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Characterization of indomethacin-loaded lipid nanoparticles by differential scanning calorimetry

Francesco Castelli [a,*], Carmelo Puglia [b], Maria Grazia Sarpietro [a], Luisa Rizza [b], Francesco Bonina [b]

[a] *Department of Chemical Sciences, University of Catania, Viale A. Doria no. 6, 95125 Catania, Italy*
[b] *Department of Pharmaceutical Sciences, University of Catania, Catania, Italy*

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Abstract

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are interesting nanoparticulate delivery systems produced from solid lipids. Both carrier types are submicron size particles but they can be distinguished by their inner structure.

In the present paper, indomethacin (IND)-loaded SLN and NLC were prepared and the organization and distribution of the different ingredients originating each type of nanoparticle system were studied by differential scanning calorimetry (DSC) technique. Furthermore, mean particle size and percentage of drug encapsulation were also determined.

From the results obtained, NLC lipid organization guaranteed an increased indomethacin encapsulation in comparison with SLN. DSC static and dynamic measurements performed on SLN and NLC showed that oil nanocompartments incorporated into NLC solid matrix drastically influenced drug distribution inside the nanoparticle system. Controlled release from NLC system could be explained considering both drug partition between oil nanocompartments and solid lipid and a successive partition between solid lipid and water.

Keywords: Indomethacin; NLC; SLN; DSC characterization

1. Introduction

Solid lipid nanoparticles (SLN) are colloidal carriers developed at the beginning of the 1990s as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles), especially for the delivery of lipophilic compounds (Hu et al., 2004; Lim et al., 2004; Tabatt et al., 2004). Although SLN are endowed with important features useful for different routes of administration (Muller and Keck, 2004; Venkateswarlu and Manjunath, 2004; Wissing et al., 2004; Kim et al., 2005) they show some potential disadvantages such as drug leakage during

* Corresponding author. Tel.: +39 095 221796; fax: +39 095 80138.
E-mail address: fcastelli@dipchi.unict.it (F. Castelli).

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storage and insufficient drug load (Mehnert and Mader, 2001).

To overcome the limitation of SLN, nanostructured lipid carriers (NLC) have been developed (Muller et al., 2002a). Both carrier types are submicron size particles (50–1000 nm) and are based on solid lipids but they can be distinguished by their inner structure. SLN consist of pure solid lipids while NLC are made of solid matrix entrapping a variable liquid lipid nanocompartments (Muller et al., 2002b). NLC can be considered as an upgrade of the solid lipid nanoparticles even though among scientists the term SLN is still intended to indicate the nanostructured lipid carriers, creating no clear differentiation.

To date, several studies about these lipid carriers and concerning optimization of production parameters, morphological characterization, long-term stability, recrystallization behaviour and in vivo toxicity have been undertaken (Muller et al., 2000; Lippacher et al., 2000, 2002; Scholer et al., 2000; Jenning et al., 2002). In addition, interesting information about drug incorporation and release were obtained comparing both SLN and NLC carriers (Muller et al., 2002b; Souto et al., 2004).

It is well known that the release of a lipophilic compound from these lipid carriers can be considered as mediated by several partition mechanisms between the aqueous medium surrounding the nanoparticles, the surfactant employed and the lipid phases. Therefore, it becomes interesting to study the organization and distribution of the different lipid ingredients originating each type of nanoparticle system, as well as how the drug is dispersed either in the lipid or in the surfactant. At this aim, in the present paper, indomethacin (IND)-loaded SLN and NLC were prepared and characterized by differential scanning calorimetry (DSC) technique.

Indomethacin, a potent NSAID, was chosen as a highly lipophilic model drug while differential scanning calorimetry as an investigation technique since it was largely employed in the characterization of pharmaceutical drug release systems. Thermoanalysis, in fact, can provide information about crystallization behaviour, the timing of polymorphic transitions, fusion temperature, enthalpy, and the degree of crystallinity of melt-homogenized glyceride nanoparticle dispersions (Siekmann and Westesen, 1994; Heurtault et al., 2003).

