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Preparation, Characterization and In Vitro Evaluation of Indomethacin Loaded Solid Lipid Nanoparticles

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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. In all systems the surfactant was sucrose fatty acid ester SP 30. The systems were characterized through particle size analyses and zeta potential measurements. The entrapment efficiency of the particles was calculated. ^{1}H-NMR of the nanosuspensions allowed investigating the crystallinity of the particles. Furthermore, release experiments of a model drug (Indomethacin) were performed and the release kinetics was 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. From the results obtained, SLNs with a narrow monomodal size distribution range from 190 to 400 nm were produced. The polydispersity results showed a polydispersity index (PdI) in general lower than 0.25. The incorporation of IND in most cases increased the sizes of the SLN particles and the particles aggregation which was represented by significantly higher Z-average (intensity weighted mean of particle sizes) and PdIs. The freshly prepared SLNs have a potential greater than -30 mV which was sufficient for a stable system in combination with the sterically stabilizing effect of the emulsifier. Glyceride SLNs showed good drug encapsulation but with a formation of aggregates. In contrast, wax- based SLNs possessed good physical stability with no aggregation but lacked sufficient drug encapsulation. These differences were attributed in part to different crystal packing. ^{1}H-NMR results showed crystalline particles at room temperature. The comparison among the release profiles of different formulations allows affirming that these systems are suitable for modified oral delivery formulations. Release studies at pH 7.4 showed that IND release exhibited a biphasic pattern with an initial burst and prolonged release over 24 hours with kinetics obeying Higuchi model. Production of IND- loaded SLNs with new solid lipids and its future formulation as tablets could be new, cost effective and commercially viable alternative to the commercial products.

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

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preparation process, a wide potential application 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 [10-13].

Common disadvantages of SLNs include particle growth, unpredictable 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. Liquid lipids usually show a better solubilization of drugs than solid lipids [14-17]. NLCs can be considered as an upgrade of the solid lipid nanoparticles even though among scientists, the term SLNs is still used to indicate the NLCs, creating no clear differentiation [5].

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 SLN suspensions 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].

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 and concentration, lipid type and crystallization pattern and the method of production. Usually, the drug substance is dissolved or dispersed in the melted lipid phase before preparation by homogenization to achieve efficient encapsulation in the lipid. However, as the lipid is in the liquid phase during the preparation process, the mobility of the drug substance is high, and it may partition between the liquid lipid and the aqueous surfactant solution dependent on the drug substance lipophilicity. The incorporation efficiency is therefore likely to strongly depend on the drug substance lipophilicity and solubility in the two phases. Additionally, the drug substance solubility in the liquid lipid during the processing may be higher than in the solid lipid.

By the oral delivery, these systems may provide enhanced and/or less variable bioavailability and prolonged plasma levels, which is postulated due to controlled, optimized release in combination with general adhesive properties of small particles [21].

Non steroidal anti-inflammatory drugs (NSAIDs) are widely used medicines for the treatment of osteoarthritis, rheumatoid arthritis, inflammations and a variety of pains [22]. One of the most potent NSAIDs, Indomethacin (IND) is widely used and the oral therapy is very effective [23], but its clinical use is often limited by the irritation and ulceration of the gastrointestinal mucosa [24], while its short elimination half-life (< 6 hrs) requires frequent

dosing. Due to these problems, several studies are aimed at the development of an efficient means for IND administration [25]. Therefore, a carrier system able to deliver the drug for prolonged periods of time after oral 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 [26-28]. 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 [29].

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 [30- 32].

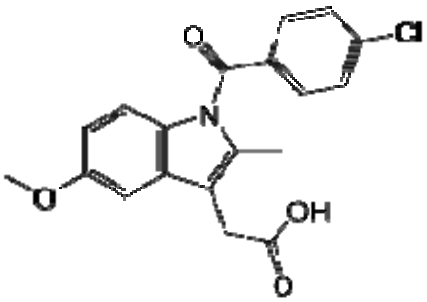

Figure 1. Introduction. Structure of Indomethacin [2-{1-[(4-chlorophenyl) carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl} acetic acid].

The aims of the study were (1) to examine the feasibility of preparing IND- loaded lipid nanoparticles using lipids and surfactants accepted as having low toxicity and excellent biodegradability. The interest in using sugar esters (SEs) as non ionic surfactants is increased includes pharmaceutical technology as emulsifiers, solubilizing agents, lubricants, penetration enhancers and pore forming agents. They can be applied in the cosmetical applications and as food additives as well [33- 34]; (2) to get experimental evidence on the ability of lipids with different compositions, hydroxyl numbers, polarities and various drug solubilities stabilized by a surfactant which can induce both electrical and steric stabilization to incorporate IND; (3) to highlight on the lipid monoglyceride contents and fatty acid chain length in governing drug incorporation and delivery. To our knowledge, there are still no reports on the study of effect of solid lipid monoglyceride contents on the entrapment efficiency of IND; (4) to investigate the physiochemical behavior of the prepared SLNs and NLCs in more detail and to compare the physicochemical behavior of Cetyl palmitate nanoparticles (waxy lipid) with SLNs prepared from Geleol™ or Compritol® 888 ATO as glyceride containing lipids; and finally (5) it was the major goal of this study to develop a nanoparticualte; lipid based drug carrier with increased payloads, increased physical stability and controlled release properties.

Nanoparticles prepared in this work were characterized by size, zeta potential, entrapment efficiency, and ^{1}H-NMR. The influence of surfactant percent, lipid type, lipid amount, and drug loading was investigated. The effect of the key properties of the lipid dispersion on the in vitro release was also examined. The physicochemical characteristics of drug loaded SLNs were compared with the empty SLNs to get optimized formulation of IND.

Even though the role of the vehicle of formulations is known, this study may provide additional features to state if the literature- mentioned effect is general or just a particular case. This work may confirm the great potential of lipid nanoparticles as carriers for prolonged oral delivery.

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), while, Sephadex® G 75 was supplied by Sigma life science (Sigma- Aldrich, Sweden).

Potassium dihydrogen phosphate and methanol were from Carlo Erba Reagenti (Rodano, Milano, Italy), while deuterium oxide was from Sigma –Aldrich (Switzerland) and Tween 80 was from 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 [35-36]. To the lipid phase melted at a temperature 10°C above its melting point, the aqueous solution (45 ml) 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 SLN formulae 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%.

Formulation no.	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.

Formulation no.	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

Particle Size and Surface Charge

The mean hydrodynamic particle size (Z- average) of the bulk population of the particles, and the polydispersity index (PdI) as a measure for the width of the distribution [ranges from 0 (monodisperse) to 0.500 (very broad distribution)] were measured by photon correlation spectroscopy (PCS) using dispersion technology Zetasizer software 6.32 (Nano ZS90 Zetasizer, Malvern instruments Corp., U.K.). The freshly prepared SLN/NLC dispersions were analyzed using a helium-neon laser with a wavelength of 633 nm. Photon correlations of three spectroscopic measurements were carried out at a scattering angle of 90° at a temperature of 25°C after equilibrium time 30 seconds in 10 mm diameter disposable polystyrene cuvettes. A 1: 100 dilution of the formulations was made using double distilled water before the measurement.

