OPTIMIZATION OF LIPID NANOPARTICLES COMPOSITION FOR SUNSCREEN ENCAPSULATION

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The aim of this study was to obtain lipid nanoparticles with appropriate characteristics, in terms of mean particle size and stability, for the co-encapsulation of two kinds of sunscreens. For this purpose, the surfactant and liquid lipid composition and concentration have been varied in order to obtain solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). Lipid nanoparticles were produced by using a modified melt emulsification method coupled with high shear homogenization. The optimized lipid nanoparticles have been loaded with butyl-methoxydibenzoylmethane and octocrylene and were characterized by dynamic light scattering technique and differential scanning calorimetry. Their in vitro UV blocking ability has been also determined.

Keywords: Lipid nanoparticles, co-encapsulation, sunscreen, UV blocking effect

1. Introduction

Lipid nanoparticles (LNs) are novel carriers systems for delivery of lipophilic compounds. They are derived from oil/water emulsion, where the lipid liquid was replaced by solid lipids (solid lipid nanoparticles – SLNs) or a blend of solid and liquid lipids (nanostructured lipid carriers – NLCs) [1, 2]. In general, LNs are submicron particles with spherical shape and average diameters ranging from 40 to 500 nm. Their solid lipid matrix has the ability to be solid at room temperature as well as at body temperature [3].

NLCs, considered the second generation of lipid nanoparticles after SLNs, were developed in order to overcome the drawbacks of SLNs. Compared to SLNs, NLCs show a higher loading capacity for a number of active compounds and limits their expulsion from the lipid matrix during storage [4]. This is due to the

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fact that the use of a lipid mixture with very different molecular structure (size) disturbs the formation of a well-ordered crystal lattice. Thus, the lipid matrix of NLCs systems contains many imperfections providing space to accommodate the lipophilic drug [5, 6].

Because LNs are easily scaled up by using existing technologies, they have been intensively studied. At the beginning of research on lipid nanostructure systems, both – SLN and NLC – were investigated in different pharmaceutical fields as intravenous/parenteral drug carrier and later, the interest was directed on the cosmetic LNs application for dermal application [7]. Many advantages of lipid nanoparticles used for dermal application were underlined. SLNs and NLCs are biocompatible systems since they are composed of well-tolerated and physiological lipids, they enhance the chemical stability of labile compounds and enable controlled release of a large class of active compounds [8, 9]. Due to the small size that ensures a close contact with the skin, they present occlusive properties and an increased skin hydration effect is observed [10, 11].

Different studies have underlined the ability of lipid nanoparticles to act as physical sunscreen and by their combination with molecular sunscreens the photoprotection can be improved [12-14]. Moreover, owing to the synergistic effect of sunscreen loaded into the particles and the UV scattering properties of LNs, the amount of sunscreen could be decreased while maintaining the same protection level of a conventional emulsion [15, 16]. Furthermore, the reduced amount of sunscreen used for the encapsulation into SLNs and NLCs leads to a lower penetration of the sunscreen and its potential photoproducts into the skin and thus limiting the side effects [17, 18].

The aim of the present study was to obtain optimized lipid nanoparticles able to sunscreen encapsulation in order to develop cosmetic formulations with broad photoprotection spectrum. In this context, the influence of surfactants combination, both qualitatively (composition) and quantitatively (concentration), and of the liquid lipid concentration on the main characteristic of LNs, namely mean particle size and zeta potential were evaluated. LNs with the best characteristics were used for a further co-encapsulation of two kinds of sunscreens. The UVA filter, butyl-methoxydibenzoylmethane and the UVB filter, octocrylene were used for determining the feasibility of LNs to encapsulate sunscreens. The LNs loaded with both UV-absorbers were characterized in terms of mean particle size, zeta potential, thermal analysis and their *in vitro* UV blocking ability has been evaluated. The efficiency of lipid nanoparticles in developing cosmetic formulations has been quantified by determining the *in vitro* sun protection factor (SPF) and UVA protection factor.

