Bioencapsulation Research Group

Bioencapsulation Innovations

March 2012

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EDITORIAL

Eric PERRIER, LVMH Recherche

ENCAPSULATION IN COSMETICS OR NOT, THAT IS THE QUESTION!

Twenty eight years years after the introduction of liposomes in cosmetic as the first delivery system, are we allowed to say that there is no room anymore to micro- or nano- encapsulation systems for the cosmetic industry? The question may look provocative in this BRG Newsletter which is most probably the most dynamic organisation in the field of delivery systems in Europe. But the question should be asked. Some companies specializing in this area, which have developed specific organisations and special business models, around R&D, production and sales, of delivery systems are now deserting this extra-ordinary subject, most probably because of the complexity of what has to be done to answer specific cosmetics needs.

And this is true; the cosmetic field is not easy to handle: ingredients and delivery systems are quickly introduced (and removed) from the market. They need to carry strong innovations, if possible visible for consumers and perceived by them as a real innovative material. They need to be perfectly known in terms of composition, traces of undesirable components such as heavy metals, traces of compounds that could be perceived as dangerous by consumers. New encapsulation technologies have emerged, which need to be quickly adaptable to any new active compounds in a very simple and dynamic way.

Last but not least, they have to fulfill cosmetic regulations for more than 140 countries where such materials are introduced through cosmetic formulations (including the new very unstable Chinese regulation), they have to be demonstrated to be toxicologically safe (without using animal tests that are banned for the cosmetic industry) and they have to be proposed in a way that will not be the subject of media and/or NGO attacks.....

But still, we are convinced that encapsulation has a tremendous potential in cosmetics:

- to enhance penetration for a better efficacy: because of the more aggressive regulations, it will be very important to use the same ingredients but encapsulated, in order to create new properties. Encapsulation will be used as one important tool for formulators in the future,
- to target some specific cells in order to deliver better: targeting melanocytes to reduce pigmentation mechanisms, or adipocytes in order to reduce fat storage is crucial for a stronger activity of cosmetic formulations.
- to protect unstable ingredients: some components are incompatible; separated in the formula (one being encapsulated for instance), this instability disappears. Many examples exist in this area.
- to reduce the side effect of molecules by increasing the biodisponibility of such molecules: for instance, encapsulated retinol reduce side effects of retinol
- to use visible spheres as a marketing tool.

Innovation is crucial in this area:

LVMH RECHERCHE

PARFUMS & COSMETIQUES

EDITORIAL (CON'T

how to increase the efficacy of active compounds without increasing their concentrations? How to increase the tolerance without decreasing their concentrations? How results are able to be compared in *ex vivo* or *in vivo* experiments with already existing encapsulation tools?

Even if the incorporation of encapsulation systems in cosmetic formulation represents a great challenge, it is subject to intensive studies and some start up. SMEs have been created around this area, more specifically to study physicochemical phenomena that are behind the problems that have to be solved, and some very new and promising technologies appear.

We believe that the way active compounds are RELEASED is more important than encapsulation and encapsulation yield themselves. In this area, not to many high quality studies have been performed, which is the key for a reasonable cosmetic use of such encapsulation systems. What are the ingredients that are used to make the membrane of such spheres? Are those ingredients (polymers, cross linking agents...) able to be stimulated to induce the opening of the particles and the release of active compounds? To which stimulations could we expect to have active compound released in a cosmetic environment? Are we able to demonstrate now or in the future, some releases linked with UV, pH, the microflora composition of the skin, or the proteolytic activities of the different part of the body? How could the new tools compared with other already existing tools, because we need to increase the efficiency, not to have something new ....

We are far away from being able to answer to those crucial questions. At this stage, we are only to the first level of the subject: answering the technical, the scientific needs of encapsulation applied in the cosmetic field. Things are becoming even more difficult when we try to reach the second step, which is "what is perceivable by consumers"? Comparative clinical trials where cosmetic products are using or not encapsulation systems provide fascinating results showing the interest of the delivery systems:

- on the intensity of the cosmetic effect of the formulations, or even more interesting,
- in the speed to reach the maximum efficacy (2 weeks with encapsulated active compound for 4 weeks with same ingredient, without encapsulation.

A tremendous hope for the future of delivery systems in the cosmetic area.

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THANKS

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COSMETIC VALLEY
FRANCE

Cosmetic Valley is the leading worldwide resources centre in perfumes and cosmetics. This cluster joins together 550 companies including Dior, L'Oréal, Guerlain, Hermès, Coty, Paco Rabanne, Shiseido, Yves Saint Laurent, Chanel, ... and 80% SMEs working for the spread of "Made in France" throughout the world. Six universities (Orléans, Tours, Rouen, Le Havre, Versailles, Cergy-Pontoise) as well as prestigious research organizations such as CNRS and INRA also participate to this cluster. All of them work in a single network to develop innovative, competitive and sustainable projects for the beauty of tomorrow.

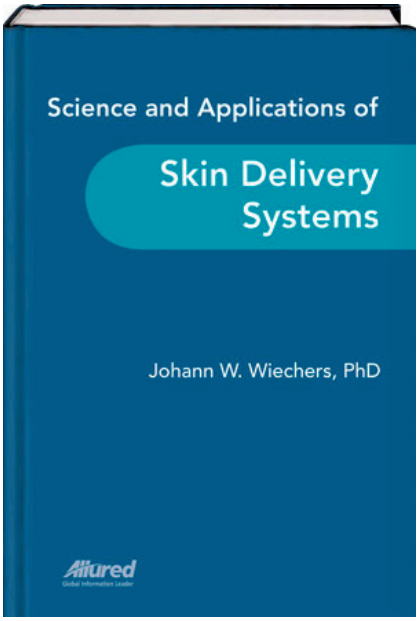
Formulation is a strategic action area for Cosmetic Valley. It is the major topic of innovation for more than 50 Research & Innovation projects in progress within the cluster. The scientists have to overcome major challenges such as ecoconception, stability and security, bioavailability and efficacy, ... Bioencapsulation plays a key role in this context and collaborations between private and academic laboratories will be a key success factor for the competitiveness of our industry.

MORE INFORMATION :

see http://www.cosmetic-valley.com or contact Christophe MASSON (PhD) cmasson@cosmetic-valley.com

BOOK

SCIENCE AND APPLICATIONS OF SKIN DELIVERY SYSTEMS



Measuring Skin Delivery

- Skin Delivery: What it is and Why We Need it
- Measuring Skin Delivery
- Methods for Studying Percutaneous Absorption
- The Application of Infrared Spectroscopic Imaging to Skin Delivery: Visualizing Molecular Localization in Formulations and in Skin

Skin Delivery from Emulsions

- The influence of Formulation Type on Skin Delivery
- The Influence of Emollients on the Skin Penetration from Emulsions
- The Effect of Changing the Polarity of the Stratum Corneum on the Dermal and Transdermal Delivery of Cosmetically Active Ingredients
- The Influence of Emulsifiers on the Skin Penetration from Emulsions
- Liquid Crystalline Emulsions as Cosmetic Delivery Systems
- The Influence of Emulsion Droplet Size on Cosmetic Delivery
- Acoustic Attenuation Spectroscopy: A New Technique to Characterize the Stability and Structure of Semi-solid Topical Delivery Systems for Cosmetic and Pharmaceutical Applications

Encapsulation Techniques

- Vesicles as Skin Delivery Vehicles
- Challenging Cosmetics—Solid Lipid Nanoparticles (SLN®) and Nanostructured Lipid Carriers (NLC®)
- Microencapsulation and Cosmetics
- Flexible Liposomes for Topical Applications in Cosmetics
- The Design of a Skin-friendly Carrier for Cosmetic Compounds Using Pheroid Technology
- Micro- and Nano-encapsulation of Water- and Oil-soluble Actives for Cosmetic and Pharmaceutical Applications
- Encapsulation and Other Topical Delivery Systems
- Delivering Chemically Labile Molecules into the Stratum Corneum: An Example of Stabilizing Linoleic Acid with Ð-Cyclodextrin

Alternative Ways to Enhance Skin Delivery

- Topical Delivery of Cosmetic Ingredients by Sonophoresis and Iontophoresis
- Principles of Iontophoresis and its Applications in Cosmetics
- Skin Electroportation

Special Delivery Routes

- Delivery from Rinse-off Products: Mission Impossible or a Reality for Cosmetic Science?
- Novel Ways of Measuring the Transfollicular Transport of Active Ingredients Across the Skin
- Significance of Follicular Delivery and Ways to Measure It

Future Perspectives

- Legal Perspective on Cosmetic and Drug Skin Delivery Systems
- Marketing Skin Delivery Systems: Despite So Many Questions, Here Are Some Answers
- Clinical Relevance of Topical Delivery Systems for Active Cosmetic Ingredients (Cosmeceuticals)

MORE INFORMATION

http://www.alluredbooks.com/Science-and-Applications-of-Skin-Delivery-Systems-p64.html

THIS ISSUE IS DEDICATED IN THE MEMORY OF ...

JOHANN WEICHERS

After earning his PhD in skin penetration enhancement from the University of Groningen, The Netherlands, in 1989, Johann W. Wiechers spent the past 18 years establishing himself in the field of cosmetic science.

He has worked for companies including Unilever Research and Uniqema. Johann was Visiting Professor at the University of London School of Pharmacy, Adjunct Professor at the School of Medicine, University of Queensland, Australia, Extraordinary Professor at Northwest University, Department of Pharmacy, Potchefstroom, South Africa

On July 1, 2007, Wiechers became Technical Advisor for Allured Business Media, serving as advisor for the magazine divisions, as well as the book division.

In July 2007 he also opened an independent consultancy business in cosmetic science and in September 2007 he became the President of IFSCC.

Johann was much appreciated in the field for his competence and kindness. He was always generous in his advice and help.

Unfortunately, Johann died in Malaysia of a sudden illness at the end of last year. This is why this special issue is devoted to him.

ARTICLE

VERSATILE DELIVERY SYSTEM BASED ON MICROEMULSIONS AND MICRODISPERSIONS - Microencapsulation of water-soluble and insoluble actives

Viladot, J.L., Fernández-Botello, A.; Delgado, R. - Lipotec, S.A., Gavà (Barcelona), Spain

WHY TO MICROENCAPSULATE WATER-SOLUBLE ACTIVES?

The in-vivo efficacy of a cosmetic active, and consequently its economical success, is highly dependent on its chemical stability and on its skin absorption. Due to that, there is a need in the cosmetic industry of delivery systems or microencapsulates able to achieve these goals.

The incorporation of encapsulated hydrophilic actives into cosmetic aqueous formulations constitutes a challenge for two main intrinsic reasons: 1) the encapsulation process normally takes place in aqueous media, and it's required to maintain the active in the core during the shell formation avoiding its migration to the continuous phase, and 2) leaking from the capsule core to the continuous phase during storage usually is a thermodynamically favored process.

Whereas several delivery systems for hydrophobic cosmetic actives have been developed [1-3], very few effective systems for hydrophilics are found in the literature, such as caffeine microspheres prepared by a W/O/W double emulsion solvent-evaporation method [4], using the biodegradable polymer poly-Ð-caprolactone as encapsulating agent and methylene chloride as solvent for hydrophobic phase or the entrappment of emulsified hydrophilic actives in solid lipid nanoparticles (SLNs) [5]. Benoit and co-workers [6] have reported on the preparation of capsules with aqueous core and lipidic shell, where the W/O nanoemulsion was prepared by phase inversion temperature, followed by interfacial polycondensation of isocyanate monomer and solvent evaporation (isopentane from the lipidic phase). However, all these methods have required the use of organic solvents or chemical reagents which eventually could react with some actives.

Here we report a novel delivery system suitable for water-soluble actives with high encapsulation efficiency and which combines the techniques of microemulsion and microencapsulation under mild conditions and in the absence of organic solvents, and its application with cosmetic peptides.

MICROENCAPSULATION TECHNIQUE FOR MICROEMULSIFIED PEPTIDES

Our approach for encapsulating hydrophilic actives was based on a strategy of complex coacervation or polymer-polymer incompatibility entrapping a W/O microemulsion.