Additionally, the surface charge of all samples was determined by measurements of the zeta potential carried out with the same instrument. The zeta potential gives information about the long- term stability. Zeta potential of –30 mV and above characterizes a stable formulation in electrically stabilized formulation, while zeta potential in the range of -25 mV in combination with steric stabilization is sufficient to physically stabilize SLN dispersions [20]. All the measurements were performed in the bore capillary applying field strength of 20 V/cm at 25°C. The electrophoretic mobility of particles in the electric field was converted into zeta potential by using the Helmholtz - Smoluchowski equation [37]. The zeta potential measurements were performed on the same dilutions used for the particle size and PdI measurements.

All analyses were performed in triplicates and the results were expressed as the mean ± S.D.

Determination of Entrapment Efficiency and Drug Loading

The percentage of IND entrapped or loaded in the lipid matrix was determined either by one of two methods: In the first method; the free drug was removed from SLN dispersions by gel permeation chromatography (GPC) using

Sephadex® G 75 column [3, 11, 24]. Five milliliters of SLN preparations were applied to the column and eluted with distilled water. The recovered nanoparticles were freeze dried. 0.40 gm of the freeze dried SLN were extracted into methanol by four times of extraction and the entrapped extracted IND concentration was analyzed by UV spectrophotometery at 320 nm (Perkin-Elmer Lambda 40 UV/VIS Spectrometer, USA). The percentage of IND incorporated in the SLNs was calculated relative to the concentration of IND extracted and analyzed from the same amount of freeze dried SLN suspensions before GPC.

The incorporation efficiency was also determined indirectly by measuring the amount of free IND using UV/VIS spectrometer at 320 nm in the supernatant [30, 38] recovered after ultracentrifugation (Thermo Scientific SoRvall WX80 Ultra Series Centrifuge, Milan, Italy) at 50,000 rpm for 3 hrs at 15°C. These conditions were varied and modified to find the best conditions to precipitate the particles from different SLN samples. The drug entrapment efficiency was expressed as percentage of the IND difference between the initial amount of IND and the free amount in the supernatant relative to the total amount used for the nanoparticles preparation [39].

IND incorporation efficiency was expressed both as drug entrapment (EE %) and drug loading capacity (DL %). The entrapment efficiency of IND in SLN was determined as the ratio between actual and theoretical loading, using the following equation:

Drug Entrapment (EE %) =

$$\frac{Amount\ of\ drug\ in\ nanoparticles}{Amount\ of\ drug\ fed\ to\ the\ system} \times 100 \qquad (1)$$

Drug loading capacity was calculated as drug analyzed in the nanoparticles (or calculated indirectly from supernatant analysis) versus the total amount of the entrapped drug and the lipid added during the preparation [40- 42], according to the following equation:

Drug Loading (DL%) =

$$\frac{Amoumt\ of\ drug\ in\ nanoparticles}{Amount\ of\ drug\ in\ nanoparticles + amount\ of\ lipids} \times 100 \qquad (2)$$

All analyses were performed in triplicates and the results were expressed as the mean ± S.D.

Calibration curve for the validated UV assays of IND was performed on six solutions in the concentration range 5 - 100 µg/ml. The squared correlation coefficient was 0.998. Each point represents the average of three measurements and the error was calculated as a standard deviation (± S. D.)

Possible lipid interferences during UV determination of IND were also investigated by comparing the standard curve of the drug alone and in the presence of the lipids [5]. The differences observed between the standard curves were within the experimental error, thus inferring that no lipid interference occurred (data not shown).

NMR Analysis

The crystallinity states of blank and IND- loaded SLNs were determined by ^{1}H-NMR analysis. The NMR spectra were obtained with a Bruker AC-400 instrument (Germany). ^{1}H-NMR spectrum was recorded on samples of the nanosuspensions (100 mg) diluted with D_2O (0.7 ml) just before the experiment [43].

Release Study

The release studies were carried out using the vertical dialysis tubes 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 [6, 13]. The solubility of IND in phosphate buffer pH 7.4 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 37°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:

$$Q=V_s \cdot \sum_{n=1}^{n} C_{n-1} + V_m \cdot C_n \quad (3)$$

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. 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.

Drug release kinetics was determined by applying three kinetic models to the data to find the best fitting equations [41]. These kinetic models were as follows:

$F = k_0 t$ Zero order equation (4)

$Ln F = \ln F - k_1 t$ First order equation (5)

$F = k_2 t / 2$ Higuchi equation (6)

Where, F is the fraction of drug released in time t, and k_0, k_1, and 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 10 hrs of release data.

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 [42].

3. RESULTS AND DISCUSSION

Hot homogenization method, being quick with simple laboratory setup, was used in the preparation of SLNs and NLCs in the present study. All formulations were produced at temperatures higher than their melting points by 10°C [44] to ensure that the lipid remains in the liquid state during the production process. After preparation, all formulations exhibited a macroscopic homogenous appearance of a milky white opalescent liquid. All formulations were of creamy texture and viscous especially Geleol™ - based systems. Blank 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 [45-46]. 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 subsequently reduced aggregation during the storage [47]. Increasing the lipid content above 5-10% in most cases resulted in larger particles, including microparticles, broader particle size distribution, and an increase in particle agglomeration [4]. Accordingly, lipid concentrations above 10% were not studied.

Tables (3) and (4) list some parameter values of SLNs characterization and the amount of loaded drug in SLN dispersions. Additionally, the pH of different preparations was measured. The pH of blank SLNs ranged from 6-7 and for IND- loaded SLNs was from 5-6.

Table 3. Results and Discussion. The characterization of blank formulations by particle size, polydispersity index (PdI) and Zeta potential (n = 3).

