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Differential scanning calorimetry studies on sunscreen loaded solid lipid nanoparticles prepared by the phase inversion temperature method

L. Montenegro*, M.G. Sarpietro, S. Ottimo, G. Puglisi, F. Castelli

Department of Pharmaceutical Sciences, University of Catania, V.le A.Doria 6, 95125 Catania, Italy

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ABSTRACT

Solid lipid nanoparticles (SLN) are regarded as interesting carriers to improve sunscreens' safety and effectiveness. In this work, surfactant effects on the physico-chemical properties of SLN loading two of the most widely used UV-filters, octylmethoxycinnamate (OMC) and butylmethoxydibenzoylmethane (BMBM), were evaluated and the interactions between SLN components and loaded UV-filters were investigated by differential scanning calorimetry (DSC). All the SLN showed a mean size ranging from 30 to 95 nm, and a single peak in size distribution. The use of isoceth-20 or oleth-20 as primary surfactants did not provide SLN with suitable physico-chemical properties since: (a) OMC loaded SLN proved unstable; (b) BMBM could not be loaded. OMC or BMBM loaded SLN prepared using ceteth-20 as primary surfactant were stable but their loading capacity lowered when both sunscreens were loaded simultaneously. DSC analyses showed that OMC distributed inside the SLN and caused a decrease of the lipid matrix molecules cooperativity while BMBM did not affect SLN calorimetric behaviour. When OMC and BMBM were loaded together into these SLN, an interaction between BMBM and OMC occurred. These results suggest that the interactions between sunscreens and between sunscreens and SLN components deserve further investigation to evaluate their effect on UV-filter-loaded SLN effectiveness.

1. Introduction

The deleterious effects of UV-radiation are well known (González et al., 2008). Recently, Lautenschlager et al. (2007) reported that UVA could play a greater role in long-term sun damages than in acute effects such as erythema or sunburn, which are basically attributable to UVB. To protect the skin from these harmful effects, the use of sunscreen products has been increasingly and widely recommended. Up to date, most sunscreen products are based on classical formulations such as oils, emulsions, lotions and gels. Recently, solid lipid nanoparticles (SLN) have been proposed as carriers for UV-filters because of their advantages compared to other topical vehicles such as good skin tolerability, improved active ingredient stability, increased bioavailability and ability to incorporate compounds with different physico-chemical properties (Muller et al., 2000; Mehnert and Mäder, 2001). Studies on sunscreen loaded SLN showed that the particles themselves act as UV blocker due to their particulate character, thus leading to a synergistic effect of both the molecular sunscreen loaded into the particles and the UV scattering by the particles (Wissing and Muller, 2002a; Cengiz et al., 2006). Wissing and Muller (2002b) reported that since SLN scattering ability depends strongly on the degree of crystallinity of their lipid matrix, SLN with a highly crystalline lipid matrix should be used to incorporate molecular sunscreens. Cetyl palmitate is one of the most widely used solid lipid for SLN preparation due to its safety and its crystal structure that leads to SLN in a crystalline form (Lukowsky et al., 2000; Wang et al., 2009). However, Xia et al. (2007) pointed out that the use of cetyl palmitate as lipid matrix to obtain sunscreen loaded SLN did not provide SLN with good physico-chemical properties. In a previous work (Montenegro et al., 2008), we observed that the type of emulsifying system can strongly affect both the physico-chemical and technological characteristics of drug loaded SLN prepared by the phase inversion temperature (PIT) method. Therefore, in this work we evaluated the effects of different non-ionic emulsifiers on the physico-chemical properties of sunscreen loaded SLN prepared by the PIT method using cetyl palmitate as solid lipid and we investigated the interactions between SLN ingredients and loaded suncreens by differential scanning calorimetry. Non-ionic surfactants (isoceteth-20, ceteth-20 and oleth-20) were used because of their low toxicity compared to ionic ones. Since an effective sunscreen has to protect against both UVA and UVB, different filters

* Corresponding author. Tel.: +39 095 738 4010; fax: +39 095 738 4211.
E-mail address: lmontene@unict.it (L. Montenegro).

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Table 1
Composition (% w/w) of SLN prepared by the PIT method.