First, peptides were pre-dissolved in an hydroalcoholic mixture, and this solution constituted the internal phase of W/O microemulsions, using soybean oil as continuous phase and isostearic acid (ISOA) and diethylhexyl sodium sulfosuccinate (DOSS) as emulsifiers. The ratios between all components were optimized by means of ternary phase diagrams in order to yield completely transparent emulsions (Figure 1). Microemulsion droplet size determined by electron microscopy showed diameters of 10-20 nm range.

These emulsions were manipulated as a hydrophobic active, incorporated into aqueous media (W/O/W) and treated with a pair of ionically countercharged polymers: quaternized soy protein and hyaluronic acid. After this complex coacervation step, lipidic phase is no longer an emulsion but a particulate phase, due to the existence of a solid membrane formed by countercharged polymers interacting ionically. The particle size of the capsules depends on the energy and the O/W surfactant system used thereby. Accordingly, we used a Microfluidizer® equipment, a high pressure homogenization system which yielded typical diameters of 150-400 nm, measured by dynamic Laser light scattering. The particle size values remained virtually unchanged during a period of three months storage at both room temperature and 40°C.

One of the common drawbacks of standard encapsulation systems is their low encapsulation efficiency for hydrophilic actives, which may com-

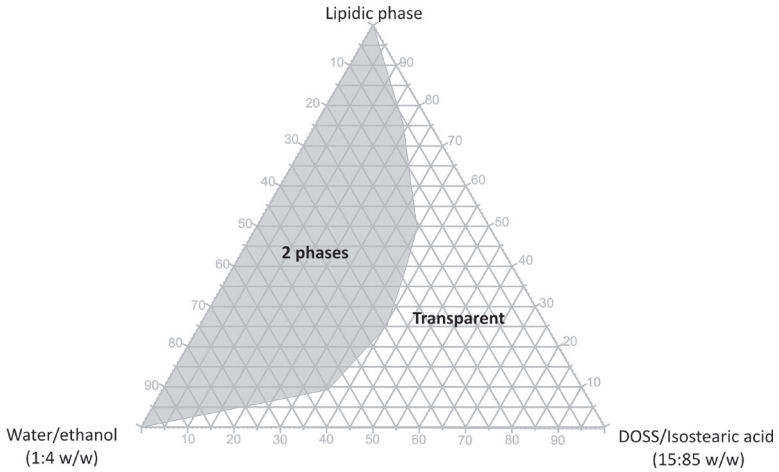


Figure 1. Ternary phase diagram for obtaining a W/O microemulsion with the following components: aqueous phase: water/ethanol 1:4 (w/w), lipidic phase: Glycine Soja (Soybean) Oil, emulsifier: Diethylhexyl Sodium Sulfosuccinate/Isostearic Acid 15:85 (w/w)

promise their industrial application. The delivery system described in this work, specially designed for hydrophilic actives, yielded high encapsulation levels (typically ca. 80%), as determined by HPLC.

In order to visualize the structure this novel delivery system, samples of capsules containing fluorescent probes were investigated by confocal fluorescence microscopy. In one of the samples, the lipophilic fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was incorporated into the lipid phase for visualization of the core. In another sample, the hydrophilic fluorescent marker carboxyfluorescein (CF) was incoporated.

Figure 2 shows confocal microscopy images of encapsulated microemulsions containing DPH (left) and CF (right). In the case of DPH, nice spherical structures are observed, with the lipophilic probe homogenously distributed through the matrix. In the sample containing CF, the image reveals that this hydrophilic probe concentrates in the surface of the spheres, and this is attributed to the interaction of anionic CF with the cationic protein of the membrane. This observation does not only constitute a proof of the existence of the membrane but, furthermore, opens the door to an alternative way for incorporation of charged hydrophilic actives.

MICROENCAPSULATION OF MICROEMULSIFIED AQUAPORIN-3 BOOSTING PEPTIDE

Aquaporins are a family of proteins involved in water transport through different tissue layers, commonly known for their essential role in the hydration of the stratum corneum. Aquaporin-3 (AQP3) is the most prominent member of this family; therefore, modulating the expression of Aquaporin-3 water channels through cosmeceutical actives constitutes a promising strategy for enhancing skin moisturization and in reducing the impact of UV-induced aging.

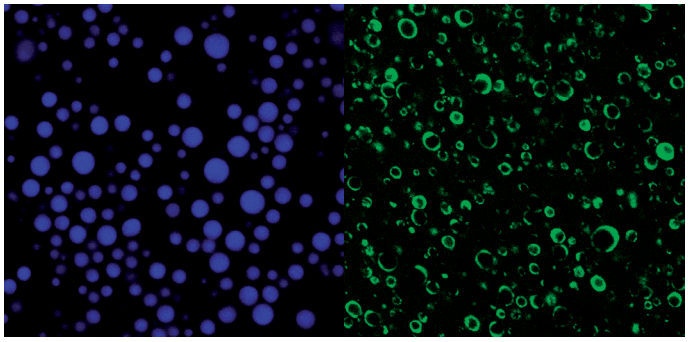


Figure 2. Confocal micros copy images of encapsulated microemulsions containing DPH for visualizing the core (left) and microemulsified CF for visualizing the membrane (right). The capsules were isolated by centrifugation and washed thoroughly with water before observation. The particle size of the capsules for confocal fluorescence microscopy was deliberately adjusted to the low µm range.

Our group has synthesized Aquaporin-3 boosting peptide for promoting skin hydration using combinatorial chemistry [7], but in order to obtain the desired in vivo boosting efficacy it's essential it reaches the basal layers of the epidermis where the expression of AQP3 takes place.

For that reason, microcapsules containing microemulsified AQP3 boosting peptide were prepared according the previously described strategy. The percutaneous absorption of encapsulated AQP3 boosting peptides in a skin biopsy was determined following the OECD guideline 428 after an exposure time of 24 h. The permeated fraction is calculated from the substance found in epidermis (except for the stratum corneum), dermis and eventually in the receptor fluid. Results for encapsulated AQP3 boosting peptide and aqueous solution forms are listed in Table 1. As shown, despite the almost negligible percutaneous absorption of the peptides in aqueous solution, the delivery system was able to enhance their absorption very efficiently, reaching a value of 26 ng/cm2/h. These high values can be tentatively attributed to the combination of several factors, namely: 1) low the particle size of microcapsules; 2) the bioadhesive properties of the coacervate membrane; 3) the occlusion caused by the lipidic phase (as reported for other lipidic nanoparticles [8]); 4) the droplet size of the microemulsion, and; 5) the chemical nature of the internal microemulsion.

As the action of the AQP3 boosters is required to take place in the epidermal keratinocytes where this protein is synthesized, the percutaneous absorption values obtained with this delivery system allow predicting satisfactory in vivo results.

The microencapsulation of microe-

Table 1. Skin distribution of AQP3 boosting peptides in different vehicles by in vitro percutaneous test after an exposure time of 24 h, given in percentages of applied dose and in terms of quantity of substance per surface and time.

ACTIVE (concentration)	VEHICLE	PERCUTANEOUSLY ABSORBED	NOT ABSORBED	TOTAL RECOVERY
Aquaporin-3 boosting peptide (0.03 %)	Aqueous solution (3 replicates)	0.0 ± 0.0 % 0.0 ± 0.0 ng/cm2/h	89.4 ± 0.6 % 141 ± 1.0 ng/cm2/h	89.4 ± 0.6 % 141 ± 1.0 ng/cm2/h
Aquaporin-3 boosting peptide (0.06 %)	Capsules (DOSS/ISOA) (5 replicates)	9.3 ± 3.7 % 26 ± 11 ng/cm2/h	83.3 ± 0.6 % 237 ± 8.0 ng/cm2/h	92.6 ± 2.6 % 263± 8.0 ng/cm2/h

Empty vehicle for each sample was used as negative control. The "percutaneously absorbed" fraction is calculated by the sum of the values in epidermis, dermis and receptor fluid, while the "not absorbed" fraction is calculated by the sum of the values in the stratum corneum and the washings of the skin surface.

All values are followed by their standard deviation. According T-tests, the difference between the values of "Peptide Aqueous Solution" and "Peptide capsules" was significant with a confidence interval of 95%.

ARTICLE

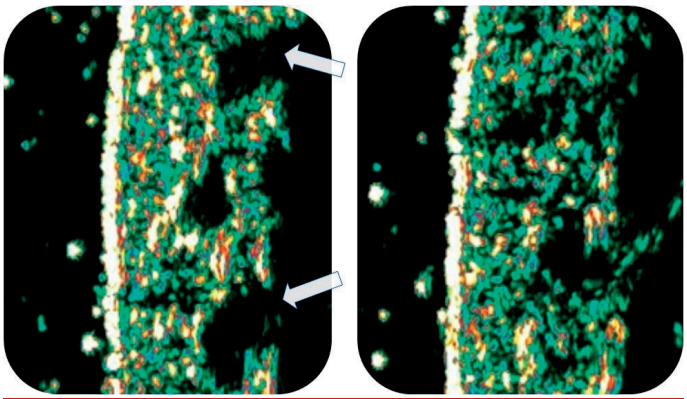


Figure 3. Images from a volunteer at the initial time (left image, with many irregularities in the junction line) and after 3 weeks applying a cream with 2% SILUSYNE™ (right picture, more regular).

mulsions containing AQP3 peptide boosters is reported in detail in reference [7].

MICROENCAPSULATION OF MICRODISPERSED ANTICELULLITIC PEPTIDES

This microencapsulation strategy was also chosen in order to vehiculize the anticellulitic peptide Acetyl hexapeptide-39, which also requires reaching inner skin layers for activity. As this active was insoluble in both lipophillic and hydrophilic media, a modification of the technology consisting on the incorporation of microdispersed peptide instead of microemulsified was chosen. With this strategy, the encapsulation yield increased to 95%. This is easily explained by the poor affinity of the active for the continuous phase. The delivery system containing Acetyl hexapeptide-39 is commercially named SILUSYNETM.

In the presence of extra lipid storage, the inner disorder of the tissue increases and the dermo-hypodermal junction line needs to be longer to surround the extra volume, becoming irregular and wavy and inducing irregularities on the skin surface (cellulite). A decrease in its length would imply a more regular junction line and better organisation of the tissue, which would improve the subcutaneous tissue uniformity and skin appearance. The anti-cellulite and slimming efficacy of this microdispersed and microencapsulated peptide, commercially named SILUSYNETM, was determined in vivo using ultrasound ecography in B-scan mode (Dermascan C) to evaluate its effects on the dermo-hypodermal junction line (Figure 3). A panel of 20 selected women (aged 25-45) presenting cellulite on their thighs (Pinch Test stage I-III) applied the placebo and the cream containing 2% SILUSYNETM in this area twice a day for three weeks. As shown in Figure 4, volunteers treated with the new material reduced their dermo-hypodermal junction line by 21% versus initial time, while placebo had no significant diminishing effect. SILUSYNETM also reduced the dermo-hypodermal junction line and its irregularities, making the subcutaneous tissue more homogeneous and regular, which facilitates the skin surface to become flatter.

This and other tests with SILUSYNETM have been reported by our group elsewhere [9]

CONCLUSION

The strategy described in this work constitutes an effective way to increase skin permeation of actives, typically hydrosoluble peptides, that require to penetrate into deep layers of the skin, named epidermis and dermis in order to make their action. In conclusion, the delivery system described is a versatile tool to increase the efficacy of actives by microencapsulation.

