International Journal of Pharmaceutics 390 (2010) 53–60

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Corticosteroid solubility and lipid polarity control release from solid lipid nanoparticles

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ARTICLE INFO

Article history:
Received 4 January 2009
Received in revised form 5 October 2009
Accepted 7 October 2009
Available online 25 October 2009

Keywords:
Solid lipid nanoparticles
SLN
Corticosteroid
Solubility
Lipophilicity
In vitro release

ABSTRACT

Solid lipid nanoparticles (SLN) show promise as a drug delivery system for skin administration. The solid state of the lipid particle enables efficient drug encapsulation and controlled drug release. The present study addresses the influence of lipid composition and drug substance lipid solubility on the *in vitro* release profile of corticosteroids from SLN for topical administration. Firstly, the effect of lipid composition on the lipid solubility and *in vitro* release of betamethasone-17-valerate (BMV) was determined by varying the lipid monoglyceride content and the chain length of the fatty acid moiety. Secondly, the effect of drug substance physicochemical properties was determined by studying five different corticosteroid derivatives with different lipophilicity. A high concentration of monoglyceride in SLN increased the amount of BMV released. The corticosteroid release rate depended on the drug substance lipophilicity and it was clear that the release profiles depended on drug partitioning to the aqueous phase as indicated by zero order kinetics. The results emphasize that the corticosteroid solubility in the lipid phase greatly influence drug distribution in the lipid particles and release properties. Thus knowledge of drug substance solubility and lipid polarity contributes to optimize SLN release properties.

1. Introduction

In the last decade, solid lipid nanoparticles (SLN), a novel colloidal drug delivery system, have gained increasing interest as dermatological and cosmetic formulations (Muller et al., 2002; Schafer-Korting et al., 2007). SLN are composed of well-tolerated excipients and the lipids used are solid at body temperature. The solid state of the vehicle lipid enables efficient drug encapsulation and controlled drug release properties (zur Muhlen et al., 1998; Jenning et al., 2000; Souto et al., 2004; Sivaramakrishnan et al., 2004; Chen et al., 2006).

Several factors will influence the drug substance distribution and incorporation efficiency in the final SLN; drug substance physicochemical properties, surfactant type and concentration, lipid type and crystallization pattern and the method of production (zur Muhlen and Mehnert, 1998; Muller et al., 2000; Kumar et al., 2007). Usually, the drug substance is dissolved or dispersed in the melted lipid phase before preparation by homogenization to achieve efficient encapsulation in the lipid (Muller and Lucks, 1996). 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 (zur Muhlen and Mehnert, 1998; Muller et al., 2000). Additionally, the drug substance solubility in the liquid lipid during processing may be higher than in the solid lipid.

Basically, the three different models for incorporation of the active compound are the homogenous matrix model (1), the drug-enriched shell model (2), and the drug-enriched core model (3) (Muller et al., 2000). A solid suspension may be another (fourth) model if the drug substance is not molecularly dispersed in the lipid. Of course a combination of the models may be relevant too. The drug release profile from SLN depends on which model the SLN resemble. Hence, release studies may be used to indirectly evaluate the incorporation model of a drug substance in SLN.

Different lipid types may cause different release profiles from SLN. A difference in entrapment efficiency and release properties were shown to be dependent on lipid lipophilicity when incorporating etoposide. Glycerol tripalmitate, a more lipophilic acylglycerol exhibited higher sustained release than a

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doi:10.1016/j.ijpharm.2009.10.022

Table 1
Compositions of solid lipid nanoparticles prepared for release studies.

Composition	Lipid 10% (w/w)	Lipid trade name	Drug substance 0.1% (w/w)
1	Trimyristate	Dynasan® 114	Betamethasone-17-valerate
2	Mono/trimyristate	Rylo™ MG 14 Pharma/Dynasan® 114	Betamethasone-17-valerate
3	Tristearate	Dynasan® 118	Betamethasone-17-valerate
4	Distearate	Precirol® ATO 5	Betamethasone-17-valerate
5	Mono/distearate	Tegin® 4100	Betamethasone-17-valerate
6	Distearate	Precirol® ATO 5	Hydrocortisone
7	Distearate	Precirol® ATO 5	Hydrocortisone-21-acetate
8	Distearate	Precirol® ATO 5	Hydrocortisone-17-butyrate
9	Distearate	Precirol® ATO 5	Hydrocortisone-21-caprylate

Polysorbate 80, 2.5% (w/w) was used as surfactant in all composition.

less lipophilic acylglycerol like glycerol monostearate (Reddy and Murthy, 2005). In another study, the lipid composition of SLN was shown to affect drug substance partitioning between lipid and aqueous phase. So, an inverse relationship between the amount of clozapine released and partition coefficient (ratio of amount clozapine in the lipids glycerol trimyristate, tripalmitate and tristearate, respectively, to the amount in the aqueous phase) was found (Venkateswarlu and Manjunath, 2004). Likewise, olanzapine partitioning between a lipid and an aqueous phase was shown to correlate with SLN entrapment efficiency (Vivek et al., 2007).