SLN	Isoceteth-20	Oleth-20	Ceteth-20	GO	CP	OMC	BMBM	Water[a]
A	10.6	–	–	3.5	8.0	–	–	77.9
A1	10.6	–	–	3.5	8.0	1.0	–	76.9
A2	10.6	–	–	3.5	8.0	3.0	–	74.9
A3	10.6	–	–	3.5	8.0	5.0	–	72.9
A4	10.6	–	–	3.5	8.0	7.0	–	70.9
A5	10.6	–	–	3.5	8.0	8.0	–	69.9
B	–	8.7	–	4.4	8.0	–	–	78.9
B1	–	8.7	–	4.4	8.0	1.0	–	77.9
B2	–	8.7	–	4.4	8.0	3.0	–	75.9
B3	–	8.7	–	4.4	8.0	5.0	–	73.9
B4	–	8.7	–	4.4	8.0	7.0	–	71.9
B5	–	8.7	–	4.4	8.0	8.0	–	70.9
C	–	–	8.7	4.4	8.0	–	–	78.9
C1	–	–	8.7	4.4	8.0	1.0	–	77.9
C2	–	–	8.7	4.4	8.0	3.0	–	75.9
C3	–	–	8.7	4.4	8.0	5.0	–	73.9
C4	–	–	8.7	4.4	8.0	6.0	–	72.9
C5	–	–	8.7	4.4	8.0	–	0.5	78.4
C6	–	–	8.7	4.4	5.0	–	–	81.9
C7	–	–	8.7	4.4	5.0	1.0	–	80.9
C8	–	–	8.7	4.4	5.0	–	0.1	81.8
C9	–	–	8.7	4.4	5.0	1.0	0.1	80.8

[a] Water containing 0.1% w/w Gram 1® and 0.05% w/w Kathon CG® was used.

have to be combined in the same solar product (Roelandts, 1998; Nohynek and Schaefer, 2001). Therefore, we assessed the feasibility of loading in the same SLN preparation two of the most widely used UV-filters, octylmethoxycinnamate (OMC), a liquid lipophilic UV-B filter, and butylmethoxydibenzoylmethane (BMBM), a solid lipophilic UV-A filter.

2. Materials and methods

2.1. Materials

Cetyl palmitate (CP, Cutina CP®) was purchased from Cognis S.p.a. Care Chemicals (Como, Italy). Polyoxyethylene-20-cetyl ether (Ceteth-20, Brij 58®) was supplied by Fluka (Milan, Italy). Polyoxyethylene-20-oleyl ether (Oleth-20, Brij 98®) was bought from Sigma–Aldrich (Milan, Italy). Polyoxyethylene-20-isohexadecyl ether (Isoceteth-20, Arlasolve 200 L®) was a kind gift of Bregaglio (Milan, Italy). Glyceryl oleate (GO, Tegin O) was obtained from Th. Goldschmidt Ag (Milan, Italy). Octylmethoxycinnamate and butylmethoxydibenzoylmethane were a kind gift of BASF (Ludwigshafen, Germany). Imidazolidinyl urea (Gram 1®) and chloromethylisothiazolinone and methylisothiazolinone (Kathon CG®) were a kind gift of Sinerga (Milan, Italy). All other reagents were of analytical grade and they were used as supplied.

2.2. Preparation of SLN

SLN, whose composition is reported in Table 1, were prepared using the phase inversion temperature (PIT) method (Montenegro et al., 2008). The aqueous phase and the oil phase (cetyl palmitate and the selected emulsifiers) were separately heated at ~95 °C; then the aqueous phase was added to the oil phase drop by drop, at constant temperature and under stirring. The mixture was then cooled to room temperature under slow and continuous stirring.

At the phase inversion temperature (PIT), the turbid mixture turned into clear. The PIT temperature was determined using a conductivity meter mod. 525 (Crison, Modena, Italy) that measured an electric conductivity change when the inversion from W/O to O/W system occurred. To find the minimum amount of surfactants needed, at the beginning we increased each time by 1% w/w the amount of primary surfactant or co-surfactant starting from a ratio 1:1, until a clear system was obtained after the phase inversion temperature. Then we lowered each time by 0.1% w/w the amount of primary surfactant or co-surfactant until a turbid system was obtained after the phase inversion temperature. The lowest amount of emulsifying systems needed using 8% w/w of cetyl palmitate as solid lipid were: 10.6% of isoceteth-20 and 3.5% of glyceryl oleate (SLN A), 8.7% of oleth-20 and 4.4% of glyceryl oleate (SLN B) and 8.7% of ceteth-20 and 4.4% of glyceryl oleate (SLN C).