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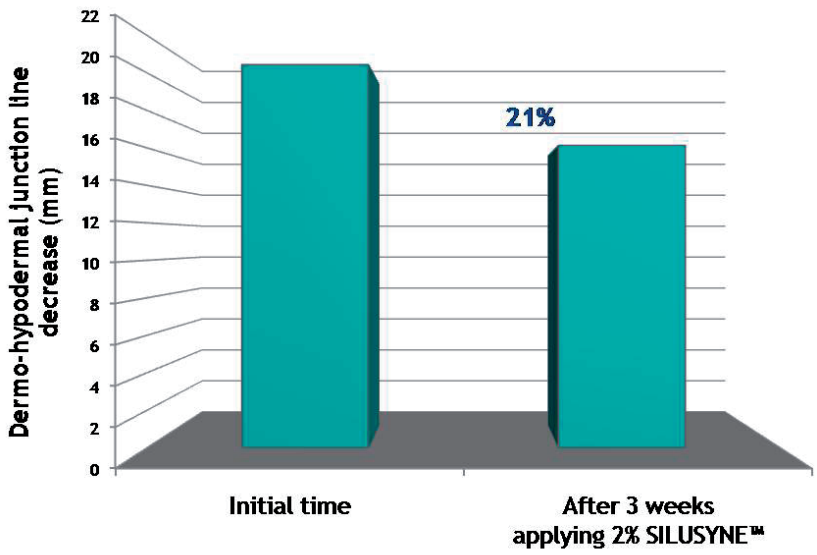

Figure 4. Length of the dermo-hypodermal junction line determined by ultrasound ecography (B-scan mode) in a panel of 20 women between 25-45 years old with cellulite. During 3 weeks, volunteers applied the cream containing 2% SILUSYNE™ on one thigh and the placebo on the other, twice a day..

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Dr. Josep-Lluís Viladot obtained its Ph. D. in chemistry in the Universitat Ramon Llull (Barcelona) in 1999. Since then he has worked in the field of microencapsulation with the companies Primacare S.L., Cognis Iberia and currently with Lipotec S.A., where he currently is the Scientific Chief Officer for Delivery Systems. His developments are in the market as constituents of cosmetic formulations of the major companies of the sector. He has published more than 100 scientific works, including papers, conference presentations and more than 60 patent application families.

OPEN POSITIONS

P&G

R&D Research fellows EU funded Academia/Industry Partnership

Together with five excellent European partners we offer young scientists a research perspective at world-class scientific level in multidisciplinary and international teams to achieve a breakthrough in the development of sustainable detergents. Thanks to the European funded Programme on Academia- Industry Partnership Pathway (CAP-IT, IAPP 251298) we are currently seeking 2 research fellows each for a period of 12 months. The research is carried out at Procter & Gamble Brussels Innovation Centre (BE).to start effective April 2012.

More information,

fernandezprieto.s@pg.co

Givaudan

Four positions opened in R&D

Givaudan is the global leader in the fragrance and flavour industry, offering its products to global, regional and local companies specialising in food, beverage, consumer goods and fragrances. In our Research and Technology Department, the Delivery System Platform, based in our European Center of Fragrance Creation, provides expertise in micro-encapsulation systems used in different consumer goods such as detergents, softeners and cosmetics, for example.

We are currently looking for a:

- Encapsulation Research Scientist
- Product Development Scientist
- Product Development Scientist
- Technology Scientist

More information, http://www.givaudan.com/Careers/ Job+Search

PDH THESIS

FACTORS INFLUENCING THE EFFECT OF MILK-BASED EMULSIFIERS ON LIPID OXIDATION IN OMEGA-3 EMULSIONS

Anna Frisenfeldt Horn
Main supervisors: Charlotte Jacobsen (DTU Food)
Date of thesis presentation: DTU Kgs Lyngby, Denmark on 15th March 2012

Intake of fish oil, and in particular the long chained polyunsaturated omega-3 fatty acids, has over the last centuries been associated with a wide range of health beneficial effects. Hence, both the industry and consumers show increasing interest in omega-3 enriched foods. The challenge when these healthy but oxidatively vulnerable lipids are added to foods is their sensitivity towards heating, metal ions and oxygen, as these factors can lead to lipid oxidation. To avoid this, a possible approach is to incorporate and thereby protect the fatty acids in an emulsion before they are added to the food product. However, the use of these so-called delivery emulsions in different food products has shown contradictory results with respect to the ability of the delivery emulsion to prevent lipid oxidation.

On this background, the overall goal of the PhD work was to increase our knowledge about factors related to the choice of emulsifier, homogenization equipment and emulsification conditions that could influence lipid oxidation in simple fish oil-in-water emulsion systems. The main focus was on the use of milk proteins alone or in combination with phospholipids as emulsifiers. In addition, the aim was to utilize the obtained knowledge for designing delivery emulsions for the addition of fish oil to foods, and thereby achieve oxidatively stable fish oil enriched products.

Overall, the PhD work showed that both the oil concentration, the type of milk protein or phospholipid used as emulsifier, the pH, the addition of iron, preheating of the protein prior to homogenization, the equipment used for homogenization and the pressure applied during high pressure homogenization influenced lipid oxidation in simple fish oil-in-water emulsions. In addition, lipid oxidation was shown to depend on combinations of these factors, and not any one of them alone. Moreover, it was shown that despite an attempt to optimize the above-mentioned factors and thereby create an oxidatively stable fish oil-in-water delivery emulsion, this was not enough to fully ensure protection of the fish oil when the delivery emulsion was added to milk or cream cheese. In fish oil enriched cream cheese, a delivery emulsion prepared with a combination of milk proteins and phospholipids gave a better oxidative stability than delivery emulsions with pure milk proteins.

More information : Charlotte Jacobsen , chja@food.dtu.dk

ARTICLE

ADAPTATION OF A CATIONIC LIPOSOME TO THE SPECIFICITIES OF THE JAPANESE SKIN CARE MARKET

Bonnet, Isabelle; Durieux, Florent; Godard, Nathalie; Boher, Aurélie; Vogelgesang, Boris; André-Frei ,Valérie

Basf Beauty Care, Lyon, France

Keywords: quasi-drug, cationic liposome, cocodimonium hydroxypropyl hydrolyzed soy protein, zeta potential

INTRODUCTION

Encapsulation offers a wide range of solutions to cosmetic issues: protection and stabilization of fragile active ingredients, decreased efficient concentrations, long lasting effects, targeted use... But one of the main problems that face topically applied cosmetic active ingredients is their ability to first cross the skin barrier (mainly the stratum corneum) and then become available for skin cells.

Liposomes first made their appearance in cosmetic research in the late 70s' and in finished cosmetic products in the mid-80s'. Liposomes are widely used to facilitate formulation of poorly soluble molecules and foremost to increase the efficacy of cosmetic ingredients. Thanks to their bi-layered membrane structure similar to that of living cells, liposomes facilitate permeation of ingredients and help increase their delivery to skin cells. It is all the more true in the case of complexion lightening or whitening ingredients whose target cells, i.e. melanocytes reside at the basal layers of the epidermis and whose targeted pathway, i.e. melanogenesis occurs within melanocytes.

In the mid-90' studies began demonstrating the ability of liposomes added with helper molecules such as cationic lipids (DOTMA, DOTAP...) or polymers (poly-L-lysine, PEI...) to efficiently deliver their content to cells [1-3]. However, most of the cationic molecules employed at that time exhibited significant cell toxicity which limited their use in cosmetics [4].

We previously developed a cosmetic cationic liposome comprised of 5% soy lecithin and 2% of a cationic soy protein hydrolyzate, namely Laurdimonium hydroxypropyl soy protein that exhibit a high ability to increase the efficacy of cosmetic ingredients such as vitamin E or ferulic acid by improving cell delivery [5]. Our purpose was to adapt this technology to the Japanese Quasi-Drug (QD) regulation and introduce today a QD additive compliant cationic liposome.

The Japanese cosmetics market is the world's largest cosmetics market. But the Japanese cosmetics regulation often makes it difficult to address. Indeed, according to the Japanese Pharmaceutical Affairs Law, cosmetics and "quasi-drugs" (QD) are basically two separated classes but quasi-drugs also comprise a class of so-called "medicated cosmetics" having a mild but yet demonstrated efficacy. Many cosmetic ingredients used as basic cosmetics worldwide enter this category of "medicated cosmetics". The regulatory requirements of these medicated cosmetics are very strict and each ingredient of a medicated cosmetic formula, be it responsible for the cosmetic claim (QD active ingredient) or a simple excipient (QD additive), must comply with precise specifications described in the corresponding monograph from the Japanese Standard of Quasi-drug Ingredients (JSQI).

Cosmetic ingredients aimed at lightening skin complexion must be approved by the relevant authority and are restricted to the claim "suppresses melanin formation and prevents freckles caused by sunburn".

We adapted our cationic liposome to the Japanese QD regulation and introduce today a cationic liposome whose ingredients all comply with the Japanese Standard of Quasi-Drug Ingredients (JSQI) and which can thus be incorporated as QD-additive in medicated cosmetics.

METHODOLOGY

Screening study

We first screened different cationic molecules in their ability to enhance the uptake of a reference fluorescent probe (pentafluorobenzoylamino fluorescein, PFB-F, previously selected for its inability to spontaneously penetrate cells) by cultured skin cells when added to the composition of liposomes. We paid particular attention to plant-derived molecules that would potentially be more acceptable for cosmetic use.

55 molecules at three concentrations (0.5, 1 and 1.5%) were tested in this experimental model to determine their capacity to promote the cell delivery in vitro of PFB-F encapsulated in modified liposomes. They were then suspended in culture medium, homogenized with a vortex mixer and deposited on normal human dermal fibroblasts grown in 24 well plates for 24 hours. The cells were incubated at 37°C in the pres-

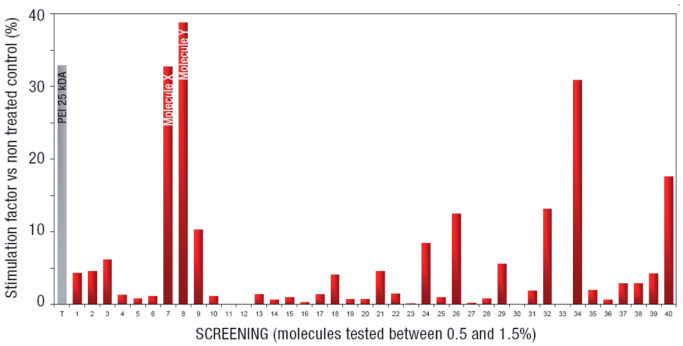


Figure 1: Extract of the screening results. Molecule Y is laurdimonium hydroxypropyl hydrolyzed soy protein

ARTICLE

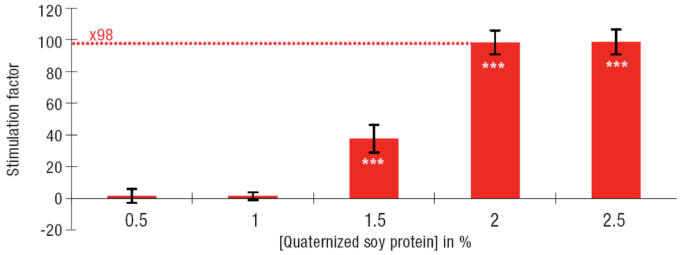


Figure 2: Stimulation factor of PFB-F probe as a function of laurdimonium hydroxypropyl hydrolyzed soy protein concentration

ence of liposomes and were then rinsed with phosphate buffer. This rinse enabled residual fluorescence to be measured, i.e. auto-fluorescence of fibroblasts. Fluorescence was evaluated on dry specimens using a Cytofluor™ (Metertech, PE Biosystem, Courtaboeuf, France). Results are expressed as stimulation factor of intracellular fluorescence signal compared to the PFB-F probe alone (negative control) standardized to a factor of 1. The results were compared to those obtained with 50µM PEI, 25kDa.

At the end of the screening step, a particular molecule, namely Laurdimonium hydroxypropyl soy protein, providing a higher cell delivery rate in vitro than 25 kDa PEI 50µM was retained for further investigations. A dose-effect protocol of the selected molecule was performed to evaluate the most efficient concentration at enhancing PFB-F uptake in vitro.

Adaptation of the cationic liposome to QD requirements

Developing a QD-compliant formula first involved substituting the original cationic protein hydrolyzate Laurdimonium hydroxypropyl soy protein by another cationic protein already listed in the JSQI, namely Cocodimonium hydroxypropyl hydrolyzed soy protein.