2. Materials

Butyl-methoxydibenzoylmethane – BMDBM was obtained from Merck as Eusolex 9020. Octocrylene – OCT was purchesd from Sigma Aldrich. Polyoxyethylene-sorbitan monooleate (Tween 80) and polyoxyethylenesorbitan monolaurate (Tween 20) were purchased from Merck (Germany). Synperonic PE/F68 (Poloxamer 188, block copolymer of polyethylene and polypropylene glycol) and L-α-Phosphatidylcholine (Lecithin) were obtained from Sigma Aldrich Chemie GmbH (Munich, Germany). The solid lipids used were: n-Hexadecyl Palmitate (CP) obtained from Acros Organics (USA) and Glyceryl Stearate (GS) from Cognis GmbH. The oil Myritol 812 (medium chain triglycerides – MCT) was purchased from Merck. The cream base (which contains stearats, glycerine, fatty alcohols, emulsifier, emollients and an antioxidant – butylhydroxyanisole) was supplied by Elmiplant S.A. Company, Romania.

3. Methods

3.1 Preparation of Lipid Nanoparticles
Lipid nanoparticles (LNs) were prepared using melt emulsification method coupled with high shear homogenization. The method is similar to that reported in a previous study of the authors [19].

Tabel I

Composition of solid lipid nanoparticles (SLNs)

Dataset*	Surfactants composition**				Surfactants concentration (% w/w)			
	Tween 80	Tween 20	Poloxamer	Lecithin	2	2.5	3	3.5
I	95	-	5	-	SLN 1	SLN 2	SLN 3	SLN 4
II	95	-	-	5	SLN 5	SLN 6	SLN 7	SLN 8
III	90	-	5	5	SLN 9	SLN 10	SLN 11	SLN 12
IV	80	-	10	10	SLN 13	SLN 14	SLN 15	SLN 16
V	70	-	15	15	SLN 17	SLN 18	SLN 19	SLN 20
VI	-	70	15	15	SLN 21	SLN 22	SLN 23	SLN 24

*All SLNs were prepared with 10 % (w/w) solid lipid mixtures, in a ratio of CP/GS = 1:1.
**The concentration in % of weight/surfactant mixture weight.

The lipid phase consisted in a mixture of CP and GS for SLNs preparation and CP, GS and liquid lipid (MCT), for NLCs preparation. The lipid phase was heated under stirring at 85°C, to ensure a total melting. For sunscreen loaded LNs, the two UV filters BMDBM and OCT were added in the lipid phase to form a clear molten solution. The aqueous phase consisting in various concentration of surfactant mixture (2%, 2.5%, 3% and 3.5%) was also heated under stirring at

85°C. The lipid phase was gradually added into the aqueous phase and stirred at 85°C for 2h. The formed pre-emulsion was subjected to an external mechanical energy by high shear homogenization with a Lab rotor-stator Homogenizer (High-Shear Homogenizer PRO250 type; 0~28.000 rpm; power of 300 W, Germany), by applying 25000 rpm for 10 min.

In order to remove the excess of water, the nanoparticles dispersions were lyophilized, using an Alpha 1-2 LD Freeze Drying System Germany. The various compositions of developed SLNs and NLCs are shown in table I and table II, respectively. In table III, the composition of sunscreen loaded into LNs and in the reference nanoemulsion is shown. The nanoemulsion was produced in the same manner as LNs, except that the lipid phase was entirely composed of MCT.

Tabel II

Composition of nanostructured lipid carriers (NLCs)

Surfactant composition (% w/surfactant mixture w*)				MCT** (% w/w)		
Tween 80	Tween 20	Poloxamer	Lecithin	1	2	3
70	-	15	15	NLC 1	NLC 2	NLC 3
-	70	15	15	NLC 4	NLC 5	NLC 6

*The concentration of surfactant mixture is 3.5%
**NLCs were prepared with 10 % (w/w) lipid mixtures, in a ratio of CP/GS of 1:1

3.2 Characterization of lipid nanoparticles

3.2.1 Particle size analysis

The particle size measurements were analyzed by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom). The mean particle size (Zave) and the polydispersity index (PdI) of the LPs in dispersion were measured at a scattering angle of 90° and at a temperature of 25°C. Prior to the measurements, the dispersions were diluted with deionized water to an adequate scattering intensity. The particle size data were evaluated using intensity distribution. The average diameters (based on Stokes-Einstein equation) and polydispersity index are given as average of three measurements.

3.2.2 Zeta potential analysis

The Zeta potential (ZP) was determined by measuring the electrophoretic mobility of the nanoparticles in an electric field, using Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom) by using the Helmholtz-Smoluchowsky equation. Prior to the measurements, the lipid nanoparticles dispersions were diluted with a sodium chloride solution (0.9%, w/v), to adjust the conductivity to 50 µS/cm. All measurements were performed in triplicate at 25°C and the average was reported.