Other studies have indicated an influence of drug–lipid structure interactions on the ability of SLN to control the release of the drug substance when applied to the skin. Thus, a difference in skin penetration between betamethasone-17-valerate and prednicarbate was believed to rely on their side chain structure (Sivaramakrishnan et al., 2004; Borgia et al., 2005; Braem et al., 2007).

Only few studies on the investigation of the drug–lipid interactions with the focus on drug substance lipophilicity and its solubility in the lipid phase can be found in the literature. The purpose of the present work was to systematically evaluate the influence of the lipid composition on the drug substance solubility in the lipid phase and on the release profile of the drug substance from SLN *in vitro*. Also, the influence of the physicochemical properties of the drug substance on the solubility in the lipid phase and on the release profile from a selected SLN lipid composition was evaluated.

The lipids applied in the solubility studies were all acylglycerols and varied with regard to monoglyceride content and fatty acid chain length. The lipids represented different lipid polarities and were selected to give various drug substance solubilities. The drug substances used in the present study were corticosteroid derivatives varying in side chain lengths and therefore in lipophilicity. Topical corticosteroids are some of the most frequently used drugs for skin diseases due to their anti-inflammatory effect. (Halvarsson and Loden, 2007; Su and Fang, 2008; Wiedersberg et al., 2008).

2. Materials and methods

2.1. Materials

The lipids used were acylglycerols except for cetylpalmitate. Glycerol distearate (Precirol® ATO 5), glycerol dibehenate (Compritol® 888 ATO) and cetylpalmitate were purchased from Gattefossé (Genas, France), glycerol monomyristate (Rylo™ MG 14 Pharma) and glycerol monostearate (Rylo™ MG 18 Pharma) were from Danisco (Grindsted, Denmark), glycerol trimyristate (Dynasan® 114), glycerol tristearate (Dynasan® 118) and hydrogenated palm oil (Softisan® 142) were kindly donated from Sasol (Germany), glycerol monostearate/distearate (Tegin® 4100) was obtained from Frankenchemie (Wendelstein, Germany), and polysorbate 80 was from Croda AB (Limhamn, Sweden).

Hydrocortisone (HC), hydrocortisone-17-butyrate (HCB) and hydrocortisone-21-caprylate (HCC) were purchased from Sigma–Aldrich (Denmark) and hydrocorticone-21-acetate (HCA) from Aventis Pharma (Denmark), betamethasone-17-valerate (BMV) was obtained from SICOR, SpA (Milano, Italy), Kleptose Crysmeb (methyl-β-cyclodextrin) was purchased from Roquette (Lestrem, France).

Sodium-acetate-trihydrate, diammonium-hydrogenphosphate and analytical solvents were all from Merck (Darmstadt, Germany).

2.2. Solubility studies

The solubility of the corticosteroids in glycerol distearate (Precirol® ATO 5) was investigated by hot stage microscopy in a Nikon Eclipse 80i microscope (USA) equipped with a Linkam PE94 heater (United Kingdom). The software used was Image Pro Plus®. An excess of drug substance was dispersed in the melted lipid at minimum 10 °C above the lipid melting point and stirred at 200 rpm using a Variomag® Multitherm thermostate and stirrer (Germany). After 30, 60 and 90 min and overnight stirring, the samples ($n = 2$) were inspected for drug crystals in a hot stage microscope heated to 80 °C to evaluate whether equilibrium was achieved or not. When equilibrium was achieved, an interval of lipid solubility was determined on the basis of the absence or presence of drug crystals.

In the case of BMV, also a range of commercially available lipid mixtures were investigated followed by a series of lipid mixtures varied systematically in monoglyceride:triglyceride ratio.

2.3. Preparation and characterization of SLN

The preparation of SLN was made by hot high pressure homogenization as described previously (Muller and Lucks, 1996). Briefly, 10% (w/w) lipid was melted at 80 °C and 0.1% (w/w) drug substance dissolved or dispersed in the molten lipid during stirring for 30 min. An aqueous 2.5% (w/w) polysorbate 80 solution of the same temperature was added to the lipid mixture and mildly homogenized for 1 min at 8000 rpm using a Silverson High Speed Mixer L4RT from Silverson Machines (Chesham, United Kingdom), to create a coarse emulsion. Then the mixture was high pressure homogenized using an EmulsiFlex C5 from Avestin (Ottawa, Canada), the homogenizer was placed in a Julabo TW 20 water bath from Julabo (Seelbach, Germany) to keep the temperature at 80 °C. The coarse emulsion was processed at 800 bar applying three homogenization cycles. These processing conditions were selected from preliminary studies which proved them to be favorable for preparing SLN with a small particle size (<500 nm) together with a low polydispersity (<0.45). The dispersions were cooled at room temperature while stirring at 200 rpm and stored at room temperature protected from light. Particle size analysis was performed by dynamic light scattering (DLS) on a Zetasizer Nano ZS from Malvern (Worcestershire, UK). The samples ($n = 3$) were adequately diluted with purified

Table 2
Solubility of betamethasone-17-valerate in different lipid compositions.