Zeta potential measurements

A series of in vitro study were then conducted to demonstrate the relevance of this new encapsulation technology, based on zeta-potential (surface electric charge) measurements.

We measured the zeta-potential of cationic liposomes made of 5% lecithin and increasing concentrations of Cocodimonium hydroxypropyl hydrolyzed soy protein.

This dose-effect study was conducted to determine the most effective concentration of quaternized soy protein.

A ZetaSizer NanoZS Zetameter (Malvern Instruments Ltd., Malvern, UK) was used on three different batches of cationic liposome. The zeta potential of each batch was the mean of 25 successive automatic measurements.

Microscopic visualization

Using transmission electron microscopy techniques (Scanning transmission microscope, CM120 Philips, Eindhoven, Holland), we next analyzed the size and structure of liposomes.

Evaluation of melanogenesis inhibitory activity in mouse B16 cells

B16 cells (murine melanoma cell line) at their 12th passage were incubated in 96-well plates and grown in DMEM medium supplemented with 10% fetal calf serum, glucose, L-glutamine and antibiotics for 24 hours at 37°C/5% CO2.

Cells were incubated for 72 hours in the presence of ascorbyl glucoside at a final concentration of 0.005% or 0.025% (148µM and 740µM, respectively) or with the cationic liposomes containing ascorbyl glucoside at the same final concentrations (0.005% or 0.025%). Kojic acid at 350µM was used as positive control. NDP-MSH, i.e. a stable derivative of Ð MSH was added to all treatment conditions.

After rinsing and cell lysis, melanin absorbance was read at 405 nm and quantified by referring to a standard curve previously obtained with synthetic melanin. At the same time, cell viability was assessed using MTT assay.

The results are expressed as % of melanin compared to untreated control standardized to 100%. Each condition was carried out in triplicate (n=3). The statistical analysis was carried out using Student's t-test for the positive control and using one way ANOVA followed by Dunnett's multiple comparison procedure for the cationic liposomes with a threshold of significance set to 5% (p←0.05).

RESULTS AND DISCUSSION

Screening study

In our in vitro screening model, PEI 25kDa at 50µM increased intracellular fluorescence of PFB F 33-fold. A hydrolyzed soy protein bearing quaternary ammonium groups, namely laurdimonium hydroxypropyl hydrolyzed soy protein, provided a 39-fold increase in PFB-F intracellular fluorescence when used at 1.5% (Fig.1).

Choice of most efficient composition

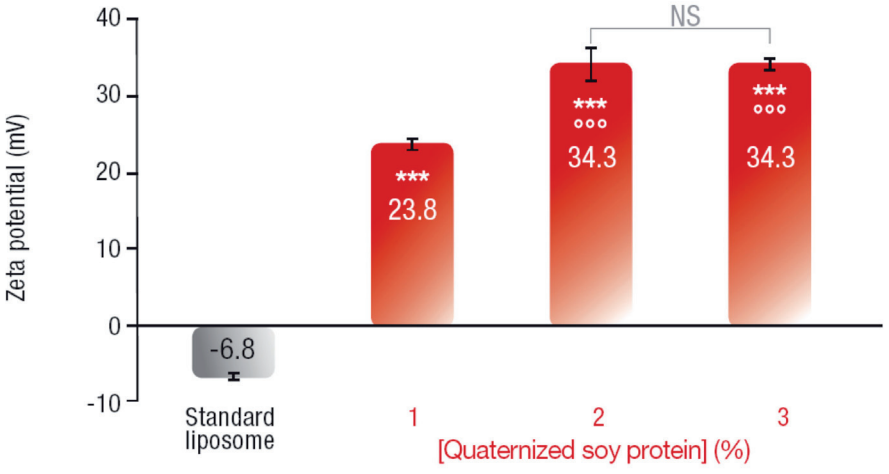


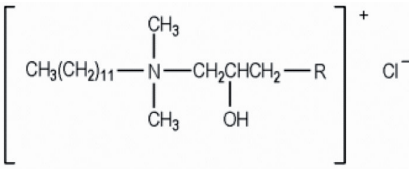
Figure 3: Zeta potential measurements of QD additive cationic liposome as a function of concentration of quaternized soy protein. ***: statistically significant vs. standard liposome, p←0.001; °°°: statistically significant vs. 1% quaternized soy protein, p←0.001

Next, we showed that laurdimonium hydroxypropyl hydrolyzed soy protein used at 2% in the manufacturing process of liposomes increased PFB-F uptake 98-fold (Fig. 2).

Zetametry studies also showed that this new vector exhibited a high zeta potential at acidic pH which basically demonstrated its positive electric charge but also provided evidence for a higher stability in colloidal solution.

QD additive compliant cationic liposome

The original cationic protein hydrolyzate, i.e. Laurdimonium hydroxypropyl soy protein bears quaternary amine groups and a C12 carbon chain (R).



The substitute Cocodimonium hydroxypropyl hydrolyzed soy protein exhibits a similar structure (quaternary amine + C12 carbon chain).

We first observed that a 2% concentration of Cocodimonium hydroxypropyl hydrolyzed soy protein conferred a mean zeta potential of +34.3mV to cationic liposomes (fig 3). Higher concentrations did not increase the zeta-potential, and lower concentrations provided a charge of less than +25mV,

Bearing in mind that a zeta potential ↗ 30mV is reported to be synonymous with good cell delivery efficiency in vitro [6] and good stability in solution – positively charged particles mutually repel and thus do not aggregate – a 2% concentration was chosen as reference concentration for the formulation of QD additive cationic liposome.

Microscopic observations

TEM pictures showed that the cationic liposomes appear as multi-lamellar liposomes with mean size of 250nm (150-400nm) (Fig. 4).

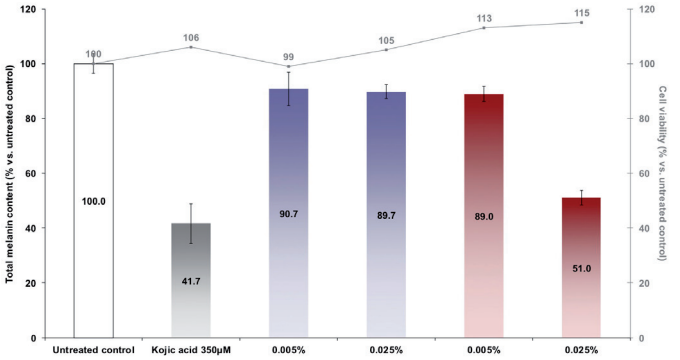


Figure 5: Melanin content of B16 cells. 72 hours of incubation. **, ***: statistically significant vs. untreated control, p←0.01 and p←0.001. °°°: statistically significant vs. free ascorbyl glucoside 0.025%

Inhibition of melanin synthesis in B16 cells

As shown in Fig.5 below, the positive control kojic acid 350µM significantly inhibited melanin synthesis by 58% (p←0.001), which validates the experiment.

Melanin content in cells incubated with 0.005% or 0.025% of free ascorbyl glucoside was significantly reduced by 9% and 11% respectively (p←0.01). This means that, despite significant inhibitions, no dose-related effect was observed and that increasing the concentration of ascorbyl glucoside 5-fold did not provide higher inhibition of melanin synthesis in cultured B16 cells.

Contrariwise, melanin synthesis in B16 cells incubated with cationic liposomes at 0.1% (final concentration of ascorbyl glucoside: 0.005%) was significantly reduced by 11% (p←0.01) and significantly reduced by 49% (p←0.001) when used at 0.5% (final concentration of ascorbyl glucoside: 0.025%). Moreover, the inhibition provided by cationic liposomes containing ascorbyl glucoside at a final concentration of 0.025% was significantly higher that of free ascorbyl glucoside at the same concentration (p←0.001).

This means that, in addition to providing dose-dependent efficacy to ascorbyl glucoside cationic liposomes could significantly outperform free ascorbyl glucoside at the same final concentration.

This demonstrates that cationic liposomes delivery system can significantly increase the efficacy of the cosmetic active ingredient it encapsulates, in the present case ascorbyl glucoside.

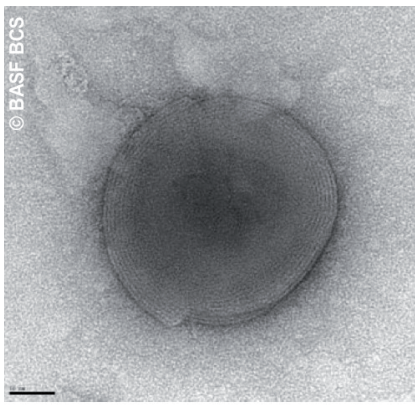


Figure 4: TEM picture of Cocodimonium hydroxypropyl hydrolyzed soy protein-based liposomes Magnification, x300,000

CONCLUSION

In summary, we developed an advanced cationic liposome able to increase the delivery of cosmetic active ingredients in vitro and whose ingredients all comply with JSQI requirements.

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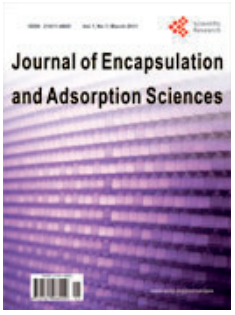

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ARTICLE

PENETRATION OF TOPICAL CHEMICAL THROUGH SKIN: ANOTHER PERSPECTIVE

Des Fernades, Environ Skin Care (Pty) Ltd, Kenilworth, Cape Town, South Africa

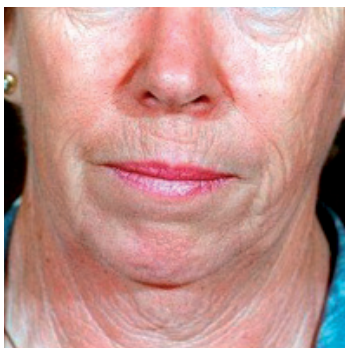
I have discovered that many scientists working with topical ingredients are unaware of what can be done with the products of their research to rejuvenate skin. Changes in the laboratory are very different from clinical changes in real skin. The inclusion of ingredients that make changes to restore or rejuvenate skin has opened up a new era of skin care.

While people like Johan Wiechers were optimising formulae to enhance penetration and effectiveness of topical actives, I was looking at another way to enhance penetration of topical products by using not only electricity (iontophoresis), and sound (low frequency sonophoresis LFS) but also simple physical holes in the stratum corneum as demonstrated by Henry with Prausnitz and others [1]. I extended that into a practical tool that could be used at home by the client themselves (superficial Roll-CIT needling). I was searching for practical techniques that could effectively enhance penetration of selected actives into the skin to make clinical changes. In the cosmetic world there is a lot of hype about active molecules and their delivery into the skin without any delivery of real changes in the skin. As a practising medical practitioner I wanted real results that my patients could clearly see. By using penetrant enhancers and optimising formulae I have been able to make changes as seen in figure 1, but I wanted more than that. That result was achieved by using vitamin A (as retinyl palmitate) plus vitamin C, vitamin E, and beta-carotene. These changes are clinically the same as would be expected from using retinoic acid.

One of the important differences between the laboratory world and the clinical, practical world is that winkles have to be refined, pigmentation marks have to be so much lighter that the client cannot easily see them, scars need to become noticeably smaller and obvious to the client. Of course clients expect more than science can now achieve thanks to unrealistic claims by the marketing divisions of large cosmetic houses. We are all well aware that one can achieve substantial histological and histo-chemical changes in the epidermis (real or synthetic) or the reticular dermis without unfortunately, a commensurate change in the appearance of the skin. Isolated skin treated for hours gives no real indication of changes that occur in living skin over weeks and months. Animal tests can't be extrapolated to human experience. However, I believe that the synergy of optimising formulae plus the special techniques that I have explored, we have opened up the cosmetic world to surprisingly excellent results that compete with the results achieved by clinicians using machines costing about $100,000.

