively, showing no difference after polymer addition. Nanoemulsions were considered monodisperse since values of PdI were lower than 0.3. Potential zeta values of N1 and N2 were -25 ± 0.76 and -26.7 ± 2.94 mV, respectively, showing physicochemical stability of formulations. Both formulations N1 and N2 presented moderate protection, with value of SPF15, demonstrating the possibility of their use as natural sunscreen. N2 showed higher Consistency Index than N1. Therefore, N2 is preferable than N1 because its higher viscosity facilitate its application and adesion in skin, boosting its efficacy.

Polyglycerol-ester based green low energy nanoemulsions – optimization of cosmeceutical formulations for antioxidant protection and skin hydration

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Abstract

INTRODUCTION

In this study polyglycerol ester-based low energy nanoemulsions (LE-NEs) were developed as skin friendly and innovative carriers for natural and sensitive molecules. Red raspberry seed oil is a rich source of anti-inflammatory polyunsaturated fatty acids, antioxidants (tocopherols and tocotrienols) and UV-protective carotenoid (yellow) pigments. Hydro-glycolic red raspberry fruit extract contains fruity acids (citric, malic), sugars (fructose, inositol) and antioxidants (vitamin C, tannins and red pigments – anthocyanins).

MATERIALS AND METHODS

LE-NEs were prepared using Phase inversion composition method at room temperature with Polyglyceryl-4 Laurate as main surfactant mixed (at 1:1 ratio) with cosurfactant blend: Polyglyceryl-6 Caprylate, Polyglyceryl-3 Cocoate, Polyglyceryl-4 Caprate, Polyglyceryl-6 Ricinoleate. Oil phase contained emollient ester Ethylhexylpelargonate – EP, Red raspberry seed oil – RO, and costabilizers: Red raspberry fragrance oil – RF or preservative blend (Phenoxyethanol (and) Ethylhexylglycerin) – PB. Antioxidant activity was tested *in vitro* with ABTS and DPPH assays. Safety profile was evaluated *in vivo* on human volunteers by measuring: transepidermal water loss (TEWL), stratum corneum hydration (SCH) and skin erythema index (EI), upon cessation of 24 h occlusive treatment. Moisturizing efficacy was investigated *in vivo* in 2 hour study by measuring SCH.

RESULTS AND DISCUSSION

Optimal LE-NE formulations with mean droplet sizes of 50 to 80 nm and narrow PDI value (< 0.1) contained: 10 wt% surfactant mix, 2 wt% RO and 80 wt% water phase (30 wt% glycerin aqueous solution). Several formulations were investigated: F1 (2 wt% RO, 7.5 wt% EP, 0.5 wt% PB) and F2 (2 wt% RO, 6 wt% EP, 2 wt% RF). Additional samples of F1 were successfully loaded with antioxidant extracts: 5 wt% Red raspberry fruit extract (F1 5% RE) and 5 wt% French oak fruit extract (F1 5% FE), or Xanthan gum gel (F1: gel = 4:1) marked as F1 gel-NE.

Antioxidant activity improved when antioxidants were present in the oil and water phase, with following order of activity: ABTS – F1 5% FE (87.9%)> F1 5% RE (33.5%)> F2 (14%)> F1 (6.2%), DPPH – F1 5% FE (93.8%)> F1 5% RE (4%)> F1=F2 (3.8%). All investigated samples showed satisfactory safety profiles and significant improvement of skin moisture 0.5, 1 and 2 hours after application, compared to both untreated skin and baseline values. Prolonged skin hydration (2 h after application) was observed for sample F2 (with fragrance) and F1 5% RE (no fragrance).

CONCLUSION

Polyglycerol-based LE-NEs could be recommended as promising and effective skin care formulations for hydration and antioxidant protection.

Ex-vivo permeation of carprofen from nanoparticles: a comparative study between porcine skin and sclera mucosa

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Abstract

Introduction: Carprofen (CP), (+)-6-Chloro-alpha-methylcarbazole-2-acetic-acid, is a non-steroidal anti-inflammatory drug (NSAID). CP is widely used in veterinary to treat symptomatic pain and inflammation [1]. The local administration of NSAIDs has been explored as a potential method in order to avoid the first pass effect and the gastrointestinal disorders [2]. When treating a topic inflammation by directly applying the NSAID on the affected area, it should be ensured that drug reaches the site of action at determined concentrations and within effective therapeutic range. Furthermore, this therapeutic concentration should remain constant throughout the time required to reduce inflammation. The use of polymeric nanospheres could be considered nowadays as a strategy to enhance the bioavailability of topically administered drugs. **Purpose:** The main objective of this work was to compare the ex vivo permeation of carprofen nanoparticles (NPs-CP) between porcine skin [3] and sclera mucosa. **Methods:** Nanoparticles with a matrix structure containing Carprofen were prepared by the method previously described [4] under optimized conditions previously determined. The transdermal and transmucosal absorption parameters: flux (J) and lag time (Tl) were estimated by means of an ex vivo permeation study using Franz Diffusion Cells and the median results were calculated [3]. The amount of carprofen in the NPs and in the samples taken in the ex vivo permeation study was determined by a validated technique of high performance liquid chromatography with UV detection. **Results:** The flux showed values of 0.13 mcg/h/cm² for skin and 1.005 mcg/h/cm² for sclera while the lag time was 2.19 h for skin and 6.18 h for sclera. **Conclusions:** It can be observed that J is higher in sclera compared to skin. However, the T1 did not show statistical significant differences. It can be concluded that NPs of carprofen are able to permeate the skin and sclera membrane of swine, thus suggesting a local antiinflammatory effect.

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Lipid nanoparticles for dermal delivery of pranoprofen loaded pluronic gels

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Abstract

Pranoprofen (PF) is a non-steroidal anti-inflammatory drug which can be used as a safe treatment in skin local inflammation. Lipid nanoparticles are one of the colloidal systems that have been most widely studied over the past few years with the objective of improving the skin penetration of the drugs. Lipid nanoparticles loaded in Pluronic gels are a novel method for the treatment of skin diseases.

Propose: PF loaded lipid nanoparticles (PF-NPs) in Pluronic gels were characterized as a means of exploring novel formulations to improve the biopharmaceutical profile of this drug for topical application.

Methods and Results: The formulations of PF-NPs loaded Pluronic R P407 exhibited physicochemical characteristics suitable for dermal application. *Ex vivo* permeation experiments were carried out using human skin obtained from abdominal region of a healthy women during plastic surgery (Barcelona-SCIAS Hospital, Barcelona, Spain), against a written informed consent. Retained amounts of PF in the skin were higher than permeated amounts in Franz vertical cells. These results might ensure that the topical application of these formulations would not have any systemic effect and ensure a local anti-inflammatory effect of this drug. The biomechanical properties of skin were studied. The transepidermal water loss (TEWL) was measured using a DermaLab R module. The measurements of stratum hydration (SCH) were carried out by a Corneometer[®] 825.

Conclusion: The dermal application of the selected PF-NPs in Pluronic gels could be an effective system for the delivery and controlled release of PF, improving the biopharmaceutical profile of this drug, facilitating the anti-inflammatory effect of the PF on the skin and improving its dermal retention. *In vivo* skin hydration and transepidermal water loss revealed an occlusive effect and low hydration power.

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Thermoresponsive nanomicelles prepared by a green emulsion-evaporation process for a cosmetic use

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Abstract

The aim of this study was to prepare thermoresponsive nanosystems for a cosmetic use. Among polymers exhibiting thermoresponsivity, Pluronics R (or Poloxamers R) 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 R 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.

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.

Three active ingredients have been encapsulated in Pluronics R 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 R nanomicelles. This technique usually used to prepare nanoparticles but barely used to prepare nanomicelles with a high stability and a small polydispersity index ($< 0,2$). The results show that the size (≈ 30 to ≈ 200 nm), the loading (up to $\approx 80\%$ for the ester) but also the 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.

Development and evaluation of carbomer gel bearing pranoprofen loaded nanostructured lipid carriers for the treatment of skin inflammatory disorders

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Abstract

Background: Pranoprofen (PF), is a non-steroidal anti-inflammatory drug (NSAID). Despite the high anti-inflammatory and analgesic potency of this drug, the oral administration of PF is somehow limited because of its inadequate biopharmaceutical profile. Recently, some articles have reported the effect of this drug for the treatment of skin diseases. Nanostructured Lipid Carriers (NLC) are one of the colloidal systems that have been most widely studied over the past few decades with the aim of improving the penetration and delivery of drugs in the skin. In chronic lesional skin affected by inflammatory disorders like rosacea the differentiation process of the keratinocytes, the biosynthesis of the stratum corneum, and organization are altered.

Purpose: The aim of this study was the development and evaluation of carbomer gel bearing pranoprofen loaded nanostructured lipid carriers with linoleic acid as a means of exploring novel formulations to improve the biopharmaceutical profile of this drug for the treatment of rosacea.

Methods: Rheological properties were analyzed at $25 \pm 2 \text{ }^\circ\text{C}$ using a Haake Rheo Stress 1 rheometer after 48 h of preparation. Franz diffusion cells was used to address the PF release studies. *Ex vivo* permeation experiments were carried out using human skin. The rabbits were subjected to the topical application of oxazolone on the back to model skin rosacea and the efficacy of the formulation was evaluated. 4 groups were evaluated: control (without treatment), control+, blank formulation, and the developed formulation. A histological analysis was conducted, sections were stained with hematoxylin-eosin and observed by microscope.

Results: The rheological evaluation of the formulations showed pseudoplastic behaviour. The release profile showed a hyperbola kinetic model. *Ex vivo* studies showed that formulations allow a great retention of PF in skin after 24 h. Observation of the histological sections showed a contiguous stratum corneum similar to control-, whereas with the control+ and blank formulation a destructuring and no contiguous stratum corneum is observed.

Conclusions: Taking into account these results, the carbomer gel including Pranoprofen loaded nanostructured lipid carriers developed, could be a new alternative and a great candidate for the treatment of rosacea.

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Berberine Chloride Dihydrate entused Nanovesicles for the management of Dermatitis and Skin Irritation

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Abstract

The present research work involved the development of Berberine chloride dihydrate (BCD) loaded ethosomal gel (EGs) for the management of dermatitis. The ethosomes were in size range 142.42-398.31 nm while polydispersity index (PDI) ranges from 0.114-1.56 and for zeta potential it was from -18.8 to -39.4. Entrapment efficiency was from 46.05-88.79 %. The optimized formulation was subjected to "anti-bacterial, dermatitis and skin irritation study. Animal studies were approved by Institutional animal ethical committee. Confocal laser scanning microscopy was used to trace the penetration depth of rhodamine entused ethosome across rat skin and it was observed that it reaches upto the depth of 110 μm in to the rat skin which was significantly higher than rhodamine solution (10 μm). In the anti-bacterial study, BCD loaded ethosomal gel (EG) showed maximum zone of inhibition of 18.5 mm against *E. coli*, 14.5 mm against *P. aeruginosa* and 23.0 mm against *S. aureus*. In dinitrochlorobenzene (DNCB) induced mice dermatitis model histopathology study showed marked decrease in amount of inflammatory cell nucleus in mice treated with BCD loaded ethosomal gel followed by 56% and 50 % increase in ear swelling and ear mass respectively in morphology study. Compared with the control, skin tissues sections of right ear lobes of disease induced animal showed increased thickening of skin layers, aggregation of an inflammatory cell comprising of neutrophils forming a neutrophil abscess in the lining epithelium in the inflamed skin layers. Conventional marketed formulation showed nominal decrease in epidermal thickness, 66.67 % increase in ear thickness and 63.64 % increase in ear mass. Further Primary irritation index was less than 0.4 indicating negligible irritation in all the groups. Hence the developed formulations are safe for topical use and free from skin irritation. It can be concluded that ethosomal gel is not only an efficient carrier for BCD but also proves its potential for the management of dermatitis. Keeping in view the fact that reaching the dermatitic tissue via topical route is difficult and limitation of its currently available treatment, incorporation of phytoconstituent into novel vesicular carriers like ethosomes could be suggested as a possible solution. These novel vesicles made of phospholipids have unique structural features that improve the topical delivery of the drug in diseased skin.