Different percentages of UV-filters were added to the oil phase prior to heating. The percentages of OMC ranged from 1% to 8% w/w when isoceteth-20 or oleth-20 were used as primary surfactant and from 1% to 6% w/w using ceteth-20. We determined the maximum amount of OMC that could be incorporated by increasing the amount added to the formulation each time by 1% w/w until the formulation remained clear after the phase inversion occurred. The same procedure was used in the case of BMBM or the association OMC/BMBM but that amount added to the formulation increased each time by 0.1% w/w.

2.3. Transmission electron microscopy (TEM)

For negative-staining electron microscopy, 5 μl of SLN dispersions were put on a 200-mesh formvar copper grid (TAAB Laboratories Equipment, Berks, UK), and allowed to be adsorbed. The surplus was removed by filter paper and a drop of 2% (w/v) aqueous solution of uranyl acetate was added over 2 min. After the removal of the surplus, the sample was dried at room condition before imaging the SLN with a transmission electron microscope (model JEM 2010, Jeol, Peabody, MA, USA) operating at an acceleration voltage of 200 kV.

2.4. Photon correlation spectroscopy (PCS)

The particle sizes of the SLN tested were determined using a Zetamaster S (Malvern Instruments, Malvern, UK), at 20 °C, by scattering light at 90°. The instrument performed particle sizing by means of a 4 mW laser diode operating at 670 nm. The values of the mean diameter and polydispersity index (PI) were the averages of results obtained for three replicates of two separate preparations.

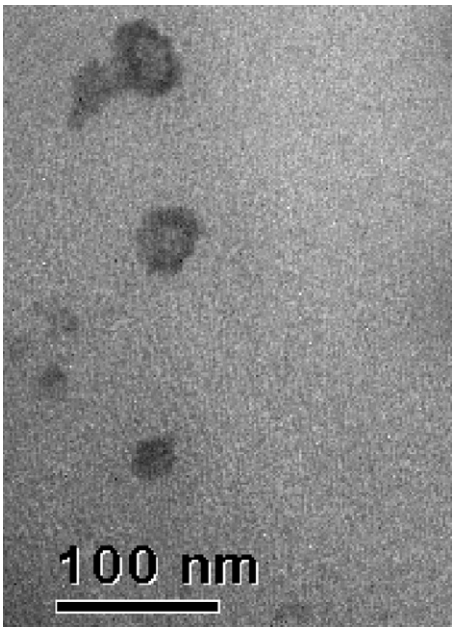

Fig. 1. TEM picture of SLN prepared by the PIT method.

2.5. Stability tests

Samples of the SLN prepared were stored in airtight jars, and then kept in the dark at room temperature and at 37 °C for two months.

Particle size and polydispersity index of the samples were measured at fixed time intervals (24 h, one week, two weeks, three weeks, one month, and two months) after their preparation.

2.6. Differential scanning calorimetry (DSC)

DSC studies were performed using a Mettler TA STARe System equipped with a DSC 822e cell and a Mettler STARe V8.10 software (Mettler Toledo, Milan, Italy). The reference pan was filled with 100 μl of distilled water. The calorimetric system was calibrated, in transition temperature and enthalpy changes, by using indium and palmitic acid (purity ≥99.95% and ≥99.5%, respectively; Fluka, Switzerland) following the procedure of the Mettler STARe software. The DSC measurements were carried out on the following SLN samples: unloaded SLN; OMC loaded SLN, BMBM loaded SLN, OMC and BMBM loaded SLN.

