Solid Lipid Nanoparticles as Indomethacin Carriers for Topical Use (2): DSC Analysis, Drug Release and Rheological Properties

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ABSTRACT

Solid lipid nanoparticles (SLNs) and nano structured lipid carriers (NLCs) containing or not containing indomethacin (IND) were prepared with either Cetyl palmitate, Geleol™, or Compritol® 888 ATO as a lipid phase by the hot homogenization technique. In all systems the surfactant was sucrose fatty acid ester SP 30 (HLB 6.0). Differential scanning calorimetry (DSC) experiments were carried out on the freeze - dried samples for all developed plain and drug- loaded formulations after one month of storage at room temperature. The recorded DSC parameters of the samples were compared with those of pure components and physical mixtures of IND with the surfactant or lipids at equivalent ratios to that in nanoparticualtes. Furthermore, rheological analyses of the empty and loaded systems, release experiments at pH 5.0 and the release kinetics were all investigated. This study focuses on the investigation of how the nature and the amount of formulation components are able to modify the properties of the system. In particular, the concentration of the surfactant used for the nanosuspension stabilization, the nature and concentration of the lipid phase used for the nanoparticles preparation, as well as the drug- lipid ratio employed in the preparation of loaded SLNs were investigated.

DSC results showed crystalline particles at room temperature exhibited a shift of the melting endotherm of the lipid phase, with the maximum at a temperature value higher than that of the empty or loaded SLNs except in Geleol™ - based systems. For the investigated formulations a percentage of crystallinity between 61.0 % and 88.33 % was found. Furthermore, IND suffered a marked reduction in its crystallinity and had better solubility in both Geleol™ and Compritol® 888 ATO as compared to Cetyl palmitate screened. Hydrogen bonding with glyceride containing lipids can be one reason for the observed loss of IND crystallinity. All formulations were of creamy texture and viscous especially Geleol™ - based systems. Rheological analysis confirmed that all systems show a complete dependence of the viscosity from the shear stress. The release profiles of different formulations at pH 5.0 allow affirming that these systems are suitable for modified topical delivery. Release studies showed that IND exhibited a prolonged slow release over 24 hours (hrs) with release kinetics in general following zero order model and there was insignificant difference in the amount of released drug depending upon the composition of the formulations.

Keywords: Solid lipid nanoparticles, nanostructured lipid carriers, sugar ester, Indomethacin, controlled release.

1. INTRODUCTION

Solid lipid nanoparticles (SLNs) 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 poorly water soluble pharmaceutical drugs and cosmetic active ingredients [1-6]. Advantages of these SLNs are the good tolerability (GRAS status for many components), the avoidance of organic solvents in the preparation process, a wide potential application

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spectrum (dermal, per oral, intravenous), low cost compared to liposomes, and high pressure homogenization as an established production method which allows large scale production [7]. Furthermore, SLNs are suitable for the incorporation of lipophilic, hydrophilic and poorly water soluble active ingredients [2]. The additional advantages of SLNs may be the possibility of steam sterilization and lyophilization [8,9], improved bioavailability, protection of sensitive drug molecules form the environment (water, light) and controlled release characteristics [10]. The controlled drug release becomes important when the drug causes irritation of the skin at high concentration and has to be delivered over a prolonged period of time [11]. SLNs possess a number of features advantageous for the topical route of application; a high inclusion rate for lipophilic substances, small particle size providing close contact to the stratum corneum and the ability to form a film on the skin surface which is desirable in chronic inflammatory diseases [12,13]. Common disadvantages of SLNs include particle growth, gelation tendency, unexpected dynamics of polymorphic transitions and inherently low incorporation capacities due to the crystalline structure of the solid lipid. To overcome this drawback, nanostructured lipid carriers (NLCs) have been developed based on a mixture of solid and liquid lipid (oil) which leads to an imperfect matrix structure and consequently higher loading capacity and controlled drug release [14-17].

SLNs composed of a high melting point lipid/s as a solid core coated by surfactants. The term lipid in a broader sense includes triglycerides, partial glycerides, fatty acids, hard fats and waxes [18]. Most of the published papers on SLNs report on particles formulated with glyceride matrix material and stabilized with block polymers or phospholipids. However, waxes and paraffins can be used as core materials as well. Waxes can be defined as simple esters of fatty acids with alcohols. In contrast to glycerides, the alcohol involved is not glycerol. Besides differences in chemical composition, glyceride and wax bulk material feature different physical properties. Waxes are plastic solids at room temperature and shine after polishing. After melting at moderately elevated temperatures, they become a low viscosity liquid. In contrast, glycerides are often obdurate and dull. Furthermore, these materials display striking dissimilarities in their crystal order. Glycerides crystallize in different subcell arrangements- hexagonal, orthorhombic and triclinic. They exhibit marked polymorphism with three and often more individual forms. The polymorphism of waxes is drastically less. Mainly, an orthorhombic subcell prevails and the polymorphic transition rate is low. Because of these chemical, physical and crystallographic differences, an influence on properties of lipid nanoparticles is expected [19]. Furthermore, most SLNs produced by hot homogenization as a simple and cost effective method are characterized by an average particle size below 500 nm and low microparticulate content [4,20]. Several factors will influence the drug substance distribution and incorporation efficiency in the final SLN; surfactant type & concentration, lipid type & crystallization pattern, the method of production, the drug substance lipophilicity and its partition coefficient.

Non steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used medicines for the treatment of osteoarthritis, rheumatoid arthritis, inflammations and a variety of pains [21]. One of the most potent NSAIDs, Indomethacin (IND) is widely used and the oral therapy is very effective, but its clinical use is often limited by its potential side effects such as irritation and ulceration of the gastrointestinal mucosa, while its short elimination half-life (< 6 hrs) requires frequent dosing [22]. Due to these problems, several studies are aimed at the development of an efficient means for IND topical administration in order to increase local soft-tissue and joint concentrations by reducing its systemic distribution so as to limit its harmful side-effects [23]. The launched topical preparations such as gels or creams also show systemic absorption and require frequent application [24]. Therefore, a carrier system able to deliver the drug for prolonged periods of time after topical administration, would be a distinct improvement on the existing delivery systems and would result in significantly fewer side effects compared to conventional chemical anti-

inflammatory compounds. Over the past years, lipidic nanoparticles are under extensive world study as promising alternative carriers for drugs and diagnostics [17,25-30]. The topical application of SLNs can avoid systemic side effects, as the permeation of drugs through the skin is reduced, possibly due to interference of the lipid nanoparticles with the epidermal lipids of the skin surface [31]. Furthermore, SLNs are known to improve skin hydration and physically strengthen the barrier, a highly relevant property when treating skin diseases involving dry skin such as atopic dermatitis [28]. Since the marketed formulations of IND are not controlled release preparations, the clinical use of IND for treating chronic pain is limited. A long acting analgesic and anti-inflammatory preparation is desirable in patients suffering from long- lasting pain [32].

IND was selected in this study as a highly lipophilic model drug because of its specific chemical structure (figure 1) and physicochemical properties (e.g. H-bonding carboxylic acid group, hydrophobic nature, and pH – dependent solubility) that were expected to affect the processes of nanoparticles formation and loading as well as the drug release rate [33-35].

