Compressed fluid based process for development of cosmetic products

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Abstract

Many aesthetic and functional problems of skin, such as cutaneous dryness and atopical dermatitis, are due to reduced amount of lipids in the external skin layer. These diseases can be resolved by using topical creams containing balanced mixtures of epidermal lipids. However, the set up of a suitable process for obtaining epidermal lipid based formulations represents a key step to yield an effective topical system. The production process must prevent material degradation, avoid the use of organic solvents and yield products with the desired properties. In this regard, we recently investigated the epidermal lipids processing by Particles from Gas Saturated Solution technique (PGSS) to obtain solid lipid nanoparticles. PGSS is a flexible solvent free technique that operates under mild conditions. In this process, the lipid mixture processed is melted under CO_2, saturated and atomized through a special nozzle in a semi-continuous apparatus.

Preliminary Differential Scanning Calorimetric (DSC) investigations were carried out to evaluate the melting point of the various epidermal lipid mixtures (Ceramide, Cholesterol and Radiacid®), which is an important parameter for process design. Then, micronization runs were performed using different formulations and processing conditions. The effects of the operative variables, such as operative temperature and pressure, were investigated to obtain nanometric particles with optimal lipid composition.

Photon Correlation Spectroscopy (PCS) analysis showed that the particle mean size was in the range of 200-500 nm. Melting temperature and enthalpy were evaluated by DSC.

The lipid nanoparticles obtained were finally formulated as cream-gel systems, which were produced using simple lipid based formulations or polymer/silicone emulsions. These systems were analyzed by optical microscopy and rheological techniques to study the effect of the nanoparticle concentration on the structure, spreadability and functionality of the final product. The cream formulations presented good microstructure (regular with droplets of about 2-5 μm) and were stable throughout 3 months of investigation.

Keywords: supercritical CO_2, lipid nanoparticles, PGSS, pharmaceutical cream

Introduction

Lipid nanoparticles, or solid lipid nanoparticles (LNP), are colloidal dispersions with excellent perspectives as both pharmaceutical and cosmetic formulations [1, 2]. They may be formulated by using a number of biocompatible materials, which make them flexible and useful for a variety of applications, such as stabilization and controlled/prolonged release of bioactive compounds. Besides their role as carrier systems for drugs and cosmetic ingredients, LNP can form a continuous film on the skin surface with occlusive properties. Reduction of Trans Epidermal Water Loss (TEWL) with the related increase of stratum corneum hydration was observed [3, 4].

Lipid nanoparticles prepared with epidermal lipids (ceramide, cholesterol and fatty acid) could represent an innovative “dermatological ingredient” for topical formulations; these lipids have, in fact, been demonstrated to be successful in treatment of common skin diseases, involving barrier deficiencies, such us atopic dermatitis, psoriasis, xerosis and pruritous in elderly patients [5].

In the present study the preparation of LNP from Gas Saturated Solution (PGSS) technique [6], has been investigated. This process is solvent-free: the lipid mixture is melted and then saturated with CO_2 under suitable pressure. Then, this gas-saturated solution is atomized by a special nozzle in a semi-continuous apparatus and particles of sub-micronic size can be obtained [7].

The CO_2 dissolution into materials leads to a reduction of the viscosity, melting and solidification temperature of the mixture. The ability of the high pressure CO_2 to reduce the melting point permits to operate at lower temperature with respect to classical methods [7, 8]. This turns to be a great advantage when processing thermolabile materials. A relatively small amount of CO_2 is required to do the job, so that the process is likely to be economically attractive.

Preliminary results were presented by Bertucco et al. (9). In this work, the preparation and the characterization of lipid nanoparticles composed with binary and ternary mixture of the Ceramide, Cholesterol and Radiacid® are discussed.

Materials and Methods

Materials

The lipids: ceramide, cholesterol and Radiacid® (a commercial mixture of fatty acids, mainly composed of stearic and palmitic acid) were kindly provided by UNI.FAR.CO Belluno (Italy).

Their chemical structure is described in Figure 1.