Lipid	Lipid trade name	Solubility parameter	Fatty acid chain length	Amount glyceride % (w/w)			Solubility % (w/w)
				Mono	Di	Tri	
Monomyristate	Rylo™ MG 14 Pharma	11.2	14	97	2	0	5.0–5.3
Monostearate	Rylo™ MG 18 Pharma	10.8	18	96	0	0	3.8–4.0
Mono/distearate	Tegin® 4100	–	18	45–55	40	5	3.0–3.2
Mono/trimyristate	Rylo™ MG 14 Pharma/Dynasan® 114	–	14	25	0	75	1.2–1.3
Dibehenate	Compritol® 888 ATO	9.3	22	12–18	52–54	28–32	1.7–2.0
Distearate	Precirol® ATO 5	9.5	16/18	8–22	40–60	25–35	1.8–1.9
Trimyristate	Dynasan® 114	9.0	14	0	4	95	0.3–0.5
Tristearate	Dynasan® 118	8.9	18	0	2	97	0.1–0.3
Cetylpalmitate	Cetylpalmitate	8.7	16	–	–	–	<0.1

water before measurement. For *in vitro* release studies, SLN with BMV were prepared with five different lipid mixtures. SLN with various corticosteroids were prepared with distearate as the lipid. The SLN compositions are shown in Table 1.

2.4. In vitro release

The release studies were set up with static diffusion cells ($n = 3$) applying a Spectra/Por® cellulose membrane (Spectrum Laboratories, Netherlands) with a cut off value of 10 kDa. Prior to use the membrane was hydrated in purified water for 12 h. To maintain sink condition during the study a 10% (w/w) solution of methyl-β-cyclodextrin in acetate buffer pH 4.5 was used as receptor medium. The solubility of the corticosteroids was determined in the receptor medium before setting up the release studies. 3 ml of SLN formulation was placed in the donor compartment after which the receptor compartment was filled with 3 ml receptor medium. The temperature was kept at 32 °C to mimic skin conditions *in vivo* and the receptor medium was stirred during the study. Samples of 1 ml were withdrawn after 1, 2, 3, 4, 5, 6, 22 and 24 h, replacing the samples with the same amount of volume of receptor medium. The cumulated amount of drug substance released Q (corrected for sampling) was calculated from Eq. (I):

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

where V_s is the volume of sample withdrawn, C_{n-1} is the drug concentration of the sample n and V_m is the volume of the receptor medium.

2.5. HPLC analysis

The concentration of BMV in the samples from the release study was analyzed by RP-HPLC on a Waters Alliance equipment using acetonitrile:phosphate buffer pH 6.0:methanol (25:45:30) as mobile phase, UV detection of BMV was done at 254 nm. For the analysis of the other corticosteroids a flow gradient method was used varying the acetonitrile concentration from 0 to 100% during 15 min, using UV detection at 254 nm. A flow rate of 1–1.2 ml/min and an injection volume of 20–80 μl were applied. Retention times were between 6.3 and 11.6 min. The column was in all cases a Licrosphere 100 RP-18 (Merck) used at room temperature.

2.6. Calculation of solubility parameters and data analysis

The solubility parameters for lipids and corticosteroids, respectively, were calculated according to Fedors substituent method (James, 1986). This method is based on the sum of energy of mixing substituent constants and the sum of molar volume substituent constants from Eq. (II):

$$\delta = \sqrt{\frac{\sum \Delta\Delta U}{\sum \Delta V}} \quad \text{(II)}$$

where δ is the solubility parameter, $\Delta\Delta U$ is the energy of mixing substituent constants and ΔV is the molar volume substituent constant. Log P values for the corticosteroids were calculated with the software SciTegic Pipeline Pilot® from Accelrys®.

3. Results

3.1. Solubility studies

Solubility of the corticosteroids in the melted lipids was determined using a hot stage microscope. The results of the solubility studies with BMV and commercially available lipid mixtures showed a tendency for BMV to be more soluble in the melted lipid with a higher concentration of monoglyceride (Table 2). This effect was investigated for a range of lipid compositions varying systematically in monoglyceride:triglyceride ratio with myristic or stearic acid as the fatty acid moiety. Plotting BMV solubility versus monoglyceride content established a linear correlation. There seemed to be a slightly higher solubility of BMV in the myristate mixtures compared to the stearate mixtures (Fig. 1, upper).

In addition, the BMV solubility versus the solubility parameter showed a linear correlation (Fig. 1, lower).