Recently, DSC technique has been used successfully to monitor drug dissolution processes in presence of liposomal systems verifying the drug ability to migrate through the aqueous medium to interact with the lipid layer (Castelli et al., 2003). This technique determining thermodynamic variations related to morphological changes could give information about drug capability to dissolve in one of the lipid phases and consequently about drug collocation inside the nanoparticles in a ternary system (drug-surfactant-lipid).

2. Materials and methods

2.1. Materials

Compritol 888 ATO (glyceryl behenate, tribehenin) is a mixture of mono-, di- and triglycerides of behenic acid (C_{22}) and was a gift of Gattefossè Italy S.r.L (Milan, Italy). Miglyol 812 (caprylic/capric triglycerides) was provided by Eingemann & Veronelli S.p.A (Milan, Italy). Lutrol F68 was a gift of BASF ChemTrade GmbH (Burgbernheim, Germany). Indomethacin was purchased from Sigma Chemical (Milan, Italy). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of SLN and NLC

SLN and NLC were prepared by ultrasonication. SLN were obtained by adding indomethacin (80 mg) to Compritol 888 ATO (4 g) previously melted at 80 °C, whilst NLC preparation required the addition of Miglyol 812 (1.47 g) to the drug and the solid lipid at 80 °C. In a second step, the hot lipid phase (IND dispersion in melted solid lipid for SLN or IND dispersion in melted solid lipid and Mygliol 812 for NLC) was dispersed in a surfactant solution (1.35%, w/w), at 8000 rpm, 80 °C for 1 min, using a high-speed stirrer (Ultra Turrax T25, IKA-Werke GmbH & Co. KG, Staufen, Germany). The obtained pre-emulsion was ultrasonified using a Labsonic 2000 (B. Braun, Melsunen, Germany). In order to prevent recrystallization during homogenization, production temperature was kept at least 5 °C above the lipid melting point. The obtained nanoemulsion (O/W) was cooled down in a bath ice to form SLN or NLC and finally diluted up to

200 ml with deionized water. Both nanoparticle dispersions were stored at 4 °C.

2.3. Determination of IND loading

The percentage of IND entrapped in the lipid matrix was determined as follows: SLN or NLC dispersions were filtered using a Pellicon XL tangential ultrafiltration system (Millipore, Milan, Italy) equipped with a polyethersulfone Biomax10 membrane. An amount of retained material was freeze-dried, dissolved in chloroform and analyzed by UV spectrophotometry at 319 nm (Jasco V-520, Rome, Italy). Calibration curve for the validated UV assays of indomethacin, was performed on five solutions in the concentration range 0.9–60 μg/ml. Correlation coefficient was >0.990. Each point represents the average of three measurements and the error was calculated as standard deviation (±S.D.).

IND incorporation efficiency was expressed both as drug recovery and drug content, calculated from Eqs. (1) and (2), respectively:

$$\text{Drug content (\%)} = \frac{\text{mass of IND in nanoparticles}}{\text{mass of IND fed to the system}} \quad (1)$$

$$\text{Drug content (\%)} = \frac{\text{mass of IND in nanoparticles}}{\text{mass of collected nanoparticles}} \quad (2)$$

2.4. SLN and NLC size distribution

Mean particle size and population distribution of the bulk particle dispersion were measured by photon correlation spectroscopy (PCS) using a Nicomp 370 autocorrelator (PSS Inc., Santa Barbara, CA, USA) equipped with a Coherent Innova 70-3 Argon Ion laser system (Laser Innovations, Moorpark, CA, USA) operating at 514.5 nm. Analyses were performed using a 90° scattering angle and at 20 (±0.2) °C. Samples were prepared by diluting 10 μl of SLN or NLC suspension with 2 ml of deionized water previously filtered through a 0.2 μm Acrodisc LC 13 PVDF filter (Pall-Gelman Laboratory, Ann Harbor, MI, USA). During the experiment, refractive index of the samples always matched liquid (toluene) to avoid stray light.