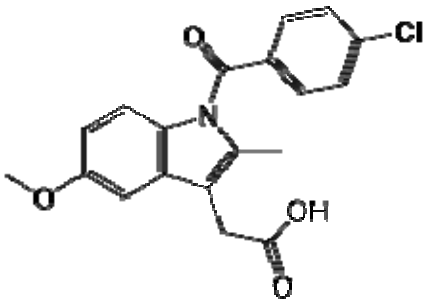

Figure 1. Introduction. Structure of Indomethacin [2-{1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl} acetic acid]

The aim of this study was to continue investigating the physiochemical behavior of the prepared SLNs and NLCs but in other aspects of characterization. The investigated SLNs/ NLCs were previously characterized (accepted manuscript) with respect to particle size, zeta potential, and entrapment efficiency. In this research paper, insightful characterization using differential scanning calorimetry (DSC) experiments was carried out. The influence of key properties of the lipid dispersions on the in vitro release at pH 5.0 and the rheological properties was also investigated. The key properties of the lipid dispersions are the surfactant percent, the drug loading and the lipid amount & its type. The physicochemical characteristics of drug- loaded SLNs were compared with the empty SLNs to get optimized formulation of IND. The present study compares between the thermal behaviors of Cetyl palmitate nanoparticles (waxy lipid) and SLNs prepared from Geleol™ or Compritol® 888 ATO as glyceride containing lipids. The lipids applied in this study were of different types and varied with regard to monoglyceride content and fatty acid chain length. The lipid represented different lipid polarities and were selected to give various drug solubilities. It was the major goal of whole study to develop a nanoparticualte; lipid based drug carrier with increased payloads and controlled release properties. This was accomplished sometimes by incorporating triglyceride containing oils in the solid core of particles. Furthermore, the study describes the production of SLN dispersions having a macroscopically appearance of creams but simultaneously maintain the nanoparticualte structure and reports on the development of SLN formulations with regard to possible topical applicability. Accordingly, rheological properties are important factors

that can influence the drug release from a vehicle, the application and performance on skin [36]. Moreover, this study was designed to evaluate the suitability of using a sugar ester as a non ionic surfactant and stabilizer of IND- loaded SLNs. Sugar esters (SEs) can be applied in the cosmetical field since they are gentle to the skin with moisturizing effect [37-38].

2. MATERIALS AND METHODS

2.1 Materials

Cetyl palmitate (Palmityl palmitate, hexadecyl hexadecanoate, m.p. 54-55°C, grade 95, EP), Geleol™ mono and diglycerides NF (a mixture of glycerol mono stearate 50.1%, diglycerides 41.0%, triesters 8.1%, free glycerol 0.7%, m.p. 58°C), Compritol® 888 ATO (glycerol dibehenate EP- glyceryl behenate NF, m.p. 72°C; is a mixture of 13-21% mono-, 40-60% di- and 21-35% triglycerides, the fatty acid fraction consists of > 83% behenic acid) and Labrafac™ Lipophile WL 1349 (caprylic/capric triglycerides NF; mainly C8 triglycerides 59% and C10 triglycerides 40%) were all gifts of Gattefossè Italy S.r.L (Milan, Italy). Sucrose fatty acid ester SP 30; HLB 6.0 (30% of stearate/palmitate monoesters) was a gift of Sisterna Company (Campodarsego, Italy). The materials were used as received without any further purification. IND was purchased from Fluka Biochemika (Switzerland). Potassium dihydrogen phosphate and methanol were from Carlo Erba Reagenti (Rodano, Milano, Italy) and Tween 80 was supplied by Riedel –de Haën (Sigma Aldrich, Italy). Other chemicals were of reagent grade and used without further purification.

2.2 Preparation of lipid nanoparticles

Different blank SLN dispersions consisting of 5-10% lipids, 2-5% sucrose fatty acid ester as an emulsifier and double distilled water added to 100% (all w/w %) were produced by hot homogenization technique [39,40]. To the lipid phase melted at a temperature 10°C above its melting point, 45 ml aqueous solution of the surfactant heated at the same temperature was added. The mixture was homogenized with Ultra-Turrax T18 Basic (IKA-WERK, Germany) for 10 minutes at 24,000 rpm and then left to cool at room temperature and the final weight (50 g) was corrected using double distilled water. These preparations were indicated as blank SLNs. By adding IND (5% w/w of total solids) dissolved in a small amount of methanol (kept at the same temperature of the lipidic phase) to the lipidic phase and following the previously described procedure, the samples IND- loaded SLNs were obtained. The composition of IND- loaded SLNs was the same as that of blank SLNs of the same number except for the presence of IND. Different SLNs were prepared, varying the amount of the surfactant needed to stabilize the nanosuspension, the type of lipid, the amount of the lipid and the drug-lipid ratio. The composition of different prepared blank SLNs and IND- loaded SLNs with their corresponding drug- lipid ratios are given in tables (1) and (2), respectively.

In case of NLCs, a fraction of the solid lipid (Cetyl palmitate) was replaced by the liquid Labrafac™ Lipophile WL 1349 oil (10 % w/w referred to the total lipid phase) and were prepared in the same way as the SLN dispersions. The samples were left to equilibrate for 24 hours (hrs) prior to further analysis.

Table 1. Preparation of lipid nanoparticles. Various ratios of lipids and the surfactant used in the preparation of blank SLN formulations in % (w/w), made up with water to 100%

Formula	Cetyl palmitate (w/w %)	Labrafac™ Lipophile WL (w/w %)	Geleol™ mono and diglycerides NF (w/w %)	Compritol® 888 ATO (w/w %)	Sucrose fatty acid ester SP30 (w/w %)
F1	10%	-	-	-	2%
F2	10%	-	-	-	3%
F3	10%	-	-	-	4%

F4	**10%**	-	-	-	**5%**
F5	-	-	**10%**	-	**2%**
F6	-	-	**10%**	-	**3%**
F7	-	-	**10%**	-	**4%**
F8	-	-	**10%**	-	**5%**
F9	-	-	**5%**	-	**2.5%**
F10	**10% total lipids (Cetyl palmitate: Labrafac™, 9:1)**		-	-	**5%**
F11	**5% total lipids (Cetyl palmitate: Labrafac™, 9:1)**		-	-	**2.5%**
F12	-	-	-	**10%**	**2%**
F13	-	-	-	**10%**	**3%**
F14	-	-	-	**5%**	**2.5%**

Table 2. Preparation of lipid nanoparticles. Various ratios of Indomethacin in % (w/w), and the drug- lipid ratios used in the preparation of loaded SLN formulations

Formula	Indomethacin (w/w %)	Drug – Lipid Ratio
INF4	**5%**	**1: 13.34**
INF8	**5%**	**1: 13.34**
INF9	**10%**	**1: 6.67**
INF10	**5%**	**1: 13.34**
INF11	**10%**	**1 : 6.67**
INF14	**10%**	**1: 6.67**

2.3 Characterization of SLN/NLC dispersions

Differential Scanning Calorimetry (DSC) measurements

DSC measurements were carried out with SETARAM DSC 131 (France), equipped with SETSOFT 2000. The samples were quickly frozen in liquid nitrogen and freeze- dried at room temperature at a pressure of 0.4- 0.5 mmHg. Ten milligrams of each sample were sealed in a DSC aluminum pan (30µl) and submitted to calorimetric analysis at a scan rate of 10 C°/min in the temperature range of 25- 200 °C. An empty sealed pan was used as a reference. A nitrogen purge at a flow rate of 80 cm^3/min was used to provide an inert gas atmosphere in the DSC cell. Prior to heating, the sample was equilibrated in the DSC pan at 25°C for 5 minutes. DSC measurements were carried out on blank SLNs and IND- loaded SLNs. The controls were the pure substances and physical mixtures of drug / lipid or drug / stabilizer at equivalent ratios used in the preparation of formulations.

The DSC parameters such as temperature onset, maximum peak and enthalpy were calculated using the in build software. The results expressed as the mean of three determinations ± standard deviation (S.D.). Furthermore, the crystallinity of the particles was quantified as so called crystallinity index [(41)], what it means is that the melting enthalpy of the lipid in the SLN dispersion was expressed as percentage of the melting enthalpy of the bulk lipid. The bulk lipid was considered being fully

crystalline, that means that it had the index of 100%. Of course, this index is only a rough measure because the particles can crystallize partially in a different modification, while peak separation is in most cases not possible.

Rheological Measurements

Rheological experiments were performed with Haake Rheostress 300 Rotational Rheometer (Germany) equipped with a Haake DC50 thermostate. Flow curves were performed at 25.0 ± 0.2°C in the range of 0.01-1000 (Pa) on aqueous solutions of different SLN samples [36,42] and in the absence or presence of IND. Enough quantity of each sample was carefully poured to completely cover the 6 cm cone- plate geometry (Haake CP 60/1, cone angle of 1°).

