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Solid lipid nanoparticles (SLN™) based on binary mixtures of liquid and solid lipids: a ^{1}H-NMR study

Volkhard Jenning, Karsten Mäder, Sven H. Gohla *

Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin, Kelchstr. 31, D-12169 Berlin, Germany

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Abstract

SLN with improved payloads and enhanced storage stability were investigated. Based on the experiences with solid lipid nanoparticles, a new type of solid lipid nanoparticle has been developed by incorporating triglyceride containing oils in the solid shell of the particle. The structure and mixing behaviour of these particles was characterised by DSC and ^{1}H-NMR. DSC yields information on the melting and crystallisation behaviour of the solid and liquid constituents of the particles. NMR is especially suited for the characterisation of the liquid oil domains inside the SLN. In this study a medium chain triglyceride oil was successfully incorporated in a matrix of a solid long chain glyceride (glyceryl behenate). The resulting particles were solid but the oil inside the particle remained in a liquid state. The relation between oil supplementation and melting point depression of glyceryl behenate proved to be linear. Mobility of the oil molecules inside the particles was considerably reduced compared to the emulsified oil. Moreover, two different chemical shifts for each of the lipid signals were observed indicating two different chemical environments. The experimental data is in line with a model describing uniform distribution of the oil molecules in the glyceryl behenate for low oil loads. However, at higher oil loads our data indicate the formation of oil clusters within the solid nanoparticle.

Keywords: Solid lipid nanoparticles; NMR; DSC; Liquid lipids; Loading capacity

1. Introduction

SLN based on pure triglycerides such as tripalmitate exhibit limited drug payloads and drug expulsion as a result of the high crystallinity of these particles (Westesen et al., 1997). By using complex glycerides like hard fats as a matrix for SLN, the incorporation of lipohilic drugs is facilitated (Siekmann, 1994). However, these hard fat SLN reveal a tendency to form supercooled melts instead of solid particles. Even if they are solidified at room temperature these particles melt at body temperature. Therefore, these particles are not suited for controlled release applications (Westesen et al., 1993). By mixing different solid lipids, e.g. trimyristin and tristearin, the crystal order is only slightly disturbed (Bunjes et al.,

* Corresponding author. Tel.: +49-30-77000478; fax: +49-30-77000475.

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1996) and thus no improvement in loading capacity can be expected. More promising appear to be mixtures of both, liquid (e.g. oils) and solid lipids (e.g. fats). The SLN in this study are produced by incorporating triglyceride containing oils in the solid shell of said particles. Medium chain triglycerides (Miglyol 812) as the oily constituent and glyceryl behenate (Compritol 888 ATO) as the solid component are used. The structure of the liquid lipids inside the matrix of a solid lipid can be characterised by ^{1}H-NMR and DSC (Müller, 1986).

It was the aim of this study to investigate the mixing behaviour of these liquid and solid lipids in the colloidal state. The DSC measurements allow the characterision of the crystallisation process of the oil upon freezing. Information about the mobility, the arrangement and the environment of the oil molecules is derived from ^{1}H-NMR experiments. The parameters utilised in this study were the line width yielding information about the mobility of the lipids and the chemical shift, which is related to the environment of the molecules. The mobility of the oil molecules is related to the width of the signal. Broad and weak signals are characteristic for molecules of restricted mobility. Decreased mobility of the molecules leads to a decrease in proton relaxation times, which results in considerable line broadening. For solid systems, such line broadening can lead to the absence of any detectable signal under the conditions used in this study. Sharp and intense signals derive from molecules with high mobility (Rücker et al., 1992). Information about the environment and arrangement of the molecules is related to the chemical shift of the signals (Holzgrabe et al., 1998). Signals at 0.9 ppm correspond to CH_3 protons while those at 1.25 ppm derive from CH_2 groups. The loser a CH_2 group is located to electronegative groups, the more the peaks are displaced to higher ppm values (Liedtke et al., 1999).

2. Materials and methods

2.1. Materials

Compritol 888 ATO (INCI: tribehenin, USP/NF: glyceryl behenate) is a mixture of approximately 15% mono-, 50% di- and 35% triglycerides of behenic acid (C_{22}) and was a gift of Gattefossé (D-Weil a. R.). Fatty acids other than behenic acid, mainly of shorter chain length, account for less than 15%. Retinol and Lutrol F 68 (poloxamer 188) were donated by BASF (D-Ludwigshafen). Miglyol 812 (caprylic/capric triglycerides, medium chain triglyceride) was provided by Hüls AG (D-Witten). The surfactant Miranol Ultra C32 (sodium cocoamphoacetate) came from Rhodia (D-Frankfurt). All other chemicals were obtained from Sigma (D-Deisenhofen).