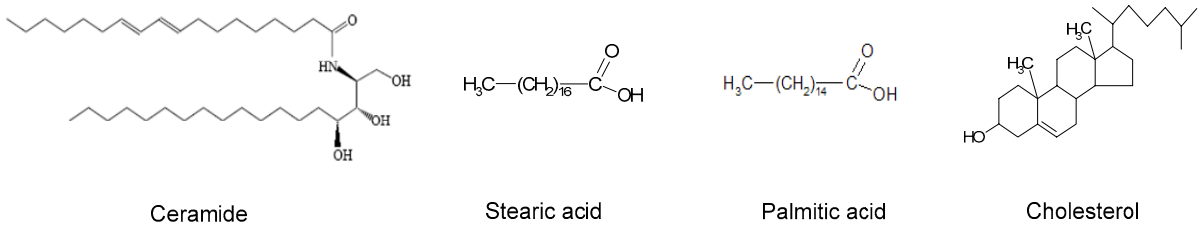

Figure 1. Chemical structures of the lipids

Carbon dioxide was supplied by Sapio Srl (Monza, Italy).

Deionized water was produced in our laboratory.

The lipid phase of the emulsions was made up with: cyclosiloxane (Mirasil CM5 - Rhodia), a caprylic/capryic triglyceride (Miritol 318 -Cognis); isohexadecane (Arlamol HD – Uniquema).

A number of emulsifiers was used: PEG-12 Dimethicone (DC 5329-Dow Corning); Polyacrylamide, C13-14 isoparaffin, Laureth-7 (Sepigel 305 – Seppic) ; sodium acrylate/ sodium acryltaurate copolymer isohexadecane, polysorbate 80 (Simulgel EG -Seppic).

Apparatus

Equipment for performing PGSS is essentially composed by a mixing chamber, a piston pump, an expansion section including a nozzle, and a system for the collection of the particles.

With reference to Figure 2, the mixture to be atomized is loaded into a mixing chamber suitably heated by a thermostat, where it is contacted with supercritical CO_2 for a suitable time at proper temperature and pressure conditions. After this, the solution is pumped through the nozzle, where it is atomised and micronized: CO_2 is vaporised and solid particles are obtained and collected on a metallic filter.

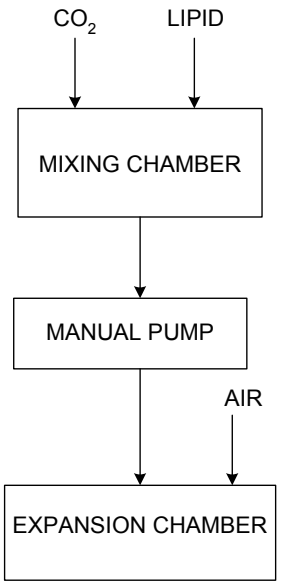

Figure 2. Block schema of the PGSS process

All the parts of the plant are kept at a temperature above the solidification temperature of the substance to avoid any precipitation of solids along the pipes. Two streams of air (one supplied by a compressor and the other by a cylinder at 200 bar) are used to improve the atomization process.

Production of lipid nanoparticles by PGSS techniques

Pure lipids and lipid mixtures were processed by PGSS to produce LNP. The lipid material in the mixing chamber was mixed with pressurized CO_2 under heating for 30 minutes. Various runs were performed using CO_2 pressures of 100 and 150 bar, and temperatures of 50 and 60°C. The melted mixture was let to flow through a nozzle into an expansion chamber. The solid particles were collected from a filter located at the bottom of this chamber.

Characterization of the materials

Differential scanning calorimetry (DSC) was performed using a Q10P/PDSC (TA Instruments) equipped with a pressure cell system that allows operating at high temperature (up to 160 °C) and under different pressure values (from 1 up to 60 bar). The unprocessed substances and the nanoparticles were analyzed both at room pressure to study the effect of the micronization process.

Particle size analysis was performed using a Nicomp 380/Dynamic Light Scattering, Submicron Particle Sizer (Particle Sizing Systems Santa Barbara-California). One mg of lipid nanoparticles was suspended in 5 mL of deionized water and sonicated in an ice bath for 45 minutes.

Preparation of emulsions

A series of cosmetic oil in water emulsions were prepared by using two polymeric emulsifiers (Sepigel 305 and Simulgel EG) and a silicone emulsifier (DC 5329) with increasing concentration of LNP (1:1:3). See Table 1 for details.