For the studies with HC, HCA, HCB and HCC the solubility in distearate was related to the lipophilicity of the corticosteroids, reported as $\log P_{calc}$ values (Table 3). When looking at compounds with $\log P_{calc}$ values from 1.28 to 4.16, the solubility increased a 100-fold from 0.1% (w/w) to more than 12% (w/w). However, there was no linear correlation between corticosteroid solubility and $\log P_{calc}$ values or solubility parameters of the corticosteroids. The solubility in distearate of the most lipophilic drug substance used, HCC, was very high compared to the other corticosteroids.

Table 3
Solubility of corticosteroids in distearate.

Drug substance	Solubility parameter	$\log P_{calc}$	Solubility % (w/w)
Hydrocortisone (HC)	13.4	1.28	0.2–0.4
Hydrocortisone-21-acetate (HCA)	12.2	1.66	0.1–0.2
Hydrocortisone-17-butyrate (HCB)	11.9	2.79	1.2–1.4
Betamethasone-17-valerate (BMV)	12.0	3.67	1.8–1.9
Hydrocortisone-21-caprylate (HCC)	11.4	4.16	>12

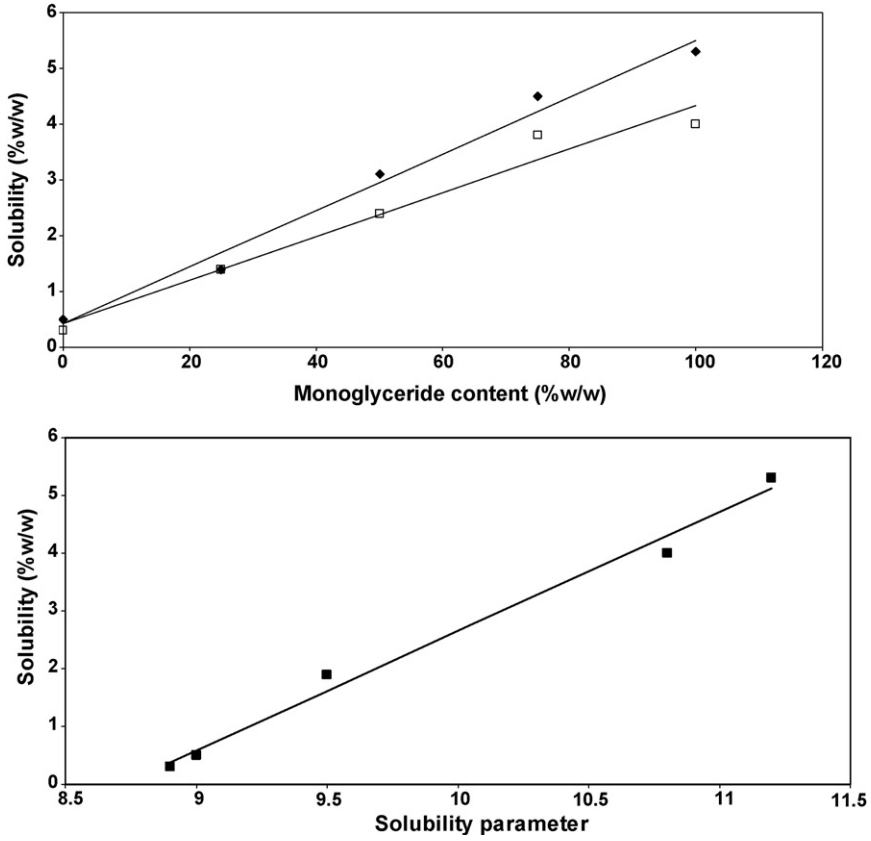

Fig. 1. Upper: solubility of betamethasone-17-valerate in mixtures of monomyristate and trimyristate (■) and monostearate and tristearate (□) correlated to lipid monoglyceride content. Lower: solubility of betamethasone-17-valerate correlated to lipid solubility parameter.

3.2. Preparation and characterization of SLN

The SLN were prepared with the hot high pressure homogenization method. In the solubility studies it was revealed that stirring more than 30 min after adding the drug substance to the lipid phase in most cases did not increase the drug substance solubility to any significant extent. Accordingly 30 min stirring time was used in the preparation of the SLN.