Nanoemulsion of apremilast for the treatment of inflammatory diseases of the skin

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Abstract

Apremilast (APR) is a small molecule that has the capacity to spread through cell membranes and whose target is intracellular signaling; modulates multiple inflammatory pathways through the inhibition of the enzyme phosphodiesterase 4 (PDE4) specific for cyclic AMP (cAMP). In 2014, the United States Food and Drug Administration (USFDA) approved this drug for the treatment of psoriatic arthritis, psoriasis, moderate to severe plaque psoriasis, atopic dermatitis, among others. The purpose of this study was to develop and characterize a nanoemulsion loaded with apremilast (APR-NE) to evaluate its effectiveness in the treatment of inflammatory skin diseases. Solubility studies and optimization of the formula were performed using four ternary phase diagrams to establish the composition of the NE. The physicochemical characterization included particle size, polydispersion index (PDI), transmission electron microscopy (TEM), viscosity and rheological behavior. In addition, *in vitro* release and permeation studies were performed on human skin using Franz diffusion cells. Study of biomechanical properties by transepidermal water loss (TEWL) and stratum corneum hydration (SCH) was performed on 12 volunteers. Finally, accelerated stability studies were carried out at different temperatures by the light transmission detection technique. According to the solubility studies, Labrasol and Transcutol-p presented the best characteristics for NE optimization, resulting in a transparent and homogeneous APR-NE. The NE presented particle size around 70 nm, PDI less than 0.4. The rheological analysis showed a Newtonian behavior with a linear relationship between the shear stress and the deformation speed. The APR release mechanism of the NE followed hyperbolic kinetics and the *ex vivo* permeation profile showed that APR does not cross the skin layers, with an amount retained therein of approximately 500 $\mu\text{g APR/g skin/cm}^2$, which suggests an effectiveness of action in the area of administration. TEWL and SCH values showed that the formulation improved the level of hydration and did not cause destabilization nor damage to the skin surface of volunteers. No changes in particle size have been observed during the three months of stability. Consequently, these results suggest that APRE-NE can be used as a local action treatment for inflammatory skin diseases such as psoriasis, dermatitis, among others.

Nanoemulsion of apremilast for the treatment of inflammatory diseases of the skin

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Abstract

Apremilast (APR) is a small molecule that has the capacity to spread through cell membranes and whose target is intracellular signaling; modulates multiple inflammatory pathways through the inhibition of the enzyme phosphodiesterase 4 (PDE4) specific for cyclic AMP (cAMP). In 2014, the United States Food and Drug Administration (USFDA) approved this drug for the treatment of psoriatic arthritis, psoriasis, moderate to severe plaque psoriasis, atopic dermatitis, among others. The purpose of this study was to develop and characterize a nanoemulsion loaded with apremilast (APR-NE) to evaluate its effectiveness in the treatment of inflammatory skin diseases. Solubility studies and optimization of the formula were performed using four ternary phase diagrams to establish the composition of the NE. The physicochemical characterization included particle size, polydispersion index (PDI), transmission electron microscopy (TEM), viscosity and rheological behavior. In addition, *in vitro* release and permeation studies were performed on human skin using Franz diffusion cells. Study of biomechanical properties by transepidermal water loss (TEWL) and stratum corneum hydration (SCH) was performed on 12 volunteers. Finally, accelerated stability studies were carried out at different temperatures by the light transmission detection technique. According to the solubility studies, Labrasol and Transcutol-p presented the best characteristics for NE optimization, resulting in a transparent and homogeneous APR-NE. The NE presented particle size around 70 nm, PDI less than 0.4. The rheological analysis showed a Newtonian behavior with a linear relationship between the shear stress and the deformation speed. The APR release mechanism of the NE followed hyperbolic kinetics and the *ex vivo* permeation profile showed that APR does not cross the skin layers, with an amount retained therein of approximately 500 $\mu\text{g APR/g skin/cm}^2$, which suggests an effectiveness of action in the area of administration. TEWL and SCH values showed that the formulation improved the level of hydration and did not cause destabilization nor damage to the skin surface of volunteers. No changes in particle size have been observed during the three months of stability. Consequently, these results suggest that APRE-NE can be used as a local action treatment for inflammatory skin diseases such as psoriasis, dermatitis, among others.

Computational Prediction of Human Skin Permeability of Chemical Compounds

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Abstract

Formulation of solvents for topical dosage forms is costly and time-consuming, but inevitable because it greatly impacts the skin permeability of active ingredients. The last few decades have witnessed the development of various quantitative structure–property relationship (QSPR) models for skin permeability. However, a majority of the models are limited for practical use, because they do not deal with the solvent effects on skin permeability. First, we compiled reliable databases of the *in vitro* human skin permeability of different compounds in different dose solutions or of different ionisation states, which are among the largest compilations to date. We generated thousands of molecular descriptors to numerically characterise the permeants in dose solution. Our databases embraced the chemical space of topical drugs sufficiently enough to suit the development of empirical QSPR models.

Subsequently, we applied promising nonlinear machine learning techniques such as support vector regression with a radial basis function kernel combined with our greedy molecular descriptor selection algorithm to our databases. The performance of our models was verified by not only internal 10-fold cross-validation but also external validation using more than 10 statistical metrics. When two datasets related to solvent formulations and pH effects were individually analysed, the obtained models satisfied all the criteria of the metrics, with the correlation coefficients (R^2) between the observed and predicted skin permeability of more than 0.85 in external validation. Thus, the fully computational models we developed can provide an effective and efficient tool for screening drug candidates during the development of topical formulations.

In this presentation, we introduce the model building processes and the performance of our prediction models, while proposing an new assessment technique of the contribution of each molecular descriptor in nonlinear models which involves covariant differentiation of the the response hypersurface.

Skin equivalents produce with an innovative hydrogel and the potential of a synthetic elastin protein to improve cell growth in dermal equivalents

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Laboratoire de Biologie Tissulaire et d'ingénierie Thérapeutique UMR 5305 – Centre National de la
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Laboratoire de Biologie Tissulaire et d'ingénierie Thérapeutique UMR 5305 – CNRS : UMR5305 – France

Abstract

An innovative hydrogel composed of poly-lysine dendigrafts (DGL) and PEG was developed and evaluated as a support for the formation of a homogenous skin equivalents. Furthermore, the potential of a synthetic elastin protein (SEP) with similar physicochemical properties as native tropoelastin to improve the dermal equivalents produced was evaluated. To allow cell colonization in 3D, hydrogels were made porous by particulate/leaching technique using paraffin microspheres as porogenes. Pore size was controlled by controlling porogen size distribution, obtaining highly porous hydrogels (up to 76%). ESEM images suggest a good interconnection between pores, confirmed by the ability of human fibroblasts to colonize the hydrogels through time. Moreover, hydrogel's mechanical properties can be modified by changing the ratio of DGL/PEG components, allowing to obtain a porous hydrogel with a complex modulus (15.2 ± 2 to 17.1 ± 2.7 KPa). Skin equivalents were obtained by seeding human dermal fibroblasts on the surface of porous hydrogels. Cells could homogeneously colonize the porous matrix after 21 days of cell culture and synthesize their own extracellular matrix (ECM). Keratinocytes were seeded on the surface of the dermal equivalents obtained previously, allowed to proliferate and subject to an air-lift interphase to allow epidermis stratification. The skin equivalents obtained were characterized by IHC, showing a proper dermis-epidermis junction and epidermal stratification. To improve the dermal equivalents, the inclusion of SEP was evaluated. SEP allowed to increase cell density of dermal equivalents, as well as an increase in ECM produced. Collagen I and fibronectin could be observed further inside the hydrogel after 21 days of cell culture. No significant effect was observed in the production of proMMP-2 or active MMP-2 between both conditions by zymography (after 8 and 20 days). To conclude, the hydrogel proposed is able to work as 3D scaffold to mimic the ECM and produce skin equivalents. The presence of EDP in the hydrogels improved cellular colonization and proliferation, resulting in a higher synthesis of ECM in dermal equivalents indicating a greater potential for skin equivalents production.

In-Silico design of permeation enhancers for topical applications

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1

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Abstract

Human skin provides an excellent opportunity for the topical and transdermal drug delivery applications. Understanding the permeation of molecules or therapeutic agents inside the skin is main key aspect of drug delivery through skin and shall lead to the development of improved or novel transdermal drug delivery systems.

Skin provides an excellent protection against harsh external environment and foreign substances. The outermost layer of skin, stratum corneum which is made up of corneocytes and lipid matrix, is mainly responsible for this barrier. The lipid matrix fills the extracellular space of corneocytes and this assembly generally referred as brick and mortar structure. The active molecules which partition either in corneocytes or lipid matrix, ultimately have to pass through the tortuous path of lipid matrix. Hence, the lipid matrix (having various kinds of ceramides, free fatty acids and cholesterol) plays major role in skin barrier function.

At TCS, we have developed an *in-silico* skin model [1] based on the multiscale modeling framework linking atomistic-molecular-mesoscale to macroscale to study molecular transport across the *Stratum Corneum (SC)*[2-3]. The model is tested against various external stimuli (nanomaterials [4-7], electric field [8], permeation enhancers [9]) and compared extensively with experiments carried out on real skin (animal or human). The model thus developed has provided a base to develop an IT enabled platform (Digital Twin) for the simulation based design and development of pharma/cosmetics products. The *in-silico* model and platform could be used for the development of better cosmetic and drug delivery product.

In this talk, we will show the development of the complex skin models [10] and their utility in design of permeation enhancers for topical and transdermal drug delivery applications.

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A molecular dynamics study of collagen to understand the mechanical properties of skin

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1

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Abstract

Human skin provides physical barrier to transport of foreign entity inside the body and also protects the body from harsh external environment. As a consequence of aging and exposure to light (photoaging), skin loses its elasticity and wrinkles occur on the surface of skin. These changes are associated with the mechanical properties of skin layer. Dermis, the penultimate layer of skin which consists of a matrix of proteins namely collagen and elastin is responsible for the mechanical behavior of skin. Aging has been known to be correlated with changes in the crosslinking of collagen molecules. Therefore understanding the mechanical properties of collagen from molecular perspective is important to understand ageing.

In this study, we performed extensive restrained and unrestrained atomistic molecular dynamics simulations of collagen molecule at various hydration levels. The mechanical property (Young's modulus) of collagen was calculated at each case and compared with available experimental data. Based on preliminary molecular simulations, there was an increased stiffness (Young's modulus) of collagen molecule with decrease in hydration levels. The increase in stiffness of collagen molecule at molecular level corroborates with the experimental finding at fibril level.

The Young's modulus of collagen molecule was obtained from the slope of the Force vs Elongation plot. In addition to this, the effect of simulation parameters such as force constant and pull rate on the Young's modulus of collagen was studied. It was found that at molecular scale, the triple helical structure of collagen stabilized by hydrogen bonds are affected due to hydration levels. The increasing stiffness observed in molecular simulations was attributed to the broken hydrogen bonds. The hydration shells and the density maps were studied to understand the effect of hydration on the microstructure. We further propose to study the effect of anti-aging molecules on the mechanical behavior of collagen molecule.

DEUTERATED CERAMIDES AS A USEFUL TOOL IN STUDY OF MICROSTRUCTURE OF SKIN BARRIER MODEL

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Abstract

Ceramides (Cer) are sphingolipids participating in various biological processes. In the mammalian skin, Cer are localized in the uppermost layer of epidermis, the *stratum corneum* (SC). In this layer, Cer along with cholesterol (Chol) and free fatty acids form multilayer lamellae of intercellular lipid matrix. The skin lipid arrangement in SC is still unclear. To evaluate the skin lipid organization, skin membrane models with labelled lipids as a useful tool in skin lipid arrangement are used. The aim of this work was to synthesize deuterated sphingosine-based Cer, *i.e.*, *N*-lignoceroyl sphingosine-*d*28 (with lignoceric acid acyl (C24); *d*-CerNS) and *N*-lignoceroyl-*d*47 sphingosine-*d*28 (*dd*-CerNS) and to study their phase behaviour in model membranes. First, the deuterated Cer were synthesized and afterwards, synthesized *d*-CerNS and *dd*-CerNS were incorporated into SC model membranes. Model mixtures contained *d*-CerNS or *dd*-CerNS, deuterated or protonated lignoceric acid, and Chol in 1:1:1 molar ratio with an addition of cholesteryl sulfate (5 wt%). Overall, four types of model membranes with different representation of deuterated methylene (CD₂) chains were studied by temperature depended infrared spectroscopy. A phase behaviour of model lipid membranes was investigated. The study of simple SC model revealed that the lipids are well ordered (CH₂ symmetric stretching under 2850 cm⁻¹). Using the deuteration we found out that the lipid chains of sphingoid base are well ordered (CD₂ symmetric stretching under 2090 cm⁻¹), but less than those of deuterated fatty acid or acyl chains. In addition, using the study of thermotropic behaviour we found out the similar miscibility of both deuterated and protonated methylene chains (*i.e.*, similar phase transition temperatures). This good mixing was also confirmed by study of methylene scissoring vibration. Lipid chains are packed in orthorhombic subcell, and deuterated sphingoid base is well mixed with protonated chains (or/and Chol). From this study, we hypothesize that the CerNS prefers the extended conformation (with chains pointing into opposite directions). The results of this study could be helpful in explaining the (patho)physiological arrangement of SC lipids. *The study was supported by Czech science foundation (19-09135J) and Charles University (SVV 260 401).*

Correlation of in vitro Skin Penetration of Ibuprofen within Franz Diffusion cell and Modified Skin Penetration Model (MSPM)

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Abstract

Aim

A new modified skin penetration model (MSPM) device with modular design for the evaluation of ex vivo skin penetration was characterized by means of the penetration of semisolid ibuprofen preparations.