All the products were prepared using a cold-cold procedure: the LNP were pre-dispersed into the lipid phase, then the aqueous phase (water and polymers) was added to the lipid phase using an Ultra Turrax T25 (IKA) homogenizer (8000 rpm for 3').

Table 1. Formulation of the cream preparation

Base formulation	**Lipid phase**	**Aqueous phase**
O/W Cream	Mirasil CM5 8% + Miritol 318 1% + Arlamol HD 1% + DC5329 4%	Sepigel 2%+Simulgel EG 2% Water q.b. 100g

Formulations with LNP	**Lipid Phase/ SLN**
CS 5	10 : 0.5
CS 10	10 : 1
CS 15	10 : 1.5
CS 20	10 : 2
CS 30	10 : 3

Cream Characterization

The rheological properties of emulsions were analyzed at 25°C using Rheostress Haake RS100 equipped with PP35 sensor (parallel plates with serrated surfaces).

Morphological analysis of emulsions were carried out using a ZEISS AXIOVERT 40CFL at 1000x, coupled with an imagine analyzer.

Results

DSC measurements

In the PGSS process, the materials are melted at suitable temperature and CO_2 pressure before to be atomized by flowing through a nozzle. The solubilization of CO_2 in the substance reduces the melting point up to 40°C depending on the chemical structure of the substance [10]. The materials employed in this study have different melting points of the starting materials: 55°C for Radiacid®, 147°C for Cholesterol and 124°C for Ceramide. Therefore, DSC experiments were carried out on the mixtures before to be processed by PGSS.

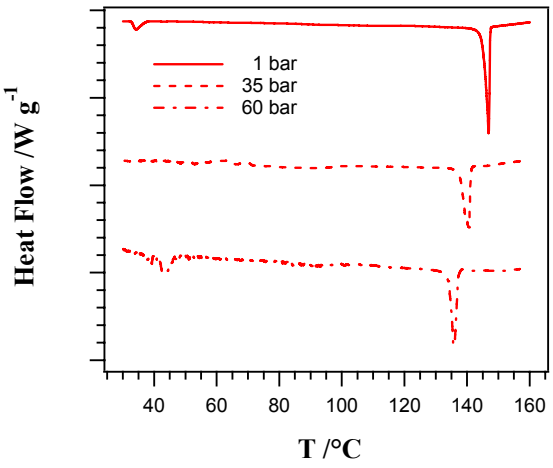

Figure 3. DSC measurement on Cholesterol as function of CO_2 pressure.

CO_2 reduced the melting and solidification temperature of the starting materials. Figure 3 shows that 10°C Cholesterol melting and solidification temperature reduction was obtained at a pressure of 60 bar. The effect of dissolving CO_2 within the materials leads to a reduction of the melting heat except for the Ceramide (Table 2).

Table 2. Temperature and heat of melting and solidification of pure lipid as function of CO_2 pressure

Sample	Pressure /bar	$T_{melting}$ /°C	$\Delta H_{melting}$ /J g^{-1}	$T_{melting}$ /°C	$\Delta H_{melting}$ /J g^{-1}	$T_{solidif}$ /°C	$\Delta H_{solidif}$ /J g^{-1}	$T_{solidif}$ /°C	$\Delta H_{solidif}$ /J g^{-1}
Ceramide	1	86.7	13.5	124.0	87.7	102.8	54.0	92.0	17.3
	35	82.3	14.0	119.8	100.8	100.0	75.7	79.6	5.7
	60			116.0	93.7	98.9	74.4	82.4	0.8
Cholesterol	1	34.3	6.8	146.9	72.5	118.6	41.3	34.3	6.8
	35			140.0	48.0	115.0	32.6		
	60	41.0	21.5	136.0	45.8	102.0	29.2	41.0	21.5
Radiacid®	1			55.0	296.0	52.5	184.5		
	35			48.0	148.5	43.9	80.0		
	60			45.0	108.0	40.0	17.2		

In Figure 4a, the results of the DSC measurements carried out on the binary mixtures at ambient pressure (heating phase) are reported. It is possible to observe that for the Ceramide-Radiacid® mixture presents two peaks in the range of 80-100 °C.