3.2.3   UV blocking ability of sunscreen loaded lipid nanoparticles

The UV blocking activity of sunscreen loaded lipid nanoparticles in dispersion was evaluated using Transpore™ tape (3M Health Care, U.S.A.) [20, 21]. An amount of 50 µL of lipid nanoparticles dispersion was spread evenly on top of the Transpore™ tape which was placed on a quartz plate. After a drying period of 15 min, the absorption spectra of the samples were registered, from 400 nm to 290 nm, using a reference support – Transpore™ tape without sample, on a UV – Vis V670 Spectrophotometer (Jasco) equipped with integrated sphere. The absorption spectrum of each sample was the result of the wavelength scan in six different points.

3.2.4   Thermal analysis

Differential scanning calorimetry (DSC) analyzes the physical and energetic properties of the formulation were measured as function of temperature. DSC was performed in order to determine the melting temperatures of the bulk lipids and SLNs and NLCs loaded with sunscreen. The thermograms were registered using a DSC Jupiter, STA 449C (Netzsch). The samples (10 mg) were weighed into a standard alumina pans using an empty pan as reference. The samples were heated from 5 to 85 °C with a heating rate of 5 °C/min.

3.2.5   *In vitro* SPF and UVA Protection Factor measurements

The in *vitro* determination of the sun protection factor (SPF) was conducted using Jasco V-530 spectrophotometric system, according to Diffey-Robson methodology [20]. The method is based on the measurement of the intensity of radiation transmitted through the substrate of the applied sample, determined by recording the photocurrent in 5 nm steps from 290 to 400 nm. The *in vitro* EUVA-PF was calculated following the SPF equation and considering the UVA wavelength ranging between 320 and 400 nm [17, 21].

The cream formulations were developed by adding the lyophilized SLNs/NLCs loaded with sunscreen into a base cream. The transmission measurements were determined in six different points for each sample and the average value was used to calculate the SPF and EUVA-PF.

4.   Results and discussion

4.1 Optimization of lipid and surfactants mixture composition

The characterization of SLNs and NLCs has been achieved in terms of mean particle size, PdI and zeta potential, immediately after the preparation of LNs dispersions by using dynamic light scattering technique.

For identifying the optimized surfactant and lipid composition with best and appropriate features for an encapsulation purpose, different lipid nanoparticles

(SLNs 1 ÷ SLNs 24) with various surfactant concentrations (2%, 2.5%, 3% and 3.5%) in different ratios of Tween 80/lecithin/poloxamer (I-V) have been prepared (table I). The obtained values of particle size and PdI of SLNs with Tween 80 as main nonionic surfactant are shown in figure 1.

The SLNs prepared with the surfactant mixture Tween 80 and poloxamer (dataset I) present the biggest values of Zave (678 nm for SLN 1) and PdI (0.625 for SLN 1). Using lecithin in combination with Tween 80 (dataset II), the particle sizes decreases to 250 nm and has a good PdI of 0.227 (for SLN 8). According to literature data, the nanoparticles with PdI lower than 0.25 are considered relatively homogeneous, with minimum predisposition to aggregation [22].

Fig. 1. Mean particle size (nm) and PdI of SLNs dispersion

The differences in the particle sizes and PdI between datasets I and II are due to the different molecular weights of the surfactants (molecular weight of poloxamer is 8400 and of lecithin is 776). Thus, the number of surfactant molecules will be lower for poloxamer than for lecithin, having the same amount of surfactant in the SLNs formulation, and this number of molecules was probably insufficient to stabilise the lipid nanoparticles in the same way as lecithin. Furthermore, the presence of the larger molecule of poloxamer on the SLNs surface should contribute to a larger SLNs size. The best results were obtained for SLNs with the surfactant mixture Tween 80/Poloxamer/ Lecithin in a ratio of 70:15:15 (set V) having the Zave of 224 nm and PdI of 0.246 (for SLN 20).

It was observed that with increase of surfactant concentration from 2 to 3.5%, a decrease in the mean particle size and PdI has occurred. This is due to the fact that utilization of a lower concentration of surfactant could be insufficient to reduce the interfacial tension. In addition, the introduction of lecithin in the surfactant mixture of Tween/Poloxamer contributes to the formation of SLNs with smaller particle size. It is well known that utilization of an appropriate surfactant

mixture could reduce interfacial tension more than one single surfactant. This phenomenon is mainly due to an increased surfactant concentration at interface which makes possible to minimize the repulsive forces used for surfactant molecules assembling [23].