Formulation no.	Z- Average* (nm) (Mean ± S.D)	PdI (Mean ± S.D)	Zeta Potential (mV) (Mean ± S.D)
F1	263.0 ± 23.30	0.201 ± 0.112	- 38.5 ± 1.49
F2	193.0 ± 11.70	0.257 ± 0.113	- 39.7 ± 2.66
F3	189.0 ± 14.70	0.194 ± 0.065	- 34.9 ± 0.96
F4	193.0 ± 3.27	0.221 ± 0.013	- 35.2 ± 0.79
F5	303.3 ± 30.56	0.245 ± 0.166	- 38.6 ± 0.80
F6	258.2 ± 5.74	0.177 ± 0.059	- 37.5 ± 0.48
F7	233.1 ± 7.49	0.191 ± 0.030	- 35.8 ± 0.55
F8	223.8 ± 5.77	0.194 ± 0.022	- 36.8 ± 0.87
F9	202.5 ± 3.24	0.121 ± 0.025	- 30.4 ± 0.60
F10	207.9 ± 9.93	0.200 ± 0.033	- 38.2 ± 1.74
F11	302.3 ± 10.50	0.245 ± 0.028	- 45.1 ± 1.35
F12	404.5 ± 40.78	0.371 ± 0.144	- 31.1 ± 0.70
F13	249.2 ± 37.33	0.539 ± 0.238	- 32.9 ± 0.78
F14	288.0 ± 21.55	0.266 ± 0.013	- 34.5 ± 1.62

Table 4. Results and Discussion. The characterization of loaded formulations by particle size, polydispersity index (PdI), Zeta potential, entrapment efficiency and drug loading of Indomethacin (n = 3).

Formulation no.	Z- Average [a] (nm) (Mean ± S.D)	PdI (Mean ± S.D)	Zeta Potential (mV) (Mean ± S.D)	Entrapment efficiency % [b] (Mean ± S.D)	Drug Loading % (Mean ± S.D)
INF4	357.6 ± 67.74	0.379 ± 0.098	- 37.9 ± 0.54	12.12 ± 0.520	0.896 ± 0.036
INF8	384.5 ± 70.80	0.381 ± 0.093	- 34.8 ± 3.35	92.37 ± 0.008	6.478 ± 0.005
INF9	242.4 ± 28.22	0.354 ± 0.117	- 27.5 ± 1.09	95.02 ± 0.733	12.469 ± 0.085
INF10	308.8 ± 45.11	0.303 ± 0.144	- 37.4 ± 2.46	96.34 ± 0.963	6.735 ± 0.062
INF11	389.6 ± 78.29	0.175 ± 0.082	- 39.6 ± 0.51	n.d. *	n.d.
INF14	211.5 ± 58.47	0.421 ± 0.087	- 25.3 ± 2.32	n.d.	n.d.

a: Z- Average refers to the intensity weighted mean of particle sizes.

b: all numbers were calculated using ultracentrifugation, except INF4.

***:** n.d. means was not determined or calculated by any of the two methods.

Particle Size and Polydispersity Index

The size of the particles depends on the production variables, the nature & concentration of the lipid matrix and the surfactant [48]. The viscosity of the lipid and aqueous phase also influence the outcome [15].

Leaving all the other parameters constant, in this part of study, the only variable was the composition of the lipid matrix, which has been changed from Cetyl palmitate to Geleol™ or Compritol®. A screening for each lipid was performed to determine the optimum surfactant concentration to yield a physically stable SLN in a narrow size range (approximately 100-300 nm). These optimized formulations were used for subsequent investigation of entrapment efficiency. The concentration of surfactant needs to be minimized due to toxicological reasons [21].

Table (3) shows that physically stable SLNs with a narrow size distribution were produced. All blank formulations show one homogenous peak and possessed a mean size range from 190 nm to 400nm (after one day of production). The particle size was the lowest ($p \leq 0.05$) for the highest concentration of surfactant and increased with decreasing the concentration taking into consideration the particle sizes with the associated inter-batch variations. The results were explained by the ability of high concentrations of the stabilizer to reduce the surface tension and facilitate the particle partition [42]. The choice of the stabilizer and its concentration is of great impact on the quality of SLN dispersions.

In addition, PCS results in table (3) show that Cetyl palmitate and Geleol™ - based SLNs were of different sizes (mean diameter 209.5 nm and 254.6 nm, respectively), while Compritol® 888 ATO- based SLNs were significantly larger (F12, 404.5 nm; F13, 249.2 nm and F14, 288.0 nm). It has generally been observed that the average particle size of SLN dispersions increased with higher melting point lipids and as the monoglyceride content of the lipids increased. The increase in particle size of SLNs in the presence of more monoglycerides content of the lipids was explained by Jensen *et al.,* 2010 [6] and related to the higher degree of interactions with water when more monoglyceride is present. Monoglycerides belong to the group of polar lipids that are water-soluble within the hydrophilic parts of their structure. In the presence of water, these lipids will recognize into the aqueous – organic interface, which accounts for their surfactant properties.

Furthermore, it was found that the particle size of the NLC system (F10) approaches the size of the corresponding SLN (F4) at oil loading of 10% (with regard to the lipid phase). There is no dependence of the particle size on the oil load. The SLN named (F4) showed a mean size of 193 nm, which was comparable ($p > 0.05$) to the NLC system (F10) of 207.9 nm. This can be explained as follows [15]: Labrafac™ is composed of caprylic/capric triglycerides; on the other hand Cetyl palmitate is a pure fatty ester. Distances between fatty acid chains can be increased by using glycerides composed of different fatty acids, resulting in particle sizes with larger spaces. Another possibility is the influence of viscosity since the trend to increase in particle size may be due to increasing viscosity. The viscosity of F10 which shows the larger size was higher than that of F4 (data not shown). Since the change in the particle size was insignificant, this suggests that NLCs are forming a liquid compartment that has strong interactions with the solid lipid [29].

Moreover, the polydispersity results showed that the particle distribution was monomodal with PdIs were in general lower than 0.25, and with slight inter-batch variations. Polydispersity indices of Compritol® - based SLNs were higher than 0.25 (F12, 0.37; F13, 0.53 and F14, 0.26). The results confirmed that solid particles and NLCs are constituted as a homogenous population except Compritol® - based systems (F12-14) where a micrometric subpopulation is present. Although these lipid dispersions show some aggregation and despite the semisolid consistency, their particle sizes are not negatively affected by these few agglomerates and the lipid dispersions preserved their colloidal particle size. The results indicate that individual particles are associated to a network structure by particle-particle interaction without sintering of the particles to larger agglomerates.

Besides, the results revealed that low concentrations of lipid (5% w/w) allow production of SLNs as small as

200-300 nm (table 3; F9, F11 and F14). Generally, the particle size increases with higher lipid amounts. This effect was not important between 5 and 10% lipid concentration, above these percentages, microparticles were always obtained. The fact that SLN formation is highly dependent on lipid concentration [10] can be explained in terms of the tendency of lipid aggregates to coalesce at high lipid concentrations. Furthermore, the high viscosity and low limiting concentration for lipid aggregation at the interface will cause a fewer lipid molecules to be carried into the aqueous phase. Therefore, the formation and stabilization of small lipid aggregates at these concentrations are reduced.

Figure (2) shows the mean particle size and polydispersity index of two lipid dispersions only differing in their lipid content (F8, F9). Compared with a 5% SLN dispersions which is still a liquid dispersion with no yield value, the lipid dispersion with increasing lipid content differ only little concerning particle size. Also, the polydispersity index increases only slightly but stays still below 0.2 indicating a narrow size distribution.