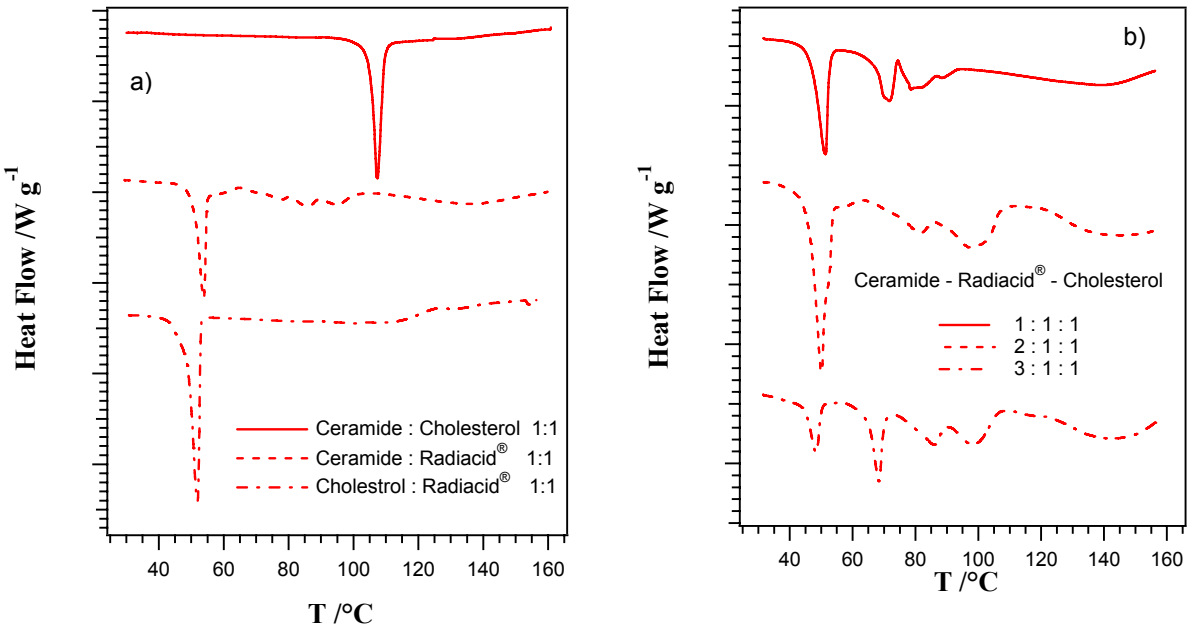

Figure 4. DSC measurement carried out at ambient pressure on a) binary and b) ternary mixtures.

The Ceramide:Cholesterol 1:1 mixture displays a melting point lower than pure Ceramide and Cholesterol, while melting points of the other binary mixtures were similar to that of the Radiacid®, thus indicating its great relevance in influencing the behaviour of the mixtures.

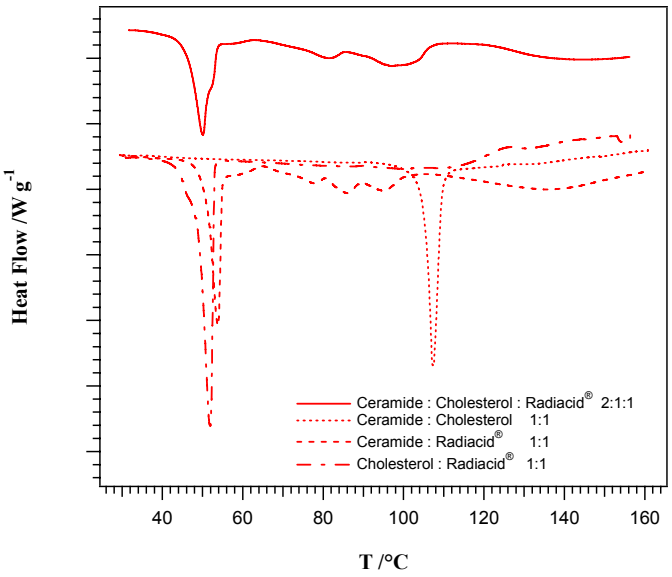

Figure 5. Comparison between the DSC measurement of ternary and those of binary mixture at ambient pressure.