2.2. Preparation of SLN and nanoemulsion

Compritol-SLN were prepared as described in detail elsewhere (Müller and Lucks, 1996). Briefly, Compritol was melted at 85°C and various amounts of Miglyol 812 and retinol were added. The hot lipid phase was dispersed in a surfactant solution (1.5% sodium cocoamphoacetate and 0.2% poloxamer 188) and a premix was formed using an ultra turrax (IKA, D-Staufen). The premix was passed through a Lab 40 high pressure homogeniser (APV Gaulin, D-Lübeck). Three cycles at 500 bar and 85°C were performed. The concentrations of Miglyol and retinol used were 0, 8, 16, 28, 33 and 38%, and 0.33, 0.8, 1.6, 2.8, 3.3 and 3.8% of the lipid phase respectively. The nanoemulsion was prepared in exactly the same manner only replacing Compritol by Miglyol only.

2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed by a Mettler DSC 821^e (Mettler Toledo, D-Gießen). Samples containing 15 mg SLN dispersions were accurately weighed in 40 μl aluminium pans. DSC scans were recorded at a heating and cooling rate of 5 K/min. Melting points and crystallisation points correspond to the maximum and minimum respectively of the DSC curves.

2.4. ^{1}H-NMR

High-resolution proton nuclear magnetic resonance (^{1}H-NMR) spectra of samples prepared in

water were obtained on an Avance DPX 400 spectrometer (Brucker, D-Rheinstätten) operating at 400 MHz and 20°C. TMS (tetramethyl silane) served as a reference for 0 ppm. Signals at 3.7 ppm derive from polyoxyethylene. The signal at 3.7 ppm was used as internal standard for quantification. These polyoxyethylene groups included in the SLN suspension using the block polymer poloxamer 188. Glycerol protons appear at 4.0 ppm and most of the protons of the model drug retinol show a chemical shift of above 4.0 ppm. However in this range a huge water signal with a maximum at 4.8 ppm can be detected masking retinol signals and possible smaller peaks in the vicinity.

3. Results

3.1. DSC investigations

DSC measurements offer a close look at the melting and crystallisation behaviour of crystalline material like lipid nanoparticles. The breakdown or fusion of the crystal lattice by heating or cooling the sample yields inside information on polymorphism, crystal ordering, eutectic mixtures or glass transition processes (Ford and Timmins, 1989). DSC experiments are useful in order to understand solid dispersions like solid solutions, simple eutectic mixtures or, as in this case, drug and lipid interactions and the mixing behaviour of Compritol and Miglyol.

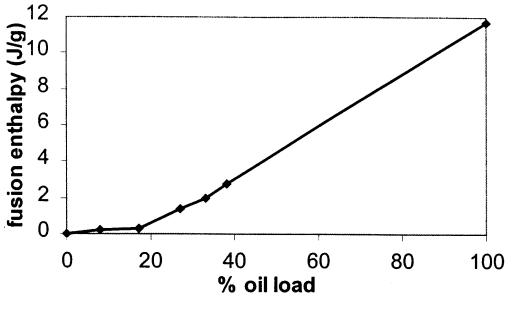

Fig. 1. Fusion enthalpy of the medium chain triglyceride fraction in the SLN and the nanoemulsion ('100% oil load').

In this study DSC is complementary measurement to the NMR experiments in the characterisation of the liquid constituents inside the lipid matrix. For this investigation, the dispersion was cooled down to −60°C and then reheated up to 25°C. Upon cooling the water crystallises at −15 to −18°C. This transition is followed by the crystallisation of the oil (Miglyol) fraction. As a reference a nanoemulsion consisting of pure oil (Miglyol) was investigated as well. Due to the polymorphism of this triglyceride, the crystallisation temperature depends on the cooling rate. The subsequent heating procedure leads to the melting of the frozen oil followed by the melting of the bulk water. The melting endotherm of the oil is very broad and interferes with the melting of water. Therefore a proper evaluation of this process was not possible.