2.5. DSC measurements

2.5.1. DSC experiments

DSC studies were performed using a Mettler TA STAR^e System equipped with a DSC 822^e cell. The scan rate was 2 °C/min in the temperature range 25–85 °C. The reference pan was filled with Tris buffer solution. Indium and palmitic acid (purity ≥ 99.95% and ≥99.5%, respectively; Fluka, Switzerland) were employed to check the calibration of the calorimetric system. Data were evaluated from the peak areas using the Mettler STAR^e V 6.10 SW software.

The DSC measurements were carried out on the following samples: (a) blank NLC; (b) NLC in which IND was added before mechanical treatment by ultraturrax; (c) IND-loaded NLC; (d) blank SLN; (e) SLN with IND added and dissolved inside at 80 °C; (f) Miglyol; (g) Miglyol with IND added and dissolved inside at 80 °C; (h) SLN added to pure Miglyol; (i) IND-loaded SLN added to pure Miglyol; and (l) NLC added to solid IND. Static (samples a–g) and dynamic (samples h–l) measurements were applied.

2.5.2. Static measurements

Briefly, 120 μl of each sample (an amount of compounds referred to the same amount of Compritol, 2.4 mg, in NLC or SLN was always used) was hermetically sealed in a 160-μl aluminum DSC pan and submitted to a scan in heating mode between 25 and 85 °C and cooled at 25 °C. This cycle was repeated at least four times.

2.5.3. Dynamic measurements

This procedure is based upon the examination at increasing incubation periods at 80 °C of the samples (a, d and e) when one of the components (Mygliol or IND) inside the DSC pan is present. Therefore, such kinetic permeation experiments were carried out on these three samples: (h) a fixed amount of SLN (containing 2.4 mg of Compritol) was added to Miglyol (0.88 mg) weighted in the bottom of the DSC crucible; (i) a fixed amount of SLN (containing 2.4 mg of Compritol) loaded with IND (0.048 mg) was added to Miglyol (0.88 mg) weighted in the bottom of the DSC crucible; a fixed amount of NLC (containing 2.4 mg of Compritol and 0.88 mg of Miglyol) was added to a defined amount of IND (0.048 mg) weighted in the bottom of the DSC crucible. The samples, hermetically

sealed, were submitted to calorimetric scans in heating, isothermal and cooling mode using the following procedure:

(1) a first scan was performed from 25 to 85 °C to detect the eventual interaction of the compound placed on the bottom of the pan with the lipid or lipid/Miglyol phase dissolving in it after its transfer through the aqueous phase;
(2) a scan from 85 to 80 °C at 4 °C/min;
(3) an isothermal period of 1 h at 80 °C was used to let the substance continue the migration processes and permeate, if able, the lipid or the lipid/Miglyol phases;
(4) a cooling scan between 80 and 25 °C was used before restarting the heating program (first step).

This process was run continuously, at least eight times, to detect the variations caused by the interaction of increasing amount of the compound with the SLN or NLC phases. Then, the same procedure was carried out for longer incubation periods (24 h).

3. Results and discussion

3.1. Preparation and characterization of SLN and NLC

As far as NLC is concerned, PCS analyses showed mainly the presence of two distinct particle populations with mean diameters of 80.4 nm and 270.2 nm. PCS analyses performed on solid lipid nanoparticles showed the presence of a particle population with mean diameter of 215.6 nm and a distinct particle population of 102.3 nm.

The NLC prepared in the present study are made of a solid lipid matrix containing Miglyol nanocompartments (Jenning et al., 2000). The presence of Miglyol was useful to increase the drug recovery (from 76 to 83.4%) and drug content (from 1.0 to 1.12%) in comparison with SLN.

These findings can be ascribed to the higher IND solubility in Miglyol 812 compared to the drug solubility observed in the lipid melt used for SLN preparation (Compritol 888 ATO). In addition, a physical exclusion phenomenon of drugs from the lipid matrix during SLN hardening with a consequent lowering of the entrapment efficiency is reported (Muller et al., 2002b).

Possible lipid interferences during UV determination of IND were also investigated by comparing the two standard curves of IND alone and IND plus lipids in chloroform solution in the range 0.9–60 μg/ml. The differences observed between the standard curves were within the experimental error, thus inferring that no lipid interference occurred (data not shown).