The DSC of the ternary mixture showed a complex behaviour (Figure 4b); it is possible to observe the presence of three-four peaks, which were shifted at higher temperature as the Ceramide concentration was increased. Mixtures 2:1:1 and 3:1:1 showed peaks at temperature higher than 80°C at 60 bar of CO_2, so they could not be processed with our PGSS plant. These peaks can be attributed to the Ceramide-Radiacid® and Ceramide-Cholesterol rich phase (Figure 5).

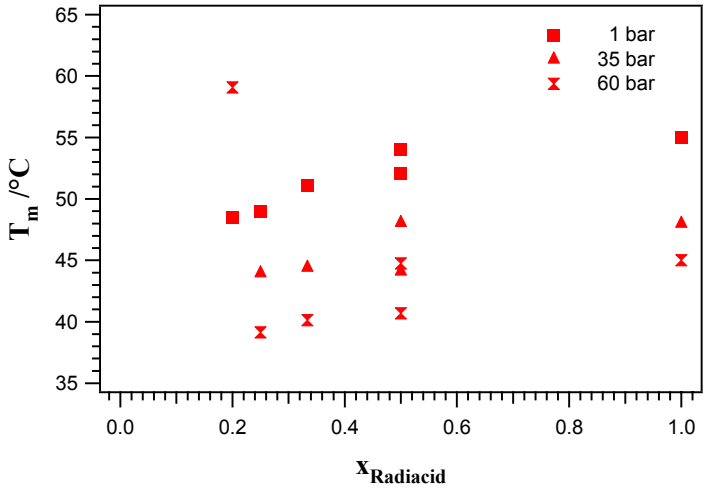

Figure 6. Melting temperature of the binary and ternary mixtures as function of Radiacid® concentration and of pressure.

Figure 6 shows that the melting temperature of the binary and ternary mixture is function of Radiacid® concentration.

PGSS processing of lipidic mixtures

Different experiments were carried out to understand the effect of temperature and pressure of the mixing chamber on particle size. Binary Cholesterol-Radiacid® mixtures were employed for this study.

Table 2. Operative condition and composition of the experiments carried out with mixtures of Cholesterol and Radiacid®

T_{CM} /°C	P_{CM} /bar	$x_{Radiacid}$
55	100	0.67
55	100	1.00
60	100	0.33
60	100	0.50
60	100	0.67
60	150	0.33
60	150	0.40
60	150	0.50
60	150	0.80

The particle size, obtained by the runs summarized in Table 2, was reduced working at lower temperature, while the effect of the pressure was quite irrelevant (data not showed). For this reason, all relevant experiments were carried out at 150 bar.

Table 3. Operative condition and composition of the experiments carried out with mixtures of Ceramide and Radiacid®

T_{CM} /°C	P_{CM} /bar	$x_{Radiacid\ e}$
70	150	0.80
70	150	0.67
70	150	0.50
75	150	0.40

The operative conditions of the PGSS runs on binary mixtures of Ceramide and Radiacid® are reported in Tables 3.

The diameter of the particles increased as the Ceramide or Cholesterol concentration increased (Figure 7). This is probably due to the lipid dimension, as Ceramide is made up by a long chain and Cholesterol is a large and rigid molecule.

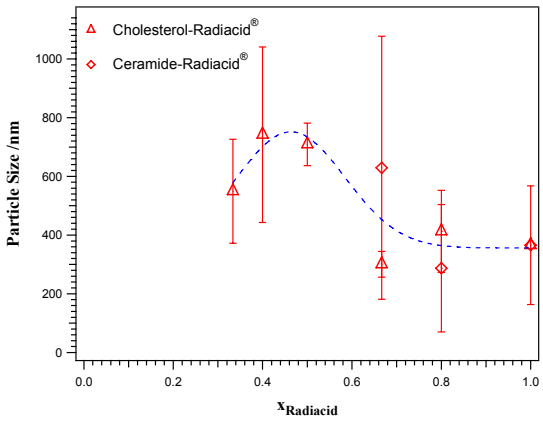

Figure 7. Particle size obtained by processing the binary mixtures.