By viewing the DLS results, it could be observed that the smaller average diameters of lipid nanoparticles prepared with Tween 80 as main surfactant (datasets I ÷ V) have been obtained as non-ionic surfactant decreases and ionic surfactant increases (dataset V). For this reason, replacing the Tween 80 by Tween 20, only the surfactant composition from dataset V (Table I) has been chosen for SLN 21 ÷ SLN 24 synthesis (dataset VI). Moreover, this last analyzed system manifests the appropriate characteristics for a further sunscreen encapsulation.

The results of SLNs prepared with Tween 20 as main surfactant are shown in figure 1. SLN 24 prepared with the 3.5% surfactant mixture, in a ratio of 70:15:15 (Tween 20/Poloxamer/lecithin) exhibited a relatively narrow size distribution, having a PdI less than 0.21, and an excellent average diameter of 157 nm (figure 1).
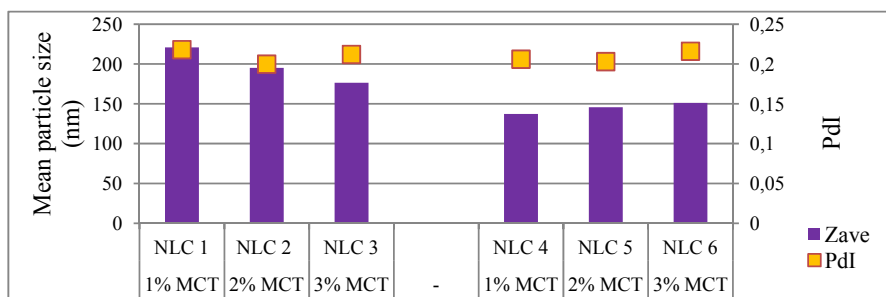

Fig. 2. Mean particle size and PdI of NLCs dispersions with main surfactant Tween 80 (NLC 1 ÷ 3) and Tween 20 (NLC 4 ÷ 6)

By comparing the particle sizes of the SLNs from dataset V and VI, the nanoparticles obtained with Tween 20 as main surfactant are much smaller than those with Tween 80, by approximately 65 nm. Having in view previous aspect about the influence of molecular weight on nanoparticles size, this could be due to the lower molecular weight of Tween 20 (1128) than that of Tween 80 (1310). This idea is also supported by a previous study that emphasized the correlation between molecular weight and particle size of lipid nanoparticles [24].

The second type of lipid nanostructures – NLCs were prepared using a liquid lipid (medium chain triglycerides) in addition to the solid lipids and by using the optimized surfactant composition from previous SLN synthesis. The particle size and PdI of NLCs prepared with Tween 80 and Tween 20/ Poloxamer/lecithin (3.5% w/w) surfactant mixture are shown in figure 2. NLC 4

with 1% MCT and main surfactant Tween 20 manifests the smallest particle size of 137 nm and an excellent PdI of 0.2.

By comparing the SLNs *versus* NLCs, the last system has led to smaller particle size. This behavior is associated to the oil content inside of NLCs that decreases the viscosity of the lipid mixture, and therefore, could reduce the surface tension to form smaller and smoother surface particles comparing with SLNs [25]. It was observed that the increase of MCT concentration from 1 to 3% leads to a decrease in the particle size of NLCs with Tween 80, and to an increase of particle size for the NLCs with Tween 20.