As an increase in the monoglyceride content increased BMV solubility (Table 2), SLN were prepared using different ratios of monoglyceride in the lipid phase. The lipid compositions contained either myristic or stearic acid as the fatty acid moiety, respectively. Some of the compositions investigated in the solubility study caused aggregation, gelation or phase separation when used for the preparation of SLN and thus were not further analyzed. It was seen that especially a high content of monoglyceride created more unstable SLN. Five compositions were subsequently selected for studying *in vitro* release profiles (Table 1). The five lipids for the SLN differed with regard to monoglyceride content, fatty acid chain length and, consequently, BMV solubility. Distearate was used for

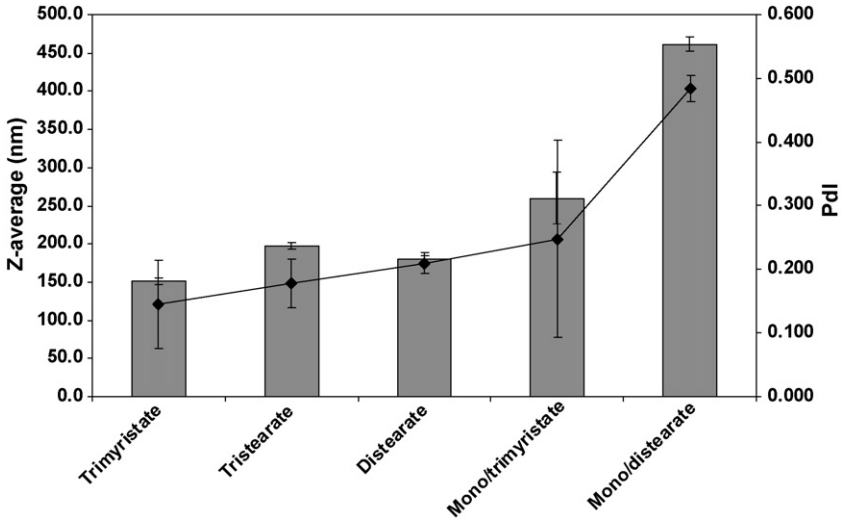

Fig. 2. Particle size measured as hydrodynamic diameter *Z*-average (grey bars) and polydispersity index PdI (■) of solid lipid nanoparticles with betamethasone-17-valerate for different lipid compositions varying in monoglyceride content and fatty acid moieties. Expressed as mean ($n = 3$) ± standard deviation.

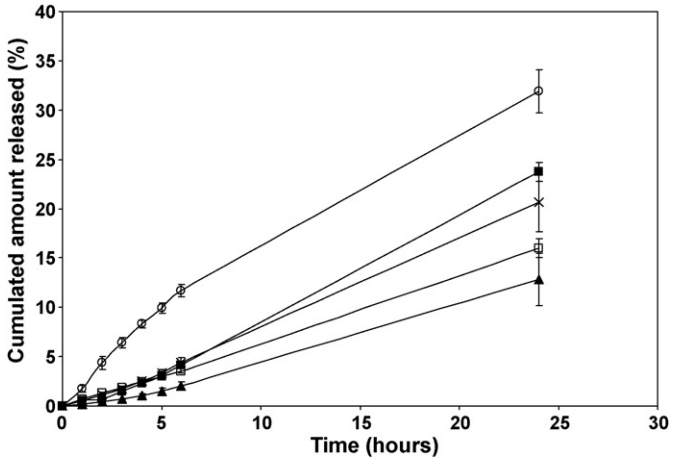

Fig. 3. Release profiles for betamethasone-17-valerate in different solid lipid nanoparticles lipid compositions. Mono/distearate (○), trimyristate (■), mono/trimyristate (×), distearate (□), tristearate (▲). Expressed as mean ($n = 3$) ± standard deviation.

preparing SLN with HC, HCA, HCB and HCC. Distearate was selected, because it was seen to create stable and homogenous particles with a mean hydrodynamic diameter of 179.5 ± 4.9 nm and a PdI of 0.210 ± 0.016 for SLN with BMV.

The prepared SLN with BMV had a mean diameter (*Z*-average, $n = 3$) from 151 ± 4.2 to 461 ± 9.2 nm, and all had a PdI below 0.484. The formulation with trimyristate resulted in the smallest mean diameter (Fig. 2). SLN with mono/distearate had the largest mean diameter and the highest PdI indicating higher heterogeneity. This formulation was also more viscous than the others. It turned out to be a rather difficult task to prepare SLN from lipids with a high content of monoglyceride. This could be reflected in the size parameters of the SLN having high monoglyceride content, e.g. the mono/distearate composition and the monomyristate/trimyristate (Fig. 2).

The SLN prepared with different corticosteroids had mean diameters from 180 ± 4.9 to 220 ± 27.6 nm and a PdI from 0.167 ± 0.016 to 0.212 ± 0.083.

3.3. In vitro release

In vitro release studies were performed with a cellulose membrane in static diffusion cells. The studies with BMV in SLN showed that the highest release was achieved with the composition having the highest monoglyceride content (Fig. 3). The release profiles followed zero order; except for the mono/distearate composition, which followed Higuchi kinetics. Higuchi kinetics was verified by plotting the cumulated amount released drug substance versus $t^{1/2}$. After 6 h from starting the experiment, there was no significant difference between the different formulations apart from the SLN with glycerol mono/distearate, which showed 11.7% cumulated amount released BMV. The other formulations all released less than 5% BMV over 6 h. Both after 6 and 24 h, a correlation between monoglyceride content and cumulated amount released BMV could be shown (Fig. 4). The higher the content of monoglyceride the more BMV was released. After 24 h, BMV was released in the highest amount (31.9%) from the composition with mono/distearate, whereas release from the other SLN was 12.8% (tristearate), 16.0% (distearate), 20.7% (monomyristate/trimyristate) and 23.7% (trimyristate), respectively (Figs. 3 and 4).

