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Physicochemical investigations on the structure of drug-free and drug-loaded solid lipid nanoparticles (SLN^{TM}) by means of DSC and ^{1}H NMR

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A solid lipid nanoparticle (SLN) formulation, based on the lipid Imwitor® 900, was developed for the incorporation of the poorly water soluble drug RMEZ98. Physicochemical investigations were undertaken to examine the structure and physical stability of the selected lipid as colloidal dispersion in comparison to the bulk material. Using differential scanning calorimetry (DSC) and proton nuclear magnetic resonance (^{1}H NMR) it could be assessed the influence of the incorporated drug on the structure of the lipid matrix. Investigation of mixtures of Imwitor® 900 and RMEZ98 showed an increasing effect on the melting/recrystallization behaviour with increasing drug content (5–30%). DSC and ^{1}H NMR results revealed the formation of a crystalline matrix of SLN when prepared by high pressure homogenization excluding, therefore, the phenomenon of supercooled melt. After preparation of RMEZ98-loaded SLN, the drug remained inside the lipid matrix; however, it exhibited only a small effect on the recrystallization behaviour of Imwitor® 900 at the lowest payload required for a therapeutic effect (4% m/m with regard to the lipid matrix). Furthermore, the incorporation of RMEZ98 revealed no distinct influence on the particle size distribution. Imwitor® 900 proved to be a suitable lipid for the drug RMEZ98, i.e. possessing a sufficient loading capacity and simultaneously physical stability.

1. Introduction

The development of a new therapeutic entity results, nowadays, in highly potent substances consisting of complex structures, which are often poorly soluble or very unstable in aqueous media. Thus, the difficulties of processing these above-mentioned substances are due to their low bioavailability. One of the major challenges of drug delivery is the incorporation of these substances into suitable drug carrier systems (such as liposomes, polymeric nanoparticles, lipid nanoemulsions), which might be suitable for the intravenous, oral, pulmonar and topical route.

Solid lipid nanoparticles (SLN^{TM}) are one of these carriers that have been exploited as an alternative colloidal dispersion by various research groups (Müller et al. 2000; Gasco 1997; Siekmann and Westesen 1992; Heiati et al. 1998; Boltri et al. 1995). Morphologically, they consist of a matrix prepared with biocompatible and biodegradable lipids, which are solid both at room and body temperature. The drug substance can be molecularily dispersed in the lipid matrix or be present in amorphous clusters (Mehnert and Mäder 2001). Otherwise, and depending on the preparation procedure, the drug can be preferentially localized in the outer shell of the carrier or in its core. SLN are easy to manufacture by high pressure homogenization (Müller and Lucks 1996) and, due to the solid physical state, prevention of drug leakage allowing prolonged drug release has been described (Heiati et al. 1997; Souto et al. 2004).

SLN were chosen as an appropriate drug delivery system for the poorly water soluble drug RMEZ98 (received from Novartis Pharma AG, CH-Basel). The amorphous substance is unstable if stored above room temperature, i.e. sensitive towards oxygen and hydrolysis. Due to its highly lipophilic character and low chemical stability, the incorporation into the lipid matrix seems very suitable, possibly providing a protection of the drug against chemical degradation. After performing a lipid screening for RMEZ98, Imwitor® 900 was chosen as lipid matrix for SLN preparation.

Although solid lipid nanoparticles are produced from crystalline raw materials, some lipids can create supercooled melts instead of solid systems, such as SLN (Bunjes et al. 1998). The crystallization of the melted lipid to form SLN differs from the bulk material due to the high dispersitivity of the lipid, the small particle size, the high surface/volume ratio, as well as the presence of emulsifying and drug substances. It has been observed that the melting and recrystallization temperatures of dispersed triacylglycerols decrease in comparison to the bulk material, requiring supercooling (Siekmann and Westesen 1994).

The purpose of this study was the investigation of the structure of the lipid matrix in the developed SLN dispersion compared to the bulk Imwitor® 900 and the examination of a possible influence of the incorporated drug RMEZ98 in the crystallization process, applying DSC and ^{1}H NMR techniques. DSC gives information about the

melting and recrystallization behaviour of crystalline material and is therefore used to evaluate the polymorphism, crystal ordering, eutectic mixtures and/or interactions between the lipid and the drug. However, in case of broad peaks interpretation of the results might be difficult due to the dependence of the melt temperature on the particle size and/or overlapping of different melt processes. Therefore, in this study complementary ^{1}H NMR analysis was also performed. Physical analysis by ^{1}H NMR is one of the most widely applied technologies in biology (Harwood et al. 1986) and is used in food industry to study the physical state of lipids. It is a non-invasive, non-destructive and fast method with a simple sample preparation. It does not require a dilution of the sample or the separation of the different components, allowing serial measurements. Since endogenous protons produce the ^{1}H NMR signals, there is no need to introduce additional molecules as for electron spin resonance spectroscopy and fluorescent techniques. Such probes might interact themselves with the lipid matrix and cause structural changes. Further characterization of the SLN dispersion was performed by laser diffractometry (LD) and photon correlation spectroscopy (PCS).

2. Investigations, results and discussion

Based on an intensive screening to find a suitable lipid for the incorporation of the drug RMEZ98 in a SLN dispersion Imwitor® 900 was chosen as hard fat. The bulk material showed a sufficient solubilization for the drug at both 90 °C and at room temperature (20 °C) and only minor polymorphic transitions investigated by X-ray diffraction (Runge 1998) and DSC were observed. Note that SLN prepared with hard fats having high polymorphic transition rates may lead to drug expulsion during storage time (Müller et al. 2000) and, therefore, they have to be observed with special regard. The high melting temperature of Imwitor® 900 (56–61 °C) decreases the risk of formation of supercooled melts during the recrystallization process of the lipid matrix, a phenomenon that has been observed for lipids such as Softisan® 601 (mixture of acylglycerols of palmitic and stearic acids), with a melting range between 40 °C and 45 °C.