100 μl of each sample was transferred into a 160 μl calorimetric pan, hermetically sealed and submitted to DSC analysis as follows: (i) a heating scan from 5 to 65 °C, at the rate of 2 °C/min; (ii) a cooling scan from 65 to 5 °C, at the rate of 4 °C/min; for at least three times. Moreover, a fixed amount (corresponding to that contained into the SLN) of CP (sample A), CP + ceteth-20 (sample B) and CP + ceteth-20 + GO (sample C) was weighted in the calorimetric pan, 100 μl of distilled water (containing Kathon CG® 0.05% and imidazolidinyl urea 0.35% as preservatives) was added, the pan was hermetically sealed and submitted to the DSC analysis reported above. Since preliminary experiments performed using water with and without preservatives to evaluate DSC behaviour of samples A, B, and C showed no significant differences of the resulting thermograms, we used the same aqueous phase used to prepare SLN. Sample C was also analyzed as follows: after DSC analysis, the sample was heated at 95 °C for 5 min and then vortexed for 1 min. After this procedure, the sample was analyzed by DSC in the range 5–65 °C. Each experiment was carried out in triplicate.

Table 2
Mean particle size ± S.D. and polidispersity index (PI) ± S.D.of SLN A–C.

SLN	Size (±S.D.)	PI (±S.D.)
A	95.3 ± 1.9	0.704 ± 0.098
A1	43.1 ± 0.9	0.434 ± 0.014
A2	46.7 ± 2.6	0.418 ± 0.003
A3	40.8 ± 0.7	0.161 ± 0.008
A4	46.0 ± 1.4	0.187 ± 0.017
A5	47.0 ± 0.5	0.199 ± 0.016
B	40.0 ± 0.1	0.310 ± 0.002
B1	46.4 ± 0.2	0.312 ± 0.009
B2	47.3 ± 0.8	0.466 ± 0.10
B3	48.7 ± 3.4	0.474 ± 0.024
B4	46.0 ± 0.6	0.217 ± 0.021
B5	48.1 ± 0.6	0.201 ± 0.005
C	30.3 ± 0.3	0.217 ± 0.045
C1	39.9 ± 1.2	0.432 ± 0.009
C2	38.5 ± 1.7	0.420 ± 0.010
C3	39.8 ± 0.7	0.410 ± 0.029
C4	39.6 ± 1.9	0.425 ± 0.006
C5	42.1 ± 1.7	0.429 ± 0.005
C6	31.5 ± 0.7	0.455 ± 0.015
C7	38.6 ± 1.3	0.443 ± 0.004
C8	40.1 ± 1.9	0.422 ± 0.012
C9	40.9 ± 2.1	0.437 ± 0.018

3. Results and discussion

3.1. SLN characterization and stability

Transmission electron microscopy (TEM) analyses of loaded and unloaded SLN showed spherical particles with no evident sign of aggregation (Fig. 1). All the formulations tested showed a mean particle diameter in the range of 30–95 nm and a single peak in size distribution (Table 2). When SLN formulations were clear, UV-filters were supposed to be completely incorporated into the lipid nanoparticles because being UV-filters poorly water soluble if they had not loaded into SLN they would have given rise to a turbid system and/or a precipitate, as reported for other lipophilic drugs loaded into SLN, such as vitamin A (Jenning et al., 2000). Therefore, the loading capacity was determined as the maximum amount of UV-filter that could be loaded into SLN leading to a clear vehicle with no sign of precipitation. SLN prepared using ceteh-20 as primary surfactant showed the lowest OMC loading capacity (6% w/w) while SLN A and B loading capacity was similar (8% w/w). The incorporation of different percentages of OMC in lipid particles resulted in a decrease of particle sizes using isoceteth-20 as primary surfactant and in an increase using ceteth-20 or oleth-20 as primary surfactants, with no relationship between OMC content and SLN particle size. Upon OMC loading, PI values changed depending on the type of surfactant used as well. According to the literature (Ghosh and Murthy, 2006), these results suggest that different interactions between OMC and SLN components could take place at the surfactant layer. However, the different surfactant lipophilicity (HLB values: oleth-20 15.3, isoceteth-20 15.5, ceteth-20 15.7) could not account for the change of particle size since no correlation between surfactant HLB values and droplet size was observed, whereas the different structure of the acyl chain of the primary surfactant (isoceteth-20: branched acyl chain; oleth-20 linear unsaturated acyl chain; ceteth-20 linear saturated acyl chain) could play an important role in determining OMC interaction with the surfactant layer. According to the literature (Izquierdo et al., 2005), formulations containing surfactants with similar HLB are expected to show similar PIT values and similar stability. Although PIT values ranged from 80 to 85 °C for all the SLN prepared, their stability was significantly different. During storage at room temperature for two months, unloaded SLN A–C and OMC loaded SLN C did not show any significant change of particle size while OMC loaded