Methods

The MSPM is made of stainless steel and allows for using full thickness skin punches (20 mm). A water soaked filter paper placed under the skin membrane ensures hydration. Bearings enable vertical contact of semisolids during incubation and constant pressure during tape-stripping procedure. Pig ear skin punches were used. Ibuprofen semisolid test formulations were obtained by dissolving the drug in triglycerides (miglyol[®] 812) before mixing with Vaseline to final drug concentrations of 1%, 3% and 10%, respectively. The incubation under infinite dose conditions was performed at 32 °C for 15 min, 60 min and 120 min. Tape-stripping occurred at a diameter of 15 mm. Ibuprofen was quantified after extraction by HPLC/UVVis. Each experiment was performed in triplicate. Franz diffusion cell experiments were run for comparative purposes (FDCs; diffusion area of 1.767 cm²).

Results

Already after 15 min, typical concentration-depth profiles of ibuprofen were found in the *stratum corneum* (SC) for all dose strengths. Increasing the dose strength from 1% to 3% or to 10% resulted in disproportionately high ibuprofen quantities in the SC. Prolongation of the incubation time to 4-fold (60 min) and 8-fold (120 min) resulted in a reduction of the disproportion. Increasing the dose strength from 3% to 10%, however, indicated dose proportionality. Very low concentrations of ibuprofen in the epidermis and dermis were found throughout. Similar results were observed for the MSPM and the FDC for all incubation times and dose strengths suggesting a vice versa interchangeability of the experimental settings for characterizing skin permeation.

Conclusions

Skin penetration testing using the MSPM enables access to concentration depth profiles and distribution already after only a few minutes (15 min). MSPM allows for hydration of the inserted skin punches like the FDC. Based on our data, a concentration of 3% ibuprofen seems to be the optimal dose strength. The MSPM prototype seems to be a promising alternative to FDC allowing for detailed information on skin permeation in a significantly shorter time than standard FDC experiments. Furthermore, the modular design allows for gathering additional information such as the change of temperature during the experiment as well as for testing different experimental settings, e.g. rubbing/massaging during incubation.

Glucosylceramide-to-ceramide processing during skin barrier formation studied by monolayers

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Abstract

The uppermost skin layer, *stratum corneum* (SC), represents the skin barrier, which protects mammalian organisms against environmental factors, and prevents body from water loss. The intercellular space of SC is composed of ceramides (Cer) together with free fatty acids and cholesterol. All Cer subtypes are synthesized from their polar precursor, glucosylated Cer (GlcCer). The removal of the polar part from the precursor is taking place by the hydrolytic enzyme β -glucocerebrosidase (GlcCer-ase). Lack of this enzyme leads to accumulation of precursors and a disturbed skin barrier function, which is manifested as e.g. Gaucher disease (subtype 2). [1] The aim of this study was to investigate the processing of GlcCer to Cer by monolayer lipid models of SC. The control monolayers contained GlcCer, free fatty acids and cholesterol in equimolar ratio. In the first part of the experiment, GlcCer was gradually (75, 50, 25, 10, 5 %) replaced by Cer. As liquid subphase, on which monolayers were formed, phosphate buffer (pH 7.4) and acetate buffer (pH 5.0) were used. Langmuir isotherms showed that the tight arrangement of lipid molecules is nonlinearly dependent on precursor's concentration. The area per molecule of the mixtures with GlcCer was higher than in the sample without the precursor, but the 50 % GlcCer had similar area per molecule as a mixture without precursor. This correlates with permeability experiments, which showed higher permeability of mixtures containing precursor with an exception for 50 % GlcCer. The lipid mixtures with GlcCer were more compressible than mixtures containing only Cer. Atomic force microscopy analysis of monolayers transferred to mica surface showed that GlcCer is causing spreading of lipidic domains. Consequently, GlcCer-ase was added in acetate buffer (pH 5.0) under the compressed monolayer of GlcCer. The enzyme addition increased the surface pressure while no obvious changes in domain formation was observed with Brewster angle microscopy.

The study was supported by the Czech Science Foundation (19-09600S) and the Charles University in Prague (GAUK 184217 and SVV 260 401).

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The effect of sphingosylphosphorylcholine and glucosylsphingosine on the barrier properties and microstructure of stratum corneum model membranes

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Abstract

In healthy patients, the lipid matrix of stratum corneum (SC) consists of equimolar mixture of ceramides, free fatty acids and cholesterol with an additional minor amount of cholesteryl sulfate. Each component contributes to the barrier function of the skin. Sphingosylphosphorylcholine (SPC) and glucosylsphingosine (GS) are metabolites of SC's constituents with increased concentration at severe skin abnormalities. SPC is upregulated in the SC of patients with atopic dermatitis [1] while Gaucher disease patients displayed elevated levels of GS.[2] In our recent approach, we tried to shed light on the role of specific metabolites of ceramides at the barrier function and microstructure of model SC. Thus, model membranes with varied concentrations of the two metabolites were prepared and their effect at the barrier function and microstructure of model SC was investigated. More specifically, permeation experiments using theophyllin and indomethacin as model drugs were performed and transepidermal water loss (TEWL) and electric impedance of model membranes were measured. The microstructure of the membranes was analyzed by X-Ray powder diffraction. Our results indicate that TEWL was increased after 20 % addition of SPC and reached values up to 1.5 times higher than the control for both SPC and GS. In addition, X-Ray diffraction suggest that long periodicity phase is clearly visible up to 10 % of GS or SPC while it disappears at higher concentrations. At 100 % concentration the microstructure of the membrane is completely different but with similar trends for the two metabolites. *The study was supported by SVV: 260401 and Czech Science Foundation (GACR 19-09135J)*

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Development of optimized animal component free and defined skin culture media for long term maintenance of functional human skin explants

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Abstract

Background: Up to now, different skin models, such as 3D-reconstructed skin or standard monolayer (2D) cell cultures, have been used for biological skin studies and for ingredient or finished product testing. However the major limitation of those models is the lack of skin native complex architecture because of containing at best 2 or 3 cell types. Skin native explants remain the relevant physiological model since they are the best mimic of the in-vivo situation, but skin explants can be normally cultivated at 37 ° C for a short period. Therefore many efforts were made on developing an optimized skin culture medium for long term maintenance of skin explants.

Objective: We aimed at developing two novel defined and animal component free skin culture media for 1) hypothermic transport/storage and 2) long term maintenance of skin explants. Those optimized media were evaluated for skin structure integrity and physiological functions Our objective was to reach 5 days of transport/storage at 4 ° C followed by 14 days of skin explant culture at 37 ° C.

Experimental Methods: The abdominal skin tissue samples were processed within 24h post-surgery. The skin disc samples were made and then either directly cultured in skin culture medium at air-liquid interface at 37 ° C up to 14 days, or in storage/transport medium immersion at 4 ° C for 5days before the skin culture period. The skin samples for each time point at different conditions were analysed for (i) structure integrity by histological staining and transepithelial electrical resistance (TEER); (ii) skin viability through MTT assay and (iii) physiological functions and location by using specific biomarkers of different types of skin cells.

Results: Our data showed that the skin disc samples could be storage at 4 ° C up to 5 days in this optimized storage/transport medium, the skin structure integrity was well maintained. In addition, the skin culture medium extended the life span of skin explants up to 14 days. Viability test at each time point showed the skin tissue is viable and hematoxylin and eosin histological staining evidenced an intact epidermal/dermal interface. The TEER values were in agreement with histological observations and functional studies.

In Silico Modelling Penetration of Xenobiotics through Human Skin

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1

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Abstract

The skin is the largest organ of our body. In particular, it is the barrier protecting the body from the uncontrolled penetration of alien substances. Originating in pharmacy, quantitative understanding of the barrier function of human skin more and more becomes crucial for a several aspects of medicine. In this context, mathematical models including detailed cellular and subcellular structures are developed. To treat problems of this complexity, novel mathematical models, methods and software tools are necessary. In recent years, such models, numerical methods and tools have been developed, allowing to attack these problems. In the talk, we present such models, discuss some of the major challenges of the problem and show the impact of the simulation results on the understanding w.r.t. penetration of xenobiotics through human skin.

The application of a skin surrogate model in screening niacinamide formulations

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Abstract

In vitro permeation study plays an indispensable role in determining skin penetration of pharmaceutical or cosmetic ingredients. However, there are ethical and practical difficulties associated with sourcing animal or human tissue. The variability between samples from different donors may also be a problem when interpreting experimental data. Hence, a reliable, robust, accessible and efficient surrogate for evaluating skin permeation is desirable. The Parallel Artificial Membrane Permeation Assay (PAMPA) model has recently been introduced and proposed as a synthetic stratum corneum model. In our previous studies, this model was shown to be a promising screening tool to predict human skin permeation of ibuprofen and niacinamide (NIA) from simple solution formulations. Here, we conducted a systematic comparison of the in vitro permeation behavior of NIA from more complex formulations in (i) conventional vertical Franz diffusion cells with excised human skin or porcine skin and (ii) the Skin PAMPA model, under finite dose conditions. The vehicles selected included dimethyl isosorbide (DMI), propylene glycol (PG), Transcutol R P (TC), t-Butyl alcohol (T-BA), oleic acid (OA), linoleic acid (LA); all of these compounds are widely used in topical and transdermal formulations. A finite dose of 5 ul/cm² was used for permeation studies conducted with human skin and porcine skin, while a finite dose of 1 ul/cm² was employed for PAMPA experiments. The Franz diffusion cell studies were run over 24 h and the PAMPA experiments were conducted up to 2.5 h. A linear correlation ($R^2 = 0.7$) was observed for the permeation data obtained in PAMPA at 2.5 h and porcine skin at 24 h. Although attempts were made to determine similar R^2 values for the data from human skin and PAMPA, no correlations were observed. Interestingly, significantly higher permeation of NIA was observed from PG:LA or PG:OA in human skin compared with all the other tested formulations ($p < 0.05$), but a very different result was observed for the PAMPA experiments. The cumulative amount of NIA that permeated from PG:LA was significantly lower than all other formulations in PAMPA ($p < 0.05$). This may reflect interaction of the lipid components of the PAMPA membrane with this formulation but further studies are needed to probe this. Future work will investigate PG and LA uptake by the PAMPA membrane. This should provide insight into which formulations are best suited to evaluation using this model.

In vitro skin models: a novel lipid-based human stratum corneum mimetic model for the optimization of skin drug formulations

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1

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Abstract

Transdermal delivery represents a very attractive administration route that provides various advantages over other methods of administration, including enhanced patient compliance via non-invasive, painless, simple application and reduced side effects. Thereby, the research on suitable drugs for this route continues to increase. Yet, alternatives to animals and human skin are impelled by economic and ethical reasons. In this context, the main goals of this study are to develop and characterize a stratum corneum model that mimics this human skin layer, inspired on a phospholipid vesicle-based permeation assay (PVPA), and to validate it using skin drug formulations. In the past few years, Nanostructured Lipid Carriers (NLCs) have been widely investigated and applied as a promising nanocarriers for skin delivery. To further unravel the skin permeation/penetration profile of NLCs a systemic investigation was conducted. To mimic the human *stratum corneum* (SC) layer, the phospholipid vesicles were prepared with a selected lipid composition, which closely corresponds to the main human lipid classes on this skin layer. The design of the developed model was optimized using dynamic light scattering, phospholipid quantification and scanning electron microscopy images. To compare the SC model developed with the porcine skin model, the storage stability was assessed as well the calcein permeation in the presence of several conditions: a pH range, co-solvents, surfactants and drugs. Also the in vitro model was applied to evaluate the permeation of NLCs with different compositions. The SC PVPA model was able to detect calcein permeability differences in the presence of different drugs commonly used in the therapy of skin-related diseases. The obtained data correlated well with the well accepted pig ear model, which highlights the potential of this new human SC model. Hence, this model can thereby constitute a valuable tool to improve the process of transdermal drugs development, as it may reduce the duration and the economic costs associated, and even replace the animal testing, during early stages of drug development.

Development of whey protein-rich shampoo and cleansing gel for personal care cosmetics

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Abstract

Nowadays, the addition of protein-rich substances in formulation for cosmetic products is of high interest and the number of marketed products with proteins increases every year. For the cosmetics purposes native and hydrolysed forms of proteins are preferred, due to their moisturizing, protecting and film forming properties. Within the cosmetic industry the proteins used are usually originated from animals, like milk proteins. Whey proteins in liquid or dry form act as emulsifiers, gelling agents, foam stabilizers and water binders. Mostly, the whey cosmetic products are used for hair and skin care, as shampoos, conditioners, cleansing lotions and gels [1, 2].