4.2 Stability of aqueous dispersions of SLNs and NLCs

The physical stability of lipid nanodispersions was evaluated by determining the zeta potential. The zeta potential (ZP) is the electrostatic potential at the plane of shear and reflects the electric charge on the particle surface [26]. The zeta potential values of the SLNs prepared with the selected surfactant systems are shown in figure 3.
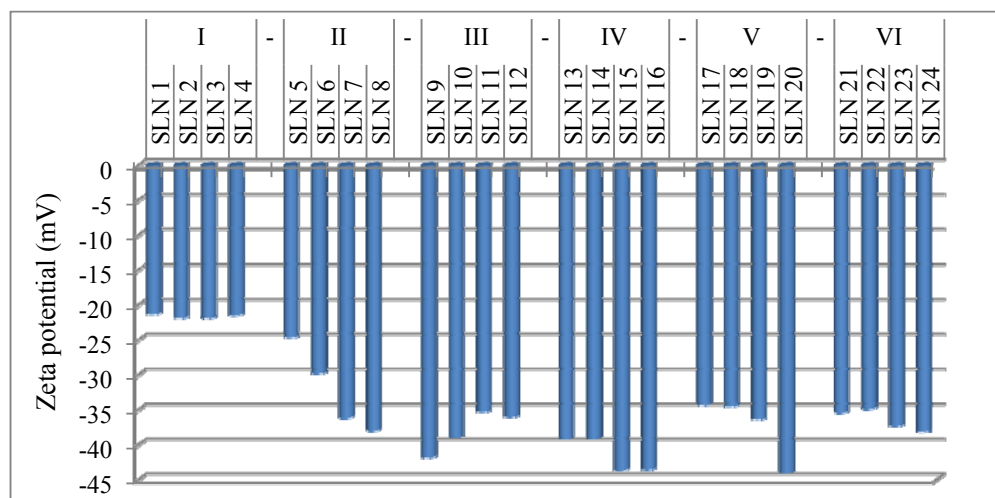

Fig. 3. Evaluation of SLNs stability by zeta potential

In general, all the developed SLNs are stable, with the exception of nanoparticles from dataset I, which have ZP values more than -22 mV. The low charges of SLN 1 - 4 are due to the use of two nonionic surfactants, Tween 20 and Poloxamer. The use of Poloxamer in combination with Tween 80 and lecithin determines the reduction of ZP to more negative values, by comparing with the surfactant mixture Tween 80/lecithin, because Poloxamer assures an electrostatic

and steric behavior. The best result was obtained with 3.5% surfactant with the system Tween 80/Poloxamer/lecithin, having a ZP of -44 mV (figure 3).
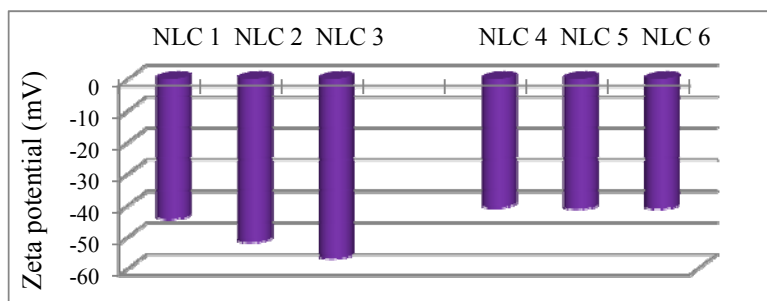

Fig. 4. Evaluation of NLCs stability by zeta potential distribution

Regarding the NLCs dispersions obtained with the modified-HSH method, they are physically stable systems and the particle aggregation is unlikely to occur due to electric repulsion (figure 4). The use of MCT as liquid lipid produces nanoparticles with bigger ZP values (-56 mV for NLC 3, -40 mV for NLC 6) comparing with the corresponding SLNs. The ZP values of NLCs prepared with Tween 80 (NLC 1 ÷ 3) are more negative than that of the NLCs with Tween 20 (NLC 4 ÷ 6).

4.3 Characterization of SLNs and NLCs loaded with sunscreens

The lipid nanostructures selected to co-encapsulate the organic filters, BMDBM and OCT, were SLN 24 produced with 3.5% surfactants mixture in a combination of Tween 20/Poloxamer/lecithin in a ratio of 70/15/15 and the NLC 6 with the same surfactants mixture as SLN 24 and 3% MCT.

The NLC 6, produced with 3% MCT was selected for sunscreens co-encapsulation even though its mean particle size (151 nm) is bigger than NLC 3 (137) with 1% MCT. This is due to the fact that a greater amount of liquid lipids incorporated into the solid lipid core leads to a more disorder in the lipid lattice, thus offering more space to accommodate the sunscreen molecules, plus the solubility of the sunscreens is greater in liquid lipids than in solid lipids. The composition of SLNs and NLCs loaded with sunscreens and of reference nanoemulsion are shown in table III.

The initial characterization of SLNs and NLCs that co-encapsulate both UV filters (BMDBM and OCT) has been achieved in terms of particle size distribution and zeta potential.