IONTOPHORESIS

Iontophoresis offers well-known effects but has the limitation that it applies only to water-soluble molecules. Furthermore, the enhancement is generally accepted at maximum 4 times better than simple topical application under ideal conditions. Obviously formulae for iontophoresis need to be optimised so that the actives are in the best milieu for ionisation and repulsion into the skin and yet are stable for storage. The ideal concentration must also be calculated to ensure that one can deliver the active molecules in effective concentrations. Schmidt first demonstrated the effectiveness of iontophoresis to make visible changes to the surface of the skin but she used medical ingredients like retinoic acid and oestriol [2]. I explored to discover if similar changes could be achieved by using simple cosmetic versions of vitamin A and vitamin C.[3]



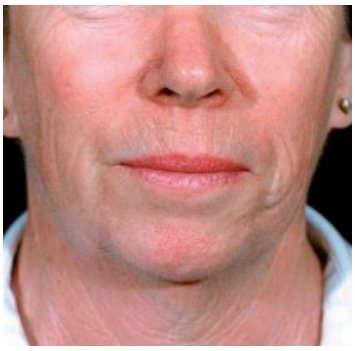


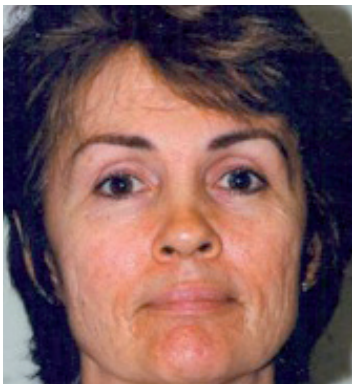
Figure 1 : Result seen from using penetrant enhancers for a cream containing vitamin A, formulated as retinyl palmitate, with the addition vitamin C, vitamin E, and beta-carotene. Changes seen at six months. These changes are the same as would be expected from using retinoic acid.

LOW FREQUENCY SONOPHORESIS

Low frequency sonophoresis (LFS) was first described by Mitragotri[4] and has the advantage that relatively low energy sounds in the region of 21kHz induce cavitation of the lipid bi-layers of the skin and thereby permit penetration of molecules up to 40 times greater than simple topical application. The special advantage of LFS is that the cavitation persists for several hours after creation. Naturally one needs water-soluble media to conduct the sound into the ski but thereafter it is possible to enhance the penetration of lipid soluble molecules. Simultaneous iontophoresis makes LFS much more effective. [5] These formulations need to meet the specifics required for iontophoresis and LFS but excellent results can be obtained (see figure 2) which in this case represent rejuvenation from weekly sessions of iontophoresis combined with LFS using vitamin A (as retinol) and vitamin C (as sodium ascorbyl phosphate) for about 50 treatments.

The major problem with LFS is that it requires specialised equipment and even then, in the clinical situation it cannot be as effective as in the laboratory. However, the results achieved with rejuvenation of skin using the combination of iontophoresis and sonophoresis are extremely gratifying. Skin care therapists can now achieve result that were previously just a pipe dream, or were available only to medical practitioners using machines costing in the region of US$ 100,000.

The problem with iontophoresis and combined LFS treatments are that they take about sixty to ninety minutes to do, which is an important drawback.



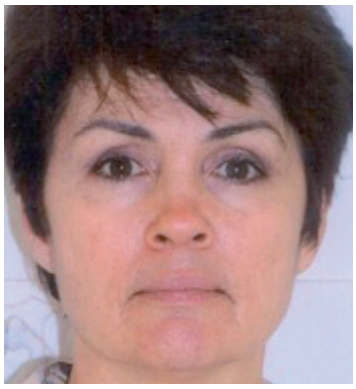


Figure 2 : LEFT shows before starting weekly sessions of iontophoresis combined with LFS using vitamin A (as retinol) and vitamin C (as sodium ascorbyl phosphate). RIGHT : shows the rejuvenation after about 50 treatments done once a week. This was a lot of work but the result compares favourably with expensive medical surface treatments.

The machines may not be expensive but it still remains a privilege only for the people who can afford the time and the cost of doing them.

SUPERFICIAL ROLL-CIT NEEDLING

This was denigrated as a medieval torture system when I first spoke about it but in fact one painlessly creates tiny holes that effectively by-pass the natural skin barrier and allow whatever is applied to the surface, to penetrate into the deeper epidermis. When one looks at superficial Roll-CIT needling, these holes in the stratum corneum are massive relative to the molecules of topical actives, which simply behave as though there has been a localised dermabrasion and all the components of the topically applied product easily enter the skin. There is, however, no guarantee that these actives can pass through the cell walls and enter the cells. That is where the formula has to be carefully considered to make the actives become bio-available.

The Cosmetic Roll-CIT tool that basically opens tiny areas of the stratum corneum should be done at leas once a day before applying the cosmeceutic type of products that will promote rejuvenation, lightening and tightening of the skin. Experience has shown that this is non-irritating and extremely effective but the importance is that the results depend on how many "holes" are created in the stratum corneum.

The major advantage of superficial Roll-CIT needling is that the client at home can do it, every day. Figure 3 demonstrate the tightening of the peri-orbital skin that was done by using the Cosmetic Roll-CIT prior to applying vitamin A (as retinyl acetate) combined with vitamin C (as ascorbyl tetra isopalmitate), vitamin E and beta-carotene as the daily skin care. The Cosmetic roll-CIT increases perfusion through the skin by penetrating through the stratum corneum and into the upper stratum spinosum (depth about 0.025 mm). Once through the stratum corneum, it is easy for molecules to enter cells and make changes. This type of skin needling will only produce a result if enough holes have been made and if, at the same time, the ingredients of the cream can make visible changes. Vitamin A is the preeminent molecule to rejuvenate the skin. Needling by itself will not make any changes.

Despite the fact that this technique is so efficient, for an idealised skin care one still absolutely needs formulae that can work independently and effectively so that the ingredients can be absorbed into the skin cells.

One drawback of using certain chemicals to enhance penetration is that they in themselves can cause reactions in sensitive clients.

These three methods potentially allow penetration far in excess of anything we could hope for with chemical optimisation but they introduce very different concepts in the design of the formula because now we have to take into account the required properties for iontophoresis or LFS that may in fact break all the recommendations for an optimised formula. Furthermore, iontophoresis and LFS have been shown to not merely optimise passage into the deeper layers of skin, but also into the cells and nuclei themselves. This far exceeds the targets for good formulation. The main drawback of these two techniques is that one needs specialised equipment to employ their powers and that means that these can only be used in clinical "treatments"

The three techniques I have mentioned merely "add the cherry on top" to optimised formulation.

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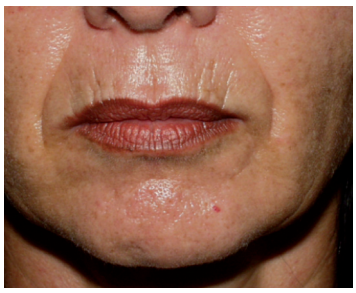

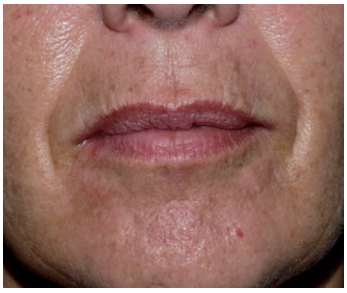

Figure 3: LEFT - shows a patient before starting the regime of using the Cosmetic Roll-CIT with needles that penetrate the skin about 0.025 mm and thereby bypass the stratum corneum. Creams applied to the surface will perfuse into the depths and if the ingredients consist of molecules like vitamin A that can make changes, then one can expect a result. RIGHT - demonstrates the tightening and thickening of the peri-oral skin after two year that was done by using the Cosmetic Roll-CIT prior to applying vitamin A (as retinyl palmitate and acetate), vitamin C (as ascorbyl tetra-isopalmitate) and other antioxidant vitamins as the daily skin care.

ARTICLE

MICROFLUIDIC PRODUCTION OF STRUCTURES FOR ENCAPSULATION AND CONTROLLED RELEASE

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INTRODUCTION

Emulsion drops have long been utilized to make capsule materials for foods, drugs, and cosmetics because of their efficient encapsulation properties. In particular, double-emulsion drops, which are drops-in-drops, have provided promising templates to produce microcapsules due to their core-shell geometry [1,2]. Through polymerization or evaporation-induced consolidation of the shell phase, a compartment between the core and continuous phase can be prepared [3-5]. Although it is difficult to make double-emulsion drops in bulk, microfluidics have enabled production of such double-emulsion droplets in a highly controlled fashion; sizes of both core and shell are precisely controlled within a limited range by flow rates. In addition, high efficiency of encapsulation can be achieved with microfluidics because the innermost phase is separately injected and emulsified and the surrounded by the shell phase. However, production of microcapsules with a well-defined membrane in submicron scales from double-emulsion drops remains a challenge, although such microcapsules are in high demand for encapsulation and controlled release for a wide range of applications.

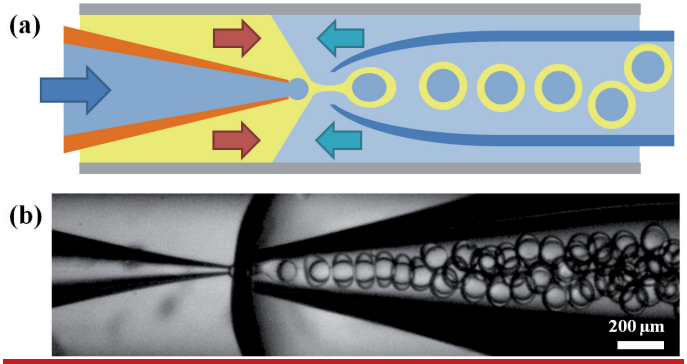

Figure 1: Preparation of double-emulsion drops in a microfluidic capillary device. (a) Schematic illustration and (b) optical microscope image.

Here, we report highly useful microfluidic approaches to produce monodisperse microcapsules with membrane thickness of tens of nanometers. Two different types of membranes are prepared: a bilayer membrane of amphiphilic diblock copolymers and a homogeneous polymeric membrane.

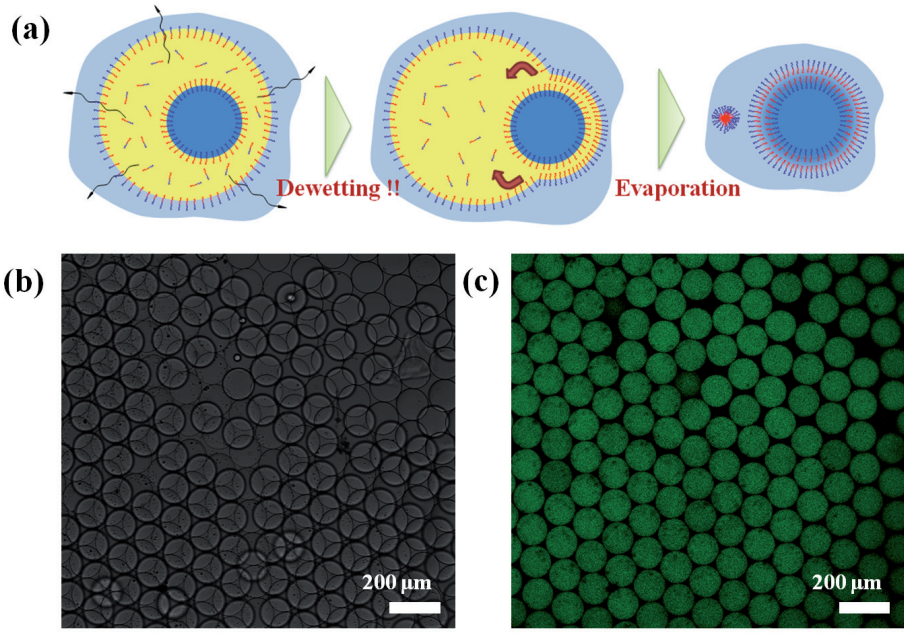

Figure 2: Formation of polymersomes from double-emulsion drops. (a) Schematic illustration of dewetting-induced formation of a bilayer membrane. (b, c) Optical and confocal microscope images of monodisperse polymersomes..