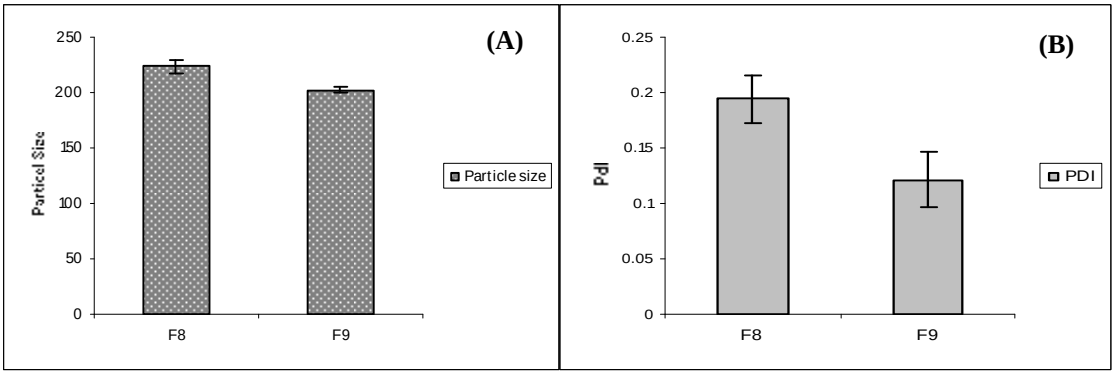

Figure 2. Particle size and Polydispersity index. (A) The mean particle size (Z-average), and (B) polydispersity index (PdI) of blank F8 and F9 measured by PCS after one day of production at room temperature.

The results of table (4) show that the incorporation of IND in general increases the sizes of the SLN particles and the particles aggregation which was represented by significantly higher Z-average and PdIs. Additionally, the aggregation was more apparent in Geleol™ - and Compritol® 888 ATO- based systems, resulting in the formation of few but large particles that could be removed by centrifugation. The results show that blank SLNs were distributed completely in the nanometer range, while small populations of particles within the lower micrometer range or diameter >1 μm were detected in IND- loaded SLNs. IND- loaded systems showed up to 3 peaks in the particle size distribution, which was also reflected by a higher standard deviation.

The physical stability of nanoparticualte dispersions can be defined by the formation of small particles having a narrow monomodal size distribution after production and by the absence of aggregates and considerable particle growth on long- term storage [19]. The results reveal that Cetyl palmitate dispersions are predicted to have excellent long- term stability (unimodal), where as glyceride containing dispersions showed particle growth and micrometer aggregates (bimodal or multimodal). This finding can be explained as follows: The used lipids in this study are with different contents of emulsifying mono- and di- glycerides (quantified by hydroxyl number; Cetyl palmitate 4-10, Geleol™ 300-330, and Compritol® 80-105) and accordingly with different emulsifying capacities for water. This might lead to different contents of water in the SLN lipid matrix which potentially can destabilize particle. The type of surfactant and its concentration can also affect the physical stability of the lipid matrix (e.g. differences in incorporation of the surfactant in the outer shell of the particles). It is reported

that sugar esters offer an effective steric stabilization upon approaching the particles [49-50].

Surface Charge

The measurement of zeta potential allows prediction about the stability of colloidal aqueous dispersions. The results in table (3) shows that freshly prepared SLNs are negatively charged on the surface and have a potential greater than -30 mV, which was higher than the critical values for pure electrostatic stabilization. It is in principle still sufficient for a stable system in combination with the sterically stabilizing effect of the emulsifier sugar ester SP30 [20-21, 50-51].

A significant difference ($p \leq 0.05$) was observed in the high and low stabilizer formulations for all lipids except Cetyl palmitate- based formulations indicating that in general the stabilizer concentration needed to cover the surface of the nanoparticles was important [52]. Furthermore, the zeta potentials changed insignificantly by decreasing the lipid load in Geleol™ - based formulations (table 3; F8 and F9, $p > 0.05$), while this change was significant in NLCs (table 3; F10, F11, $p \leq 0.05$). Moreover, the addition of drug results in a minimal reduction in the negative values of zeta potential except in SLN named F9, where the reduction of zeta potential due to the addition of IND was significant ($p \leq 0.05$). All systems showed unimodal zeta potential distribution before and after the addition of IND. For both unloaded and loaded formulations, the absolute value of zeta potential is higher than - 25 mV and it can be concluded that they are electrochemically stable in the presence of steric stabilizer.

The magnitude of aggregation was dependent on the used lipid and reached from almost no aggregation in Cetyl palmitate system to strong one in Geleol™ and Compritol® 888 ATO systems. It was more apparent in the presence of IND and was accompanied by a significant reduction of zeta potential (table 4, INF9). Or as a consequence of this lower zeta potential, aggregation is facilitated [13]. Previous studies [21] show that the stability of the SLN formulations is dependent on the optimized combination of the lipids with the surfactant. This is attributed to differences in the affinity of the emulsifier to the lipid- water interface during the SLN production. The affinity of the surfactant to the particle surface is less entropy driven, but very much influenced by the hydrophobicity of the lipid surface. Due to these differences in hydrophobicities of different lipids, there are different affinities of the surfactant towards the surface, and as a result leading to differences in surface coverage (no. of surfactant molecules per nm^2). Without complete coverage of the interface with emulsifier, particle aggregation is enhanced; the contact of the uncovered liquid crystalline surfaces can lead to crystal growth between the particles.

Furthermore, the affinity of the emulsifier for the lipid– water interface during SLN production also leads to differences in the anchoring of the lipophilic surfactant parts in the outer shell of the lipid particles, which are liquid during the hot homogenization procedure. These differences are a function of the compatibility and structural similarity of the lipid and the surfactant moiety. Both affinity of the surfactant to the lipid and anchoring of the surfactant in the particle surface result in a different thickness of the stabilizing layer and a different zeta potential. Thus, the same surfactant can lead to different stabilities in combination with different lipids and vice versa [21].

Entrapment Efficiency and Drug Loading

The systems with the smallest sizes and PdIs with notable small standard deviations were extensively studied for entrapment efficiency. Tables (2) & (4) show the drug entrapment efficiencies and loading capacities of the investigated nanoparticles with their corresponding drug – lipid ratios. A more efficient encapsulation of IND was achieved with the more polar glyceride containing lipid Geleol™ compared to the less polar glyceride free Cetyl palmitate, may be due to a higher solubility of IND in this lipid component [28]. Table (4) shows that drug entrapment efficiency and loading capacity of nanoparticles were increased from 12.12 ± 0.52 to 92.37 ± 0.01 % and from 0.90 ± 0.04 to 6.48 ± 0.01%, respectively, by changing the lipid from Cetyl palmitate

to Geleol™ at the same drug lipid ratio (1:13.34). This finding can be ascribed to the strong bond between the carboxyl group of IND molecule and the hydroxyl in glycerides. In addition, less ordered crystal form of the lipid is characteristic of higher drug loading [53].