Samples analyzed after 6 and 24 h showed that HC, HCA, HCB, BMV and HCC were released to a lesser extent with increasing lipophilicity, and showed zero order release profiles. One exception was HCA which showed a biphasic release profile as only 7.5% was released after 6 h whereas 65.8% was released after 24 h (Fig. 5).

4. Discussion

4.1. Solubility of betamethasone-17-valerate increases with monoglyceride ratio

The solubility of BMV in the melted lipid phase could be correlated to the ratio of monoglyceride to triglyceride. A linear correlation was also observed with solubility parameters, which is caused by the solubility parameter being closely linked to polarity (Fig. 1). The solubility parameter is a measure of cohesive energy and may be used when looking at interactions and compatibility between different chemical substances in solution (Vaughan, 1988). As such the solubility parameter can be useful when selecting lipids for preparation of SLN loaded with a specific drug substance. The increase in BMV solubility with a higher concentration of monoglyceride and a smaller content of triglyceride can be explained by the polarity of the lipid phase because monoglycerides are more polar than triglycerides (Small, 1986; Garti and Sato, 1998). In addition, monoglycerides are known to possess surfactant properties, which may contribute to dissolving BMV (Small, 1986). However, the solubility determined in the molten lipid does not necessarily correlate directly to the incorporation of the drug substance in the SLN. There may be an exchange of drug substance between lipid and water phase during processing

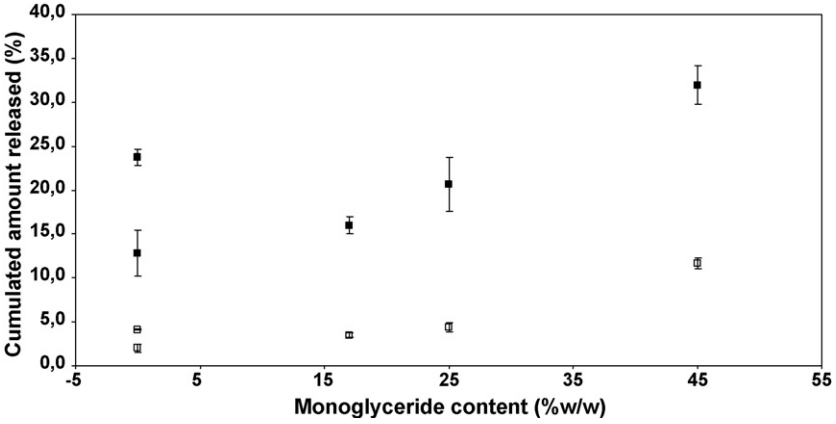

Fig. 4. Correlation of betamethasone-17-valerate release to monoglyceride content in the different solid lipid nanoparticles compositions. Release after 6 h (□), release after 24 h (■). Expressed as mean ($n = 3$) ± standard deviation.

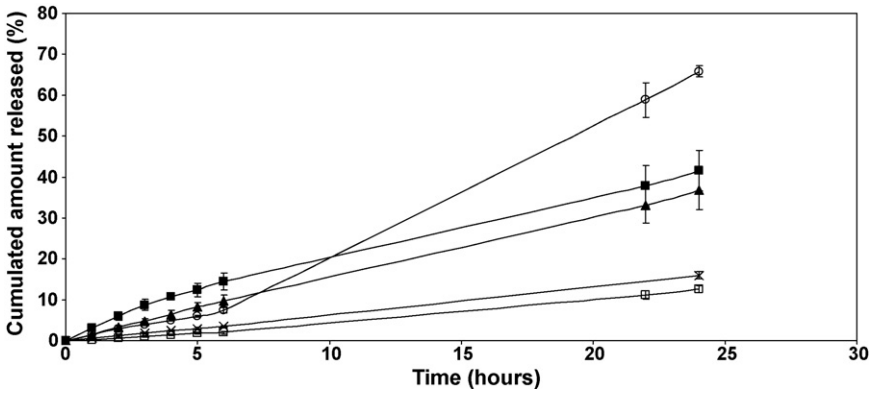

Fig. 5. Release profiles for different corticosteroids in distearate solid lipid nanoparticles. Hydrocortisone-21-acetate 45 (○), hydrocortisone (■), hydrocortisone-17-butyrate (▲), betamethasone-17-valerate (×), hydrocortisone-21-caprylate (□). Expressed as mean ($n = 3$) ± standard deviation.

because it is performed above the lipid melting point in which the mobility of the drug substance in the lipid phase is presumed to be high (zur Muhlen and Mehnert, 1998; Muller et al., 2000). To avoid risk of redistribution of drug to the aqueous phase, the same surfactant and surfactant concentration was used in all preparations. Thus, the BMV solubility in the aqueous phase was considered comparable between the formulations. Moreover, the solubility was determined in the melted lipid mixtures, whereas the SLN represented the solid lipid below the melting point at which the drug substance solubility is expected to be lower. This is partly because of the decrease in temperature and partly because of the solid structure leaving less room for accommodation of the drug substance (Bunjes et al., 1996).