Both membranes are highly stable for encapsulation of water-soluble or water-dispersible materials; moreover, both membranes are simultaneously biocompatible and biocodegradable. In addition, hierarchical microcapsules, capsules-in-capsules, are produced by re-injection of capsules with microfluidic devices, which enables encapsulation of multiple distinct components and their controlled sequential release while avoiding cross-contamination.

POLYMERSOMES TEMPLATED BY DOUBLE-EMULSION DROPS

Polymersomes are vesicles with membranes composed of bilayers of amphiphilic block-copolymers. Because of the relatively high molecular weight of the amphiphiles, polymersomes exhibit enhanced stability in comparison with liposomes whose membranes consist of bilayers of phospholipids. Thus, polymersomes are promising delivery vehicles for drugs, cosmetics, and nutrients, encapsulating and storing the actives for a long time. Conventional approaches to their production, such as electroformation or bulk hydration, produce polymersomes with poor control of size and

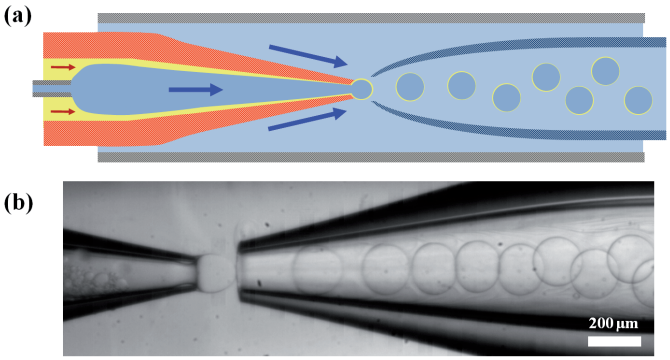

Figure 3: Preparation of double-emulsion drops with ultra-thin shell in a microfluidic capillary device. (a) Schematic illustration and (b) optical microscope image.

low efficiency of encapsulation. The use of microfluidic techniques based on double-emulsion drops overcomes the shortcomings of conventional approaches, enabling fabrication of highly monodisperse polymersomes which can encapsulate active materials with very high efficiency [6-8].

Capillary microfluidic devices can be used to make water-in-oil-in-water (W/O/W) double-emulsion drops. The coaxial geometry and robustness of glass channels facilitate the generation of double-emulsion drops, regardless of solvents [1]. We design a capillary device composed of two tapered cylindrical capillaries inserted in a square capillary as shown in Figure 1a; the red-colored left cylindrical capillary is treated to be hydrophobic and is used for injection of an aqueous innermost phase and the blue-colored right cylindrical capillary is treated to be hydrophilic and is used for collection of double-emulsion drops. To make double-emulsion drops, an aqueous solution containing actives is injected through the injection capillary to become the innermost drop phase and a mixture of chloroform and hexane containing amphiphilic diblock copolymer, poly(ethylene glycol) (PEG)-b-poly(lactic acid) (PLA), is injected through the interstices of the square and the injection capillaries to become the middle oil phase. An aqueous continuous phase is injected through the interstices of the square and the collection capillaries, forming a counter-flow to the innermost and middle phases. These three streams flow coaxially through the orifice of the collection capillary, making double-emulsion drops in a dripping mode, as shown in Figure 1b.

The resultant double-emulsion drops show dewetting of the middle oil layer on the surface of the innermost drops as chloroform evaporates, as shown in Figure 2a; because hexane is a poor solvent for the PEG-b-PLA diblock copolymer, the middle layer dewets as the concentration of hexane increases, leaving a bilayer of the diblock copolymers on the surface of the innermost drops [7]. The middle layer finally separates from the innermost drops, producing monodisperse unilamellar polymersomes as shown in Figures 2b and c, where polymersomes contain green dye molecules in their interior as a model encapsulant. The polymersomes are biocompatible and biodegradable; PEG in the copolymer is typical biocompatible polymer and PLA is typical biodegradable polymer. In addition, organic solvents are removed during the dewetting and subsequent separation and any small amount of residual oil is also removed by incubation in an open bath. The polymersomes are stable for at least 15 days and then spontaneously degrades as PLA block is hydrolyzed. The stability can be further enhanced by adding PLA homopolymer in middle oil phase, which incorporates into hydrophobic region of the bilayer; the reinforced polymersomes are stable for at least two months. This stability can be further increased by slightly increasing the thickness of the shell through the incorporation of small amounts of additional homopolymer

MICROCAPSULES WITH ULTRA-THIN SHELL

Double-emulsion drops are unstable and show coalescence of the innermost drops to the continuous phase. Therefore, double-emulsion drops frequently rupture before the consolidation of the middle layer is completed. Significant enhancement of the stability can be achieved by making the middle layer very thin; the lubrication effect on the middle layer slows the migration of inner drops, making long-live double-emulsion drops [9]. In addition, the thin middle layer facilitates fast consolidation. Therefore, double-emulsion drops with ultra-thin shell are excellent templates to produce microcapsules.

To produce such W/O/W double-emulsion drops with an ultra-thin shell, we use biphasic flows in a capillary microfluidic device. When two immiscible fluids flow through a single capillary, one fluid which has higher affinity to the wall flows along the surface of the capillary, while the other fluid flows through the center of the capillary. This core-shell flow can be emulsified into the third continuous phase in a capillary device as shown in Figure 3a [10]. Because the flow velocity of sheath stream is relatively low, the resulting double-emulsion drops have very thin shells as shown in Figure 3b, where the shell is too thin to observe. The thickness of shell is controllable

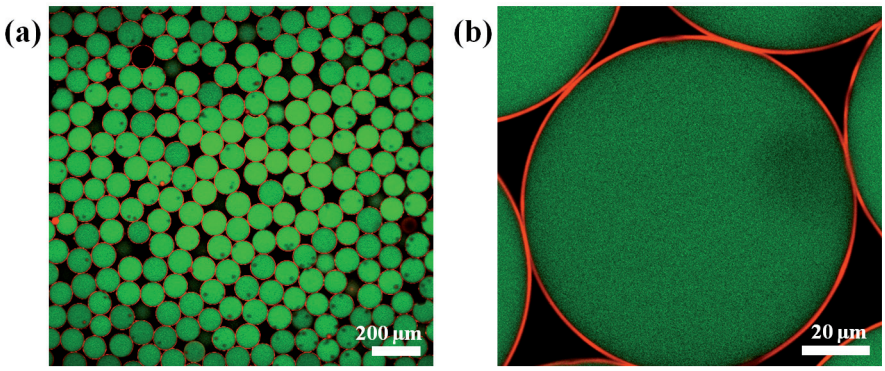

Figure 4: Confocal microscope images of monodisperse microcapsules with ultra-thin membrane.

ARTICLE

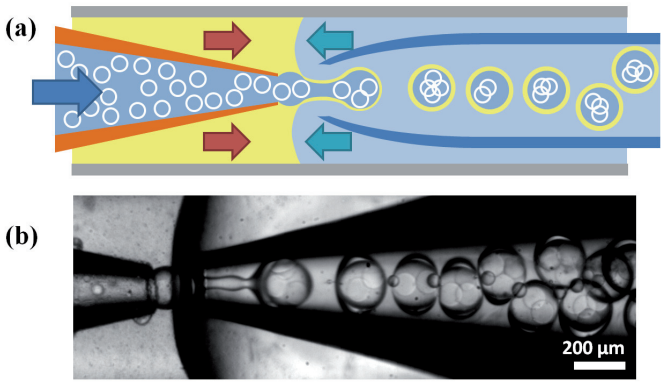

Figure 5: Preparation of polymersomes-in-polymersomes. (a) Schematic illustration and (b) optical microscope image showing injection of polymersomes into the innermost drops of double-emulsion drops.

by flow rates and viscosity of the middle phase, which is in the range of 0.2-2 µm.

By using a volatile organic solvent containing polymers as the middle phase of W/O/W double-emulsion drops, we can make microcapsules through evaporation of the solvent. For example, when we use toluene containing PLA homopolymers, microcapsules with homogeneous membrane of 80 nm in thickness are prepared as shown in Figure 4, where the interior of the capsules contain green dye molecules and the membrane has red dye molecules. During evaporation of the middle oil layer, the thickness decreases, resulting in ultra-thin membrane with a thickness of tens of nanometers. The resultant microcapsules are highly stable because the polymers are entangled with each other in the membrane. In the case of biodegradable polymers, the stability of the capsules depends on type of polymers, molecular weight of the polymers, and thickness of the membrane; for example, capsules made of PLA with a molecular weight of 20K Dalton, and with a membrane thickness of 80 nm, show release of encapsulants for approximately three month at room temperature and capsules made of 108K poly(lactic-co-glycolic acid) (PLGA) with the same thickness show slower release for approximately five month.

POLYMERSOMES-IN-POLYMERSOMES

If polymersomes are surrounded by the second level of polymersomes, we can efficiently encapsulate multiple distinct components without cross-contaminations between components. Microfluidics enable the production of such valuable polymersome-in-polymersome structures. The monodisperse polymersomes can be prepared from double-emulsion drops as seen in Figure 2. When the polymersomes are re-injected into a microfluidic device and are encapsulated in the innermost drop of the second level of double-emulsion drops, polymersomes-in-polymersome structures can be produced though a formation of bilayer membrane from the double-emulsion drops [11]. For this, we make a capillary device with similar design as the one for single polymersomes, but with larger orifice for the injection and collection capillaries as shown in Figure 5a. By using the same middle oil phase and the continuous phase, we can produce double-emulsion drops whose innermost drops have monodisperse polymersomes as shown in Figure 5b. The dewetting of the middle oil layer and subsequent separation of the oil produces polymersomes-in-polymersome structure, as shown in Figure 6, where we inject a mixture of three different polymersomes of the same size encapsulating a red dye, a green dye, and a mixture of red and green dyes, respectively. The polymersomes-in-polymersomes provide sequential release of multiple encapsulated actives in a programmable fashion. For example, as the simplest means of release, we can use mechanical strain. When the polymersomes-in-polymersomes suffer the strain, outer polymersomes will rupture first due to their large size and inner polymersomes will rupture second; therefore, encapsulated materials in the outer polymersomes will be released upon rupturing and subsequently, the materials in the inner polymersomes will be released. The sequential release under mechanical strain is very useful property in cosmetic applications of the structures. To achieve sequential release in a more elaborate fashion, we can control the stability of bilayer membrane of the inner and outer polymersomes; this can be accomplished through the addition of PLA homopolymer. Dramatic change of the stability depending on contents of PLA in the bilayer membrane enables the programmed rupture of membrane and therefore programmed release of encapsulated materials.

POLYMERSOMES-IN-MICROCAPSULES

Polymersomes-in-microcapsules with ultra-thin shell provide much higher stability of the structures than that of polymersomes-in-polymersomes. Because the microcapsules with ultra-thin shell are highly stable due to entanglement of homopolymers in the membrane, they can protect inner polymersomes from external stimuli. Therefore, the polymersomes-in-microcapsules are useful for long-term storage of multiple components. To

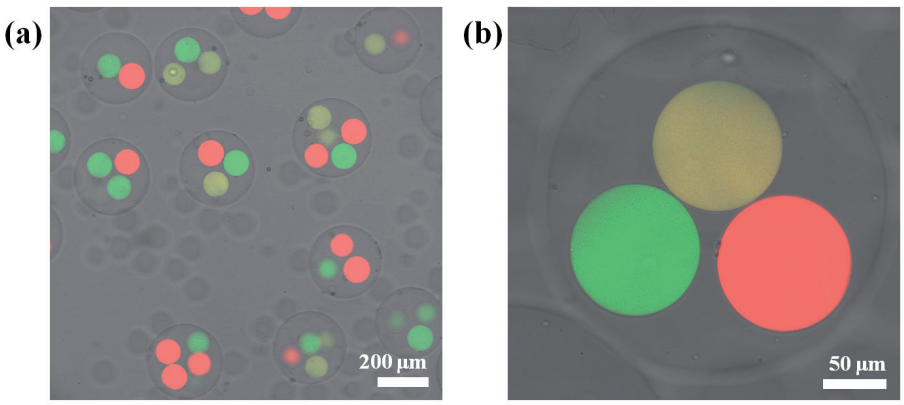

Figure 6: Confocal microscope images of polymersomes-in-polymersomes with three different inner polymersomes.

make such structures, monodisperse polymersomes are injected into the innermost drops of double-emulsion drops with ultra-thin shells using a capillary device as shown in Figures 7a and b. Upon evaporation of middle oil phase, the double-emulsion drops make ultra-thin membranes, resulting in polymersomes-in-microcapsules as shown in Figure 8.