The presence of Labrafac™ in INF10 was useful to increase the mean drug entrapment and the drug loading % from 12.12 ± 0.52 to 96.34 ± 0.96 and from 0.90 ± 0.04 to 6.74 ± 0.06%, respectively, if compared to INF4 with an equal drug –lipid ratio of 1:13.34. These findings in table (4) can be ascribed to the higher IND solubility in Labrafac™ compared to its solubility in sole Cetyl palmitate. Furthermore, in the presence of IND, Labrafac™ addition tends to promote the formation of a small particle population as a result of a higher molecular mobility of the matrix.

Additionally, the entrapment efficiency was not changed significantly ($p > 0.05$) for samples with lower lipid concentrations. Although, the drug loading was increased from 6.48 ± 0.01 (INF8) to 12.47 ± 0.09 (INF9) and the drug- lipid ratio was increased from 1:13.34 to 1:6.67, the entrapment efficiency was not changed significantly (INF8; 92.37 ± 0.01 and INF9; 95.02 ± 0.73). This could be explained by the high solubility of IND in the lipid Geleol™, even if the lipid concentration presents at a low concentration (5% w/w). This is in contrary with other studies, which shows that as the lipid concentration decreases, the entrapment efficiency decrease [42].

The entrapment efficiency of systems INF11 and INF14 were not measured due to difficulties in separating the free drug. However, their entrapment efficiencies are expected to be high. This is confirmed later by the results of ^{1}H-NMR and release studies. In addition, nanoparticles are characterized by a high entrapment capacity especially for lipid soluble drugs, probably in a consequence of their melting points. It is likely that nanoparticles solidify very quickly, encapsulating the drug almost quantitatively [22, 54].

NMR Analysis

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].

^{1}H-NMR spectra of unloaded SLNs containing different lipids are reported in figure (3). The SLNs scan reveals the crystalline solid character of the nanoparticles at room temperature. The signal at 4.6 ppm is related to the huge water signal. The group of signals in the range 4.84 - 4.92 ppm is characteristic of the hydrophilic portion of sugar ester SP30 emulsifier. The signals of both the lipophilic portion of the surfactant and the lipid phases are totally absent in the spectra. These results agree with the hypothesis that the sugar ester SP30 has the lipophilic portion inside the colloidal particles, which are almost completely in the solid state. On the contrary, the spectrum of Labrafac™ containing SLN (F10) displays signals in the range of 0.81-2.30 ppm that can be assigned to the glycerides, indicating the high mobility of these molecules, characteristic of a liquid state. This result indicates that the colloidal dispersion prepared with Labrafac™ may contain an emulsion of a supercooled melt [10].

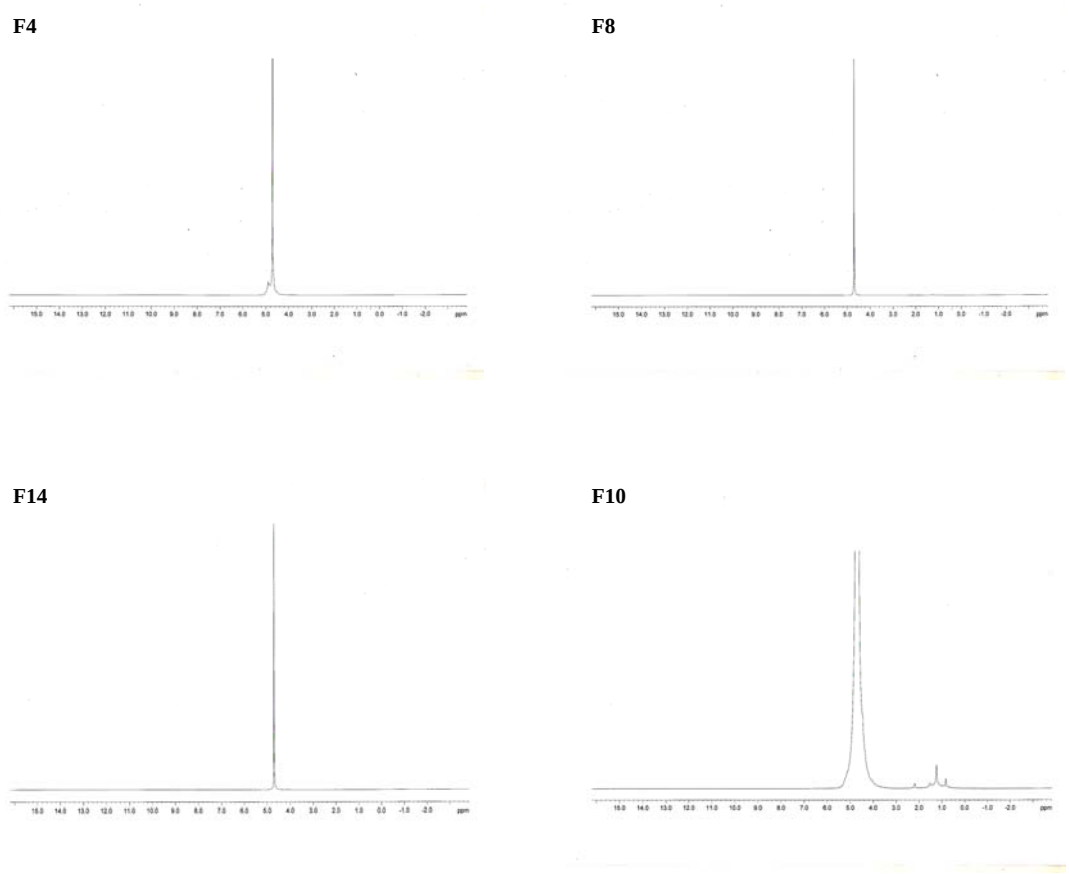

Figure 3. NMR analysis. ^{1}H-NMR spectrum of blank SLNs in D_2O.

The physical state of systems F4 and F10 could account for their physicochemical characteristics (sizes and polydispersity indices reported in table 3). The use of Labrafac^{TM} oil mixed with Cetyl palmitate (F10) as a lipid phase gave rise to particles with an average size and PdIs larger than the SLN named F4. Although of the semisolid state of the lipid matrix of NLC, this increment in particle size was not significant ($p > 0.05$). Possibly, this can be explained by the small percent of the Labrafac^{TM} oil (10%, referring to the lipid phase) used in F10. The trend is not the same for the samples containing IND and can be explained by the possible interaction of IND with Labrafac^{TM} Lipophile WL 1349.