3.2. DSC characterization

In Fig. 1, the DSC curves of the samples a–c are reported. It is possible to observe as the blank NLC (curve (a)) show a peak and a plateau between 67 and 71 °C (indicated by an arrow). The same shape is observed when IND is introduced by mechanical vorticization in NLC (curve (b)) while it changed into a peak shape (curve (b′)) after 24 h incubation at 80 °C. The NLC loaded with IND (following the procedure described in Section 2.5.3) show a calorimetric curve with a well-defined two-peak structure (indicated by the arrow; curve (c)) instead of the plateau, as men-

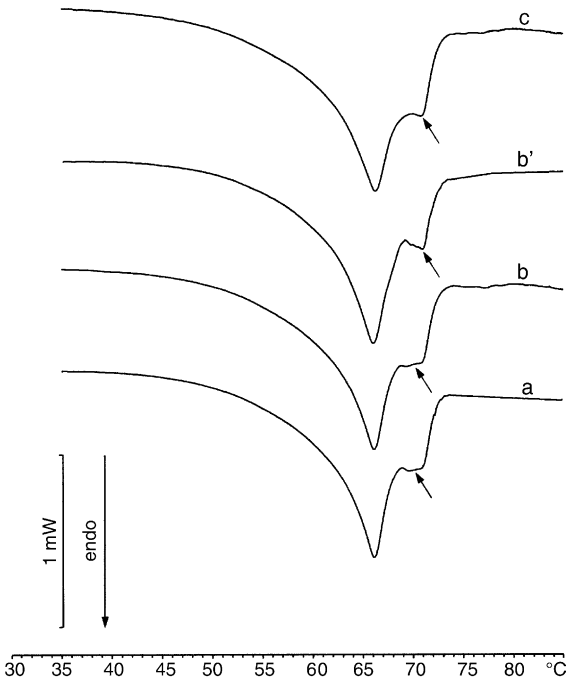

Fig. 1. Differential scanning calorimetric heating curves of (a) blank NLC; (b) blank NLC in presence of IND added before mechanical treatment by ultraturrax; (b′) as curve (b) after incubation at 24 h at 80 °C; and (c) NLC loaded with IND.

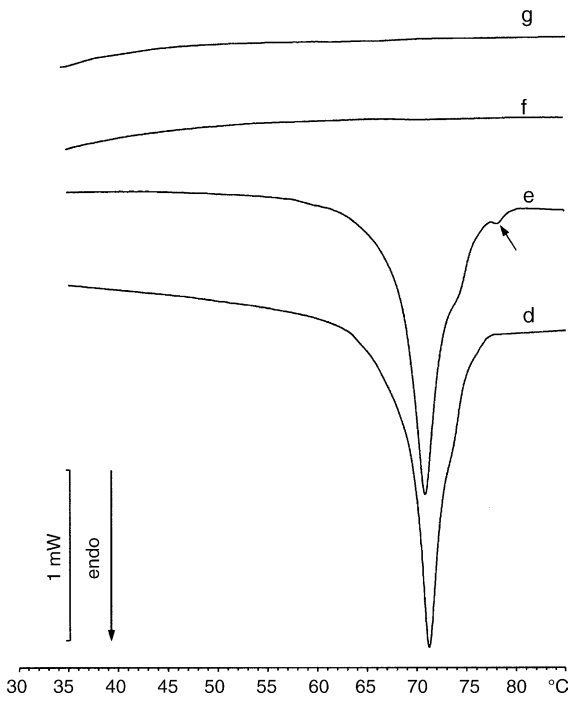

Fig. 2. Differential scanning calorimetric heating curves of (d) blank SLN; (e) SLN with IND added and dissolved inside at 80 °C; (f) Miglyol alone; and (g) Miglyol with IND added and dissolved inside at 80 °C.

tioned above. It clearly indicates an effect of IND on the Miglyol lipid phase aggregation.

This change in the calorimetric peak shape will be employed to demonstrate the preferential dissolution of IND in the Miglyol phase dispersed in the lipid solid phase.