Table 4 reports the main results obtained with ternary mixtures. It is possible to see the increase of the temperature in the mixing chamber with the decrease of the Radiacid® concentration.

Table 4. Operative condition and composition of the experiments carried out with ternary mixtures.

T_{CM} /°C	P_{CM} /bar	$x_{Cholesterol}$	$x_{Radiacid}$	$x_{Ceramide}$
60	120	0.10	0.80	0.10
60	120	0.20	0.78	0.02
60	150	0.09	0.73	0.18
65	150	0.14	0.57	0.29
65	150	0.17	0.50	0.33
65	150	0.29	0.43	0.29
70	150	0.20	0.60	0.20
70	150	0.25	0.50	0.25
75	140	0.20	0.40	0.40
75	150	0.29	0.43	0.29

The diameter of particles produced did not change with the mixture composition. The particle size obtained with ternary mixtures (800-1000 nm) did not depended on their composition. DSC measurement of these particles showed a reduction of the melting temperature and heat of fusion with respect to the lipid mixture employed in their production. These results demonstrated that that the PGSS process solidified the lipid in different solid states.

Effect of LNP on cosmetic formulations

The effect of LNP on the cosmetic formulations were first investigated by optical microscopy, in order to confirm the expected morphology and mean size of the emulsions droplets (Figure 8).

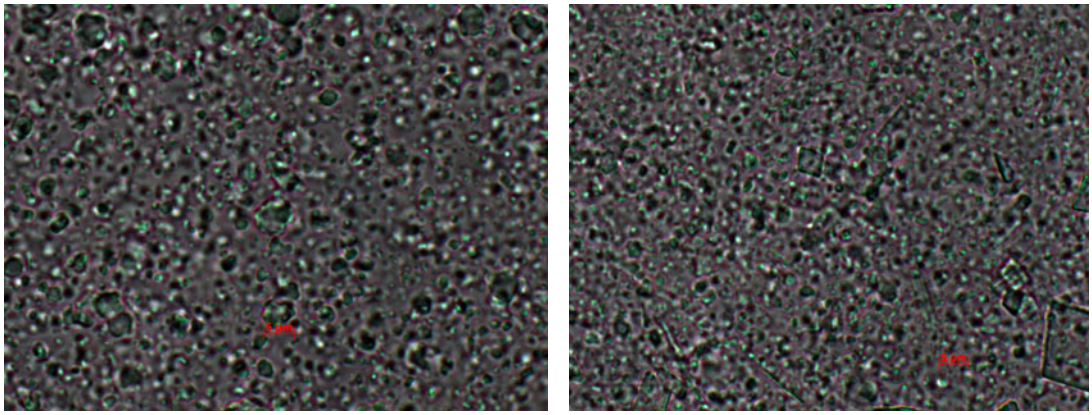

Figure 8: *Base formulation without (on the left) and with LNP (2% w/w) (on the right)*

The rheological characterization of the products, performed at low shear conditions (oscillatory flow conditions), showed that the LNP addition did not affect the cream-gel system structure and its stability in time; elastic (G') and viscous (G") moduli trends appear slightly affected by oscillation frequency indicating the formation of a weak-gel network. The absolute values of G' and G" did not significantly change with the increasing concentration of LNP, as reported in figure 10.

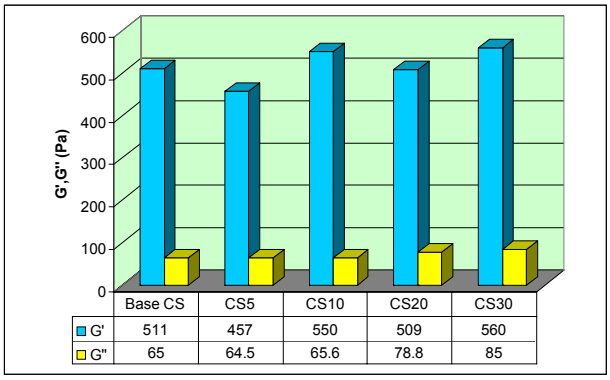

Figure 9: Elastic (G') and viscous (G") moduli values at fixed frequency (1.47 Hz).