The aim of the present research work was to develop cosmetic products with whey to ensure zero waste from the processing of milk. Liquid and spray-dried form of whey in two different cosmetic products, i.e. hair shampoo and gel for face cleaning were under investigation. Three different whey fractions, acid whey, permeate, and flow-through whey, were incorporated in the products instead of the amount of water. The technological process for producing cosmetic products was newly developed and adapted to the incorporation of whey. Preliminary stability studies at different temperatures to evaluate physico-chemical properties (pH, viscosity) and organoleptic evaluation were also carried out. Regarding shampoos the effect of NaCl on product viscosity and stability was tested.

The results show that addition of all three whey fractions leads to production of shampoo and gel in a concentration dependent manner. While for liquid whey the highest amount is up to 20 % (w/w), the optimal concentration for dried whey is 2 % (w/w). Use of amount in excess of that resulted in product instability and microbiological contamination. Shampoo stability was also affected by NaCl, since amount above 2 % (w/w) led to formation of larger lamellar micelles of primary surfactant used, which resulted in increased viscosity but lower stability of shampoos seen as sedimentation. Prepared gels and shampoos expressed high pH values, therefore the addition of citric acid was needed to adjust pH to 4.5-5.5.

According to results, we can conclude that by incorporating whey, the stable cosmetic products with added value can be formed.

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The effect of personal hygiene products on the skin barrier function

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Abstract

The skin function as a protective barrier layer of the stratum corneum is intended to prevent the loss of fluids from the body and simultaneously preventing the penetration of infectious agents, xenobiotic and chemical substances. It also plays a key role in the percutaneous absorption of lipophilic and hydrophilic drugs. An indirect assessment of this function is achieved by evaluating Transepidermal Water Loss (TEWL). It is a non-invasive method that quantifies the flux in water vapor density when the water evaporate passively through skin to the external environment. The TEWL value shows changes in case the skin barrier is affected by various pathologies or in case it is influenced by chemical substances or personal hygiene products. The aim of this study is to assess the impact of daily personal hygiene products on the integrity of the skin barrier function in case of healthy, female subjects, aged between 41 and 59 years old. The essential exclusion criterion was represented by dermatological diseases. 50% of the subjects included in the study presented daily professional exposure to chemical substances of pharmaceutical use. The influence factors studied were the soap type used and the age of the subjects. The values of the temperature and humidity were recorded constantly, using the specific detectors of the determination device. The obtained results indicated lower TEWL values to the group of subjects who used soap formulated in liquid form (9.14 ± 0.95 and $18.9 \pm 2.47 \text{ g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$), compared to the group who used solid soap (8.28 ± 3.32 and $20.64 \pm 11.86 \text{ g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$). The study demonstrates that the ingredients used for solid and liquid soaps have direct influence on the skin protection barrier; products' pH also plays a major role. The Transepidermal Water Loss is in direct relation with age. Thus, the study results indicated that the TEWL values varied between 8.28-18.46 $\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ for the subjects aged between 40-50 years, while for those over 50 years the TEWL values increased considerably. Although in the study was used an open-chamber device, temperature and humidity variations were minimal. In conclusion, it can be stated that there are no direct measuring devices or a direct measuring method for the Transepidermal Water Loss and consequently, for the skin's barrier function. Nonetheless, with the help of TEWL determinations, it can be demonstrated the influence of certain external factors on skin barrier integrity.

Cosmetic compounding as a model of modern skin care – examination of attitudes and prevalence of use among pharmacy patients

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1

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Abstract

Healthy and attractive skin is of great value to the well-being of individuals. (1) Due to variations in the genetic structure, habits, and the influence of external factors, the assessment of the physiology of the skin cannot be generally defined, but it requires testing on individuals. The compounding of skin care products in pharmacies, based on the individual approach to each user could represent one of the latest trends in skin care. (2) The knowledge obtained through instrumental assessment combined with the patient's own analysis of his/her expectations and habits can play an important role in better understanding of skin care personalization. (3) The aim of this research was to examine the attitudes of individuals and the self-assessment of the skin condition through the questionnaire before and after the use of compounded cosmetics.

The research was conducted in the form of a questionnaire at the Dona Pharmacy, Nis, Serbia, where compounded products were formulated; the questionnaire was based on the subjective examination of the skin and attitudes of the individuals before, as well as three weeks after the first use of a compounded cosmetic product. The analysis included 55 distributed questionnaires that contained 16 open and closed questions.

The initial problems encountered by the users/respondents were dehydration of the skin and increased sebum level, despite the long use of different commercial skin care products. The largest number of respondents (61%) decided to use compounded cosmetics because they liked the fact that it was prepared for their individual needs, 26% of them had a bad experience with commercial cosmetics, while 13% of them considered that compounded cosmetics contained less parabens and other "harmful" substances. After three weeks of using compounded cosmetics, respondents have reported the improvement of skin hydration (90% of them) and sebum reduction (68%).

Despite ambiguous regulatory requirements on compounding of cosmetics in pharmacies in Serbia, pharmacists have traditionally compounded skin care products, and this is considered to be a valuable aspect of our practice. Individualization seems to be one of the most promising concepts in modern skin care because of better compliance and attitudes of users towards the effects of compounded cosmetics vs. commercial cosmetics particularly regarding skin hydration and the use on oily skin.

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Fuller's earth: from a dry powder to an innovative formulation for skin decontamination

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Abstract

Fuller's earth (FE) as a dry powder is a well-known skin decontaminant used by the French Army in order to limit systemic damages caused by chemical warfare agents, such as organophosphorus. But, as cosmetic clay, it has also been largely used as a traditional and natural tool to detoxify skin. When this clay is formulated as a water suspension (9% w/w) its decontamination efficiency is highly improved regarding the penetration of 4-cyanophenol through pig ear skin [1]. By using VitroPharma cells diffusion an analog of Franz 'cell [2] new FE water suspension (with different concentration from 5% to 15%) were tested to determine the decontamination efficiency and the improvement of the functional prognosis. Permeation profile of an organophosphorus pesticide for 24 hours was assessed through both pig ear and human abdominal skins. After kinetic analysis, skin was separated in three compartments, respectively *stratum corneum*, epidermis and dermis, in order to determine the depth of the pesticide contamination, using HPLC-UV detection. Kinetic of contamination in the receptor compartment follows the same pattern for each suspension and a plateau is reached after 10 hours meaning that this decontamination process is indeed effective, using FE powder as read-out. After assessing toxicity of the formulation *ex vivo* using dermal fibroblasts and *in vivo* with *Danio rerio*, *in vivo* experiments on SKH1 hairless mice were realized. Decontamination efficiency was evaluated through physiological parameters, such as acetylcholinesterase levels, in addition to local and systemic inflammation profiles.

Taken together these results leads us to propose the development of a new cosmetic skin care, based on Fuller's earth suspension, in order to protect skin against environmental pollutant.

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Sunscreen formulation protective effect against environmental aggressions (pollution, ozone, UVs) on skin

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Abstract

Skin is exposed to external stress including UV or pollution such as particulate matter (PM) or ozone generated by human activities. When adsorbed on the skin, molecules like polycyclic aromatic hydrocarbons penetrate, regulate XME activity and generate deleterious effects (oxidative stress, lipids peroxydation...). UVs exposition leads to oxidative stress and photoaging.

To evaluate protective effect (shield effect, anti-oxydant, lipids protection) of a formulation against environmental aggressions different complementary assays were performed.

Shield effect against pollution was studied based on the modulation of the skin penetration of a mix of radiolabeled B(a)P and Urban Dust PM_{2.5}&PM₁₀ with and without application of the formulation on human skin explants. The formulation protects from pollutants penetration into the skin and divided by a factor 3.5 the skin penetration of the pollutants.

To study protection against oxidative stress induced by ozone, squalene degradation was quantified by GC/MS *in tubo*. The oxidization of squalene, a specific human skin lipid, bring important consequences at various levels and appears a reliable marker of various sources of pollution-induced assaults. The tested formulation is more protective (37.4%) than positive control (21.5%) from squalene degradation induced by ozone.

Effect of UVA and Urban dust on reconstructed skin model was studied based on malondialdehyde (MDA) level by GC-MS. MDA level allows to evaluate lipid peroxidation induced by UVA and pollution. Formulation significantly protects by 20,9% lipid peroxidation induced by environmental aggressions exposure (UVA/Pollution).

In conclusion, the studies performed allow to conclude on the protective effect of the formulation against several environmental factors like ozone, UVs and Urban dust by creating a shield effect and reducing oxidative stress.

The importance of 12R-lipoxygenase and transglutaminase activities in the hydration-dependent ex vivo maturation of corneocyte envelope's

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Abstract

Corneocyte protein envelopes (CPE) are assembled in the late stage of keratinocyte differentiation and coated covalently with lipids to form the corneocyte lipid envelope (CLE). In this process 12R-lipoxygenase (12R-LOX) and other enzymes process linoleoyl acylceramides before transglutaminase 1 (TG) attaches the omega-hydroxy fatty acid-containing ceramides covalently to the CPE while forming isodipeptide bonds. This work demonstrated a) the effect of relative humidity on ex vivo cornified envelope (CE) maturation, b) the effect of a mixture of broad spectrum protease inhibitors (PI) on TG activity and on the first and ninth tape strippings of the photo-exposed (PE) cheek and photo-protected (PP) post auricular sites from healthy Chinese volunteers (n=12, age 25 ± 3 years). CE maturity was assessed by CE rigidity and CE hydrophobicity per unit of CE surface area. Irrespective of tape stripping depth, PE samples had an increase in CE rigidity to the same extent as more mature PP samples, but such improvement was lacking for CE hydrophobicity. Ex vivo CE maturation was optimal at 70% RH but CE rigidity reduced by the TG inhibitor LDN-27219. CE hydrophobicity remained unchanged irrespective of ex vivo conditions. The second and eighth tape strippings were examined in order to understand the effect of RH on the activity of TG. High hydration diminished TG activity more than the commercially available inhibitor LDN-27219. Furthermore, a protease inhibitor mix was able to overcome the negative effect of over-hydration. Both enzymes showed a similar pattern of activity in both anatomical sites where PP samples were enzymatically more active especially in the deeper SC layers. This study highlights the impact of relative humidity with special focus on the roles of TG activity in CE maturation.

Humidity-dependent ex vivo corneocyte maturation and the importance of transglutaminase activity

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Combined mechanical and chemical skin damage: effect of everyday skin care on skin properties and barrier function

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Abstract

The skin represents our primary barrier against ingress of chemicals and loss of water. Skin penetration/permeation studies to estimate the penetration potential of applied chemicals normally involve intact human or animal skin in ex-vivo or in-vivo experimental setups. However, under real-life conditions, human skin is subject to numerous small stresses on a constant basis that might add up to impair barrier function. Little is known about the impact of combined mechanical, chemical and light-induced everyday stresses, e.g. the combined effect of hair removal, washing processes and exposure to sunlight. Since ex-vivo models are important for penetration studies and risk assessment, such a model involving real-life conditions would be of high practical interest.

As one step of this project, the aim of this work was to assess the effect of different washing procedures alone and in combination with razor shaving on physiological skin parameters and skin penetration of model substances using porcine ear skin ex-vivo and human forearm skin in-vivo. The tested procedures simulating potential chemical skin damage included brief washing for 60 seconds with sodium dodecyl sulfate 15% w/v, with the lecithin mixture S LPC65 15% w/v, with a sodium lauryl ether sulfate-based commercial washing product, with ethanol 70% v/v and with purified water as control. These procedures were conducted alone and after razor shaving with a commercial shaving foam. The skin was investigated by biophysical techniques beforehand; after the procedures, the skin was analysed again or incubated with a sodium dodecyl sulfate solution to evaluate the skin penetration behaviour of this model substance into treated and untreated skin by non-invasive confocal Raman spectroscopy. Physiological skin parameters were assessed before and after treatment of the skin; the parameters of interest included skin hydration, transepidermal water loss, stratum corneum permittivity, sebum content and pH.

First results showed minor effects of the individual tested brief washing procedures. In general, the determined values showed high variations due to inter- and intra-individual differences. Final results will reveal if the individual effects are potentiated by combination with razor shaving.

Alcohol gels can strengthen skin-barrier function and reduce skin susceptibility to irritating soaps

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Abstract

The use of disinfectants is crucial to prevent the spreading of nosocomial infections in healthcare workers. As many as 25 applications of hand disinfectants is a realistic default value during a working day according to the European Chemical Agency (ECHA) (1). Besides efficacy, the safety for the workers is crucial. However, skin irritation reduces the acceptance of disinfectants (1,2).