Tabel III

Composition of sunscreen loaded lipid nanoparticles (LNs)

Sample	Composition of LNs dispersion* (% w/w)				
	CP	GS	MCT	BMDBM	OCT
SLN_BMDBM-OCT	5	5		0.7	0.3
NLC_BMDBM-OCT	3.5	3.5	3	0.7	0.3
NE_BMDBM-OCT			10	0.7	0.3

* All samples were prepared with 70% Tween 20, 15% poloxamer and 15% lecithin
from the 3.5% concentration of surfactants mixture.

The results obtained by **dynamic light scattering technique** showed that LNs loaded with sunscreens present a monomodal profile of size distribution, with mean particle size of 170.1 nm for SLN_BMDBM-OCT (figure 5) and 162.4 nm for NLC_BMDBM-OCT (figure 6). By co-encapsulation of the sunscreen molecules into the lipid matrix, the particle size of loaded LNs increases slightly, by approximately 10 nm, when comparing to the unloaded lipid nanoparticles.
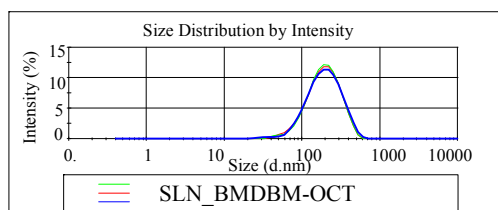

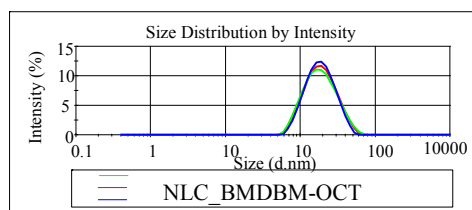Fig. 5 Particle size distribution of SLN_BMDBM-OCT dispersion

Fig. 6 Particle size distribution of NLC_BMDBM-OCT dispersion
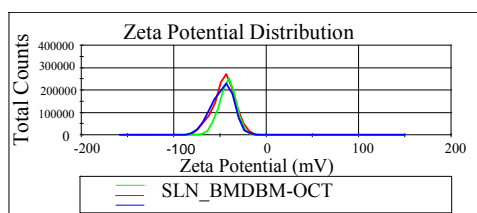

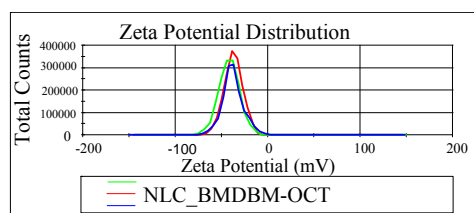Fig. 7 Zeta potential distribution of SLN_BMDBM-OCT dispersion

Fig. 8 Zeta potential distribution of NLC_BMDBM-OCT dispersion

The **electrokinetic potential evaluation** of SLNs and NLCs loaded with both UV-A and UV-B filters has revealed an excellent physical stability with zeta potential values of -38 mV for SLNs (figure 7) and -41 mV for NLCs system (figure 8).

Diferential scanning calorimetry analysis can be used to determine the physical state and cristallinity of the lipid core of LNs. The DSC curve of

sunscreens loaded NLCs (1) show a single melting peak at 49.5 °C and the physical mixture (3) shows two melting peaks at 50.6 °C and at 59.8 °C corresponding to the presence of liquid lipid, MCT (fig. 9). It is observed that lipid nanoparticles present a narrow melting range and a shift in the melting temperatures to lower values than those of physical lipid mixtures. This is due to the formation of the lipid matrix in the nanocrystalline size. This information is also sustained by a previous research [27]. The decrease in the endotherm peak intensity and peak temperature (49.5 °C) of NLC_BMDBM-OCT, as compared to that of loaded SLN (50.4 °C), indicates a less ordered lipid matrix of NLC and a smaller particle size, fact confirmed by the DLS measurements. These results confirm that lipid nanoparticles loaded with BMDBM and OCT were successfully prepared.
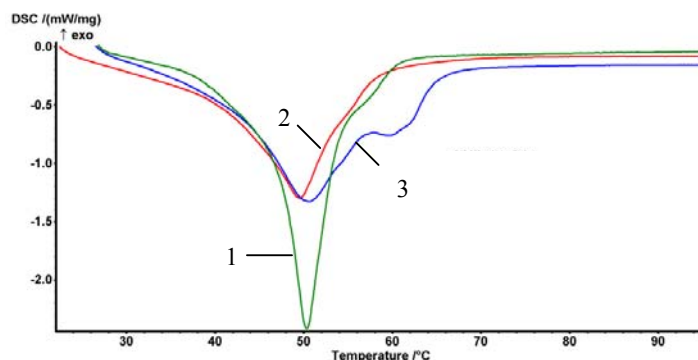