4.2. Particle size and stability of betamethasone-17-valerate loaded SLN depend on monoglyceride content

The sizes of the different SLN varied, with trimyristate having the smallest diameter. Previous studies with acylglycerol SLN have shown that this was likely to be caused by the low melting point of trimyristate resulting in the homogenization process being more effective (Siekmann and Westesen, 1992; Mehnert and Mader, 2001). As the monoglyceride content of the lipids increased, the size of the SLN increased (Fig. 2). This may be explained by a higher degree of interaction with water the more monoglyceride is present (Small, 1986). According to Small (1986), lipids may be classified by their interaction with water. Monoglycerides belong to the group of polar lipids, that are insoluble in water but water soluble within the hydrophilic parts of their structure, which can cause swelling. In the presence of water, these lipids will reorganize into the aqueous–organic interface, which accounts for their surfactant properties (Small, 1986). The swelling property is likely to cause the higher observed viscosity of the mono/distearate SLN.

4.3. Release profiles of betamethasone-17-valerate loaded SLN depend on monoglyceride ratio

In the current study, it was observed that the more the monoglyceride present or the higher the polarity of the lipid, the better the solubility of the drug substance and the more BMV was released from the SLN (Table 2 and Fig. 4), most probably due to differences in loading capacity and drug incorporation model. The correlation between monoglyceride content and the amount of drug released during the first 24 h showed that the higher the content of monoglycerides, or the higher the solubility, the more drug substance was released. The order of cumulated amount released during 24 h was: mono/distearate > trimyristate > mono/trimyristate > distearate > tristearate. A higher solubility of BMV may increase the concentration of BMV in the lipid particle and cause a greater driving force for the release of BMV to the aqueous phase because of a larger concentration gradient. A similar result was reported with nitrendipine in SLN. Highest drug release was achieved with SLN composed of glycerol monostearate despite a large particle size (Kumar et al., 2007). Incorporation of etoposide in different acylglycerols also showed a tendency for the monoglyceride content to increase the total amount released. This effect was attributed to the influence of lipid polarity on incorporation efficiency (Reddy and Murthy, 2005).

The effect of monoglycerides on the BMV release profiles may also be explained by the monoglyceride surfactant properties and ability to interact with water molecules. When employing lipids with a polar character, other colloidal structures besides SLN may be present (Mehnert and Mader, 2001; Mader, 2006). These could be lipids forming liquid crystalline phases or mixed micelles formed by the emulsifier and the lipids. Since the monoglycerides possess surfactant properties, they may increase the amount of drug substance in the aqueous phase or cause a higher mobility in the lipid particle surface from where the drug substance is readily released. As seen from the correlation between BMV solubility, monoglyceride content and cumulated amount released it is important to consider the polarity of the lipid and the possibility of interaction with water.

The release profiles were all zero order during the studied 24 h except for the mono/distearate composition (Fig. 3). Had the drug substance been distributed in the lipid particles as a solid solution, then diffusional release kinetics would be expected due to the effect of entrapment and particle diffusion length. The mono/distearate composition shows diffusional Higuchi kinetics but this is likely to be related to the more viscous character of this SLN dispersion. The zero order release profile from SLN may be explained by several factors. The size of the SLN is small, so particle diffusion length may not be reflected in release kinetics. The enormous surface area may contribute to the zero order release profile since the rate of exchange of drug substance with the aqueous phase is high. Smaller particles also mean a shorter diffusion length through the particle in order to release the drug substance. Furthermore, the lipid particles are not monodisperse in size (PdI between 0.146 and 0.484), i.e. the total amount released may be considered the sum of releases from different surface areas. The release profiles indicate that BMV is incorporated in the particle surface layer (drug-enriched shell model) from where it is readily released following zero order. Of the five SLN with BMV used in the *in vitro* release studies, BMV was dissolved in the melted lipid in distearate, mono/trimyristate and mono/distearate. In tristearate and trimyristate, BMV was not completely dissolved in the melted lipid. If not dissolved in the melted lipid, small BMV crystals may be present in the aqueous

phase or attached to the surface of the lipid particles after cooling. It may also create a solid suspension (model 4). Dissolution rate of any BMV crystals may thus influence release profiles and a saturated solution of BMV in the aqueous phase may be created.