Release study

The release studies were carried out using the vertical dialysis tubes [43] applying *Sic* cellulose membrane (Eastleigh, Hampshire, UK) with a cut off value of 12-14 kDa. Prior to use, the membrane was hydrated in purified water for 12 h. The final concentration of the drug was always lower than 10% (w/w) of its solubility in the receptor medium. The solubility of IND in a mixture of Tween 80 in phosphate buffer pH 5.0 (2% w/v) [6,13] was determined before setting up the release studies, so that sink conditions could be assumed. Five grams of SLN formulation was placed in the donor compartment after the receptor compartment was filled with 300 ml of receptor medium. The temperature was kept as 32 ± 0.1°C and the receptor medium was stirred with the help of magnetic stirrer at 100 rpm. Samples of 5 ml were withdrawn after 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 24 hrs, replacing the samples with the same amount of volume of receptor medium kept at the same temperature. The cumulative amount of drug substance released Q (corrected for sampling) was calculated from equation:

$$Q = V_s \cdot \sum_{n=1}^{n} C_{n-1} + V_m \cdot C_n \qquad (1)$$

Where, V_s is the volume of sample withdrawn, C_n is the drug concentration of the sample n and V_m is the volume of the receptor medium. The amount of released drug was measured by UV analysis at 320 nm (Perkin-Elmer Lambda 40 UV/VIS Spectrometer, USA). The percentage of release was calculated by dividing the cumulative amount released at a given time by the amount of total IND present in 5.0 grams of SLN. The release profiles were obtained correlating time (hr) versus drug release %. All the experiments were carried in triplicates and the results were reported as mean ± S.D. Calibration curve for the validated UV assays of IND was performed on six solutions in the concentration range 1.1-110 µg/ml. The squared correlation coefficient was 0.999. Each point represents the average of three measurements and the error was calculated as a standard deviation (± S.D.)

Drug release kinetics was determined by applying three kinetic models to the data to find the best fitting equations [27]. These kinetic models were as follows:

$F = k_0 t$ Zero order equation (2)

$Ln F = \ln F - k_1 t$ First order equation (3)

$F = k_2 t / 2$ Higuchi equation (4)

Where, F is the fraction of drug released in time t, and k_0, k_1, & k_2 are the apparent rate constants for zero order, first order, and Higuchi release constants, respectively.

The data were fitted and the linear regression of the mathematical models was evaluated using the squared correlation coefficient (R^2). The linear regression was applied in the range of 0.5 to 24 hrs of release data at pH 5.0.

2.4 Statistical analysis

Statistical analysis was performed with SPSS 13.0 software package. Results are expressed as the mean ± standard deviation (X ± S.D.). Statistical significance was determined using paired sample t-test and the analysis of variance (One- way ANOVA) followed by Tukey's-b multiple range test with $p \leq 0.05$ as a minimal level of significance [44].

3. Results and Discussion

After preparation by hot homogenization method [45], all formulations presented a macroscopic homogenous appearance, like a milky white opalescent liquid. All formulations were of creamy texture and viscous especially Geleol™ - based systems. Unloaded or IND-loaded formulations were obtained without any subsequent step of filtration or centrifugation. The lipid-surfactant ratio and the lipid core material were found to affect the extent of drugs loading in SLNs and their physical stability [46,47]. Accordingly, the amount of sugar ester SP30 was optimized in the range of 2-5% (w/w) against a constant amount of lipids (10% w/w). Another lower ratio 5% (w/w) of lipids was also investigated against a constant amount of surfactant 2.5% (w/w) in order to obtain a lower viscosity SLNs. Reducing the lipid concentration from 10% to 5% improved the stability of the SLN dispersions although the ratio lipid-surfactant was not changed. Lower particle concentration means a lower probability of particle collision and subsequent reduced aggregation during the storage [48]. Increasing the lipid content above 5-10% in most cases results in larger particles, including microparticles, broader particle size distribution, and an increase in particle agglomeration[4]. Accordingly, lipid concentrations above 10% were not studied. The results of characterization of the SLN/ NLC dispersions by DSC, rheological behaviour, and release at pH 5.0 are shown below.

Differential Scanning Calorimetry (DSC) measurements

The physical state of the particles is very important from the technological as well as from the biopharmaceutical point of view. By the use of particles with solid lipid matrix, stability problems, e.g. drug leakage or coalescence, often observed for lipid dispersions such as emulsions or liposomes may be overcome. Moreover, drug release from solid matrix is supposed to be degradation- controlled and thus slower than diffusion – controlled release from emulsions [4]. DSC experiments were performed to characterize the thermal behavior and the crystallinity of the developed SLNs. Supercooled melts are not unusual in SLN systems; they describe the phenomenon that lipid crystallization may not occur although the sample is stored at a temperature below the melting point of the lipid. As the advantages of SLN drug –carrier systems are essentially based on the solid sate of the particles [4], the solidification of the particles after the preparation of nanodispersions has to be ensured. Furthermore, information about the interactions among IND and the different lipid particles were also obtained. DSC analysis was performed for all developed plain and drug- loaded formulations after one month of storage at room temperature. The samples were heated above their melting point and the recorded DSC parameters are presented in table (3). The results were compared to that of pure components and physical mixtures of IND with the surfactant or lipids at equivalent ratios to that in nanoparticualtes.

Table 3. Differential Scanning Calorimetry (DSC) measurements. DSC results of pure substances, of physical mixtures of Indomethacin with the lipids or the surfactant, of blank and loaded SLNs (n = 3)

	Formula	Mean Peak ± S.D.	Onset Point ± S.D.	Enthalpy (Joule/g) ± S.D.	Crystallinity Index%*
Pure Substance	Indomethacin	161.51 ± 0.01	157.97 ± 0.18	93.54 ± 0.86	100
	Cetyl palmitate	54.10 ± 0.77	47.82 ± 0.16	196.01 ± 11.26	
	Geleol™	58.73 ± 0.41	51.94 ± 0.31	101.06 ± 1.82	
	Compritol®	74.43 ± 2.47	68.34 ± 0.71	93.35 ± 11.26	
	Sugar ester SP 30	50.74 ± 0.01 63.52 ± 0.04	48.11 ± 0.01 58.26 ± 0.00	23.41 ± 0.75 9.30 ± 0.04	

Blank SLN	F4	52.15 ± 0.34	47.46 ± 0.38	133.70 ± 0.87	68.20
	F8	62.10 ± 0.47	53.89 ± 0.96	81.04 ± 21.86	80.19
	F9	61.65 ± 0.05	53.31 ± 0.12	88.66 ± 4.51	87.73
	F10	52.16 ± 0.30	46.62 ± 0.41	134.12 ± 7.71	68.42
	F11	51.87 ± 0.13	44.12 ± 0.24	127.46 ± 2.32	65.02
	F14	70.81 ± 0.18	64.25 ± 0.05	80.17 ± 5.18	85.88
IN SLN	INF4	52.16 ± 0.09	47.62 ± 0.62	134.02 ± 6.25	68.37
	INF8	61.14 ± 0.09	53.05 ± 0.26	87.70 ± 11.37	86.78
	INF9	60.86 ± 0.06	51.97 ± 0.34	89.27 ± 0.01	88.33
	INF10	52.13 ± 0.50	44.92 ± 0.64	124.71 ± 7.44	63.62
	INF11	52.27 ± 0.12	44.51 ± 0.65	119.58 ± 1.20	61.00
	INF14	69.43 ± 0.09	63.55 ± 0.09	78.04 ± 3.41	83.60
Physical Mixtures	IN- Cetyl palmitate (1:13.34)	54.29 ± 0.13	47.81 ± 0.71	200.62 ± 1.11	
	IN- Cetyl palmitate (1:12)	54.58 ± 0.20 157.45 ± 0.18	47.75 ± 0.54 154.71 ± 1.93	187.71 ± 13.13 1.92 ± 1.25	
	IN- Cetyl palmitate (1:6)	54.29 ± 0.13 157.55 ± 0.24	47.73 ± 0.26 155.31 ± 0.38	176.34 ± 2.48 4.84 ± 0.35	
	IN- Geleol™ (1:13.34)	59.12 ± 0.03	52.15 ± 0.71	103.24 ± 0.20	
	IN- Geleol™ (1:6.67)	59.02 ± 0.21	52.14 ± 0.35	92.82 ± 10.35	
	IN- Compritol® (1:6.67)	73.29 ± 0.09	68.16 ± 0.47	98.60 ± 6.57	
	IN- Sugar ester SP 30 (1:6.67)	51.16 ± 0.30 63.93 ± 0.11 141.51 ± 1.41	48.4 ± 0.45 59.02 ± 0.09 133.96 ± 3.27	20.43 ± 2.31 7.74 ± 1.61 12.64 ± 3.74	
	IN- Sugar ester SP 30 (1:3.34)	51.38 ± 0.19 63.93 ± 0.04 143.87 ± 0.04	48.79 ± 0.19 59.00 ± 0.18 135.65 ± 4.48	15.81 ± 3.53 7.05 ± 0.01 26.54 ± 1.09	