Fig. 1 shows the enthalpy of fusion for the oil inside the SLN shell. For comparison a nanoemulsion comprised of the oil only was investigated as well and included in Fig. 1 as '100% oil load'. Depending on the mixing ratio of oil and solid lipid, the enthalpy of fusion varies. For 8 and 16% oil load, only a very weak crystallisation process is observed. With increasing oil load of the SLN suspension the fusion process increases. However, yet at 38% oil load the fusion energy is significantly smaller for the oil inside the SLN compared to the emulsified oil. The emulsion yields roughly 11.7 J/g oil free whereas the oil inside the solid matrix yields only less than 2.8 J/g oil (38% oil load).

To ensure quantitative incorporation of the oil in the glyceryl behenate matrix the melting points of the mixtures were determined. As a result of the incorporation of guest molecules into the crystalline lattice melting temperature was depressed (Müller, 1986). A linear correlation between the oil concentration of the SLN dispersion and the melting point depression should be a good indicator of complete incorporation. Indeed, for the tested concentration range of 0–38% oil load a linear relationship between oil load and melting temperature was found ($R^2 = 0.993$, slope −0.176 K/% oil).

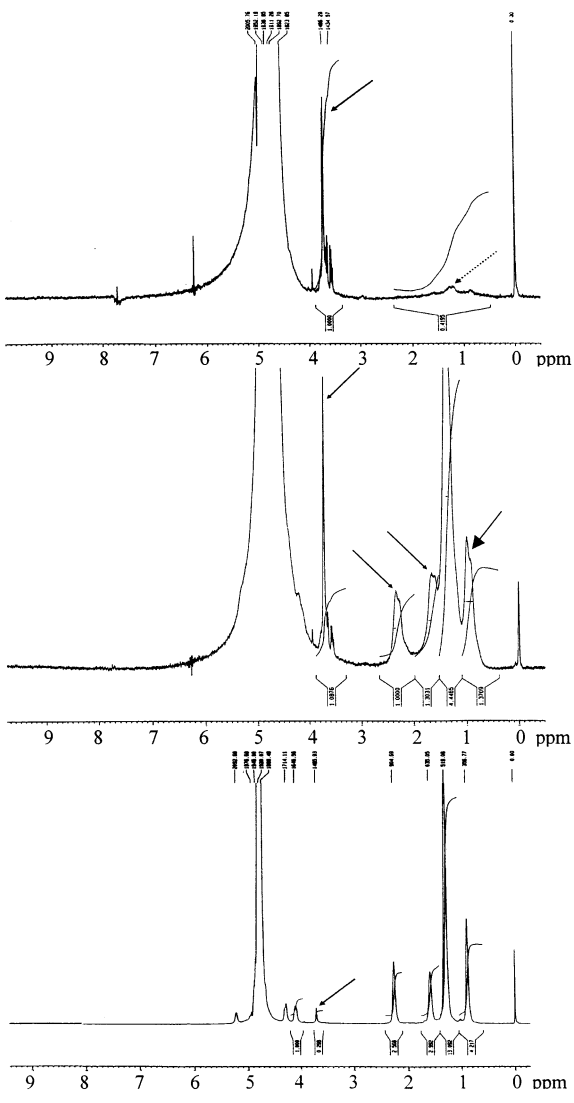

Fig. 2. ^{1}H-NMR spectra of SLN suspensions and nanoemulsion. (Top) SLN based on 100% glyceryl behenate. The dashed arrow is pointing at the signals corresponding to the fatty acids of the glycerides. The solid arrow is pointing at the internal standard. (Middle) SLN based on 16% medium chain triglyceride and 84% glyceryl behenate. The arrows are pointing at the splitting of the signals and the internal standard at 3.7 ppm. (Bottom) Nanoemulsion based on 100% medium chain triglyceride.

3.2. ^{1}H-NMR

Fig. 2 shows the NMR spectra of SLN based on either glyceryl behenate only (top) or 16% medium chain triglycerides and 84% glyceryl behenate (middle). In the lower part of Fig. 2 proton signals of a comparable nanoemulsion consisting of medium chain triglycerides only are shown. Since glyceryl behenate contains no fractions of lipids, which are liquid at room temperature, only very weak and broad signals corresponding to the lipid are detected in this SLN suspension (dashed arrow). Thus, these colloidal particles are practical completely solid. The nanoemulsion shown at the bottom can be characterised by intensive, clearly resolved and sharp signals of the lipid protons between 0.9 and 2.5 ppm. The oil incorporated in the nanoparticle displays an intermediate intensity and width of the signals. This oil can be clearly distinguished from solid lipids (top) and emulsified liquid lipids (bottom).