Disinfectants are usually based on alcohols. Alcohols enter membranes and increase the sensitivity of the living parts to other agents, which is advantageous when it comes to microorganisms, but is bad for sensitive skin (1, 2). Skin conditioning agents, such as urea, may therefore be a useful additive in disinfectants, similarly to its beneficial effect in moisturizing creams (3).

The influence of a urea-containing alcohol gel* on the skin barrier-function was therefore evaluated by studying changes in skin barrier function and sensitivity to soap-exposure in a randomized and controlled study (n=22). The contralateral forearm served as an untreated control.

Twice daily treatment with the 70% ethanol-gel did surprisingly not cause dryness (measured as skin capacitance) but resulted in an improved barrier function at day 18, measured as lower transepidermal water loss (p=0.02). Furthermore, the sensitivity to the irritating soap (sodium lauryl sulfate) decreased and the participants showed lower degree of visible irritation in the treated area compared to the control area. The instrumental measurements confirmed reduced sensitivity to the soap (lowered TEWL p=0.007 and redness p=0.02) by the treatment.

In conclusion, twice daily treatments with the urea-containing ethanol gel prevented dryness, reduced TEWL, and decreased skin-sensitivity to soaps. These findings may have relevance for healthcare workers in their daily disinfectant procedures.

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*Patent pending

Cleaning and repairing the sensitive skin in dogs

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Abstract

The use of disinfectants or antibiotics reduces colonization of microorganisms, responsible for skin infections or exacerbations of skin diseases. Colonization by *Staphylococcus aureus* is often found in humans with atopic dermatitis (AD). Patients with AD also have a defect barrier function, which contributes to eczema. Not only humans, but also dogs suffer from AD, where microorganisms are common causes of flares (1). Barrier repair is fundamental in the management of AD in humans, and is becoming increasingly important also in the management of atopic dogs.

The treatment of bacterial and yeast infections is recommended to consist of topical and/or systemic antimicrobials by the International Committee on Allergic Diseases of Animals (ICADA) (1). However, veterinarians and pet owners are recommended to watch for an irritating effect of topical antimicrobials, as these might induce flares of AD in their patient (1). The increased use and resistance to antibiotics are problematic.

A new 70% alcohol-gel without antibiotics has been tested on dogs with irritated skin in an open user-study. The gel contained skin conditioners, such as the barrier-improving urea* (2).

The dog owners were recruited from the company registers of customers (www.allergenius.se) and social media and were asked to test the gel* on affected areas on their dogs. In total 62 tubes were dispensed for testing. The results from the treatment were reported in a questionnaire and photos of the areas were submitted. Areas treated with the gel includes skin folds around the mouth, groins, feet/pads, but also in more openly-exposed areas on the skin.

Most of the dogs (> 90%) improved by the treatment with the ethanol gel. The pet owners became satisfied. The areas showed a healthy appearance and previous signs of irritation subsided. Only a few dogs tried to lick the treated areas, or showed discomfort, due to e.g. stinging, itching.

In conclusion, the purifying, antimicrobial and skin conditioning effects from the ethanol gel, free from antibiotics, resulted in a healthy appearance of the skin. Future studies will address the skin barrier improvements in the dogs.

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Ability of the skin to maintain its physiological pH after application of cosmetic products

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Abstract

The importance of the acidic pH of the skin surface as a regulating factor for the maintenance of the stratum corneum (SC) homeostasis and integrity has been recognized in the last two decades (1). The influence of cosmetic products on skin pH is an important part of every research on skin biophysical parameters. In our work we have investigated the effects of three moisturizers on skin pH, together with their effect on SC moisturization (SCM) during 5 hours after application.

Three formulations were prepared: placebo" sample (P), sample with 2% of glycolic acid – GA (G2%) in P sample, and sample with 10% of GA (G10%) in P sample. The pH and moisturizing effects were investigated in 5-hours short-term *in vivo* study on 14 healthy volunteers with normal skin. Once basal values were measured, samples were applied and measurements were repeated after 30 minutes, 1, 2, 3, 4 and 5 hours. Skin parameters were measured with Cutometer R MPA580 (Courage+Khazaka, Germany).

Sample P had pH=4.7 and samples G2% and G10% had pH adjusted to 3.7. Basal pH values, prior to application, on treated sites were similar; 5.37 ± 0.44 (max=6.01, min=4.48), 5.49 ± 0.49 (max=6.38, min=4.55) and 5.43 ± 0.51 (max=6.14, min=4.41). Corresponding pH values on treated sites after 5h were significantly different between placebo and GA samples ; P: 4.61 ± 0.29 (max=4.96, min=4.15), G2%: 4.19 ± 0.06 (max=4.28, min=4.09) and G10%: 4.09 ± 0.07 (max=4.25, min=3.98). After application, pH decreased significantly when compared to basal values on each site in every time point (30min, 1, 2, 3, 4 and 5h). SCM significantly increased in every time point after samples with GA were applied, while placebo sample increased SCM after 30 min and 1h, all compared to basal values. Samples with GA increased SCM when compared to placebo sample after 2 and 3h, while only G10% succeeded to increase moisturization after 4 and 5h, when compared to placebo samples' effect at the same time points.

The study showed that the skin pH could be significantly altered when samples with different pH were applied. Nevertheless, skin has an ability to reverse these changes, faster and easier with samples containing lower acid concentration/being less acidic. Correlations between the effect of samples on skin pH and SCM should be additionally investigated.

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Evaluation of *Alchemilla vulgaris* L. extract as a promising cosmetic active: chemical characterization, antioxidant activity and skin barrier repair potential

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Abstract

Introduction

Botanical extracts represent one of the largest categories of cosmetic actives found in the marketplace today due to the rising consumer demand for natural products. Taking into account traditional application of Lady's mantle (*Alchemilla vulgaris* L., Rosaceae), as the plant with beneficial effects on skin, and the results of our previous investigations which have shown its safety on skin and wound healing potential, the aim of this study was to promote its ethanolic extract as effective cosmetic active.

Materials and methods

The total phenols, tannins and flavonoids content of leaf ethanol extract and its antioxidant activity in lipid peroxidation inhibition test *in vitro* were determined. Besides, HPLC analysis was performed. We used carbopol-based hydrogels as model vehicles for chemically characterized *A. vulgaris* extract in different concentrations. We estimated their skin barrier repair potential *in vivo* by measuring the following biophysical parameters: transepidermal water loss (TEWL) and *stratum corneum* hydration (SCH) on the human skin pretreated with sodium lauryl sulfate (SLS) as a common test substance for induction of skin barrier damage.

Results and discussion

Performed HPLC analysis revealed the presence of six phenolic compounds, while isoquercetin (4,0 mg/g), ellagic acid (3,4 mg/g) and morin (1.9mg/g) were the main compounds. Lady's mantle extract *per se* showed satisfying lipid peroxidation inhibition ability and inhibited this reaction significantly even in the concentration of 12.5µg/ml. Regarding biophysical measurements, significant changes of both TEWL and SCH were recorded before vs. after SLS induced skin impairment, and after subsequent treatments with investigated gels, confirming satisfying barrier repairment potential of investigated samples.

Conclusion

Our study offers evidences about the benefits of the potential use of *A. vulgaris* ethanolic extract as cosmetic active intended for dry and irritated skin care. Also, this study suggest that *A. vulgaris* extract could be used to protect dry as well as normal skin against damage caused by free radicals and reactive oxygen species. It is likely that antioxidant activity of *A. vulgaris* play a significant role in the skin barrier repair potential and may be attributed to its phenolic content.

Monitoring of active ingredients penetration by Raman imaging of skin

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Abstract

Encapsulation of active cosmetic ingredients (ACI) in delivery systems has emerged as a novel strategy to overcome the skin barrier function, ensuring an increased penetration through the *Stratum Corneum*. For instance, Alginate nanocarriers (ANC) developed by our laboratory are very attractive vehicles to encapsulate hydrophobic active molecules that need to reach the deep layers of the skin in order to exhibit maximum efficiency [1]. Although major advancements have been made in the area of physico-chemical characterization of the nanocarriers, biological assessments remain particularly limited due to a lack of suitable and label-free analytical techniques. Confocal Raman imaging has evolved as an important noninvasive approach [2] providing the distribution of topically applied ACI in skin layers which is a decisive prerequisite to evaluate the *Stratum Corneum* permeation to a given nanocarrier and consequently optimize the encapsulating protocols of active molecules. The study aims to develop a reliable approach based on Raman micro-imaging coupled with powerful statistical method NCLS (Non Negative Least Squares) for monitoring Delipidol R (BioEurope) loaded on alginate nanocarriers (ANC-D). The ANC-D were applied for periods of time between 1h and 3h in order to measure the kinetics of diffusion through the skin. Using different references spectra the NCLS algorithm informs about the contribution of each compound in the original spectrum then cartography of the spatial distribution of different physiological constituents (lipids) and of the ACI can be constructed. The penetration profiles obtained clearly support the accumulative effect of the Delipidol R in the *Stratum Corneum* but also highlight increasing levels in the deepest layer of the epidermis for extended exposure [3]. Ultimately, Raman imaging is a promising tool for both industrial and research applications in the field of cosmetic that can be adapted to *in vitro* screening of newly identified ACI and/or newly developed encapsulating systems.

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INFLUENCE OF PLASMID DNA CONFORMATION IN PRIMARY HUMAN SKIN CELL TRANSFECTION: COMPLEXATION WITH LIPOSOMES.

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Abstract

Although viral vectors are becoming safer over time, their immunogenicity and ability to do insertional mutagenesis still curtail their large-scale use in gene therapy. Non-viral gene delivery systems, such as liposomes, are less efficient but they are less immunogenic, less toxic and more suitable for long term expression¹, especially in the context of topical application on the skin. While liposome formulations are studied to improve transfection efficiency, there is less information about the conformation of plasmid DNA (pDNA) in lipoplexes. In the laboratory, several lipoplex formulations are being tested to efficiently transfect primary cells located in the skin. Meanwhile, pDNA conformational changes occurring during complexation with liposomes is under investigation. Even if it is commonly accepted that pDNA conformation impacts the transfection rate efficiency^{2,3}, it is still unclear whether the supercoiled (SC), open-circular (OC) or linear form is best to use in terms of transfection efficiency.

This study aims to determine if cationic liposome (DOTAP/DOPE) can affect pDNA conformation and to define the best ratio between SC, OC or linear forms to enhance the transfection efficiency in terms of percentage of cells expressing the plasmid and of intensity of this expression.

Analysis of conformational changes of pDNA occurring upon complexation indicate that the concentration of DOTAP modifies SC/OC/linear pDNA ratios. Moreover, we demonstrated by FACS that pDNA conformation has a higher impact on the percentage of cell transfected and the expression intensity in primary dermal fibroblasts than in cell lines such as HeLa cells.

Our preliminary results highlight that conformation of pDNA impact transfection efficiency in primary human skin cells. Complexation of pDNA with high concentration of DOTAP increases the OC form compared to the SC form. It seems however that the SC form of pDNA is the more efficient to transfect primary cells. In conclusion, this study aims to define the best compromise between liposome chemical content and pDNA conformation to efficiently transfect primary human skin cells.

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INFLUENCE OF PLASMID DNA CONFORMATION IN PRIMARY HUMAN SKIN CELL TRANSFECTION: COMPLEXATION WITH LIPOSOMES.

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Non-destructive quantitative analysis of semi-solid forms loaded with encapsulated active molecules (by vibrational spectroscopy)

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Abstract

Recent evolutions in formulation strategies have seen increasing numbers of vectorisation or encapsulation approaches for active pharmaceutical or cosmetic ingredients administration to the skin. Encapsulation systems are then dispersed in appropriate galenic forms like gels or creams. To achieve quantification of the active ingredients in those complex mixtures a combination of chemical extraction protocols followed by separation analytical techniques such as High Performance Liquid Chromatography (HPLC) can be used. However, this method remains fastidious and generates considerable amounts of chemical waste which is in conflict with current concerns aiming to develop green chemistry alternatives. Infrared and Raman spectroscopy deliver molecular fingerprint of samples enabling both characterisation of the composition but also to define concentrations of specific compounds.

Alginate based-nanocarriers (ANC) were selected as a model to demonstrate their potential to accurately quantify Omegalight R (Bioeurope, France), a lightening agent loaded in ANC for the purpose of the study. Additionally, the loaded ANC were incorporated in a hydrogel matrix containing additives such as Cosgard[®] (0.5% w/w), an antimicrobial preservative, and glycerol (10% w/w), as a humectant, to mirror commercialised end products.