* The Crystallinity index was calculated as a percentage of the enthalpy of SLN relative to the enthalpy of the corresponding

Figure (2) shows the thermographs of a Cetyl palmitate- based SLNs (blank and loaded F4). For a better observation all the thermographs have been displaced along the same ordinate. The thermograms show a melting point of Cetyl palmitate at expected temperature of 54.09°C. Small portion of another polymorph might be present, resulting in a shoulder with melting point of ≈ 45°C [49]. When it is employed for the preparation of blank F4, the melting peak decreases to 52.15°C, and the melting shoulder is reduced and reappears at approximately 58°C, probably for the presence of the surfactant (two melting points 50.74°C, 63.51°C). Regarding the surfactant thermogram, the first peak related to the melting of the acyl chain, and the second melting transition corresponding to complete melting of the surfactant including the head group [50,51]. Both peaks are well separated and their melting enthalpies can be evaluated. These results are matching with studies [41,52] state that the transformation of lipid bulk material into lipid nanoparticles leads to changes of the melting behavior of the lipid accompanied by potential occurrence of lower melting α and βi modifications and in general, the onset temperature and the melting peak of the lipid nanoparticles are 2- 5°C lower compared to the bulk material. This decline can be explained by the nanometric small particles associated with an increase in surface curvature, high specific surface area and the presence of the surfactant. This melting point depression can be attributed to the Kelvin effect and is described by the Thomson equation. In INF4 samples, the melting point is 52.16°C showing that very little interaction between the lipid phase and the drug

(m.p. 161.50°C) has taken place and that this latter is at least in part not loaded into the particles. In confirmation of this hypothesis, the physical mixtures Cetyl palmitate-IND and surfactant – IND (at the same ratios used in the formulation of INF4) show endothermic peaks at 54.29°C and 51.16, 63.93°C, respectively. The behavior is already described in the literature for similar systems [49] and matches with the low entrapment efficiency of INF4 (data not shown). Moreover, due to the fact that the melting enthalpies remain almost unchanged, absence of dissolution of F4 in the presence of IND and the crystalline character of the nanoparticles were assumed.

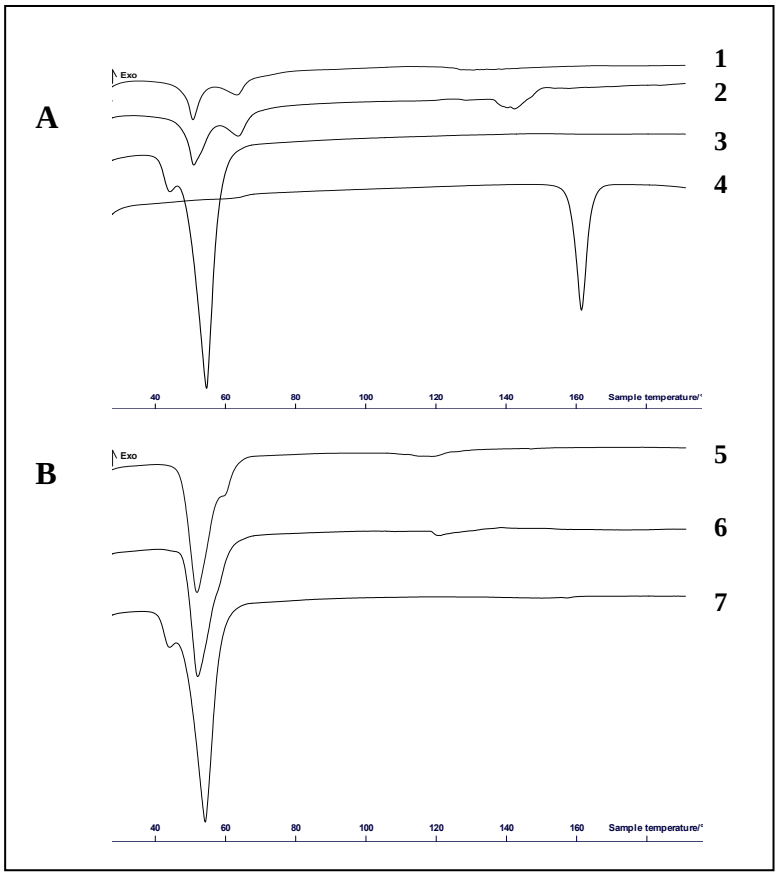

Figure 2. Differential Scanning Calorimetry (DSC) measurements. (A) DSC thermographs of (from top) (1) bulk sugar ester SP30, (2) physical mixture of the surfactant with Indomethacin (1:6.67), (3) bulk Cetyl palmitate, and (4) bulk Indomethacin. (B) DSC thermographs of (5) blank F4, (6) INF4, and (7) the physical mixture of Indomethacin with Cetyl palmitate (1:13.34)

Furthermore, table (3) illustrates the melting properties of the prepared NLC dispersions. The status of Labrafac^{TM} Lipophile WL 1349 was not examined by the DSC because the liquid oil could not be registered using the described temperatures and conditions. As shown in figure (3), pure Cetyl palmitate exhibited a sharp main transition peak at 54.09°C. When this lipid was formulated as a mixture with Labrafac^{TM} Lipophile WL

1349 (F10), the peak occurred at a lower temperature (52.16°C). The melting peak temperature of the bulk lipid decreased with an addition of liquid lipid. Besides, Labrafac^{TM} Lipophile WL 1349 incorporation into the matrix provoked an additional shift of the melting point onest to lower temperatures, with a decrease of Cetyl palmitate crystallinity. The degree of crystallinity of the lyophilized nanoparticles was calculated by comparing the melting enthalpies (ΔH) based on the total weight taken of the nanoparticles relative to the melting enthalpy of their corresponding bulk lipids. DSC uses the fact that different lipid modifications possess different melting points and enthalpies. Enthalpy decreased in SLNs, which was attributed to emulsifiers intercalated in the particle surfaces and the dispersed state of the lipid. A depression of the transition can be mentioned also for the NLCs. Previous reports [32,53] explain the reduced enthalpy following an oil addition by the smaller size of the oil containing systems. A smaller particle size leads to a higher surface energy, which creates an energetically suboptimal state causing a decrease in melting point. This is not a general rule since the SLNs had the smalles diameter in our study and the results show insignificant change in the melting enthalpies of lipid dispersions F4 and F10; may be due to the small percent of liquid lipid used in the formulation of F10 dispersion. Moreover, no new melting peaks were observed upon application of IND (INF10) but the loading of the drug; provoke a considerable effect on the melting point onest and crystallinity under these experimental conditions. This melting temperature reduction of solid lipid may be explained by the disturbance of the lipid crystal structure in the presence of both Labrafac^{TM} Lipophile WL 1349 and IND [17, 28]. Analogous types of results were obtained for the Cetyl palmitate/ Labrafac^{TM} Lipophile WL 1349 based NLCs with a lower lipid concentration 5% w/w (F11, INF11). Notably, IND- loaded NLCs based on mixture of Cetyl palmitate and Labrafac^{TM} Lipophile WL 1349 (INF10, INF11) showed the lowest degree of crystallinity, in comparison to the other developed formulations. This result might indicate the presence of a metastable polymorphic matrix, which is responsible for a higher loading capacity; in comparison to the more stable polymorph of pure solid lipid [53].