The signals in the SLN formulated with liquid and solid lipids might result from the oil or from liquefied glyceryl behenate molecules. These two types of molecules can be distinguished by their ratio of CH_2 (1.25 ppm) protons to CH_3 (0.9 ppm) protons. The behenic acid (C_{22}) in glyceryl behenate possesses more CH_2 protons than medium chain triglyceride (C_{8-12}) and, therefore, the ratio of CH_2 to CH_3 protons is higher for glyceryl behenate. Using this ratio, the signals of the SLN with 16% oil can be attributed to the oil and not to liquefied glyceryl behenate molecules. The experimental results using the integrated line intensity (area under the curve) are listed in Table 1. The oil shows a ratio of 3.1 whereas the glyceryl behenate reveals a ratio of 12.2. The detected signals of the SLN based on the binary mixtures reveal ratios of 3.2 and below and result therefore from the oil molecules only.

As mentioned above, the signals arising from oil molecules inserted in the solid nanoparticle matrix are significantly broadened compared to the emulsified molecules. The cumulated half-line widths of the signals between 0.9 and 2.5 (= fatty acid protons) as a function of the oil load of the SLN particle is plotted in Fig. 3. Cumulated half-line widths range from 0.24 and

Table 1
Ratio of the integrated line intensity of the 1.25 ppm signal to the 0.9 ppm signal[a]

Oil load	8%	16%	28%	38%	100%	Glyceryl behenate[b]
Ratio	2.5	3.2	2.6	2.6	3.1	12.2

[a] Measurements of the SLN suspensions, nanoemulsion and pure glyceryl behenate. One hundred percent oil load corresponds to the nanoemulsion.
[b] Measured in $CDCl_3$.

0.83 ppm. The line widths are considerably broader for the oil inside the glyceryl behenate matrix compared to the emulsion ('100% oil load'). A maximum can be detected for 16% oil load. As discussed in more detail below, this maximum can be attributed to a line splitting of these signals. The line splitting can be seen in the spectra of Fig. 2 for the SLN based on liquid and solid lipids (middle) as well (arrow). With increasing oil load, the signals show a better resolution and increase in intensity and sharpness.

The chemical shift of the signal results from the chemical neighbourhood of the active nucleus (Holzgrabe et al., 1998). It was shown that the chemical shift of glyceride protons could be used to distinguish the distribution of these lipid molecules among monomers, aggregates or glyceride molecules in micelles (Zhou and Roberts, 1997). Similarly, in this study two distinct chemical shifts for each of the fatty acid peaks are observed. For the CH_2 protons next to the glycerol moiety (α-CH_2) the chemical shifts are either 2.35 or 2.25 ppm. The chemical shift of the dominating peak for the CH_3 and CH_2 protons of the fatty acids as a function of the oil concentration inside the particle is demonstrated in Fig. 4. Basically, the chemical shifts for the 8 and 16% load levels differ from the chemical shifts of the higher loads and the nanoemulsion. At 8% oil load the shift at 2.35 ppm clearly dominates for the α-CH_2 protons. The 16% level shows a splitting into two signals, however the 2.35 ppm is still dominating. At 28% oil load the 2.25 ppm prevails and the 2.35 ppm peak is reduced to a peak shoulder. At 38% the peak shoulder at 2.35 ppm can be almost neglected. The resemblance of the spectra of the 38% load and the nanoemulsion is most pronounced.

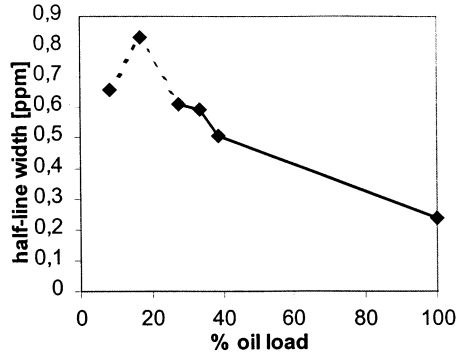

Fig. 3. Cumulated half-line widths (ppm) of the signals corresponding to the fatty acids of the glycerides. * The maximum for 17% oil dotation results from line splitting and not from line broadening.