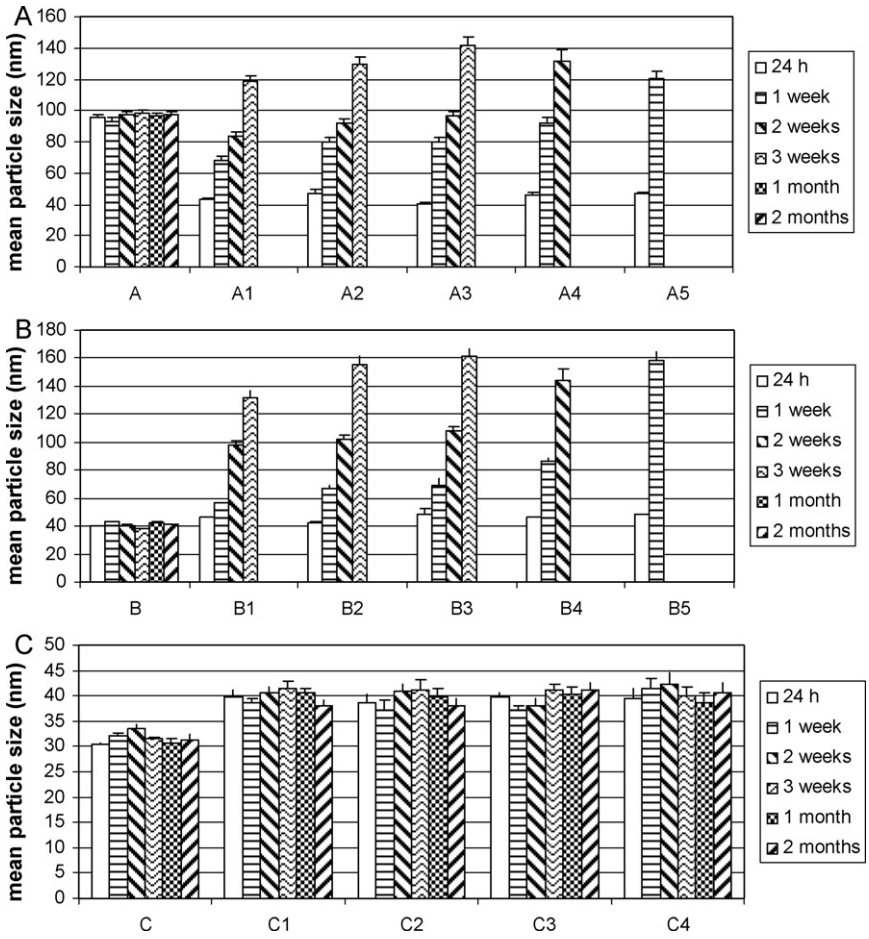

Fig. 2. Stability data of loaded and unloaded SLN during storage at room temperature. (A) SLN 5 A prepared using isoceteth-20; (B) SLN B prepared using oleth-20; (C) SLN C prepared using ceteth-20. Particle size was not determined when a precipitate was observed.

SLN A and B particle size increased and a precipitate was observed at different times of storage depending on the percentage of loaded OMC (Fig. 2).

The lower stability of OMC loaded SLN A and B could be attributed to the different surfactant's structure rather than to their lipophilicity since the most stable formulation was obtained using the least lipophilic surfactant. During storage at 37 °C, less stability in terms of particle size for all the formulations tested was observed (data not shown). As reported by Mehnert and Mäder (2001), less stability at higher temperature could be due to the introduction of energy into the system, that leads to particle growth and subsequent aggregation.