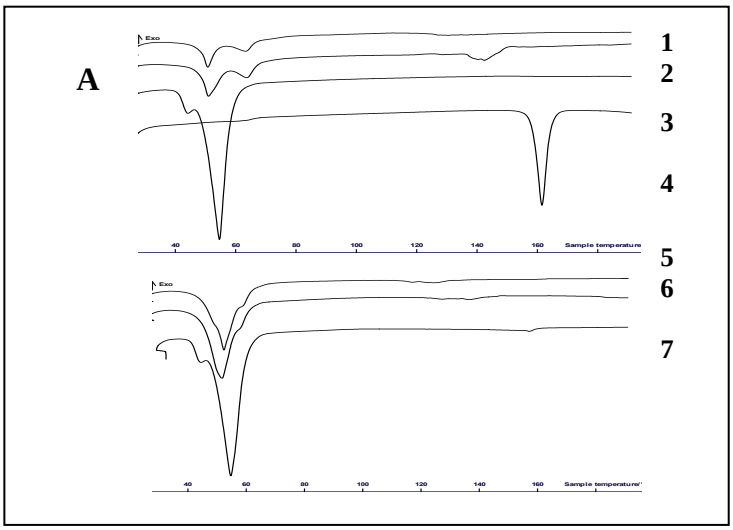

Figure 3. Differential Scanning Calorimetry (DSC) measurements. (A) DSC thermographs of (from top) (1) bulk sugar ester SP30, (2) physical mixture of the surfactant with Indomethacin (1:6.67), (3) bulk Cetyl palmitate, and (4) bulk Indomethacin. (B) DSC thermographs of (5) blank F10, (6) INF10, and (7) the physical mixture of Indomethacin with Cetyl palmitate (1:12)

DSC analysis of Geleol™ - and Compritol® - based SLNs were also carried out. In Geleol™ - based thermographs (figure 4), the melting point of bulk Geleol™ is 58.73°C. Interestingly, when this lipid is formulated as SLN (F8), a shift of melting point towards a higher temperature was observed. The melting point increases to 62.1°C, probably due to the presence of the surfactant and the freeze drying which may increase the melting point of SLNs. It may be caused by that some components such as surfactants were expelled out from SLNs during freeze drying process. It is quite difficult to explain this increase; it may be possible that crystallization of lipid after lyophilization lead to different chemical composition and/or different lipid modifications and other analytical methods are necessary in the future to support any hypothesis. Anyway, according to DSC results for the considered systems, it must be assumed that crystallization of the prepared particles started at room temperature [4]. Additionally, in the sample of INF8, the melting point of the bulk lipid increased to 61.14°C, showing that an interaction between the lipid phase and the drug (having melting point 161.50°C) has occurred and that this latter is at least in part loaded into the particles. The existence of drug in the lipid matrix of INF8 increases the number of lattice defects, and thus the melting point decreases if compared to F8. In confirmation of this hypothesis, Geleol™ - IND and surfactant – IND physical mixtures (at the same ratios used in the formulation of INF8) show endothermic peaks at 59.12°C and 51.16, 63.93°C, respectively. Analogous types of results were obtained for Geleol™ - based SLNs with a lower lipid concentration 5% w/w (F9, INF9).

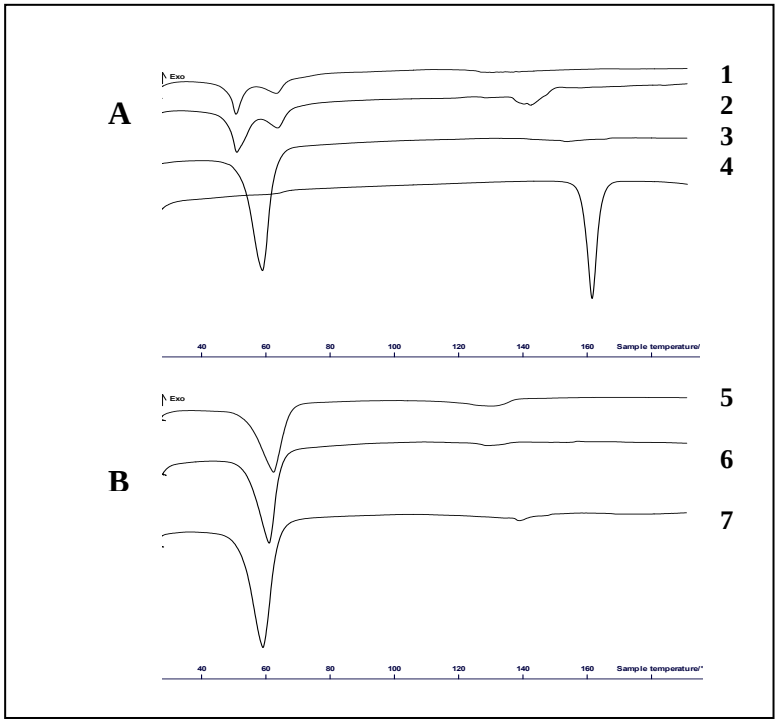

Figure 4. Differential Scanning Calorimetry (DSC) measurements. (A) DSC thermographs of (from top) (1) bulk sugar ester SP30, (2) physical mixture of the surfactant with Indomethacin (1:6.67), (3) bulk Geleol™, and (4) bulk Indomethacin. (B) DSC thermographs of (5) blank F8, (6) INF8, and (7) the physical mixture of Indomethacin with Geleol™ (1:13.34)

The thermal curve of unloaded and loaded Compritol® 888 ATO nanoparticles showed endothermic peaks at 70.81°C and 69.43°C, respectively (figure 5). The melting endothermic peak of drug- loaded nanoparticles appeared at a slightly lower temperature. The decrease in melting temperature of nanoparticles compared with the bulk lipid has been attributing to their nanometric small size and the presence of stabilizer [17,44]. For the crystalline Compritol® 888 ATO bulk material, a single sharp endothermic melting peak at 74.4°C was observed, in agreement with the melting point of β' modification[13,48,50,52].

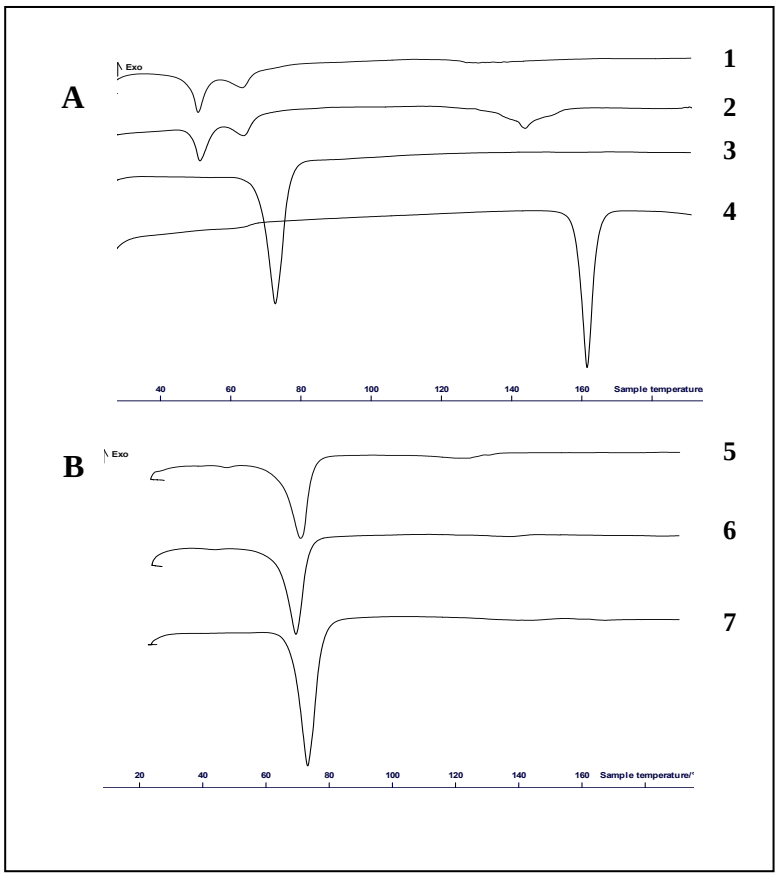

Figure 5. Differential Scanning Calorimetry (DSC) measurements. (A) DSC thermographs of (from top) (1) bulk sugar ester SP30, (2) physical mixture of the surfactant with Indomethacin (1:3.34), (3) bulk Compritol® 888 ATO, and (4) bulk Indomethacin. (B) DSC thermographs of (5) blank F14, (6) INF14, and (7) the physical mixture of Indomethacin with Compritol® 888 ATO (1:6.67)

Additionally, the thermal analysis performed can be used to investigate the degree of crystallinity of SLNs [41]. The production of nanoparticles reduces the degree of crystallinity. For the investigated formulations a percentage of crystallinity between 61.0% and 88.33% was found (table 3). The reduction in crystallinity is due to partial formation of lower energy lipid modifications, in addition, the surfactant distributed to the melted lipid phase during the production process can distort crystallization resulting in a lower melting enthalpy [50]. The presence of other colloidal structures apart from SLNs can be supposed and is supported sometimes by multimodal particle size distribution (data not shown). The effect of surfactant on lipid crystallization can be seen when comparing SLN named F9 with F8 (the same lipid- surfactant ratio). After production, the percentage of crystallinity decreased from 87.73% to 80.19% with an increasing of surfactant concentration. Furthermore, it was noticed that all loaded SLNs/NLCs were of lower crystallinity indices if compared to their corresponding blank formulations except Geleol™ - based systems may be due to the additional hydrogen bonding interactions between the hydroxyl groups present in the structure of Geleol™ (50% monoglycerides) and the carboxylic groups of IND molecules.