Data handling remains a critical aspect to successfully extract relevant spectral features from the complex data sets collected and ultimately construct reliable regression model. Coupling multivariate analysis such as Partial least square regression (PLS-R) with preprocessing methods such as Extended Multiplicative Signal Correction (EMSC) generate RMSECV of 0.0923% (w/w) with predicted experimental concentrations with relative errors below 5% compared to the true concentration. Infrared and Raman spectroscopy are label free and nondestructive methods perfectly suited for analysis of complex cosmetic formulations, however development of robust protocols for quantitative analysis is the next milestone to reach for transposition of the techniques as quality control tools in industrial environment.

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Study of the effects of storage conditions on the barrier function of reconstructed human epidermis by confocal Raman spectroscopy

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Abstract

The development and characterization of reconstructed human epidermis (RHE) is an active area of R&D. RHE can replace animal tissues in cosmetic, pharmaceutical and toxicological sciences, yielding scientific, economical and ethical advantages. To date, little is known on the effects of storage conditions on the barrier function of RHE. Confocal Raman spectroscopy (CRS) is fast becoming an established method for non-invasive analysis of skin. It has not, however, been widely used with lab-grown skin equivalents. Here we use CRS to quantify the effects of different storage (freezing) conditions on the barrier function of the commercially available EpiSkin R RHE. In parallel, we use conventional skin penetration methodology and quantification by HPLC. Preliminary data indicate an effect of storage conditions on the RHE's barrier function. The permeability of resorcinol applied in aqueous solution is significantly higher in EpiSkin R RHE stored at -20 °C and -80 °C, compared to fresh RHE, suggesting a decreased barrier function in the former. These data contradict published results on the effects of freezing on excised human and animal skin. We are currently investigating the effects of storage conditions on the RHE's barrier to a range of actives and vehicles (hydrogel, emulsion). This project yields novel practical insight into the usage of RHE, while at the same time further establishing CRS for non-invasive investigation of the skin barrier in cosmetic and pharmaceutical R&D.

3D visualization of ambient effects on skin and topical formulations – a synchrotron X-ray tomography investigation

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Abstract

In-depth understanding of skin barrier function and interactions between the skin and specific excipients comprised in topical formulations clearly benefits development of efficacious medications for various skin diseases. Since many formulations are heterogeneous and often contain volatile components (e.g. water) they will inevitably suffer reformulation when applied, leaving a residual film on the skin surface. This may change the thermodynamic activity of the active substance in the residue and affect its permeation across skin. Moreover, film formation may induce occlusion, which subsequently increases skin hydration - a key aspect that changes the physical and mechanical properties of the skin^{1, 2}. In order to understand how the physicochemical properties of skin depend on skin topography, formulation residue formation as well as excipient interactions with skin and effects of ambient humidity, we perform synchrotron X-ray tomographic experiments on porcine skin and topical formulations.

X-ray tomography allowed us to capture high-resolution 3D images of the skin and topical formulations at different controlled humidity. The skin topography and the detailed anatomical features of the (epi)dermis were visualized in details without using any contrasting agents. We were able to measure the thickness of the epidermis in response to hydration and also when formulations are applied on top of the skin. Further image analysis on the 3D formulation stack reveals detailed features of the reformulation layer at various time intervals. The geometrical size, shape, distribution and preferential orientation of the formulation ingredients were obtained and revealed characteristics when applied on hydrophilic lipophilic substrates and on the skin. Our results offer new tools and method to examine skin anatomical features in near native form and the skin interaction with pharmaceutical formulations and reformulation kinetics in controlled ambient conditions that are beneficial in the development of topical formulations and to increase their therapeutic efficacy.

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Development of nanoprobes for *in situ* redox potential measurements in skin models

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Abstract

The cosmetic industry commonly supplements most types of skin care products with UV filters and antioxidant molecules to limit the oxidative stress damages on the skin. The claims of cosmetic products need to be justified by biological tests. The methods available to bring out the antioxidant properties of cosmetic products have to be developed. In contrast to destructive methods, analysis of oxidation-sensitive fluorescence is applicable *in situ* but suffers from the signal dependence on fluorophore concentration. To overcome this drawback, we attempt to develop nanoprobe (NP)-based ratiometric fluorescence approach for an objective *in situ* evaluation of the oxidative stress in skin.

Two fluorescent labels were encapsulated in lipid-based nanosystems, already known for their ability to improve the skin distribution of fluorescent molecules¹. The first label served as a redox probe (RP), the second one, resistant to oxidation, played the role of an internal standard (IS). Systematic assays allowed identifying the optimal concentrations of each fluorochrome to encapsulate. The NPs prepared by the method of phase inversion temperature, show a hydrodynamic diameter of 61 ± 1 nm, a polydispersity index < 0.1 and a slightly negative zeta potential of -8 ± 1 mV.

The approach was tested on keratinocytes in culture: the fluorescence microspectroscopy study demonstrated that the RP/IS signal ratio increased significantly when the cells were stressed, while the signal of the IS remained comparable.

Further steps of the study include application to human skin explant coming from plastic surgery and kept alive, submitted or not to physical or chemical stress. In view of the promising results, we conclude that the optimized nanoprobes could be used as a tool for screening antioxidant effect of skin care products.

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1 H.T.P. Nguyen, E. Munnier, E. Allard-Vannier, X. Perse, F. Yvergnaux, F. Vial, T. Perrier, M. Soucé, I. Chourpa. J Pharm Sci, 2016, 105: 3191–3198 Qualitative and quantitative study of the interest of lipid nanocapsules of 120nm for the topical administration of hydrophobic molecules.

Quantitative *in vivo* analysis of skin penetration of topically applied products based on non-invasive confocal Raman spectroscopy.

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Abstract

When a product is applied to the skin, which constituents are penetrating, how much, how fast, how deep? Answers to these questions are important in product development, in toxicology and in dermatological research.

Raman spectra reflect the molecular composition of the skin, consisting of intrinsic skin constituents and of products that have penetrated into the skin.

We introduce a simple calibration method and data analysis software, based on *in vivo* Raman spectroscopy. It uniquely enables non-invasive quantitative determination of *in vivo* skin penetration of molecular substances, expressed in micrograms/cm² of skin surface.

Quantitative *in vivo* analysis of dermal product penetration is an important extension of our tool kit for skin analysis, with applications in development and testing of cosmetic and personal care products, transdermal drug delivery and in toxicology. It will help to bridge the gap between the *ex vivo* or *in vitro* conditions, under which most experimental penetration results are currently obtained, and the actual *in vivo* situation.

It will also increase our understanding of differences in skin properties at different locations on the body and between people and it provides new avenues for exploring the effect of skin conditions on skin barrier properties.

The method is applicable to water-soluble, alcohol-soluble and lipid-soluble compounds, as will be illustrated with results for salicylic acid, glycerol, caffeine, and other compounds. A growing library of calibrated spectra of actives, fragrances, sun screens, drugs and excipients is available, which can be readily included in spectral analyses to quantify product uptake.

Quantitative *in vivo* analysis of skin penetration of topically applied products based on non-invasive confocal Raman spectroscopy.

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Ex-vivo depth profiling of tazarotene a third generation retinoid via confocal raman spectroscopy

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1

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Abstract

Topical retinoid treatments have proven to be effective in managing plaque psoriasis of any severity, either as a monotherapy or in combination with other treatments. Non-selective retinoids have a multitude of physiologic effects and are usually associated with toxicity problems that limit their therapeutic usefulness. Tazarotene is the first of a new generation of receptor-selective retinoids. Its actions are focused and target two specific retinoic acid receptors (RARs), RAR- β and RAR- γ improving its therapeutic index compared to nonselective retinoids. However, In-vivo studies of topically administered tazarotene gel (Zorac) have highlighted its limited ability to penetrate across human skin. Crystallization of actives on and in the skin is proposed as one of the reasons underlying the limited amount of active which may be delivered to the skin. By enhancing the penetration of tazarotene through rational formulation design, tazarotene delivery to the skin could be optimised improving its therapeutic effect. Many of the current methods used to obtain information about the presence and concentration of molecular compounds in the skin are to a greater or lesser extent invasive. They destructively alter the system under investigation either by extraction of compounds from the skin or by physically disrupting cell layers. Non-invasive techniques such as confocal raman spectroscopy (CRS) can provide detailed information on the penetration of actives and excipients through the skin without disturbing the system being investigated. The main objectives of our recent work were to track tazarotene in the skin and examine the affects penetration enhancers have on its deposition. Conventional Franz diffusion cell studies were conducted using porcine skin to determine the penetration of tazarotene from the commercially available Zorac gel and simple solvent systems after a 6-hour finite dose application. Raman measurements were then performed on the porcine skin using a Skin Composition Analyzer (River Diagnostics) to track both tazarotene and the excipients driving its penetration into the skin. Compared with the commercially available Zorac gel, tazarotene in all solvent systems examined could be detected in greater proportion further into the skin. The findings have provided further support for the use of confocal raman spectroscopy to monitor drug delivery into the skin non-invasively.

Investigating tactile friction and residual film properties of Pickering formulations on skin

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Abstract

Consumer perception of topical formulations plays a central role in the cosmetic industry and there is a constant demand on formulators to develop new formulations that appeal to costumers in terms of functionality as well as sensory feel. Even in the pharmaceutical industry, there is a requirement for cosmetic appeal of the formulations for better patient-compliance. Film formation upon application and subsequent reformulation due to evaporation of volatile excipients will have a major impact on tactile (i.e. sensory, cosmetic) perception of the skin.

Recently, health and environmental concerns have led to an increasing demand for safe, natural and sustainable ingredients. Particle stabilized (Pickering) formulations offer the possibility to create sustainable, surfactant-free topical formulations (1). Whilst starch Pickering emulsions have shown promising performance in creating surfactant-free creams (2–4), it remains interesting to understand the residual film formation properties and sensorial attributes of such formulations.

In the current study, we introduce excised skin in parallel to previously used Vitro-Skin R as model skin. Excised skin was believed to be more representative than a skin simulant. Finger friction measurements were performed using a ForceBoard™ (5,6) to evaluate the perception of touch and possible effect of film formation under controlled ambient conditions. Results were compared against subjective tests by a human sensory panel. Friction measurement of starch Pickering formulations were compared to commercial creams and corresponding residual films were visualized using microscopic techniques. The present investigation presents a method to perform friction measurements on excised skin and evaluate formulation properties responsible for a pleasant sensory perception ex vivo.

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Developing cream formulations: renewed interest in an old problem

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Abstract

Background: The pharmaceutical industry has entered in a new era, as there is a growing interest in increasing the quality standards of dosage forms, through the implementation of more structured development and manufacturing approaches. For many decades, the manufacturing of drug products was controlled by a regulatory framework to guarantee the quality of the final product through a fixed process and exhaustive testing. The emergence of Quality by Design (QbD) as a systematic and risk-based approach introduced a new quality concept based on a good understanding of how raw materials and process parameters influence the final quality profile. Although the QbD system has been recognized as a revolutionary approach to product development and manufacturing, its full implementation in the pharmaceutical field is still limited. This is particularly evident in the case of semisolid complex formulation development. This work aimed at establishing a framework to screen and understand the product variability deriving from factors that affect the quality features of cream formulations.

Research design and methods: As per quality by design-based approach, cream quality target profile and critical quality attributes were identified, and a risk assessment analysis was conducted to qualitatively detect the most critical variables for cream design and development. A Plackett-Burman design was used to screen out unimportant factors, avoiding collecting large amounts of data. Accordingly, two design of experiments (DoE-1 and DoE-2) were performed and the effects of independent variables on the cream formulations responses estimated. The statistical analysis allowed determining the most influential factors.

Results: The most influential process and formulation parameters were identified and at different factors combination, significant variability was observed in droplet size, consistency, hardness, compressibility and adhesiveness from 2.60 ± 0.88 to 9.91 ± 5.63 μm , 7.93 ± 0.05 to 13.53 ± 0.14 mm, 27.60 ± 0.30 to 58.39 ± 1.10 g, 37.98 ± 6.20 to 447.18 ± 37.20 g.s and 25.66 ± 2.12 to 286.30 ± 32.86 g.s, respectively.

Conclusions: This study successfully implemented QbD principles to cream design and development. Such approach assisted in the reduction of practical issues in pharmaceutical technology research by forecasting the most critical formulation and process parameters on product quality and performance. The promising outcomes corroborates the applicability and relevance of QbD industrial implementation in the early stages of pharmaceutical development, since it improves the fundamental understanding of cream formulation and manufacturing process, minimizing product variability and assuring its final quality.

UV imaging of the drug permeation process in a developed Franz cell

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1

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Abstract

The analysis of drug behaviour in the drug development process is essential. Whole body autoradiography (WBA) is used in the determination of drug and its metabolites in animal tissues. Some of the limitations associated with this current methodology means techniques such as MALDI-IMS-MS has gained popularity due to its ability to image a range of drugs and metabolites without the need of expensive radiolabelling (1). Terahertz imaging, optical coherence tomography, confocal microscopy and photoacoustic microscopy have also been used as imaging tools for the skin (2-5).