As regards BMBM, only SLN prepared using ceteth-20 as primary surfactant were able to load BMBM, although their loading capacity was very small (0.5% w/w). BMBM incorporation into SLN caused an increase of particles size and PI values. These results could be attributed to BMBM physico-chemical properties. This UV-filter is a white crystalline powder whose melting point is 80–85 °C and whose log *P* (calculated using Advanced Chemistry Development Software Solaris V 4.67) is 4.80. Since BMBM is less lipophilic than OMC (Log P 5.60, calculated using Advanced Chemistry Development software Solaris V 4.67), a better interaction with the most hydrophilic surfactant (ceteth-20) used to prepared our SLN could be expected. However, its chemical structure could affect its loading into SLN as well. Our results suggest that a linear surfactant like ceteth-20 could allow a better intercalation of BMBM compared to a branched or an unsaturated surfactant. Furthermore, the crystalline nature of BMBM could affect its ability to be incorporated into SLN since SLN were prepared at a temperature (higher than the PIT) that caused the melting of BMBM but during the cooling process BMBM could re-crystallize, diffusing out of the particles. As reported in the literature (Muller et al., 2000), when particles can form relatively perfect lipid crystals, like particles obtained using cetyl palmitate as solid lipid, their loading capacity can be limited and it can be lowered further by the incorporation of a crystalline active compound. As observed for OMC loaded SLN, BMBM loaded SLN were stable at room temperature but their stability was lower at 37 °C (data not shown).

Preliminary experiments showed that only SLN prepared using ceteth-20 as primary surfactant were able to load OMC and BMBM at the same time but to incorporate both UV-filters in the same SLN formulation, the amount of cetyl palmitate had to be reduced to 5% w/w (SLN C6). No significant difference of particle size and PI values was observed comparing SLN C with SLN C6, prepared using a lower amount of cetyl palmitate. As shown in Table 2, when OMC and BMBM were loaded simultaneously, their loading capacity was 1% w/w and 0.1% w/w, respectively. Upon loading OMC and/or BMBM into SLN C6, a behaviour similar to that obtained for SLN C was observed, i.e., particle size increase without significant changes of PI values, good stability at room temperature and less stability at 37 °C (data not shown). These results suggest that an interaction between these UV-filters could occur when they were loaded together in the same SLN formulation. Therefore, DSC studies were performed to better elucidate the mechanism of these interactions.

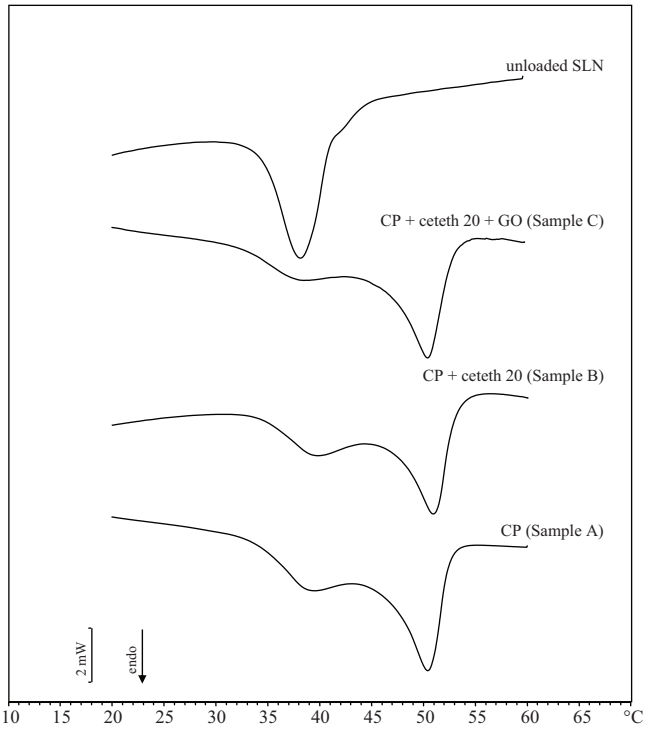

Fig. 3. DSC curves of cetyl palmitate (CP), CP + ceteth-20, CP + ceteth-20 + glyceryl oleate (GO), and unloaded SLN.