Furthermore, DSC analysis can be used to check the physical status of IND within the lipid matrix of SLNs, by comparing the DSC thermograms of pure compounds and loaded SLNs. For the crystalline IND, a single sharp endothermic melting peak at 161.5°C was observed, in agreement with the melting point of γ-crystalline IND. The thermoanalysis of IND- loaded SLNs did not show any endothermic change at 161.5°C (table 3), confirming its complete dissolution or amorphisation in the semi solid matrix of the nanoparticles[54]. DSC thermographs of the surfactant as a physical mixture with IND had a further broadened peak at 141.51 - 143.86°C (table 3), may be due to the presence of crystalline IND[55]. This IND broadened melting peak appears in the physical mixtures of IND with Cetyl palmitate at the highest and the intermediate ratios, but suffered a marked reduction in crystallinity reflected in the decreased AUC of the melting peak at 161°C with a shift of the melting peak to approximately 157°C. The melting peak at 157°C corresponding to the meta-stable α form may be attributed to a thermodynamic transition from amorphous to meta-stable crystalline IND during the heating process in the DSC operation[56]. Additionally, IND displayed the greatest reduction in crystallinity, with no trace of an endothermic peak at 161°C observed in the DSC curves of the physical mixtures of IND with Geleol™ or Compritol® at any studied ratio. The observed amorphous IND in Geleol™ or Compritol® 888 ATO physical mixtures could be attributed to the space confinement or the hydrogen bonding, which occurred between the hydroxyl groups present in the structure of Geleol™ or Compritol® lipids and the carboxylic groups of IND molecules. Hydrogen bonding between different compounds has been reported in the literature as one reason for the observed loss of crystallinity [56].

Moreover, the solubility of IND in the used lipids was evaluated by measuring DSC thermograms of IND/ lipid physical mixtures. As shown in table (3), although the physical state of the drug was not confirmed by other analytical means, the absence of the melting point peak suggested that IND had better solubility in both Geleol™ and Compritol® 888 ATO as compared to Cetyl palmitate screened. Solubility of the drug in the lipid melt is known to be an important precondition to obtain sufficient entrapment efficiency[57]. This observed loss of endothermic peak at 157°C in IND-Cetyl palmitate physical mixture at a ratio of 1:13.34 could be ascribed to the small undetectable amount of crystalline IND and the insufficient sensitivity of the DSC instrument.

Rheological Measurements

Rheological measurements were performed on SLN dispersions in the presence or absence of IND. It is well known that rheological properties are important factors for the application and performance on the skin[7,12,18]. In the literature, it is reported that a nanosuspension with a concentration of the lipid phase of 10% w/w shows a solution behavior and that in order to reach a consistency comparable to values commonly used in cream systems,

the lipid concentration must raise to 30% w/w[10]. In accordance with these data, it is expected that SLN samples prepared with 10% of the lipid phase will show no dependence of the viscosity from the shear stress. On the contrary, SLNs prepared in this research shows a complete dependence of the viscosity from the shear stress.

Figures (6) and (7) demonstrate the flow curves of blank and IND- loaded systems. A dependence of the viscosity from the shear stress is evident for all samples, in particular the viscosity (η) decreases when the shear rate (γ) increases, according to a behavior characteristic of pseudo-plastic systems. In blank formulations, a NLC named F11 shows the lowest value of viscosity while F8 SLN dispersion has the highest viscosity. The viscosity seems to be strictly dependent of the lipid phase of the nanoparticles and increases with the melting point of the latter. Figure (6) shows that the viscosity of F8 (Geleol™- based SLN, m.p. 58°C) is higher than that of F4 (Cetyl palmitate – based SLN, m.p. 54°C) and the viscosity of F14 (Compritol®- based SLN, m.p. 72°C) is higher than that of F11 (Cetyl palmitate- based SLN, m.p. 54°C). Similar trends are already reported in the literature[58]. The SLN named F9 was an exceptional case; may be because of the presence of aggregates if compared to dispersions of F11 and F14. In addition, the results in figure (6) revealed that higher concentration of lipids (10% w/w) allows production of SLNs of a higher viscosity if compared to systems of a lower lipid concentration (5% w/w); the viscosity can be ranked as follows: F8 > F9 and F10 > F11. Furthermore, the results show that the addition of Labrafac™ Lipophile WL 1349 increased the viscosity of the corresponding SLN dispersion (i.e. F10 > F4).

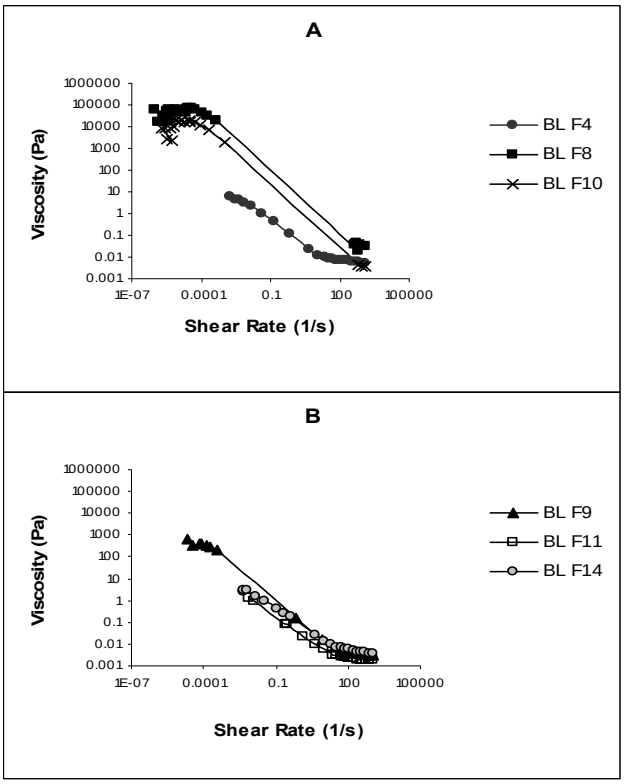

Figure 6. Rheological measurements. Flow curves of blank SLNs contain (A) 10% (w/w) or (B) 5% (w/w) lipid phase

Moreover, figure (7) illustrates that IND- loaded formulations are in general with higher viscosities than their corresponding blank one except a dispersion named INF8. The effects of melting point of lipids, lipid phase concentration, and the oil addition on the rheological properties are similar to blank formulations. The viscosities can be ranked as follows: INF14> INF9> INF11; INF10 > INF11 and INF10> INF4.

Notably, Geleol^{TM} - based systems characterized with high content of monoglycerides show the highest degree of aggregation than any other formulations.