SUMMARY

Microfluidics provide novel and practical methods to produce simple and multiple microcompartments which are very useful for encapsulation and controlled release of active materials for drugs, cosmetics, and foods. For example, double-emulsion drops produced in microfluidic devices can be used as efficient templates to make monodisperse unilamellar vesicles through dewetting of the middle oil phase. In addition, double-emulsion drops with ultra-thin middle layer are also excellent template to produce microcapsules with homogeneous membrane composed of any materials.

In particular, microfluidics enable the production of hierarchical structures such as polymersomes-in-polymersomes and polymersomes-in-microcapsules, which have highly valuable as delivery vehicles of multiple distinct components because of their abilities for efficient encapsulation and programmable release.

In addition, the biocompatibility and the biodegradability of the polymersomes and microcapsules as well as the high encapsulation efficiency of this microfluidic approach, provide new opportunities to use these capsule materials in practical biological delivery systems for active ingredients such as drugs, cosmetics, and nutrients. In particular, these structures have great potential for encapsulation of active for cosmetic applications.

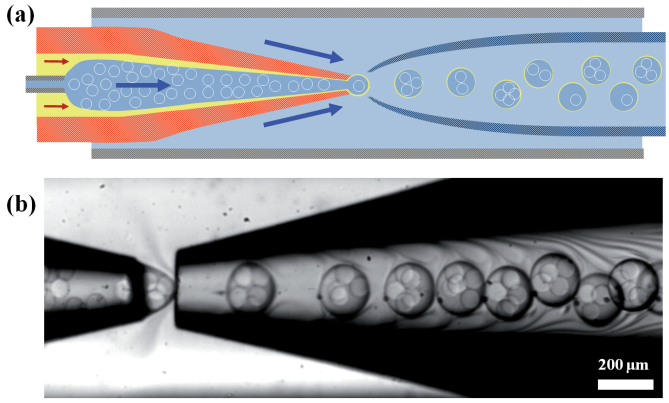

Figure 7: Production of polymersomes-in-microcapsules. (a) Schematic illustration and (b) optical microscope image showing injection of polymersomes into the innermost drops of double-emulsion drops with ultra-thin shell.

ACKNOWLEDGEMENT

This work was supported by Amore-Pacific and Capsum.

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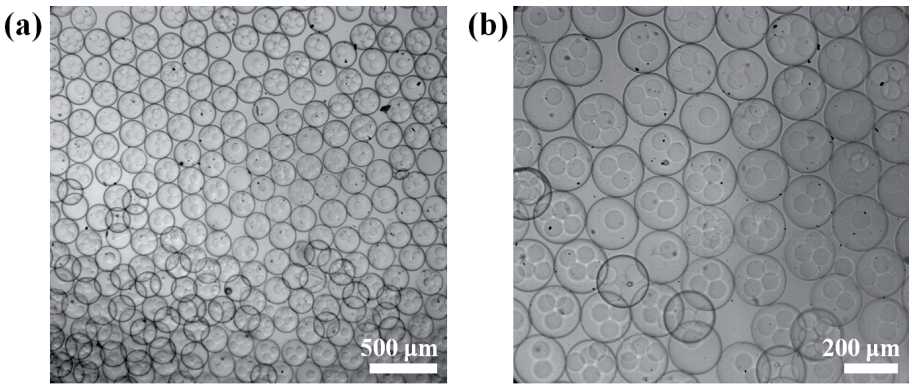

Figure 8: Optical microscope images of polymersomes-in-microcapsules.

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Coffee break and poster session

Session 3 : Biomaterials

Chairperson: Prof. Harald Stover, McMaster University, Canada

Lunch and poster session

Session 4 : Nutrition, food and feed

Chairperson: Prof. Muriel Subirade, Laval University, Canada

Coffee break and poster session

Session 5 : Emerging nanomaterials

Chairperson: Dr. Arthur Carty, Waterloo University, Canada

Gala dinner

September 23, 2012

Session 6 : Delivery of biopharmaceuticals

Chairperson: Prof. Frank Gu, Waterloo University, Canada

Coffee break and poster session

Session 7 : Biopolymers

Chairperson: Mary Ann Augustin, CSIRO, Australia

Lunch and poster session

Session 7 : Environment and agriculture

Chairperson: Luz de Bashan, CIBNOR, Mexico

Coffee break and poster session

Session 8 : Green technologies

Chairperson: Luigi Ciampi, Valdivia University, Chile

Dinner

September 24, 2012

Session 9 : Innovative technologies

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Coffee break and poster session

Session 10 : Cell immobilization

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ARTICLE

PICKERING EMULSION ENCAPSULATION FOR COSMETIC INDUSTRY

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INTRODUCTION

Encapsulation technique provides protection to the active substances, enhance their stability, can be used to transport and deliver them to specific destinations.

The most important goal in the delivery of an active principle is to bring the active principle concentration to a specific level and maintain it at that level for a specified period of time. Stability and solubility are two key physicochemical properties that must be considered when designing a successful active ingredient formulation . The main challenge is to formulate a product that has sufficient chemical and physical stability to not degrade

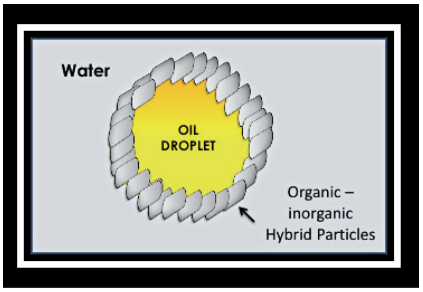

FIG 1 Schematic illustration of a Pickering emulsion stabilization

during the shelf life of the product and yet has sufficient dissolution rate to reach the required dose effect. Typically biodegradable polymers, liposomes or emulsions are used as vectors. This latter ensures the transport across the biological barriers and the protection of the active substance in the living medium.

Encapsulation has been successfully used in various fields of manufacturing: cosmetic, pharmaceutic, food, agriculture, paint and inks industries.

PICKERING EMULSION ENCAPSULATION

In this study we would like to focus on emulsion encapsulation and especially emulsion for the cosmetic industry.

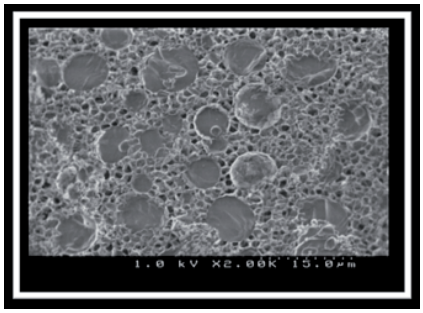

FIG 2. O/W pickering like emulsion, oil droplets formation

Most of cosmetic preparations like cream or lotion are emulsion based systems, mainly composed by a water phase, an oil phase and an emulsifier.

Emulsifiers are typically low molecular weight molecules, having both hydrophilic and lipophilic character. This dual character allows the stabilization of droplet emulsion. Unfortunately, emulsifiers are generally processed at high temperature. Moreover, they could cause several problems because of irritation potential especially for sensitive skins.

In order to overcome this problem, emulsifier-free emulsions have been prepared by substituting the classical emulsifiers by solid particles of nanometric/micrometric size that adsorb at the oil-water interface (FIG 1). This kind of emulsion is generally called Pickering emulsion.

A wide range of solid particles have been used as emulsifiers for Pickering emulsion, including silica, clay, latex, etc. Recently, such emulsions have been received increasing level of attention, due to a variety of reasons such as, their remarkable stability against coalescence and their high stability against changes to processing conditions (pH, salt concentration, temperature, etc.). Many researchers have reported that the effectiveness of particles in stabilizing emulsions depends on various parameters, such as the particle wettability, particle concentration , shape and size .

The formation of a dense shell of solid particles around the droplets acts as a barrier against material transfer. Therefore, Pickering emulsions can be viewed as capsules which form a densely packed and rigid shell-like structure that could be used for a controlled delivery purpose.

RECENT DEVELOPMENTS

Frelichowska and co-workers have recently reported an investigation of skin penetration of a hydrophilic model active substance (retinol) encapsulated in a w/o Pickering emulsion and compared the penetration to that given by a classical emulsion stabilized by surfactant. The distribution of retinol inside the skin layers depended significantly on the emulsions type: the classical emulsion allowed easy diffusion through the stratum corneum, so that large amounts reached the viable epidermis and dermis. Conversely, high storage of retinol inside the stratum corneum was favored by the Pickering emulsion. The retinol content in stratum corneum evaluated by skin stripping, demonstrated the increased retinol accumulation from pickering emulsion. In this study has been highlighted the use Pickering emulsions are new drug penetration vehicles with specific behavior; they are well-suited either for targeting the stratum corneum or aimed at slow release of drug from stratum corneum used as a reservoir to the deeper layers of skin.

Ephyla has developed a new organic-modified clay able to form particle-stabilized emulsion, using a cold process. This hybrid material, called

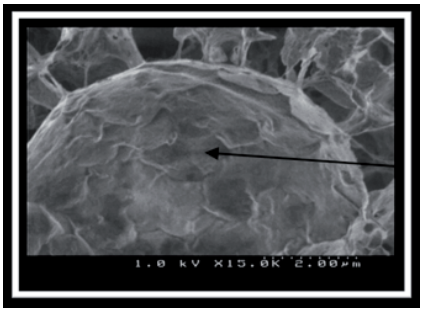

FIG 3 Magnification O/W emulsion : Hybrid material platelets form a layer surrounding the oil droplets preventing their coalescence

Frametime®, is obtained via the organic modification of a phyllosilicate type smectite, using an organo-modifier. Frametime® has been especially designed to stabilize oil in water emulsions. Organic modified clays have been studied.The emulsions obtained, have been characterized by rheological analysis and observed by different microscopic and cryogenic techniques. Emulsions obtained using Frametime show a homogenous dispersion. Oil droplet diameters range between a few micrometers up to 10 µm (FIG. 2). As highlighted in magnification FIG. 3, the hybrid material forms a layer surrounding the oil droplets preventing their coalescence and leads to an effective core-shell like microcapsulae formation. In addition, the organic-modified clay forms a network in the continuous phase. The network formation increases the stability of the emulsion reducing the movement of the discontinuous phase.

Stability against creaming and flow properties has been extensively investigated by rheological analysis. Whether a cream will have a good skin feel depends on several rheological factors. In this study we have shown the unique shear-thinning behavior and the thixotropic properties (FIG 4) of Frametime stabilized emulsions.

Microcapsulae, using Frametime, are produced by a simple and fast physical process that does not require additional heat sources. Consequently, costs and environmental impact are dramatically reduced. The process is cost-effective, reproducible and easily up-scalable. Unlike most of classic encapsulation technique where the resulting capsule shell material is a cross-linked polymer network, Frametime encapsulation does not require the use of such polymers or other chemical modifications. This allows to preserve the biological properties of each ingredient.

Because of the easy-of-use and the versatility of Frametime microcapsulae process, skin, hair and mucosal surfaces could useful targets for the delivery of active lipolytic ingredients. Ingredients so encapsulated include active ingredients, colors, fragrances, flavors, essential oils and vegetable oils. Especially for these latters a possible application includes lipid oxidation prevention. Lipid oxidation is a major concern for both cosmetic and food manufactures since oxidation has negative effects on cosmetic and food qualities such as, taste, appearance, texture and shelf-life, and also leads to the formation of off-flavours and even toxic compounds .

Moreover Frametime is especially suitable for heat-sensitive product protection, since the encapsulation process is a cold process.