Fig. 9. DSC curves of lyophilized (1) SLN_BMDBM-OCT and (2) NLC_BMDBM-OCT compared with (3) physical lipid mixture CP+GS+MCT

The **UV blocking effect** of lipid nanoparticles in dispersion was evaluated using Transpore[TM] tape. The maximum absorption peak of BMDBM and OCT is at 356 nm and at 303 nm, respectively. The absorption spectrum of NLC_BMDBM-OCT appears shifted to higher values, when compared with the absorption spectra of SLN_BMDBM-OCT and of reference nanoemulsion (fig. 10). NLCs show the best UV blocking effect, the presence of liquid lipid in the lipid matrix ensuring a good incorporation of the lipophilic filters.

In some previous studies, this behavior is attributed to the synergistic effect caused, firstly by the incorporation of UV filters into lipid nanoparticles and secondly, by the UV protective effect of the lipid matrix [12, 15, 28].

The efficiency of both kinds of lipid nanoparticles (loaded SLNs and NLCs) in developing cosmetic formulations was evaluated after incorporation of lyophilized nanoparticles loaded with BMDBM and OCT into a base cream model and determining the SPF and EUVA-PF values (figure 11). As a reference, an

emulsion was used, which was prepared by solubilizing amounts of 2.5% BMDBM and 1% OCT into the base cream (in similar amounts as in the formulation with solid lipid nanoparticles). The cosmetic formulation based on loaded NLCs provides the best photoprotection, both in UVA and UVB domain, with EUVA-PF of 39.7 and SPF of 13.7, higher than for the reference emulsion (EUVA-PF of 23 and SPF of 10.2). The high EUVA-PF of the NLC_BMDBM-OCT based formulation ensures the absorption of 96% UV-A radiations.
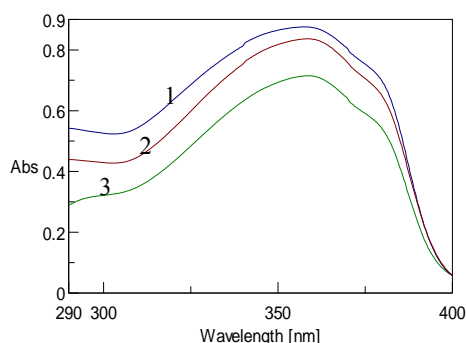

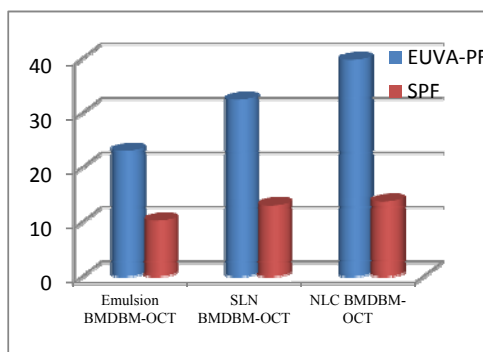Fig. 10 Electronic spectra of 0.7% BMDBM and 0.3% OCT in: NLC; (2) SLN; (3) nanoemulsion, in dispersion.

Fig. 11 EUVA-PFs and SPFs of cosmetic formulations with 2.5% BMDBM and 1% OCT from loaded -NLC, -SLN and emulsion

5. Conclusion

The results described in this study indicate that the modified high shear homogenization method could be used for the preparation of some free lipid nanostructures suitable for co-encapsulation of both kinds of UV-A and UV-B filters (BMDBM and OCT). The SLNs obtained with surfactant system Tween 20/Poloxamer/lecithin in a concentration of 3.5% surfactant and the NLCs obtained with the same surfactant mixture and 3% liquid lipid were chosen for the sunscreens co-encapsulation.

The incorporation of the sunscreens improves the UV blocking effect of the lipid nanoparticles in dispersion and, also, of the developed cosmetic formulations. The presence of liquid lipid (MCT) in NLCs has a positive influence on the UV blocking activity of lipid matrix and organic UV filters.

By increasing the UV blocking activity of sunscreens encapsulated into lipid nanoparticles, the concentration of organic filters in the final cosmetic formulation could be reduced while maintaining a good photoprotection level and limiting their side effects.

R E F E R E N C E S

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