In Fig. 2, the DSC curves (samples d–g) of SLN as well as the Miglyol ones, either with or without IND, are reported. SLN (curve (d)), according to the data reported in literature (Jores et al., 2003), exhibit a peak at 71.2 °C with a shoulder at 73 °C. The calorimetric curve of SLN loaded with IND (curve (e)) shows when compared to SLN curve:

(1) an increase in the shoulder intensity;
(2) a slight decrease of the main peak temperature;
(3) a new peak at 78.4 °C due to the presence of IND.

Differently, when submitted to calorimetric scan (curves (f) and (g)), pure Miglyol as well as the Miglyol loaded with IND at 80 °C did not show any calorimetric peak.

In Fig. 3A–C, the kinetic measurements curves are reported. They show the induced formation of empty or IND-loaded NLC and also the effect of IND on NLC. This effect was obtained incubating at 80 °C, for increasing time period, pure Miglyol with relative amounts of blank SLN and IND loaded SLN, respectively or blank NLC added to pure IND.

Such a kind of kinetic experiments are reported (Castelli et al., 2000, 2002; Librando et al., 2004) as a valid way to determine if a compound in contact with a lipid system in an aqueous medium is able to migrate through it and dissolve inside the lipid phase. Therefore, they let us to follow all the steps of the lipophilic enrichment by incubating the samples at the same temperature of preparation. If a migration happens, it can be detected following the changes induced on the calorimetric peak shape due to the increasing amount of compounds which leaves the bulk, migrates through the aqueous medium and dissolves in the lipid phase.

The calorimetric curves of samples (h–l) are compared with SLN and NLC curves of the samples a, c, d and e. The simple contact between Miglyol and SLN (Fig. 3A), under periods of increasing incubation at 80 °C (curves (h)), induces no changes in the calorimetric peaks. Instead, with incubation time increasing, the contact of IND-loaded SLN with Miglyol (curves (i) of Fig. 3B), causes a little change of the peak shape in comparison with the curve of NLC loaded with IND (curve (a)) and, at the same time, we observed the gradual disappearance of the IND peak at 78.4 °C.

When NLC were added to IND (curves (l) of Fig. 3C), it was observed that the characteristic NLC plateau (curve (a)) is changed in a well-defined peak structure that became similar to that of loaded NLC (curve (c)).

Considering the difference on the shape of the calorimetric curves caused by the presence of IND in the NLC (samples a and c), the DSC results suggest that IND is preferentially dissolved in the Miglyol rather than in the solid lipid phase.

These conclusions can be drawn by considering that:

(a) DSC curves, of Miglyol alone or in the presence of IND (curves (f) and (g) of Fig. 2), did not show any calorimetric peak (implying a complete IND dissolution in Miglyol);
(b) the SLN lipids (curve (d) of Fig. 2) showed a peak (71.2 °C) with a shoulder at higher temperatures.