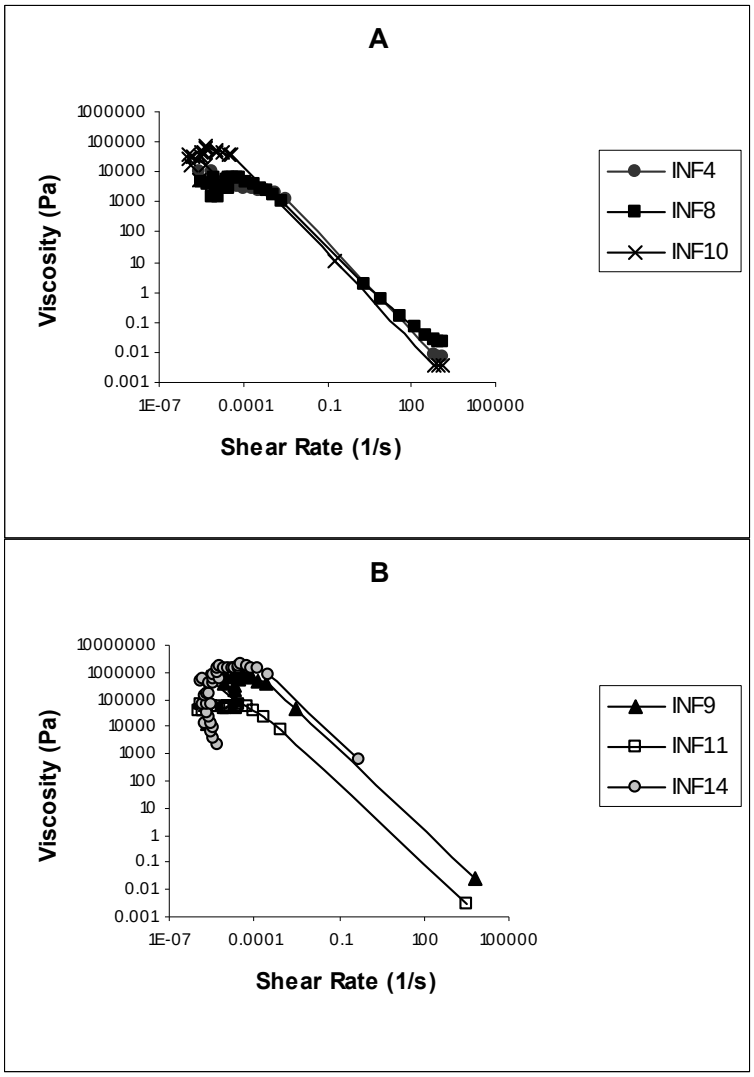

Figure 7. Rheological measurements. Flow curves of Indomethacin- loaded SLNs contain (A) 10% (w/w) or (B) 5% (w/w) lipid phase

Release study

Dialysis tubes were used to evaluate the controlled release potential of IND- loaded dispersions[43,59] and the diffusion of IND was investigated over 24 hrs. The obtained data and profiles release of IND loaded in different formulations at pH 5.0 are shown in figure (8). Figure (8) confirmed that IND- loaded nanoparticles produced very slow and sustained release profiles over 24

hrs. The release pattern revealed small initial burst effect within the first hour (less than 2%) followed by a controlled release of drug for several hrs. The initial burst could be due to the free drug and the superficially entrapped molecules in the outer layer of nanoparticles [29,60]. The process of IND release and the burst release fraction in pH 5.0 was slower and more sustained than in pH 7.4 (data not shown).

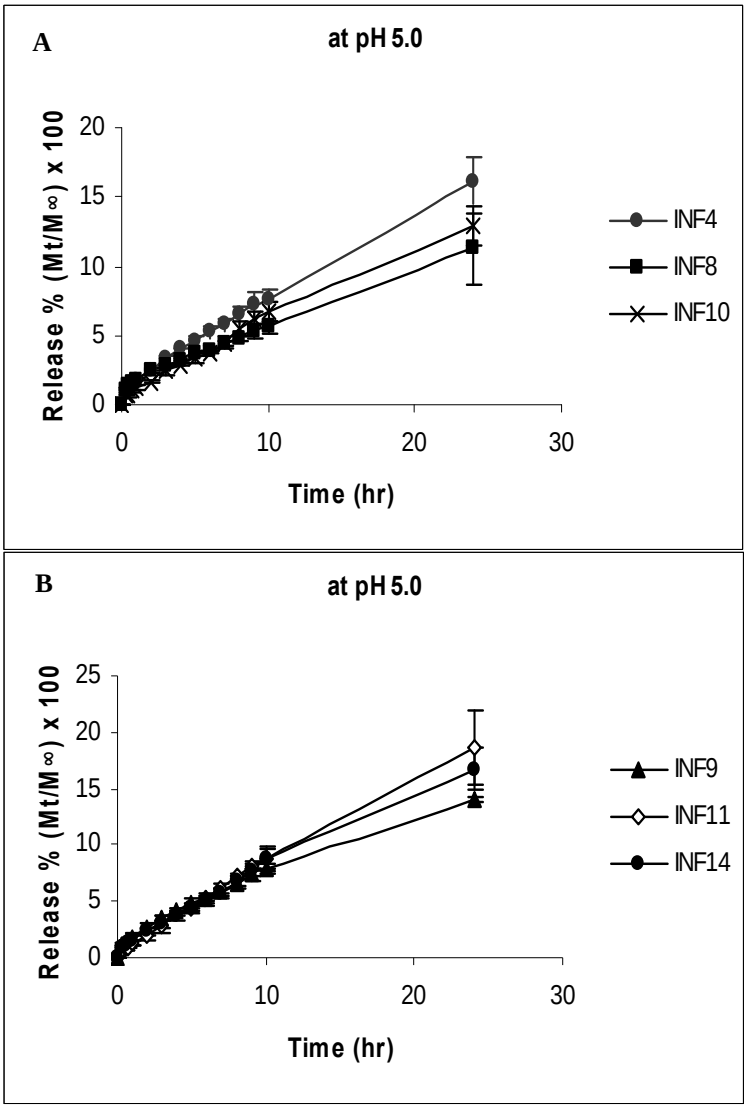

Figure 8. Release study. In vitro release percentage (%) – time profiles of Indomethacin from different lipid nanoparticles using the in vitro dialysis tubes in pH adjusted to 5.0 and surfactant containing aqueous media at 32 ± 0.1°C. SLNs contain (A) 10% (w/w) or (B) 5% (w/w) lipid phase. Each value represents the mean ± S.D. (n = 3)

Statistical analysis of 10 and 24 hrs data release of IND (table 4) show that after 10 hrs from starting the experiment, there was a significant difference ($p < 0.05$) in the amount of released IND depending upon the

composition of SLN dispersions. In addition, the quantity of IND released from the nanoparticles over the tested period of 24 hrs doesn't reach 20% and there was insignificant difference ($p > 0.05$) in the amount of released drug depending upon the composition of the formulations.

Table 4. Release study. One Way - ANOVA analysis of 10 and 24 hrs data release of Indomethacin loaded in SLNs at pH 5.0

	At 10 hours Release			At 24 hours Release		
Formula	**Mean Release ± S.D. (%)**	**F-value**	**p- value**	**Mean Release ± S.D. (%)**	**F-value**	**p- value**
INF4	**7.53 ± 0.85 [ab]**	**5.151**	**0.035***	**16.15 ±1.69 [a]**	**3.402**	**0.084**
INF8	**5.63 ± 0.45 [a]**			**11.24 ± 2.55 [a]**		
INF9	**7.90 ± 0.34 [ab]**			**14.11 ± 0.24 [a]**		
INF10	**6.73 ± 0.64 [ab]**			**12.95 ± 1.47 [a]**		
INF11	**8.72 ± 0.90 [b]**			**18.61 ± 3.22 [a]**		
INF14	**8.73 ± 1.05 [b]**			**16.76 ± 1.96 [a]**		

* = there is a significant difference between groups by using One- Way ANOVA.
The same letter (a, b) means no significant difference between the formulae.

The drug enriched shell model was proposed for drug distribution within the lipid matrix and although of factors such as large surface area, high diffusion coefficient (small particle size), short diffusion distance of the drug, the slow drug release at pH 5.0 was determined by the low solubility of IND in the dissolution medium and the presence of hydrogen-bonding between IND molecules and the hydroxyl groups of glycerides- containing lipids in the nanoparticle core[33]. In addition, it was noticed that the more monoglycerides present, the better the solubility of the drug substance (monoglycerides have surfactant properties)[6] and the less IND was released from the SLNs. The Cetyl palmitate- based SLN (INF4) differed from the other formulations with IND, because it released IND in a relatively high amount. This could be due to the lower melting point of lipid (Cetyl palmitate, m.p. 54°C), which may result in a higher mobility at the temperature used in the release experiment. A difference in release profiles caused by a difference in lipid melting points was also suggested by Paolicelli et al. 2009[58] in a study with ibuprofen and acylglycerols differing in melting points. Furthermore, the presence of other colloidal structures apart from SLNs can be supposed with melting points may be lower than that of the bulk [6]. Another reason could be the absence of monoglycerides resulting in low solubility of IND in Cetyl palmitate. Moreover, table (4) shows that a SLN named INF4 and a NLC named INF10 sustained similar levels ($p > 0.05$) of IND release at both 10 and 24 hrs; means that there was no statistical difference related to the presence of Labrafac™ Lipophile WL 1349 in the SLN. Additionally, the drug - lipid ratio insignificantly affects IND release from either SLNs or NLCs at both 10 and 24 hrs.