Furthermore, ^{1}H-NMR spectra of loaded SLNs were obtained. The spectra show the absence of dissolution of nanoparticles in the presence of IND and the crystalline character of the IND- loaded nanoparticles was ensured. Additionally, it was expected that crystallization of the lipids in the inner phase reduces the capacity to accommodate foreign molecules [29] and causes the explosion of IND. For example, the highly crystalline particles of F14 may result in stronger expulsion of drug compared to NLC (F11). However, the spectra (figure 4) show no signals for IND protons (chemical shifts of heterocyclic rings are supposed to appear in the range of 6.6-7.6) which indicates the high IND entrapment efficiency. Furthermore, the spectra show the absence of signals of the hydrophilic portion of the sugar ester surfactant, which indicates the interaction of the carboxylic group of IND through a strong bond with the hydroxyl groups of the hydrophilic head of the surfactant. Additionally, although the presence of a small amount of

mobile glycerides in INF10 and INF11, the mobility of the drug is still weak and it's partitioning to the water phase inhibited.

Notably, a signal at 3.26 ppm appeared in the spectra of IND- loaded SLNs was due to methanol protons. Methanol was used in the solubilization of IND before its addition to the lipidic phase.

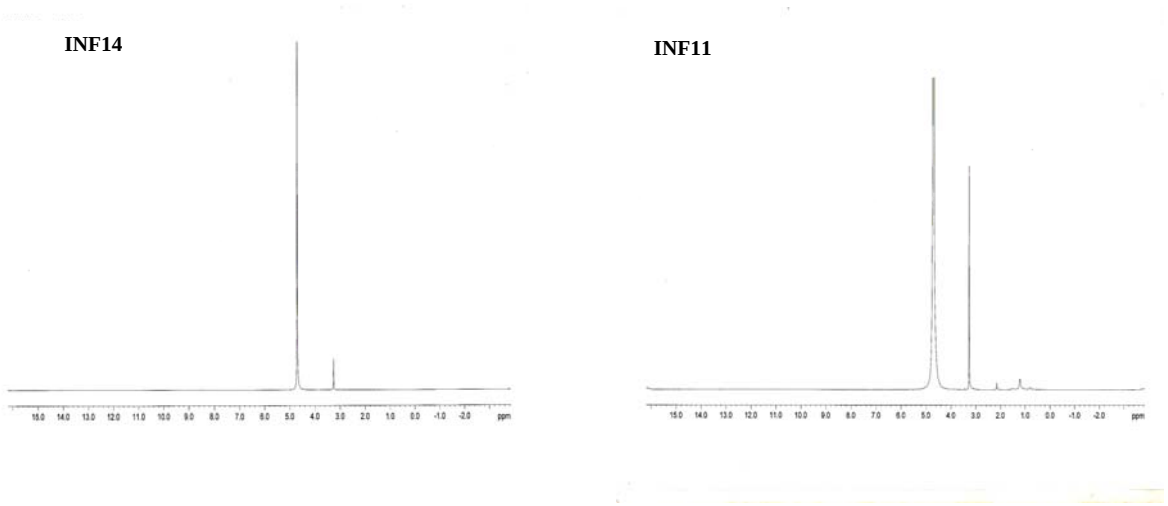

Figure 4. NMR analysis. ^{1}H-NMR spectrum of Indomethacin loaded SLNs in D_2O.

Further information about the encapsulation efficiencies of IND within different systems were further confirmed by the results of release experiments.

Release Study

Many research groups used dialysis bag/ tubes for the study of drug release from solid lipid nanoparticles [6, 13,38, 55]. IND is a weak acid (pK_a of 4.5) and its solubility depends upon the pH of the dissolution medium. IND solubility in phosphate buffer pH 7.4 at 25°C was 5.7 mg/ml. To maintain sink conditions and to prevent the saturation of the receptor medium during the release study, the final concentration of the drug was always lower than 10% (w/w) of its solubility in the receptor medium. The obtained IND release data and profiles from different formulations at pH 7.4 are shown in figure (5).

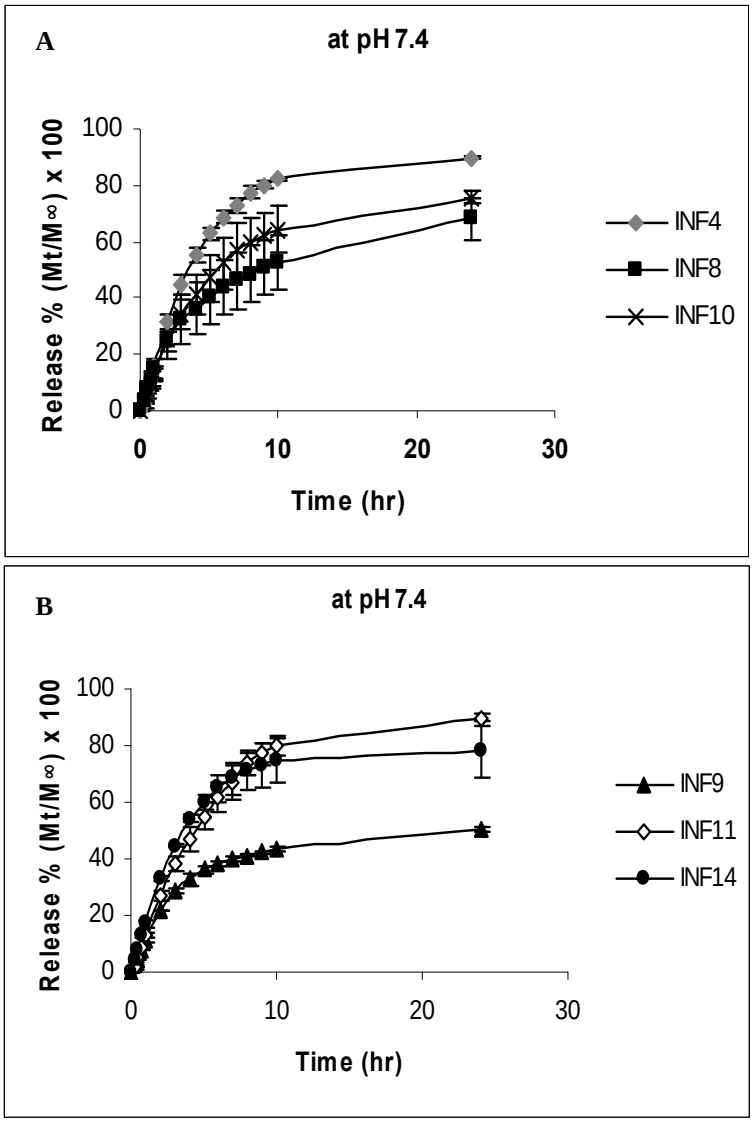

Figure 5. Release study. In vitro release percentage (%) – time profiles of Indomethacin from different lipid nanoparticle systems using the in vitro dialysis tubes in pH adjusted to 7.4 aqueous media at 37 ± 0.1°C. (A) SLNs contain 10% (w/w) lipid phase. (B) SLNs contain 5% (5%) lipid phase. Each value represents the mean ± S.D., n = 3.