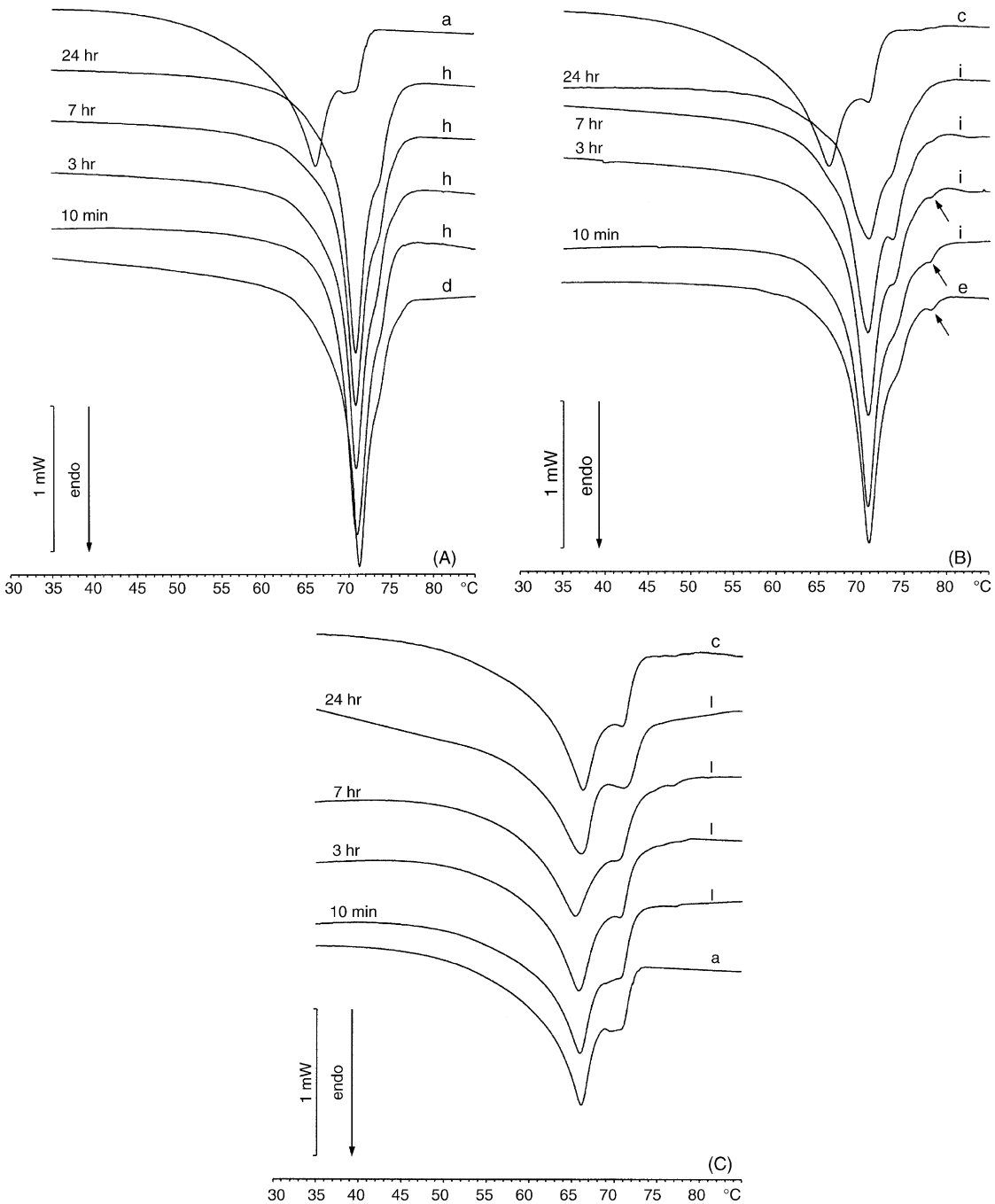

Fig. 3. Differential scanning calorimetric heating curves at increasing incubation periods at 80 °C of: (A) blank SLN added to Miglyol weighted in the DSC pan (curves (h)) compared with curves (d) (blank SLN) and (a) (blank NLC); (B) SLN loaded with IND added to Miglyol weighted in the DSC pan (curves (i) compared with curves (e) (SLN loaded with IND) and (c) (NLC loaded with IND); (C) blank NLC added to IND weighted in the DSC pan (curves (l)) compared with curves (a) (blank NLC) and (c) (NLC loaded with IND).

The main peak remains nearly constant in the presence of IND, and a small endothermal event is displayed at 78.4 °C (curve (e) of Fig. 2, indicated by the arrow);

(c) comparing NLC (blank, curve (a)) and SLN (curve (d)), we observed that the peak of the pure lipid changed in a two-component calorimetric peak with a shoulder at the same temperature of the pure lipid and a well-defined peak at lower temperature (changes caused by the presence of the Miglyol inside the lipid structure).

This shoulder showed a characteristic shape that would be represented in a well-defined plateau every time IND was not present. Instead, when IND was added to the preparation of NLC the above mentioned shoulder was transformed in a peak and a well-defined two-peak system was originated (curve (c) of Fig. 1).