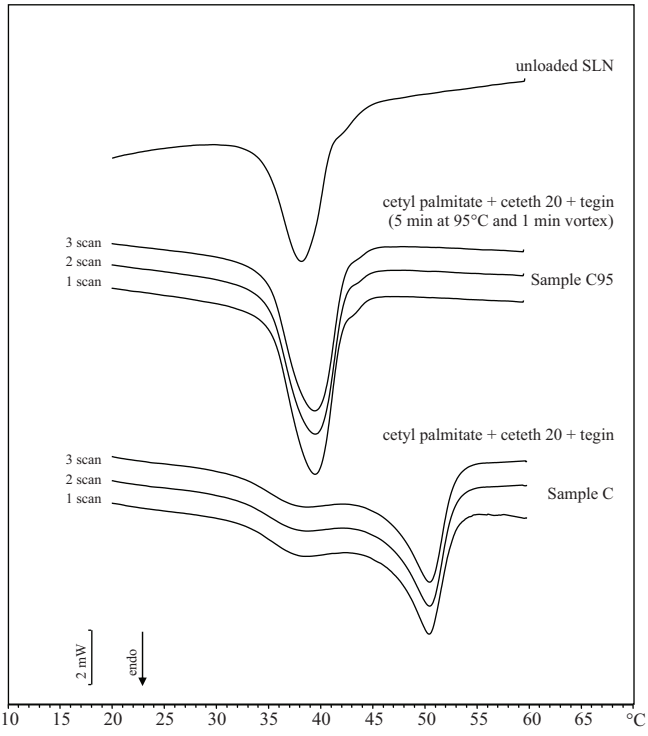

Fig. 4. DSC curves of unloaded SLN, sample C, Sample C95.

3.2. DSC analyses

DSC analyses were performed only on loaded SLN that proved stable, i.e.,SLN C1 and SLN C5–C9. The corresponding unloaded SLN (SLN C) were analyzed to determine the effects of drug loading on SLN calorimetric behaviour. As reported in the literature (Muller et al., 2000; Mehnert and Mäder, 2001), DSC analysis can be used to determine the physical state of the core lipid in SLN. In general, the melting peak of the lipid core of the SLN is observed at a lower temperature than that of bulk lipid mainly due to the nanocrystalline size of the lipids in the SLN (Westesen and Bunjes, 1995). Fig. 3 shows the DSC curves for CP bulk powder (sample A), CP and ceteth-20 (sample B), CP and ceteth-20 and GO (sample C), and unloaded SLN (SLN C) prepared by the PIT method. DSC analyses of samples B and C were performed since they could provide useful information on SLN ingredient interactions. The calorimetric curve of CP bulk (sample A) was characterized by a broad peak at about 39 °C and a main peak centred at about 50.5 °C, due to the compound melting. The addition of ceteth-20 to CP did not produce any variation on the calorimetric curves, suggesting that these compounds did not interact each other. When ceteth-20 was added together with GO to CP bulk (sample C), a modification of the calorimetric curve with respect to that of CP bulk occurred since the first peak shifted towards lower temperatures suggesting an initial interaction among the components that could lead to SLN formation upon heating. The calorimetric curve of unloaded SLN exhibited a well defined peak at about 38 °C and a shoulder at 42 °C. These data suggest that the interactions among ceteth-20, GO and CP molecules during the preparation process of SLN produced a new ordinate structure, different from that obtained when the single components were added each other in the calorimetric pan. Unloaded SLN showed main melting transition peak temperature about 12 °C lower than that of the CP bulk. The lowered melting peak of the SLN suggests that CP located in the core of the SLN had been successfully solidified by the PIT method we used to prepare our SLN. As previously reported by others (Lee et al., 2007), these results confirmed that solid lipid nanoparticles were prepared.