At pH 7.4, the results show that the diffusion of free IND through the cellulose membrane was sustained over the 24 hrs. Statistical analysis showed differences ($p \leq 0.05$) in the diffusion of free drug from different formulations at 10 and 24 hrs (table 5). Table (5) shows that after 10 and 24 hrs, the lowest release was related to the SLN named INF9 (43.67 and 50.74%, respectively) and the highest release was correlated with the SLN named INF4 (82.35 and 89.75%, respectively).

Table 5. Release study. One- Way ANOVA analysis of 10 and 24 hrs percent release of the prepared SLNs at pH 7.4.

Formulation no.	At 10 hours Release			At 24 hours Release		
	Mean Release ± S.D. (%)	F-value	p-value	Mean Release ± S.D. (%)	F-value	p-value
INF4	82.35 ± 0.53 ^{c}	12.283	0.004*	89.75 ± 0.37 ^{c}	15.170	0.002*
INF8	52.67 ± 9.43 ^{ab}			68.05 ± 7.70 ^{b}		
INF9	43.67 ± 0.68 ^{a}			50.74 ± 0.82 ^{a}		
INF10	64.38 ± 8.50 ^{abc}			75.61 ± 2.25 ^{bc}		
INF11	79.84 ± 3.55 ^{c}			89.34 ± 2.16 ^{c}		
INF14	74.43 ± 7.86 ^{bc}			78.31 ± 9.97 ^{bc}		

* = there is a significant difference between groups by using One- Way ANOVA.

The same letter (a, b, c) means no significant difference between the formulae.

The release pattern revealed that there was an initially burst effect within the first hour (11.03-17.28%) 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 [56-57]. The drug enriched shell model is proposed for drug distribution within the lipid matrix and factors such as large surface area, high diffusion coefficient (small particle size), short diffusion distance of the drug and the pH of the receptor medium contributing to a relatively fast release [42, 56, 58]. Nearly 89% (INF4 and INF11) of drug was released during the 24 hrs study.

At 10 and 24 hrs, the release of IND from Geleol™ - based SLNs was significantly less than from Cetyl palmitate- based SLNs or Compritol® - based SLNs. Table (5) shows that IND release was from INF8 < INF4 and from INF9 < INF14. It was noticed that the more the monoglycerides present, the better the solubility of the drug substance (monoglycerides have surfactant properties) [6] and the less IND was released from the SLN.

The Cetyl palmitate- based SLN (INF4) differed from the other compositions 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. It is well known that the melting point of the colloidal structures may be lower than that of the bulk due to the influence of surface energy [6]. A difference in release profiles caused by a difference in lipid melting points was also suggested by Paolicelli *et al.,* 2009 [54] in a study with ibuprofen and acylglycerols differing in melting points. Another reason could be the absence of monoglycerides and the consequently the low solubility of IND in Cetyl palmitate.

Furthermore, Table (5) shows that the SLN (INF4) and the NLC (INF10) systems sustained similar levels ($p > 0.05$) of IND release at 10 and 24 hrs; means that there was no statistical difference related to the presence of Labrafac™ oil in the SLN. Additionally, the drug - lipid ratio insignificantly affects IND release from SLNs or NLCs at both 10 and 24 hrs, with an exception of IND release from INF8 and INF9 after 24 hrs ($p \leq 0.05$) where, IND release was more quickly when using lower concentration of drug because of the drug-enriched shell model proposed for these particles [56]. Interestingly, the particle size had no influence on the invitro release of IND at pH 7.4.

Data from the first 10 hrs of the experiments were fitted to zero- order, first- order, and Higuchi equations and the results were exhibited in table (6). Release data from all samples fit a Higuchi model that provided the highest value of squared correlation coefficient (R^2). Higuchi kinetics was verified by plotting the cumulated amount released drug substance versus $t^{1/2}$. The Higuchi release constants which are the slopes of the equations are listed in table (6) and considered to be characteristic of the formulation. The Higuchi model has been based on

Fick's law where the release occurs by the diffusion of drugs within the delivery system [41]. The mechanism of the IND release from the nanoparticles in phosphate buffer pH 7.4 can be considered as follows: water penetrates into the lipidic matrix of the nanoparticles through small porous channels, slowly dissolving the drug and IND is released by diffusion to acceptor solution [57]. Moreover, the pH sensitivity of the hydrogen bonding interactions between the carboxylic acid group of IND and the hydroxyl groups of glyceride- containing lipids in the core have a bearing on the release rate. At pH 7.4, IND is in a dissociated state, its solubility increases and the physical interactions within the structure significantly weaken due to the disruption of the drug-lipid hydrogen bonding, which enhances the drug release rate [30, 59].

Table 6. Release study. Squared correlation coefficients (R^2) obtained in case of application of different kinetic models to the data release of the Indomethacin loaded in the nanoparticles.

Formulation no.	Release at pH 7.4 [d]			
	Zero Order [a]	First Order [b]	Higuchi Order [c]	Higuchi Release Constant
INF4	0.9323	0.6698	**0.9795**	30.59
INF8	0.9092	0.6217	**0.9863**	18.38
INF9	0.8811	0.6477	**0.9685**	15.98
INF10	0.9430	0.6871	**0.9849**	23.34
INF11	0.9639	0.6961	**0.9814**	29.22
INF14	0.9105	0.6354	**0.9769**	27.51

[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.

[d] The kinetic model was applied to the data of the first 10 hrs release.

Bold print indicates the best fits.

It was concluded that the release from SLNs can be influenced and optimized by considering (1) the nature of the lipid matrix such as the overall lipid polarity and the lipid interaction with the aqueous phase, (2) the solubility of the IND in the lipid excipients plus the partition coefficient of the drug, and (3) the surfactant concentration [58]. Furthermore, in vitro release studies of SLNs may be a valuable tool as an indirect determination of the drug substance distribution in the SLNs. These release studies will be followed up by other measurements in order to deeply investigate the drug distribution in these nanoparticles.

Additionally, the entrapment efficiencies of two systems (INF11, INF14) were difficult to be determined by GPC or ultracentrifugation and therefore, the release studies used were used as indirect method to expect their entrapment efficiency [6]. It is well known that glyceryl tribehenate SLNs form highly crystalline particles with a perfect lattice leading to drug explosion and particle aggregation of the nanoparticles is normally accompanied by a transition from the metastable β' polymorph to the more stable β_i polymorph with rapid drug explosion [13]. On the contrary, in our study, Compritol® 888 ATO consists of mono, di, and triglycerides lending slight

emulsifying properties to the lipid (HLB=2) [60] and the contents of partial glycerides favors successful drug inclusion and avoids drug expulsion as described for pure triglycerides. Consequently, in the presence of an emulsifier such as sugar ester SP30 with its thickening properties, Compritol® - based SLN transforms slowly into the stable polymorph and sustained release potential is still high. The same discussion can be applied to SLN named INF11, where the presence of glyceride containing oil (Labrafac™ Lipophile WL 1349) increases the IND inclusion and retards its expulsion.