A simple experiment allowed us to attribute this trend to the IND dissolution into Miglyol. Empty NLC were mixed mechanically with IND, submitted to ultra-turrax and successively to a calorimetric scan. The plateau shape that appeared in the calorimetric curves (curve (b) of Fig. 1) suggested that IND, in spite of its lipophilic character, was not able to dissolve inside the lipid phase when added in the aqueous phase. If the same sample is heated at 80 °C for 24 h (curve (b′) of Fig. 1), we can observe that the shape slowly changes into a two well-defined peak form. This evidence together with the successive kinetic experiments suggested that IND came into the lipid matrices and more exactly in the Miglyol component.

In such kind of experiments when the SLN were incubated at 80 °C for more prolonged periods in presence of pure Miglyol (curves (h) of Fig. 3A), we observed that the shape of the SLN remains nearly unaltered. In particular, the shoulder was not evidenced and this was probably due to a small transfer and dissolution of the Miglyol in the lipid matrix. When the SLN loaded with IND were incubated at 80 °C for more prolonged periods in presence of pure Miglyol (curves (i) of Fig. 3B), the latter was able to diffuse inside the lipid matrix and this phenomenon suggested that drug promotes the Miglyol absorption. Anyway, the shape of the peak appeared only partially similar to the one obtained by adding IND to NLC (curve (c)). The curve changes and the absence of a two-peak system could suggest the presence of a continuous phase between Miglyol and lipid. The hypothesis of Miglyol dissolution in the lipid is also supported by the disappearance of the small peak at 78.4 °C that we attributed to the IND presence in the lipid matrix.

More indications were drawn by considering the kinetic data obtained by the interaction of blank NLC with pure IND after prolonged incubation periods at 80 °C (curves (l) Fig. 3C), in particular, we observed that the plateau shape of the curves remained initially unchanged, but after four calorimetric scans it started to change.

After 24 h, finally, it produced a two-peak structure which indicated the presence of IND in the NLC structure.

In Fig. 3B it was reported the small peak attributed to the IND dissolved in the blank SLN (curve (e)) that gave useful indications about the Miglyol distribution inside the solid lipid particles. The rapid disappearance of the peak demonstrated that IND present in the lipid phase was dissolved in the Miglyol and that it comes inside the lipid phase after the addition of IND-loaded SLN (10 min to 24 h; curves (i) of Fig. 3B). Differently, when IND was added to NLC (10 min to 24 h; curves (l) of Fig. 3C), we had no evidence of the small peak at 78.4 °C. Such a peak should have been produced by the preferential dissolution of IND in the lipid phase compared to Miglyol phase, as it appears in Fig. 3B.

We can suppose that inside the lipid particles, during the preparation, the segregation of Miglyol brought to the formation of zone rich of this lipid oil where IND prefers to dissolve in. Furthermore, a small amount of drug remained in lipid phase to consent the migration responsible for the drug release.

In conclusion, this calorimetric study demonstrated that NLC systems are constituted by oil nanocompartments incorporated into a solid matrix. Miglyol nanocompartments contain a higher amount of active compound, but the release is strongly influenced by the IND amount dispersed in the surrounding solid lipid barrier. The experimental evidences regarding a drug controlled release from NLC system (Ricci et al., 2005) could be explained considering both drug partition between oil nanocompartments and solid lipid and its successive solid lipid–water partition. We demonstrated indirectly this behaviour by loading SLN with IND or with Miglyol and following time by time the changes in the calorimetric peak shape of the nanostructured lipid systems.

References

Castelli, F., Pitarresi, G., Giammona, G., 2000. Influence of different parameters on drug release from hydrogel systems to biomembrane model. Evaluation by differential scanning calorimetry technique. Biomaterials 21, 821–833.

Castelli, F., Librando, V., Sarpietro, M.G., 2002. Calorimetric approach of the interaction and absorption of polycyclic aromatic hydrocarbons with model membranes. Environ. Sci. Technol. 36, 2717–2723.

Castelli, F., Sarpietro, M.G., Messina, C., De Lazzari, A., Di Rosa, D., Giannetto, A., 2003. Differential scanning calorimetry differences in micronized and unmicronized nimesulide uptake process in biomembrane model. Eur. J. Pharm. Sci. 19, 237–243.