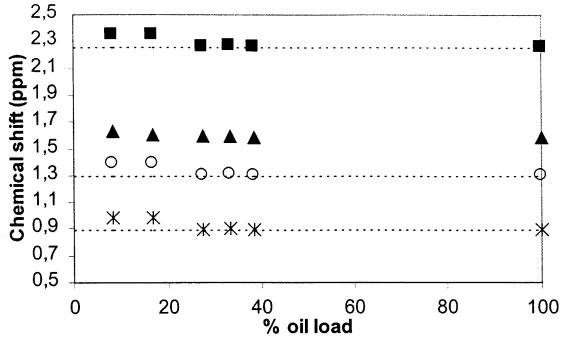

Fig. 4. Chemical shifts (ppm) of the signals corresponding to the fatty acids of the glycerides in the SLN suspensions and nanoemulsion. The dashed lines show the chemical shifts of the pure oil.

4. Discussion

The fusion process of the oil molecules is greatly disturbed as a result of their incorporation into the solid matrix of the lipid nanoparticles. Especially for the 8 and 16% oil supplememtation only a very limited heat of fusion is observed following freezing of the suspension. Obviously the crystal lattice of the glyceryl behenate prevents the proper arrangement of the oil molecules required for crystallisation. Most likely, in the case of these oil loads the molecules are widely distributed within the glyceryl behenate matrix. Since these glyceryl behenate molecules are fixed in their crystal lattice, they prevent relocation of the oil molecules to form individual small oil crystals. The distribution for the C_{8-12} and C_{22} mixture of this study is in good agreement with results for C_{12} or C_{14} and C_{18} mixtures (Precht et al., 1978).

With increasing oil loads easily detectable amounts of crystallisation enthalpy are observed. Thus for these oil load levels clusters of oil molecules that can form crystals must be present in the particle. This result might be expected because lipids of different chain length show only a limited mutual solubility (Bunjes et al., 1996). Therefore higher oil concentrations result in (partial) phase separation or cluster formation.

The melting point depression for the glyceryl behenate matrix corresponds well with the oil load in a linear fashion. From these data it seems likely that the oil is completely incorporated in the matrix of the nanoparticles. Combining these results with the crystallisation behaviour of the oil, it is obvious that at higher oil loads a phase separation of the two components *within* one particle occurs. From the DSC experiments there are no indications for the co-existence of liquid oil droplets and solid glyceryl behenate particles separately.

This view is further strengthened by the NMR measurements. The oil molecules show a restricted mobility as can be seen by the increase in line width and loss of clear resolution of the different signals. With increasing oil loads the mobility increases. However, even at 38% oil load the line widths are still significantly broader for the oil inside the nanoparticle compared to an emulsion of the oil. These signals can be clearly identified as oil molecules and not liquefied glyceryl behenate molecules by the ratio of CH_2 to CH_3 protons. The determined ratio of CH_2 to CH_3 protons fits in all cases the ratio for pure oil. Thus the glyceryl behenate molecules remain in the solid state and do not contribute to the detected lipid protons. Therefore, a certain phase separation between liquid oil areas and solid glyceryl behenate areas inside the particle can be assumed. Furthermore the CH_2: CH_3 ratio for the oil inside the SLN is slightly smaller than for the emulsified oil. Possibly the CH_2 groups in the chain of the fatty acid are more immobilised than the CH_3 end groups. Such behaviour has been observed as well for the α polymorphs of triglycerides. Experimental and computer modelling results indicate greatest movement (or rotation) at the methyl end of each chain of the α form. Furthermore, the glycerol adjacent proportions of the chains are relatively motionless (Hagemann, 1988).

Retinol yielded no observable signals in the NMR-spectra. One reason is the low concentration (0.5% in the suspension) of this model drug. Furthermore, most retinol signals appear above 4 ppm. However, above 4 ppm signals are masked by a huge water signal with a maximum at 4.7 ppm.

The chemical shifts of the lipid protons differ in nanoparticles with high and low oil load. Therefore, the chemical neighbourhood of the oil molecules must be different in particles with either high or low oil load. This is in line with the DSC data indicating uniform distribution of the oil inside the nanoparticle at low oil loads. It can be assumed that for the 8 and 16% oil supplementation the oil molecules are surrounded by a matrix of solid glyceryl behenate molecules. However, in particles containing 28–38% oil,the oil molecules form clusters inside the particle. These two different locations should explain the two different chemical shifts observed for each lipid proton.

In summary this study contributes an observation on the distribution of oil molecules in colloidal lipid nanocrystals. Low concentrations of the oil are distributed randomly among the solid molecules and at high concentrations the phases partly separate and the oil forms cluster inside the nanoparticle.

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