The formulations were further subjected to release kinetics studies. The data from the 24 hrs of the experiments were fitted to zero- order, first- order, and Higuchi equations and the results were exhibited in table (5). Release data from all samples fit a zero order model that provided the highest value of squared correlation coefficient (R^2), apart from the formula INF9, which followed Higuchi kinetics. Zero kinetics was verified by

plotting the cumulative amount released drug versus time. The zero order release constants which are the slopes of the equations are listed in table (5) and considered to be characteristic of the formulation[44]. The zero order release profiles from SLNs may be explained by enormous surface area provided by small monodisperse particles (data not shown). The Higuchi model of IND release from a SLN named INF9 has been based on Fick's law where the release occurs by the diffusion of drugs within the delivery system[27]. Moreover, the pH sensitivity of the hydrogen bonding interactions between the carboxylic acid groups of IND molecules and the hydroxyl groups of glyceride-containing lipids in the core have a bearing on the release rate. On the contrary to what happens at pH 7.4 (data not shown), IND is in undissociated state at pH 5.0, and as a consequence, its solubility will not be affected and the physical interactions within the structure insignificantly affected. Therefore, the drug release rate from glyceride containing lipids at pH 5.0 will be slower than the release rate at pH 7.4 [33,61]. Finally, it was concluded that IND release from SLNs can be influenced and optimized by considering (1) the nature of the lipid matrix such as the overall lipid polarity and its interaction with the aqueous phase, (2) the solubility of IND in the lipid excipients, (3) the surfactant concentration, and (4) the nature of the receptor medium[62].

Table 5. Release study. Squared correlation coefficients (R^2) obtained in case of application of different kinetic models to the data release of Indomethacin loaded in the nanoparticles

Formula	Release at pH 5.0			
	Zero Order [a]	First Order [b]	Higuchi Order [c]	Zero Order Rate Constant
INF4	**0.991**	0.772	0.936	0.64
INF8	**0.979**	0.794	0.947	0.43
INF9	0.964	0.731	**0.975**	2.80 *
INF10	**0.985**	0.804	0.931	0.54
INF11	**0.995**	0.785	0.920	0.78
INF14	**0.989**	0.774	0.930	0.69

[a] Extent of a linear relationship between F and time.
[b] Extent of a linear relationship between positive values of log F and time.
[c] Extent of a linear relationship between F and square root of time.
* The number refers to Higuchi release constant.
Bold print indicates the best Fits.

4. CONCLUSION

In this study, several blank and IND containing SLN dispersions were prepared. The physicochemical properties of the resulting nanoparticles were characterized and compared with respect to crystallinity of lipids, possible interaction between the drug with the lipids, rheological characteristics, and release profiles at pH 5.0. IND was used as a model lipophilic drug and loaded at two different drug – lipid ratios. Insightful characterization by DSC experiments showed crystalline particles at room temperature exhibited a shift of the

melting endotherm of the lipid phase, with the maximum at a temperature value higher than that of the empty or loaded SLNs except in Geleol™ - based systems. The production of nanoparticles reduces the degree of crystallinity. For the investigated formulations a percentage of crystallinity between 61.0 % and 88.33% was found. Furthermore, IND suffered a marked reduction in its crystallinity and had better solubility in both Geleol™ and Compritol® 888 ATO if compared to Cetyl palmitate. Hydrogen bonding can be one reason for the observed loss of IND crystallinity and the solubility of the drug in the lipid melt is considered to be an important precondition to obtain sufficient entrapment efficiency. The prepared nanoparticles show a creamy viscous texture and a complete dependence of the viscosity from the shear stress. Besides, in vitro experiments revealed a promising delayed and sustained activity of IND- loaded SLN/NLC formulations. Release studies at pH 5.0 showed very slow and controlled IND release with kinetics following zero order model. The pH sensitivity of the hydrogen bonding interactions between the carboxylic acid groups of IND molecules and the hydroxyl groups of glyceride- containing lipids in the core have a bearing on the release rate. The active compound may be enriched in the shell of the particles. Slow drug release observed with the developed IND-loaded SLNs would be advantageous over the commercial products and the present results suggest a new opportunity for IND to be employed in a well controlled and modern topical formula. Production of IND- loaded SLNs with new cosmetical solid lipids and its future formulation as a topical dosage form could be new, cost effective and commercially viable alternative to the commercial products.

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الجسيمات الدهنية الصلبة النانوية كناقلات الإندوميثاسين للاستخدام الموضعي: المسح التفاضلي الحراري، انطلاق الدواء والخواص الريولوجية

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ملخص

في هذه الدراسة تم تحضير جسيمات دهنية صلبة و ناقلات دهنية نانو تركيبية تحتوي أو لا تحتوي على الإندوميثاسين من دهون السيتيل بالميتات أو الجيليول أو الكمبريتول ATO 888 باستخدام تقنية التجانس الساخن. في جميع النظم كانت المادة النشطة سطحيا المستخدمة هي استر السكروز مع حمض دهني SP 30 (HLB 6.0).

في هذه الورقة البحثية تم إجراء مسح تفاضلي حراري (DSC) على العينات المجففة تجميدا، وأظهر جسيمات بلورية على درجة حرارة الغرفة، وأظهرت النتائج تحولا في درجة حرارة ذوبان الدهون، مع الحد الأقصى بقيمة لدرجة الحرارة أعلى من تلك الظاهرة في الجسيمات الفارغة أو المحملة باستثناء الأنظمة المستندة إلى دهن الجيليول. إن إنتاج الجسيمات النانوية قد خفض درجة التبلور للدهون المستعملة، وبالنسبة للتركيبات المفحوصة تم الحصول على نسبة تبلور ما بين 61.0٪ إلى 88.33٪ وعلاوة على ذلك، فقد تبين انخفاض ملحوظ في تبلور الإندوميثاسين وكانت درجة ذوبانيته أفضل في كل من الجيليول والكمبريتول بالمقارنة مع السيتيل بالميتات. إن الرابطة الهيدروجينية بين الإندوميثاسين والدهون المحتوية على الجليسيريدات يمكن أن تكون أحد الأسباب المسؤولة عن الفقدان الملاحظ في تبلور الإندوميثاسين. وتعتبر ذوبانية الدواء في منصهر الدهون أحد الشروط المسبقة المهمة للحصول على حوصلة كافية للدواء. ولقد كانت كل التركيبات المحضرة ذات ملمس كريمي ولزج، خصوصا تلك الأنظمة المستندة إلى الجيليول وأكدت تحاليل الانسيابية أن جميع النظم تظهر اعتمادا كاملا للزوجة على إجهاد القص (shear stress). وإن المقارنة بين منحنيات انطلاق الإندوميثاسين من التركيبات المختلفة عند درجة حموضة 5.0 تثبت أن هذه النظم مناسبة للتركيبات الجلدية المتطورة. وأظهرت الدراسات عند درجة حموضة 5.0 على مدى 24 ساعة أن انطلاق الإندوميثاسين يكون بطيئا لفترات طويلة مع حركية انطلاق تتبع نموذج النظام الصفري. إن كمية الإندوميثاسين الصادرة من الجسيمات النانوية وعلى مدى فترة اختبارها (24) ساعة لم تصل إلى 20٪ من أصل الكمية، ولم يكن هناك اختلاف ذو دلالة إحصائية بين الكميات المنطلقة تبعا لتكوين التركيبات.

إن إنتاج الجسيمات الدهنية الصلبة المحملة بالاندوميثاسين من دهون صلبة جديدة ذات استخدام تجميلي، وصياغتها في المستقبل كشكل صيدلاني يمكن أن يكون جديدا، وبديلا فعالا من حيث التكلفة، ومجدياً تجاريا عن منتجات تجارية أخرى.

الكلمات الدالة: الجسيمات الدهنية الصلبة، ناقلات دهنية نانو تركيبية، استر سكري، إندوميثاسين، انطلاق متحكم.

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