Heurtault, B., Saulnier, P., Pech, B., Proust, J.E., Benoit, J.P., 2003. Physico-chemical stability of colloidal lipid particles. Biomaterials 24, 4283–4300.

Hu, L., Tang, X., Cui, F., 2004. Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs. J. Pharm. Pharmacol. 56, 1527–1535.

Jenning, V., Lippacher, A., Gohla, S.H., 2002. Medium scale production of solid lipid nanoparticles (SLN) by high pressure homogenization. J. Microencapsul. 19, 1–10.

Jenning, V., Thünemann, A., Gohla, S.H., 2000. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int. J. Pharm. 199, 167–177.

Jores, K., Mehnert, W., Mader, K., 2003. Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: a nuclear magnetic resonance and electron spin resonance study. Pharm. Res. 20, 1274–1283.

Kim, B., Na, K., Choi, H., 2005. Preparation and characterization of solid lipid nanoparticles (SLN) made of cacao butter and curdlan. Eur. J. Pharm. Sci. 24, 199–205.

Librando, V., Sarpietro, M.G., Castelli, F., 2004. Role of lipophilic medium in the absorption of polycyclic aromatic compounds by biomembranes. Environ. Toxicol. Pharmacol. 14, 25–32.

Lim, S., Lee, M., Kim, C., 2004. Altered chemical and biological activities of all-trans retinoic acid incorporated in solid lipid nanoparticle powders. J. Control. Release 100, 53–61.

Lippacher, A., Muller, R.H., Mader, K., 2000. Investigation on the viscoelastic properties of lipid based colloidal drug carriers. Int. J. Pharm. 196, 227–230.

Lippacher, A., Muller, R.H., Mader, K., 2002. Semisolid SLN dispersions for topical application: influence of formulation and production parameters on viscoelastic properties. Eur. J. Pharm. Biopharm. 53, 155–160.

Mehnert, W., Mader, K., 2001. Solid lipid nanoparticles: production, characterization and applications. Adv. Drug Deliv. Rev. 47, 165–196.

Muller, R.H., Mader, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Muller, R.H., Radtke, M., Wissing, S.A., 2002a. Nanostructured lipid matrices for improved microencapsulation of drugs. Int. J. Pharm. 242, 121–128.

Muller, R.H., Radtke, M., Wissing, S.A., 2002b. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv. Drug Deliv. Rev. 54, S131–S155.

Muller, R.H., Keck, C.M., 2004. Drug delivery to the brain—realization by novel drug carriers. J. Nanosci. Nanotechnol. 4, 471–483.

Ricci, M., Puglia, C., Bonina, F., Di Giovanni, C., Giovagnoli, S., Rossi, C., 2005. Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies. J. Pharm. Sci. 94, 1149–1159.

Scholer, N., Zimmermann, E., Katzfey, U., Hahn, H., Muller, R.H., Liesenfeld, O., 2000. Preserved solid lipid nanoparticles (SLN) at low concentrations do cause neither direct nor indirect cytotoxic effects in peritoneal macrophages. Int. J. Pharm. 196, 235–239.

Siekmann, B., Westesen, K., 1994. Thermoanalysis of the recrystallization process of melt-homogenized glyceride nanoparticles. Colloids Surf. B 3, 159–175.

Souto, E.B., Wissing, S.A., Barbosa, C.M., Muller, R.H., 2004. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm. 278, 71–77.

Tabatt, K., Kneuer, C., Sameti, M., Olbrich, C., Muller, R.H., Lehr, C.M., Bakowsky, U., 2004. Transfection with different colloidal systems: comparison of solid lipid nanoparticles and liposomes. J. Control. Release 97, 321–332.

Venkateswarlu, V., Manjunath, K., 2004. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J. Control. Release 95, 627–638.

Wissing, S.A., Kayser, O., Muller, R.H., 2004. Solid lipid nanoparticles for parenteral drug delivery. Adv. Drug Deliv. Rev. 56, 1257–1272.