The SLN formulation with trimyristate differed from the other compositions with BMV, because it released a relatively high amount considering the low BMV solubility in the lipid and the absence of monoglycerides. The reason that trimyristate SLN released a higher amount of drug substance could be the lower melting point of this lipid (53–58 °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 colloidal structures may be lower than that of the bulk due to the influence of surface energy (Mader, 2006). A difference in release profiles caused by a difference in lipid melting points was also suggested by Paolicelli et al. (2008) in a study with ibuprofen and acylglycerols differing in melting points. The higher amount released from trimyristate particles may also reflect the smaller size of these particles. The mean diameter was 152 nm, which makes the particles the smallest of the SLN tested. Release of olanzapine from acylglycerol SLN was shown to increase with a decrease in size (Venkateswarlu and Manjunath, 2004).

The release profiles observed in the present study are used as an indirect method of determining corticosteroid distribution in the SLN. Another common method of evaluating drug entrapment in SLN is ultrafiltration (Sivaramakrishnan et al., 2004; Venkateswarlu and Manjunath, 2004). This was also tried here, but the entrapment efficiency determined by this method was more than 98% for all of the compositions and the ultrafiltration method used here was therefore decided to be of little value for interpretation of the results.

4.4. Lipophilicity of corticosteroids determines solubility and release from SLN

Solubility studies with different corticosteroids in distearate demonstrated that the solubility is strongly related to the lipophilicity of the corticosteroid, but that a linear correlation between log P_{calc} and solubility could not be established.

The particle size was very similar for the SLN prepared with different corticosteroids, i.e. the difference in solubility was not reflected in particle size. The concentration of corticosteroids in SLN (0.1%, w/w) was probably too low to affect the particle size measured.

Results from *in vitro* release studies showed that the more lipophilic the corticosteroid, the less is released during the 24 h experimental period. However, more HCA was released after 24 h than HCC, even though HCC is more hydrophilic than the HCA (Fig. 5). This may be related to the entrapment not only being dependent on solubility in the lipid phase but also on the chemical structure, e.g. of side chains in the drug substance (Sivaramakrishnan et al., 2004; Schafer-Korting et al., 2007).

The reason that the more lipophilic corticosteroids caused less release may be that lipophilic substances are more effectively incorporated in the solid lipid than the more hydrophilic substances (Muller et al., 2000; Mehnert and Mader, 2001; Keck et al., 2008). This reflects their increased solubility in the melted lipid. Conversely, the more hydrophilic substances are more likely to be dissolved in the water phase, especially during the preparation process during which the temperature is high (zur Muhlen et al., 1998; Muller et al., 2000).

As in the study with BMV and different lipids, zero order release profiles for the different corticosteroids were observed. The zero order kinetics was likely to originate from the same effects as discussed for the study with the different lipids. The SLN with corticosteroids were all similar in size and homogeneity. Thus, any differences in release profiles were not believed to relate from differences in particle size.

The results from the release studies can be used to indirectly describe the corticosteroid distribution in the lipid particles. Referring to the different SLN models, the results from the present release studies indicate that the SLN with BMV, HC, HCA, HCB and HCC in distearate most likely resemble a drug-enriched shell or a solid suspension considering that the drug substance is not completely dissolved in all of the different lipids.

When interpreting the release profiles it should be kept in mind that only 31% BMV was released from the SLN in the lipid study and 65% HCA was released in the corticosteroid study. The release studies were, however, not run for a longer period than 24 h, because hereafter the SLN tended to gel.

5. Conclusion

In the present study it was shown that the corticosteroid solubility in the lipid mixtures used for SLN depends mainly on the monoglyceride content, i.e. the polarity of the lipid. The release profiles for BMV with various lipids were dependent on BMV solubility in the lipid mixture. The cumulated amount of BMV released followed zero order kinetics, which is explained by the fact that the release profiles are highly dependent on a fast drug substance partitioning and exchange between the lipid particles and the aqueous phase. The release study with five different corticosteroids showed a decreased release with increasing lipophilicity of the corticosteroids. This is likely to be caused by a more efficient incorporation in the lipid particle. Release profiles of corticosteroid loaded SLN were zero order. Thus, it was concluded that the SLN with corticosteroids most likely resemble a drug-enriched shell model possibly combined with a solid suspension. The release from SLN may be optimized by determining the drug substance solubility in the lipid excipients as well as the overall lipid polarity in SLN and considering the lipid interaction with the aqueous phase. Moreover, *in vitro* release studies of SLN may be a valuable tool as an indirect determination of the drug substance distribution in the SLN. These release studies will be followed up by *in vitro* skin penetration and permeation studies in order to evaluate the influence of drug substance solubility and lipid polarity on skin penetration and permeation profiles.

Acknowledgement

The Danish Ministry of Science Technology and Innovation is thanked for financial support to this project.

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