A critical point of the PIT method is represented by the temperature at which the oil and the aqueous phase are mixed together as demonstrated by the following simple experiment. CP, ceteth-20 and GO were weighted in the calorimetric pan (the same amounts used for SLN preparation), water was added and the sample was analyzed by DSC in the range 5–65 °C. To determine result reproducibility, three scans of the same sample (in heating mode) were carried out (sample C). Then, the same sample was heated at 95 °C for 5 min, shaken for 1 min and again analyzed by DSC (sample C95). The calorimetric curves obtained were compared with that of unloaded SLN (Fig. 4). The calorimetric curves of sample C and sample C95 are quite different. Upon heating at 95 °C (the temperature used to mix the oil and the aqueous phase in SLN preparation procedures), the peak at 50.5 °C disappeared, whereas the broad peak at about 38 °C became more evident and more shaped; in addition a shoulder on its right side appeared. It is interesting to note that the calorimetric curves of sample C after heating at 95 °C were very similar to that of unloaded SLN. This indicates that heating at 95 °C is necessary to form the SLN structure The calorimetric curves of unloaded and loaded SLN are shown in Fig. 5. Compared to unloaded SLN, OMC loaded SLN showed a less intense peak shifted towards lower temperature. These data suggest that OMC distributed inside the SLN and caused a decrease of the CP molecules cooperativity; in addition, the shoulder became more evident. Loading BMBM into SLN no significant difference was observed suggesting that: (a) BMBM inserted into the SLN but, due to its low amount, did not affect SLN calorimetric behaviour; (b) BMBM could form cluster inside the SLN without interacting with CP domains. This last hypothesis could be supported by CP arrangement into a lamellar lattice structure in SLN. Lukowsky et al. (2000) demonstrated, by crystallographic studies on SLN prepared using CP as solid lipid, that cetyl palmitate was arranged in a lamellar lattice structure and suggested that crystalline compounds could be stored between these layers. The crystalline nature of BMBM at room temperature

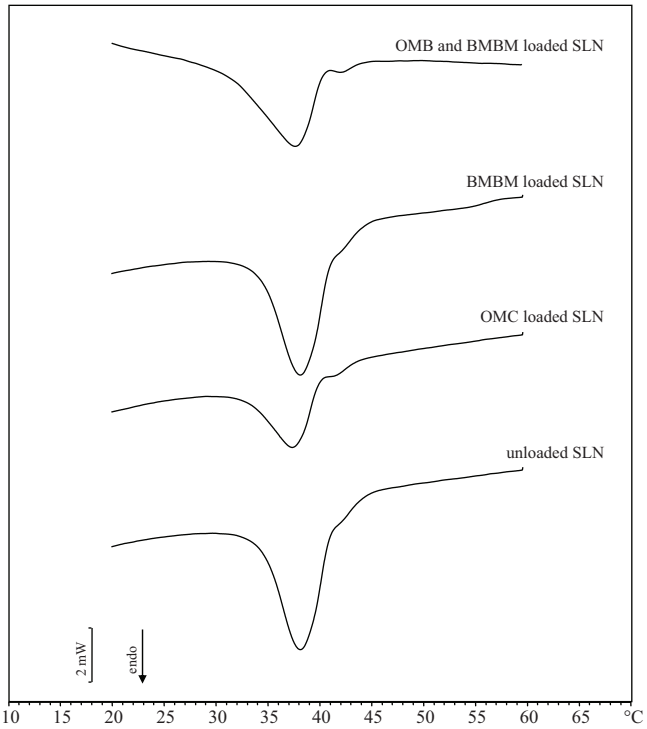

Fig. 5. DSC curves of unloaded SLN, OMC loaded SLN, BMBM loaded SLN, OMC and BMBM loaded SLN.

could lead to its insertion between CP lamellar layers without any interaction with the solid lipid.

Compared to the calorimetric curve of OMC loaded SLN, OMC and BMBM loaded SLN calorimetric curve was characterized by a more evident shoulder that actually became a peak, thus indicating an interaction between BMBM and OMC that modified SLN calorimetric behaviour.

Many authors reported that active ingredient solubility into the lipid used to obtain SLN is an important parameter in determining drug loading into SLN (Muller et al., 2000; Mehnert and Mäder, 2001). In our study, OMC could have worked as vehicle that solubilized BMBM and the resulting interaction between these two components could have led to the observed changes of SLN calorimetric curves.

4. Conclusions

The results of our study suggest that when cetyl palmitate was used as solid lipid to prepare SLN, primary surfactants like isoceth-20 or oleth-20 did not provide suitable SLN to load OMC and/or BMBM while using ceteth-20 as primary surfactant the resulting UV-filter loaded SLN were stable but BMBM loading capacity was very low.

DSC analyses showed that OMC distributed inside the SLN causing a decrease of the lipid matrix molecules cooperativity while no change of SLN calorimetric behaviour was observed after loading BMBM. Furthermore, when OMC and BMBM were loaded together into these SLN, an interaction between BMBM and OMC occurred. Further studies are planned to investigate the effects of such interactions on OMC and BMBM release from these SLN.

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