UV-vis spectrophotometry is a technique widely used as most drugs absorb light in the 190-800 nm range (6). This wide ranging technique has been expanded to UV imaging (specifically surface dissolution imaging, SDI). This technique has provided a lot of added insights to the drug dissolution process, swelling, diffusion and precipitation processes (7-9).

Here' the authors report the development of an instrument in the similitude of a Franz cell capable of UV imaging drug permeation through a membrane. The Franz cell was 3D printed with dimensions allowing for insertion into the SDI instrument system. A 2.5 % w/w Ketoprofen (KTP) gel formulation was used as the model topical formulation in this proof of concept study. Prior to assessment the lower 28 mL acceptor compartment of was filled with a phosphate buffer. Next, the silicone membrane (0.13 mm) was placed on a divider between donor and acceptor compartments providing a diffusion area of 3.14 cm². The donor compartment of the cell was filled with approximately 1 mL of the KTP gel administered using a 5mL syringe. A method was constructed using the data collection software to record images using the 255 nm LED and the 520 nm LED. This proof of concept purpose built Franz cell was successful in imaging permeation of the KTP gel through a membrane.

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Changing spreading behavior of O/W emulsions with the physical properties and the ratio of the emollients

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1

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Abstract

Ease of spreading is one of the sensory characteristics commonly evaluated during skin care products application. Understanding the spreading behavior of the products and their interaction with skin during application is a key issue [1]. The aim of the study was to investigate the impact of two different emollients, stearic acid (polar, semi-solid at room temperature) and isohexadecane (non polar, liquid at room temperature), and their mixtures in the spreadability and formation of residual films obtained from oil-in-water emulsions. Rheological, textural, sensory and tribological analysis were performed on human skin and artificial substrates. The sensory analysis showed that emollients ratio influences significantly the spreading behavior of emulsions: more isohexadecane in the oil phase easier to spread the product on the skin. High significant correlations were obtained for the spreading behavior obtained by textural measurements on artificial substrates and sensory analysis. The tribological data obtained on human skin, also showed that physical properties and the ratio of emollients highly influence the spreading behavior in time. The friction values increase with the stearic acid concentration in emulsion and the residual film is formed faster. First, spreading was governed by the consistency of the emulsion, particularly impacted by the emollients ratio. But then, in a long-time spreading, when the emulsion broke down and residual film was formed, a particular interaction with skin influenced the spreadability [2]. It appears that not only the physical state of the emollient but also its chemical nature, physical state, polarity, temperature might explain these phenomena. This study showed the importance to consider the emollient properties when one emollient is used in the emulsion, but especially their interactions in mixtures, to better understand and anticipate their behavior. These results are very promising when it comes to anticipate the formulations and show very good predictive opportunities to replace sensory analyses by instrumental ones.

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Role of physical interactions in membrane-drug compatibility during *in vitro* performance tests of topical products

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Abstract

In vitro performance tests (IVRT) are studies monitoring drug release from a semi-solid dosage form. Based on release data, IVRT have been used to assess sameness and quality of product, following Level 2, scale up and post-approval changes(1). In recent years, IVRT with other supplementary *in vitro* permeation tests have been recommended to demonstrate bioequivalence of a few generic topical products(2,3). Also, IVRT are a valuable tool in development of topical formulations(4).

Nowadays, IVRT have taken a great attention because of aforementioned reasons, but there is no standard procedure applied to all semi-solid dosage forms. Especially, membrane selection is crucial due to its requirement of minimal diffusion resistance to drug and only acting as a support to separate formulation from receptor phase(5). Commonly, drug recovery test in which a standard drug solution is passed through membrane to appoint the most proper membrane(6). Nevertheless, this method is time-consuming and costly. Thus, we purposed to predict membrane-drug compatibility, based on physical interactions (*e.g.* hydrogen bonds) among functional groups of drug and membrane, whether there is a correlation between a number of hydrogen bonds and membrane-drug incompatibility. In this concept, IVRT were performed using drugs with various numbers of acceptor and donor groups (acyclovir, ketoprofen, flurbiprofen, alclomethasone). Regenerated cellulose (RC) and mixed cellulose (MC) filters were used as membrane because of having and not having of hydroxyl groups, which can form hydrogen bonds, respectively.

IVRT results revealed that numerous hydrogen bonds occurred between RC membrane and acyclovir/alclomethasone due to its plenty of acceptor and donor groups. On the other hand, an incompatibility between RC membrane and flurbiprofen/ketoprofen was not expected due to a small number of acceptor and donor groups. However, hydrogen bonds generated more strongly between hydroxyl groups in RC membrane and fluorine atom in flurbiprofen, in contrast to oxygen atom of ketone in ketoprofen.

Consequently, MC membrane can be more useful because of its molecular structure for IVRT of most drugs with strong and plenty of acceptor and donor groups in comparison with RC membrane.

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In vitro assessment of bioequivalence of topical products for dermatological indications

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Abstract

For systemically acting drugs, therapeutic equivalence can be demonstrated using bioequivalence studies in a small healthy volunteer population by comparing pharmacokinetic parameters of the RMP (reference medicinal product) and the generic product. This method cannot be used for topically applied and locally acting medicinal products since systemic absorption is undesirable, often undetectable and, more importantly, occurs after passage through the target compartment. In light of this, the European Medicines Agency (EMA) has expressed the need for a guideline on the quality and equivalence of topical drugs. Nevertheless, the *in vitro* assays recommended in the guideline do not necessarily reflect the quantitative drug bioavailability in the viable skin tissues. In this work, we propose a new methodology to assess bioequivalence of topically applied dermatological formulations. Topical biodistribution of econazole nitrate (ECZ) from a reference medicinal product (RMP) and two generic products was compared in terms of the amounts of ECZ present as a function of depth in the different skin layers – i.e. descending from the stratum corneum to the dermis. A different and non-equivalent ECZ product (econazole solution) was used as a negative control.

Experiments were performed with porcine skin using vertical Franz diffusion cells under finite dose conditions (100 $\mu\text{g}/\text{cm}^2$ of ECZ). Formulations were applied to the skin for 12 h. Then, skin samples were mounted in a cryotome to obtain horizontal lamellae (2 lamellae of thickness 20 μm and 19 lamellae of 40 μm). ECZ deposition in each individual lamella was evaluated by UHPLC-MS/MS. Bioequivalence was assessed by comparing the biodistribution profile of the different products and the results were evaluated statistically using analysis of variance (ANOVA) or Student's T-test, as well as using the recommendations in the EMA guideline.

Quantification of the amounts of ECZ as a function of depth in the skin showed that there was no statistically significant difference between the biodistribution profile (i.e. the deposition of ECZ in each skin lamella) when comparing the RMP and the generic products. Moreover, the results were found to be reproducible (i.e. with respect to inter-day variability). The selectivity of the method (i.e. ability to identify bioequivalent products) was demonstrated by the biodistribution profile of the negative control formulation, which was found to be statistically different from that of the RMP.

The results obtained in this study are promising for the assessment of bioequivalence *in vitro* and can complement the assays described in the draft guideline.

Activity versus efficacy for topical product presentation; guidelines and regulations.

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Abstract

Products for topical use include different galenical dosage forms, such as for example liquids and semi-solid formulation types (creams, ointments, sticks and gels) etc. Products may have similar appearance, similar indications, similar composition, and be placed next to each other on the shelf at pharmacies or drug stores but comply with different regulations. Those regulated as medicinal products or medical devices are marketed for prevention or treatment of diseases, such as for example warts, atopic dermatitis, and acne, whereas those regulated as cosmetics are not allowed to be marketed for diseases. Furthermore, if the combination of substances modifies the physiological function by exerting a pharmacological action, then the product should be regulated as a medicinal product. The bases to determine which sets of rules that apply for a certain product is governed by the intended use, physiological effects, mode of action and the risks at use.

Efficacy of medicinal products is based on interventional trials that determine the difference in efficacy between the product containing an active drug substance and its placebo. Such measures enable a quantitative description of the pharmacological properties of drugs, where the obtained level of statistical significance is determined by the measured difference in efficacy and sample size.

Substance based medical devices exert their locally acting effects via chemical interactions, such as those mediated by acid-base reactions, chelating, solubility due to precipitation- complexation reactions, diffusion, etc. Medical devices may use several ingredients and have multiple actions contributing to the therapeutic effect, making it difficult to determine the efficacy of active ingredients in placebo-controlled trials. Similar challenges are encountered for cosmetics, where the entire formulation may contribute to the effect.

However, all topical products, irrespective of regulatory classification, are dependent on the formula and its changes during storage and application for the final effect. Ingredients may be "active" but lose efficacy if presented to the skin in a non-suitable vehicle, i.e. "activity" of certain ingredients may not match "efficacy". Therefore, the transfer of "activity" from e.g. *in vitro* studies needs studies, or careful validation of data-transfer, before claims on efficacy can be expressed. This is carefully considered for medicinal products but is less controlled for medical devices and cosmetics. The new Medical Device regulation will fully apply in May 2020 and the technical guidance for cosmetic claims (1) will be applicable as of 1 July 2019.

Reference

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aQbD as a Framework for In Vitro Release Testing Development

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Abstract

Introduction: The new EMA draft guideline on quality and equivalence of topical products encourages the establishment of a solid, harmonized regulatory background of *in vitro* release testing (IVRT). In this context, the present work describes a novel framework applicable to the development of a discriminative IVRT method, according to the aQbD principles. A commercially available diclofenac emulgel formulation was used as a "model product".

Methods: The IVRT analytical target profile was established, followed by the performance of a risk analysis. Both critical analytical attributes (CAA)– In vitro release rate (IVRR), Cumulative amount released in the beginning (Qi) and end of the assay (Qf) and Dose depletion; and critical method variables (CMV) – dose, membrane, release medium, were selected. Two 2k full factorial designs were performed. The first design aimed at assessing the impact of the cosolvent ethanol, whilst the second one addressed the behavior of propylene glycol. For each medium, the influence of membrane type, as well as dosing regimen was assessed. A total of 16 analyses were conducted. The experiments were made using static vertical Franz diffusion cells with a diffusion area of 0.636 cm² and 5 mL capacity.

Results: Irrespective of the release media, the membrane and the release media are the main CMV that affect the IVRR. The applied dose influences the system negatively, meaning that when moving from finite dose to infinite dose conditions, a proportional increase in the IVRR is not necessarily observed. Nevertheless, this effect is mitigated when the receptor media is PBS-Ethanol. Similarly, tuffryn membranes are more permeable compared to dialysis membranes. Moreover, these results indicate that the addition of a cosolvent promotes an increase of IVRR.

Qi and Qf are obviously interrelated with IVRR, nevertheless in Qi the coefficients are higher. Moreover, when the release media comprises PBS-ethanol, there is a sharper definition of the profile.

A statistical analysis was made, as well as the performance of additional experiments in the so called "test points". These, supported the predictive capabilities of the model. The most suitable IVRT parameters were: PBS:Ethanol (80:20), tuffryn membranes and 300 mg.

These conditions were validated in light of the new EMA requirements [1].

Conclusion: The application of aQbD reduces method development time and cost, besides offering a robust and regulatory-oriented platform for IVRT predictive development.

Acknowledgements:

FCT PhD grant PD/BDE/135075/2017 and Project UID/QUI/50006/2013 LAQV/REQUIMTE.

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Differences in shear viscosities in the non-plateau region of the viscosity curve and implications for rheological method development and release testing of semisolid products

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1

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Abstract

In the course of the development process of a generic semisolid formulation, containing Polawax NFTM (mixture of cetearyl alcohol and polysorbate 60) as consistency agent, a trend for higher viscosities of the produced batches in comparison to the originator was observed when the shear viscosity was recorded at a single shear rate. Initially, it was thought that the manufacturing process or the use of different excipient batches were the cause of those variations. However, temperature sweeps did not show a huge impact of the cooling rate on the viscosity of the product and DSC data showed low batch-to-batch variability of melting and solidification temperatures of Polawax NFTM. Comprehensive rheological characterization has revealed that no systematic differences between development and originator batches exist. The problem was artificially created due to the use of an inappropriate rheological method that measures the time-controlled viscosity at a constant shear rate. Our results show that the shear viscosities and the ranking of shear viscosities of the different formulations changed with different shear rates. Due to the shear-thinning behavior of the formulations, there was a trend for lower shear viscosities at shear rates between 10 1/s to 100 1/s. A completely different viscosity ranking was obtained at very low shear rates (0.00001-0.001 1/s) that are related to storage conditions. The plateau region at such low shear rates is known as zero viscosity. Here, the development batches exhibited lower zero viscosity values than the originator batches. Additionally, oscillatory measurements at a constant frequency with increasing shear deformations were conducted to get more insight into the inner structure and the viscoelastic behavior of the formulations. Interestingly, the zero viscosities obtained from the viscosity curve showed nearly the same behavior as the complex viscosities measured with the amplitude sweep. Differences between development and originator formulations were less prominent. From this, it can be concluded that a viscosity method that determines shear viscosity at a single shear rate bears the risk of generating wrong-negative or out of specification results if the plateau region is not reached. The determination of the viscosity with the flow curve over a wide range of shear rates might be an alternative to single shear rate measurements. Ideally, the viscosities are determined at a plateau of the flow curve (either zero viscosity or shear viscosity at higher shear rates) to have a method that is feasible for the comparison of different formulations and for release testing.