Ephyla is very open to collaboration providing to future customers or partners flexibility and an exciting opportunity to drive innovation.

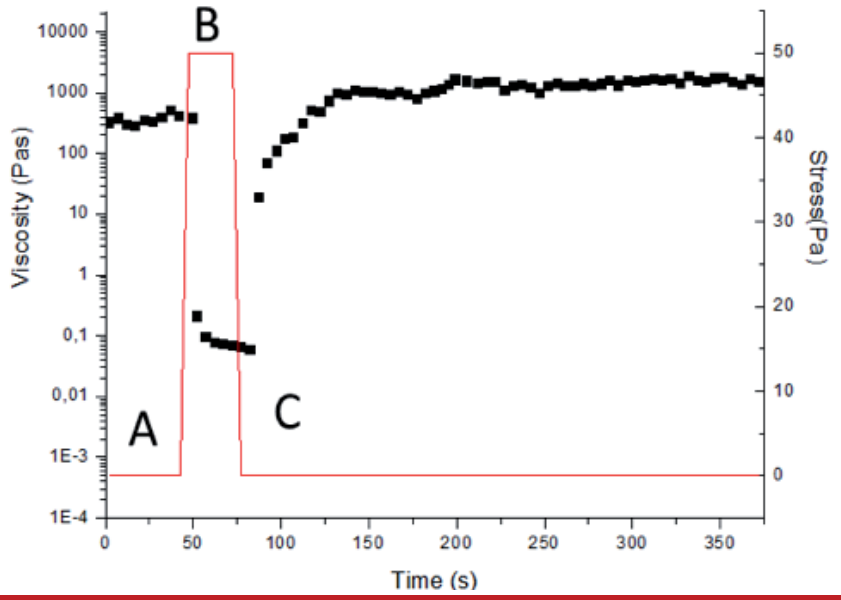

FIG 4. Structure breakdown and build up: most cosmetic emulsions are deformed when a small amount is removed. How quickly the structure is restored can be crucial. In point A the emulsion is at rest. Then in point B the amplitude is suddenly increased (100-fold over the Linear Viscoelastic Region): structure breakdown. In point C: the amplitude is returned into the LVR: structure build-up

ACKNOWLEDGEMENT

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Fabrizio FORDIANI has grown up in Italy where he received his basic education in Material Science. He then moved to France where he obtained a Master in Polymer Science and then a PhD working on bio-based composite materials. During his doctorate he had the opportunity to collaborate with Vincent BOURGETEAU from the EPHYLA company, a cosmetic start-up specialised in natural solutions development. He almost immediately joined the team as Materials Department Project Manager. Working with EPHYLA on the design of new materials, his research ranges from oleochemistry to the development of new formulation concepts and the vectorization of active ingredients.

ARTICLE

ENCAPSULATION: MAKING THE IMPOSSIBLE POSSIBLE.

Incorporating Therapeutic Acids into Alkaline Products. - Case Study on Salicylic Acid

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INTRODUCTION

Acne vulgaris (also known as acne) is a form of skin disease, which affects the porous intrusions of the follicular-sebaceous unit. Acne is the most common skin disorder in the United States afflicting 40 to 50 million Americans. The primary source in the development of acne begins with the human sebum (a naturally produced oil within the sebaceous gland), which causes a plug/cap to form within the pores of the epidermis. As the inside components (dead skin particles, oils, etc.) begin to saturate and congest the pores, it becomes a hospitable environment for pathogenic bacteria (known as P. Acnes), to trigger an inflammatory cytokine response. Once inflammation of the sebaceous gland becomes prevalent, signs of comedones, papules, pustules, along with occasional nodules appear onto the outer surface of the skin.

While there are several ingredients for treating the infectious components of acne, SA has been widely accepted as an anti-acne agent, due to its high efficacy. SA is one of the safest and most commonly used for patients with mild to moderate acne. Concentrations of SA ranging from 0.5% to 10% have been recommended for acne, but the maximum strength allowed in non-prescription acne products, in the United States, is up to 2% by the FDA Final Acne Monograph. SA acts as an exfoliant, causing the cells of the epidermis to shed more readily, opens clogged pores, and prevents them from clogging again. The mechanism of action for SA is disruption of intercellular adhesions and inhibiting an inflammatory cascade associated with acne formation.

Figure 1: MultiSal™ SA as a (A) raw material and (B) in a commercial soap bar.

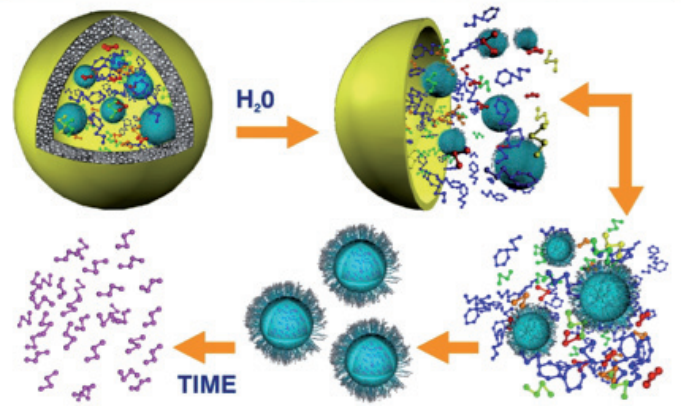


Figure 2: Mechanism of action of MultiSal™ SA.

However, the challenge in utilizing SA as an anti-acne medication is incompatibility with alkaline soap bars. In an alkaline soap bar, the SA is converted to a salt form. Therefore, the main challenge becomes basically to protect SA in an alkaline environment, while delivering it effectively.

Another issue is that through the attempts of delivering free SA to the skin surface using a rinse-off application such as soap bar, much of the product is lost, reducing deposition on skin from rinse-off applications. Therefore, the primary goal in delivering SA is to stabilize the compound in an applicable complex, which can efficiently be deposited onto the skin, and specifically to the pimples or comedones.

MICROENCAPSULATION TECHNOLOGY

MultiSal™ technology is based on double-layered encapsulation of functional ingredients. The functional ingredients are contained in sub-micron spheres, which are re-encapsulated in larger microspheres. These microspheres stabilize the active, preventing them from neutralization and facilitating their use in alkaline soap-bar products.

The multi-layer technology is based on double encapsulation of SA. The acid is contained in tiny sub-micron spheres (0.1-0.5 microns in diameter), which are then re-encapsulated into larger microspheres (30-50 microns in diameter). The wall of the microsphere ruptures when the product is rubbed into the skin in the presence of water (Figure 2). At this point, the sub-micron spheres are released and deposited onto the skin. The release rate of SA from the sub-micron spheres is modulated by the properties of the spheres that give them the ability to partition into the outer layer of the skin. As they disintegrate, the encapsulated SA is slowly released, providing longer-lasting benefits to the user. It does this without the typical drying of the skin.

METHODS

Application Procedure

Using the extraction apparatus (circular bulb 15.5 cm2 in area), the applied areas were marked. Approximately 0.3 g of the soap bar was applied to each

sample area, rubbed for 15 seconds and rinsed-off. After 15 mins, each application area was rinsed with 4.5 ml of ethanol (1.5ml of ethanol rinse for 30 sec., extraction repeated for a total of three times with an extraction cup until most free SA was extracted from the skin.

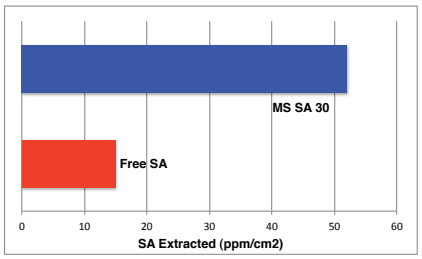


Figure 3: Enhanced deposition studies after treatment with MultiSalTM SA at 2 % SA vs. control using soap bar.

pH of MultiSalTM in Solution

Skin pH is typically used as a criterion to predict the possible irritation capability in a product. A pH range of 4.5 – 5.0 is considered to be safe for normal skin. Three different formulations were prepared: 1. 2 % free SA in water, 2. soap-bar containing (2 % SA from MultiSalTM SA) and 3. soap-bar containing 0.5 % concentration of free SA. The pH was tested with a Hanna, HI99181 pH meter.

RESULTS

Clinical Study of Deposition of MultiSal™

Traditional rinse-off products deposit only 2-5 % of SA onto the skin, wasting over 95%. MultiSal™ technology significantly enhances the deposition from a rinse-off through both lipophilic and electrostatic forces. This increases the overall efficacy of the product. Deposition of SA onto skin was determined by clinical testing following analytical procedure. Clinical studies show that the MultiSal™ SA technology ensures significantly greater deposition (→3 times) of SA onto the skin compared to products containing free SA (Figure 3).

pH of MultiSalTM in Solution

MultiSal™ SA helps to retain acid form and extend the shelf life of SA in a soap bar with an alkaline pH (Table 1). SA in water has an acidic pH of 2.5. When this solution is applied on the skin, it may cause dryness. MultiSal™ SA in a soap base has a pH of 4.1, indicating the majority of acid was not released into the water solution. The pH of the soap solution with free SA is 10.2, indicating all the SA has been titrated to form a salt, and is not in an active form.

Table 1: pH of solution containing free and MultiSal™ SA in a Soap Bar.

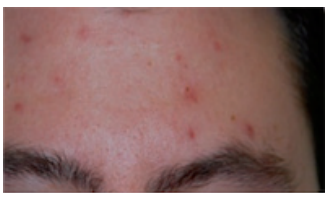
Sample Description	pH
free SA at 2% in Water	2.5
Veggie Soap - 2% SA from MultiSal™ SA	4.1
Veggie Soap - 0.5% free SA	10.2

Clinical Study of MultiSal™ in Soap Bar

In a clinical study, we showed that MultiSal™ is most effective to treat severe cases of acne in which the consumer has →20 blemishes. Washing the face three times a day with this system produces significant effects within 4 weeks of the initial application (Figure 4). As shown, there was a 54 % reduction in number of blemishes using the technology vs. only 30 % reduction for the control after 30 days (n=57) (Figure 5).

FUTURE PERSPECTIVES

MultiSalTM technology is being utilized to enhance compatibility of various functional ingredients such as alpha-hydroxy-acid for acne and general skin exfoliation, kojic acid for skin brightening, azelaic acid and others as anti-bacterial agents. The outer layer of the micro-spheres provides stability from outside factors while the sub-micron spheres allows for skin deposition and time-release kinetics. The combination makes this encapsulation technology unique and effective.



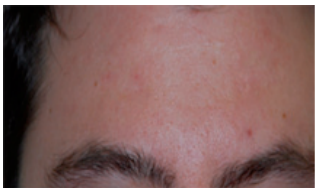


Figure 4: Photos illustrating the ability of MultiSal™ SA to reduce blemishes. After a period of treatment (left) there are significantly less blemishes than at the beginning (rigth).

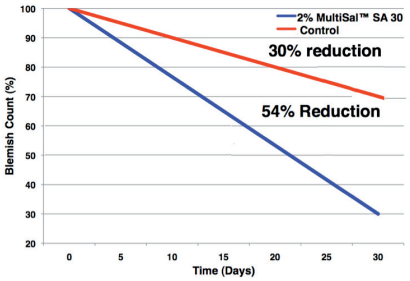


Figure 5: Reduction in blemish count over 30 days for the technology vs. control sample.

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Dr. Sam Shefer earned a BS and MS in Bio Chemistry and PhD in Bio-chemical and Chemical Engineering from Ben Gourion University in Israel, with a post doc experience at the Massachutes Institute of Technology in Biomedical Engineering. He has over 20 years of experience with developing commercial products based on microspheres and nano spheres, for pharmaceutical and industrial applications. Sam is an expert in the fields of micro and nano encapsulation, drug delivery and controlled release technologies, published multiple papers and was granted with over 100 US & worldwide patents.

Dr. Shefer co established Salvona Technologies, as a center for development and commercialization of nano and micro spheres enabling technologies utilized for dermatology and beauty products. The Company has successfully launched over a dozen of innovative products for topical applications over the last 6 years.

Bioencapsulation Research Group

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