In the future, additional information about possible interactions between the drug and different lipid particles, and the encapsulation efficiencies of IND within different systems will be further confirmed by DSC experiments. Moreover, the potential of these lipid nanoparticles as carriers for prolonged topical delivery will be investigated. The prepared SLN dispersions will be further processed to tablets, hydrogels, or oil/water creams to mimic professional use in medical or cosmetic carriers.

4. CONCLUSION

In this study, several blank and IND containing SLN dispersions were prepared and characterized. IND was used as a model lipophilic drug and loaded at two different drug – lipid ratios. The physicochemical properties of the resulting nanoparticles with respect to particle size, surface charge, and crystallinity of lipids were determined and compared. Drug entrapment, loading capacities, and release characteristics were also investigated. The effect of solid lipids monoglyceride content on the entrapment efficiency of IND was examined. In conclusion, the presented data gave evidence that it was possible to produce SLNs/ NLCs endowed with high encapsulation efficiency and with a well determined size distribution. It is possible to produce IND stable SLN dispersions by optimizing the surfactant concentration, lipid type and concentration and drug – lipid ratios in vitro. The prepared nanoparticles were crystalline at room temperature. Besides, in vitro experiments revealed a promising delayed and sustained activity of IND- loaded SLN/NLC formulations. Release studies at pH 7.4 showed that IND release exhibited a biphasic pattern with an initial burst and prolonged release over 24 hrs with kinetics obeying Higuchi model. The pH sensitivity of the hydrogen bonding interactions between the carboxylic acid group of IND 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. Therefore, the present results suggest a new opportunity for IND to be employed in well controlled and modern oral formula.

Production of IND- loaded SLNs with new solid lipids and its future formulation as tablets could be new, cost effective and commercially via7ble alternative to the commercial products.

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إعداد، تمييز وتقييم مختبري لجسيمات دهنية صلبة نانو تركيبية محملة بالإندوميثاسين

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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. وفي النظم جميعها كانت المادة النشطة سطحيا المستخدمة هي استر السكروز مع حمض دهني SP 30 (HLB 6.0). وتميزت النظم من خلال تحليل حجم الجسيمات وقياسات زيتا ((Zeta potential). وتم حساب كفاءة انحباس الإندوميثاسين داخل الجسيمات. أما تقنية H1-NMR فسمحت بالتحقق من بلورة الجسيمات. وعلاوة على ذلك، فقد أجريت تجارب إطلاق سراح الإندوميثاسين من الجسيمات وجرى التحقق من حركية الانطلاق. تركز هذه الدراسة على التحقيق في كيفية تأثير طبيعة وكمية مكونات التركيبات على تعديل خصائص النظم، و على وجه الخصوص، جرى فحص تأثير تركيز المادة النشطة سطحيا المستخدمة لتحقيق استقرار المعلقات ذات حجم النانو وكذلك طبيعة وتركيز الدهون المستخدمة في إعداد النانوجسيمات، وأيضا النسبة بين الدواء والدهون المستخدمة في إعداد الجسيمات الدهنية الصلبة المحملة. وكذلك تمت مناقشة تأثير هذه المتغيرات على الخواص الفيزيائية والكيميائية للجزيئات وملامح إطلاق نموذج للدواء (إندوميثاسين). ومن النتائج التي تم التوصل اليها أنه تم إنتاج جسيمات دهنية صلبة ذات توزيع حجمي ضيق، وكانت كل التركيبات الفارغة من الإندوميثاسين تظهر منحنى ذا ذروة واحدة وهو متجانس وتمتلك متوسط حجم مابين 190 –400 نانوميتر بعد يوم واحد من الإنتاج.وأظهرت نتائج التشتت المتعدد أن توزيع الجسيمات كان أحادي المنوال مع وجود اختلافات طفيفة بين التشغيلات، وكان مؤشر التشتت المتعدد بشكل عام أقل من 0.25. وإن إدماج الإندوميثاسين بشكل عام يزيد من أحجام الجسيمات الصلبة الدهنية وتجمعها، مما انعكس على حجمها ومؤشر التشتت المتعدد. وأظهرت النتائج أن الجسيمات الدهنية الصلبة المحضرة حديثا تمتلك قيما للزيتا أعلى من -30mV وكانت أعلى من القيم الحرجة لتحقيق الاستقرار الكهربائي النقي، ولكنها لا تزال من حيث المبدأ كافية لنظام مستقر إذا ما أخذ في الاعتبار التأثير الفراغي المثبت لإستر السكروز SP30. وأظهرت النتائج أن الجسيمات الدهنية الصلبة المحتوية على الجليسيريدات ذات قدرة جيدة على حوصلة الدواء ولكن مع تشكيل مجاميع. وفي المقابل، تمتلك الجسيمات الدهنية الصلبة المستندة الى الشمع في تركيبها استقرارا فيزيائيا جيدا مع عدم وجود مجاميع ولكنها تفتقر القدرة على تغليف الدواء. وتعود هذه الاختلافات في جزء منها إلى ترتيب الأشكال الكريستالية المختلفة للدهون المستعملة. فالترتيب الأقل انتظاما للأشكال الكريستالية لمادة الجيليول والكمبريتول تفضل حوصلة الدواء، أما الشكل المنتظم للكريستالات المكونة للجسيمات الدهنية الصلبة المستندة الى شمع السيتيل بالميتات فتؤدي إلى طرد الدواء، ولكنها تصل إلى استقرار فيزيائي عالٍ. أما دراسات الرنين المغناطيسي النووي، فأظهرت الجسيمات بلورية عند درجة حرارة الغرفة (25°م). وكذلك أظهرت المقارنة بين أشكال انطلاق الإندوميثاسين من التركيبات المختلفة أن هذه النظم مناسبة للتركيبات الفموية المتطورة. فدراسة انطلاق الإندوميثاسين عند درجة حموضة 7.4 أظهرت أن الانطلاق يأخذ نمطا ثنائي الطور مع انفجار أولي، وانطلاق لفترات طويلة على مدى 24 ساعة مع حركية تتبع نموذج هيجوتشي. يمكن لهذه الجسيمات الدهنية الصلبة المتقدمة التغلب على الآثار السلبية لدواء الإندوميثاسين وتقديم فعالية في علاج الأمراض الالتهابية و تقبل المريض العلاج . إن إنتاج الجسيمات الدهنية الصلبة المحملة بالاندوميثاسين من دهون صلبة جديدة وصياغتها في المستقبل على شكل أقراص يمكن أن يكون جديداً، وبديلاً فعالا من حيث التكلفة، ومجديا تجاريا عن منتجات تجارية أخرى.

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

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