Study of a new gel for dermal use based on a pollenic macrolide antibiotic with antifungal action

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Abstract

The pollenic macrolide antibiotics, as in the case of amphotericin B (AmB), have the capacity to generate the opening of the channels in the ergosterol membrane present in some fungi (*Candida* spp.) causing their death. These compounds are insoluble in most organic and inorganic solvents, that is we have proposed to formulate an AmB gel based on sepiigel

³⁰⁵®(gel-AmB). **Propose.** In this work we evaluate the impact of a gel-AmB on Transepidermal water Loss (TEWL) and on the Stratum Corneum Hydration (SCH) after topical application, predicting the rheological behavior and evaluating of short-term stability. **Methods and Materials.** A total of 10 healthy volunteers, were recruited and the Cutometer R MPA 580 was used. Measurements were carried out on forearm. **TEWL:** the measurement was carried out with a Tewameter R TM 300 and **SCH:** the measurement was carried out were carried out by a Corneometer® 825, Courage and Khazaka, Electronic GmbH, Cologne, Germany).

Rheological behavior. The rheological measurements were performed using a rotational rheometer Thermo Scientific Haake RheoStress 1 (Thermo Fischer Scientific, Kalsruhe, Germany) equipped with cone plate geometry (60 mm diameter, 2° angle) with mobile upper cone Haake C60/2° Ti (0.105 mm gap) at 25 °C.

Finally, destabilization phenomena (coalescence, flocculation, sedimentation, creaming, etc.) were tested by multiple light scattering analysis of transmission (T) profiles during 24 h using the TurbiScanLab R (Formulation Co., L'Union, France) at 25 °C.

Results. The values of TEWL and SCH at 2 hours after the application of the gel-AmB did not show a statistically significant difference ($p \leq 0.05$) (TEWL basal: 8.47 ± 1.74 and TEWL after 2 h: 8.12 ± 1.72 g/m/cm², SCH basal: 33.58 ± 6.71 and SCH after 2 h: 36.42 ± 9.11 UC). Regarding the rheology, the AmB-gel presented trixotropy (value: 194.4 ± 47.09 Pa/s) and a pseudoplastic behavior (Cross equation = $r^2: 0.9996$).

Conclusion. Our formulation will not cause any alteration in the biomechanical parameters of the skin. The gel-AmB could be applied to the skin, especially in mycosis processes since it was easily tolerated. None of the volunteers manifested burning, itching or redness. No instability was observed.

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Are Current Vehicle Designations Up-To-Date?

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Abstract

Topical treatment of the skin is as old as human existence. Over the centuries, a myriad of mixtures has been created and modified along with new scientific discoveries. Innovative materials useful in topical formulations have been discovered and developed, leading to a broad spectrum of preparations or vehicles that patients and consumers apply to their diseased or healthy skin. These vehicles range in physicochemical and textural properties from liquids to semisolids to powders and even to patches. There is also convincing evidence that the skillful selection of vehicle ingredients may multiply the bioavailability of topically applied drugs. In parallel the terminology to classify vehicles has also developed and ancient designations such as decocts, lards or liniments have been replaced by more fanciful terms such as serums, liposomes or foams etc. Also, regulatory bodies have tried to classify vehicles. Varying definitions in compendia documents, use of fanciful names created by marketers and one's own personal experience with typical topical vehicle formats (e.g., ointment is greasy, gel is translucent) has led to a Babylonian confusion of linguistic designations that impedes scientific, patient and consumer related communication regarding vehicle formats and vehicle effects. Analyses of currently used terminologies and vocabularies to describe vehicle formats and vehicle effects are presented. Furthermore, a new practical guide to describe vehicle properties and vehicle effects that will serve consumers, patients, healthcare professionals and scientists alike is presented and discussed.

Dermal pharmacokinetic endpoint studies to evaluate bioequivalence of topically applied lidocaine and prilocaine drug products

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Abstract

A promising approach to evaluate the bioequivalence (BE) of topical dermatological drug products compares the in-vivo dermal pharmacokinetic (PK) profiles for a prospective generic product and its reference listed drug (RLD) product using dermal open flow microperfusion (dOFM). The feasibility of dOFM to evaluate BE was previously demonstrated for acyclovir creams. The current study assessed the feasibility of dOFM to evaluate the BE of drugs that are more hydrophobic and more protein bound than acyclovir.

A pilot in-vivo study was conducted with 6 subjects to evaluate the dermal PK of lidocaine (moderately hydrophobic, moderately protein-bound) and prilocaine (moderately hydrophobic, highly protein-bound) using dOFM, and to verify/optimize the parameters for a pivotal in-vivo BE study. Each subject had 9 test sites (8 on the thighs; 1 on the upper arm). Two dOFM probes were inserted into each test site and dermal interstitial fluid was continuously sampled for 24 hours to characterize dermal PK profiles. The reference product, EMLA R topical cream (2.5% lidocaine, 2.5% prilocaine) was administered on 6 test sites at different cream doses (5, 10 or 15 mg/cm²) to evaluate the dose-response relationship. On another test site, 10 mg/cm² of Oraquix R dental gel (2.5% lidocaine, 2.5% prilocaine) was administered as a negative control for BE relative to the reference product. To investigate the influence of potentially confounding factors (e.g., lateral "cross-talk" between adjacent test sites, or recycling of the drug back into the skin by the systemic circulation) non-dosed test sites were monitored by dOFM, and blood samples were drawn to monitor the systemic PK of lidocaine and prilocaine.

The reference product showed a dose-dependent response, confirming that dOFM was sensitive to changes in the bioavailability (BA) of lidocaine and prilocaine. The dermal area under the curve (AUC) values for the test gel (AUCLidocaine: 2,444 ng*h/mL; AUCPrilocaine: 3,218 ng*h/mL) were well-differentiated from those of the reference cream (AUCLidocaine: 6,036 ng*h/mL; AUCPrilocaine: 10,520 ng*h/mL) and failed to demonstrate BE, as the 90% confidence interval of the mean AUC ratios did not fall within the BE limits of 0.80–1.25 (AUCLidocaine: 1.51–2.65, AUCPrilocaine: 2.14–3.63) suggesting that the gel is a reasonable negative control for BE.

The results of this pilot study supported the sensitivity and reproducibility of in-vivo dOFM to characterize the dermal PK profiles of moderately hydrophobic and highly protein-bound compounds for BE evaluations.

Cytotoxicity and *in-vitro* anti-melanoma responses to Rose Bengal following enhanced topical delivery by a Self-Emulsifying Microemulsion system

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An effective topical delivery system must be capable of exposing the target site of action to a sufficient concentration of the active pharmaceutical ingredient (API). In addition, the formulation and its inactive excipients must be, safe, non-toxic and non-irritating. In practice, excipients are usually selected from a list of ingredients that are generally regarded as safe (GRAS).

The aims of this work were to investigate: (i) The safety of the excipients incorporated in Self-Emulsifying Micro Emulsion (SEME) systems on human melanoma (WM164, WM1366, and D24) and normal keratinocyte (HaCaT) monolayers, (ii) The antitumor activity of Rose Bengal (RB) and RB loaded SEME in enhancing the growth inhibitory effect of RB on tested melanoma and normal cell lines, (iii) *In-vitro* human skin penetration of RB from SEMEs and control solution.

SEME systems containing Labrasol and Transcutol as surfactants/co-surfactants, Labrafac PG as the oily phase and propylene glycol were systematically characterized by physicochemical and rheological methods. For cytotoxicity studies, cells were treated with various concentrations of RB alone and in SEMEs and the MTT assay was used to derive IC₅₀ values. Flow cytometry served to investigate the growth inhibitory mechanism of RB aqueous solution and in SEMEs. Skin delivery of RB aqueous solution and RB loaded SEMEs was examined with Multiphoton Microscopy with FLIM analysis (MPM-FLIM).

The blank SEME systems reduced cellular toxicity in comparison to the single excipient, Labrasol. RB loaded SEMEs enhanced cell growth inhibition activity compared to the RB aqueous solution. Apoptotic cells were determined using PI staining of DNA fragmentation by flow cytometry. RB in SEMEs induced a sub-G₀G₁ peak in the histogram of treated cells, indicating that apoptosis may be one of the mechanisms of cell death and pointing towards involvement of other cell death mechanisms such as necrosis and autophagy. Preliminary results of MPM-FLIM analysis showed deeper penetration with greater skin concentrations of RB delivered from SEMEs compared to the RB aqueous solution. Images indicated that SEMEs delivered RB to the stratum basale, a target site for melanoma.

This study highlights the enhanced skin penetration and anti-melanoma effects of RB when loaded in a SEME system. The self-emulsifying microemulsion shows promise as a RB delivery system for the chemotherapeutic treatment of cutaneous melanoma.

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75	chemical characterization, antioxidant activity and skin barrier repair potential	Tasic-Kostov Marija, Nesjia Ivana, Pavlovia Dragana, Tadia Vanja
76	Monitoring of active ingredients penetration by Raman imaging of skin	Bonnier Franck, Stella Aline, Tfayli Ali, Yvergnaux Florent, Chourpa Igor, Tauber Clovis, Munnier Emilie

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76	INFLUENCE OF PLASMID DNA CONFORMATION IN PRIMARY HUMAN SKIN CELL TRANSFECTION: COMPLEXATION WITH LIPOSOMES.	Reusch Céline, Piel Géraldine, Debacq-Chainiaux Florence, Mottet Denis
77	Non-destructive quantitative analysis of semi-solid forms loaded with encapsulated active molecules (by vibrational spectroscopy)	Bonnier Franck, Miloudi Lynda (1), Henry Sandra, Perse Xavier, Yvergnaux Florent, Byrne Hugh, Chourpa Igor, Munnier Emilie
78	Study of the effects of storage conditions on the barrier function of reconstructed human epidermis by confocal Raman spectroscopy	Dancik Yuri, Eklouh-Molinier Christophe, Kichou Hichem, Soucé Martin, Miloudi Lynda, Munnier Emilie), Chourpa Igor, Bonnier Franck
79	Mean Sensing Depth for Skin Hydration Measurement of the Epsilon Capacitance Contact Imaging System	Imhof Bob, Berg Elliott
80	3D visualization of ambient effects on skin and topical formulations using a synchrotron X-ray tomography investigation	Mojumdar Enamul, Mokso Rajmund, Ali Abdullah, Valetti Sabrina, Morin Maxim, Lind Tania, Engblom Johan
81	Development of nanopores for in situ redox potential measurements in skin models	Munnier Emilie, Buchy Eric, Lombardo Sonia, Soucé Martin, Le Truong Phuong Nha, Boucaud Alain, Chourpa Igor
82	Quantitative in vivo analysis of skin penetration of topically applied products based on non-invasive confocal Raman spectroscopy.	Puppels Gerwin, Caspers Peter, Nico Claudio, Bakker Schut Tom, De Sterke Johanna
83	Ex-vivo depth profiling of tazarotene a third generation retinoid via confocal Raman spectroscopy	Sherif Solomon, Lane Majella E., Taylor Kevin, Uchegbu Ijeoma
85	Investigating tactile friction and residual film properties of Pickering formulations on skin	Ali Abdullah, Ringstad Lovisa, Skedung Lisa, Wahlgren Marie, Engblom Johan
86	Developing cream formulations: renewed interest in an old problem	Simoës Ana, Veiga Francisco, Vitorino Carla
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90	Role of physical interactions in membrane-drug compatibility during in vitro performance tests of topical products	Kocabasyözge, Aydılek Neriman, Kahraman Emine, Gungor Sevgi
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92	Activity versus efficacy for topical product presentation; guidelines and regulations.	Loden Marie
93	QbD as a Framework for In Vitro Release Testing Development	Miranda Margarida, Cardoso Catarina, Vitorino Carla
94	curve and implications for rheological method development and release testing of semisolid products	Köllmer Melanie, Puschmann Julia, Herbig Michael E.
95	Study of a new gel for dermal use based on a pollenic macrolide antibiotic with antifungal action	Marcella Sessa
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97	Dermal pharmacokinetic endpoint studies to evaluate bioequivalence of topically applied lidocaine and prilocaine drug products	Manfred, Raml Reingard, Augustin Thoma, Kanfer Isadore, Raney Sam G., Sinner Frank

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