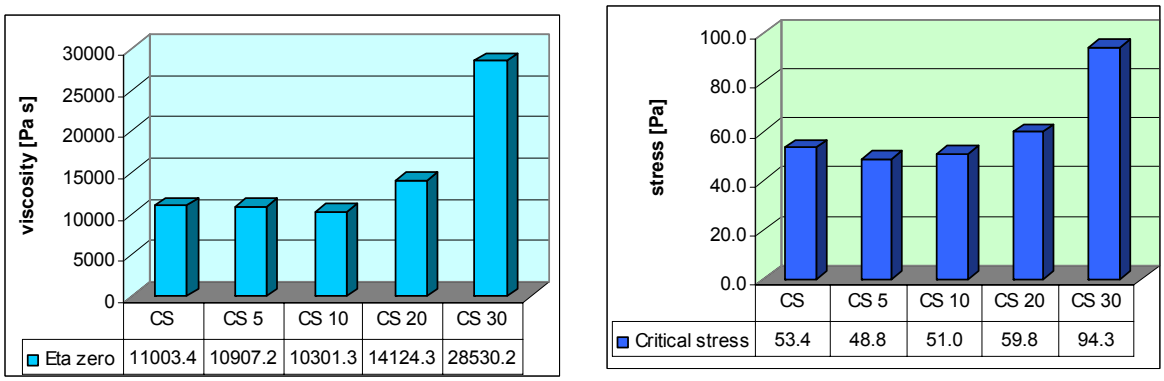

Figure 10: Eta zero values and critical stress values

Structural evaluations were performed by continuous flow analysis. The pseudoplastic behaviour of materials was calculated by fitting the experimental viscosity data as a function of stress with the Carreau equation. The data.show that that the structural parameters (η_o , τ_{crit}) increase when nanoparticle concentration is higher than 2% w/w.

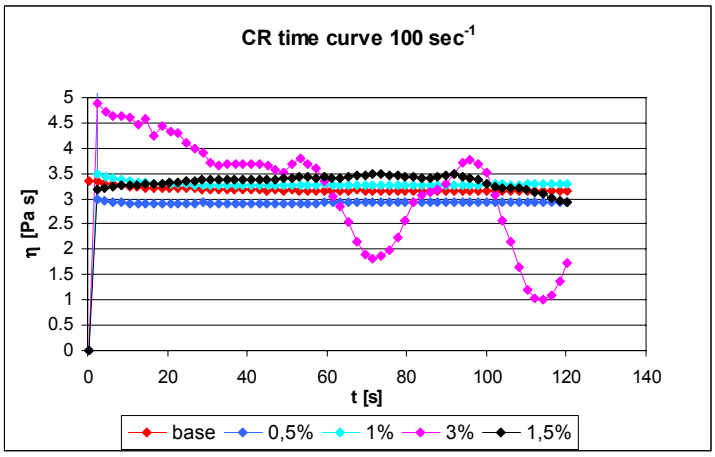

Figure 11: Viscosity trends in time (shear rate 100 sec^{-1})

Controlled rate time analysis suggested that the spreading properties of the cosmetic formulations can be significantly compromised by high concentration of nanoparticles; as shown in figure 11. The viscosity trends in time (obtained at 100 sec^{-1}) are linear for all the samples up to the 10:3 lipid phase/LNP critical ratio (Figure 11).

Conclusions

A CO_2-based PGSS process was set up to produce lipid microparticles and nanoparticles without the use of organic solvent and formed by binary or ternary mixture. Differential Scanning Calorimetric (DSC) investigations were carried out to evaluate the melting point of the various epidermal lipid mixtures (Ceramide, Cholesterol and Radiacid®) to optimize the operative condition of the PGSS technique. The particles obtained were analyzed by Photon Correlation Spectroscopy and DSC: they had a mean particle size below 1 micron in all the composition analyzed and a melting point lower than the pristine mixture.

Both the mean size and the surface properties of these lipid particles are well-suited for the formulation of cosmetic emulsions if a cold-cold procedure is adopted. The rheological studies show that the addition of the solid fraction up to a critical ratio lipid phase:LNP 10:3 does not compromise the stability of the products and does not affect the compliance of the formulation.

These data suggest that lipid particles produced with PGSS could be an interesting cosmetic ingredient for dermatological products.

Acknowledgment

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