2.1. DSC analysis

To investigate the interaction of the drug RMEZ98 with the lipid matrix, the melting and recrystallization behaviours of physical mixtures of Imwitor® 900 with increasing amounts of RMEZ98 (0%, 5%, 10%, 15%, 20%, 25% and 30%) were examined by DSC. The first heating/cooling process with the following isothermal storage at 20 °C simulates the production of SLN dispersions, including solubilization of RMEZ98 in the melted lipid and expected recrystallization processes at room temperature. To investigate the mixture behaviour of drug and lipid, evaluation was focused on the second heating and cooling process of the mixtures, i.e. after dissolution of the drug in the lipid during the first heating run.

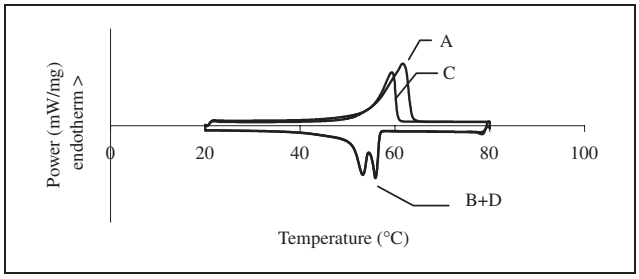

Fig. 1: DSC scans of bulk Imwitor® 900. (A) first heating run from 20 °C to 80 °C; (B) second heating run from 20 °C to 80 °C; (C) first cooling process, and (D) second cooling process. Heating and cooling rates at 5 K/min

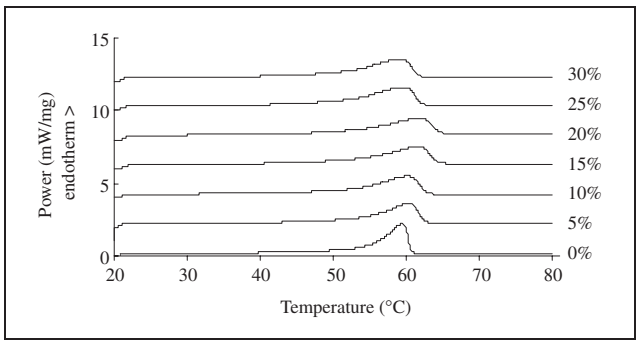

Fig. 2: DSC scans of physical mixtures of Imwitor® 900 containing (from bottom) 0, 5, 10, 15, 20, 25 and 30% of RMEZ98 (second heating process from 20 °C to 80 °C at a rate of 5 K/min)

Fig. 1 shows the first and second heating and cooling runs of Imwitor® 900 as bulk material. From the first to the second heating curves a decrease in onset temperature and peak maximum of the bulk Imwitor® 900 could be observed. This decline might impair the polymorphic transition from the β′-modification to the α-modification of the lipid. On the contrary, the cooling curves (first and second runs) showing recrystallization in two steps, are superposed revealing the absence of polymorphic transitions.

With regard to the physical mixtures of drug and lipid, Table 1 and Fig. 2 show, respectively, the obtained DSC

Table 1: DSC data of physical mixtures containing 0, 5, 10, 15, 20, 25 and 30% RMEZ98 (second heating curve from 20 °C to 80 °C at a heating rate of 5 K/min)

DSC parameters	Physical mixtures of Imwitor® 900 with increasing amounts of RMEZ98 (m/m)						
	0%	5%	10%	15%	20%	25%	30%
Melting parameters:							
Onset (°C)	54.4	52.9	51.5	51.9	51.3	50.2	50.1
Peak maximum (°C)	58.9	59.5	59.3	60.7	61.0	58.8	58.4
Δ maximum-onset (K)	4.5	6.6	7.8	8.8	9.7	8.6	8.3
Enthalpy (J/g)	121	123	124	123	123	123	124
Recrystallization parameters:							
Onset (°C)	56.9	56.4	55.0	55.9	55.6	54.8	54.1
Minimum (°C) 1st peak	56.3	53.6	53.3	–	–	–	–
2nd peak	53.5	51.4	50.7	49.2	48.6	50.0	49.6
Enthalpy (J/g)	−121	−120	−124	−128	−130	−128	−123

parameters and melting curves. The lowest curve represents the pure Imwitor® 900. The curves above record the physical mixtures with increasing amounts of drug. The extrapolated onset of the melting curve decreased with increasing drug loading (Table 1). The peak maximum of the curves first increased from 58.9 °C (bulk material) with increasing drug loading to 61.0 °C (20% drug), then decreased with further increasing drug loading to 58.4 °C (30% drug). The melting enthalpy of all samples was very similar to pure Imwitor® 900. Additional melting peaks were not observed. Up to 20% of incorporated drug, the difference between the onset and the peak maximum increased from 4.5 K (bulk material) up to 9.7 K. Further increase in drug loading slightly decreased this difference to 8.3 K. A broadening of peaks (lower onset and higher peak maximum temperatures) indicates crystal order disturbance.

Regarding the recrystallization of the physical mixtures after the second heating process (Table 1), a decrease of the onset temperature could be observed as well. The pure bulk material and the physical mixtures containing 5% and 10% of RMEZ98 recrystallized in two peaks, while increasing amounts of RMEZ98 from 15% to 30%, the physical mixture recrystallized only in one peak. The temperature in the minimum of the main peak decreased with increasing drug content from 53.5 °C to 49.6 °C, whereas an increase of the melting enthalpy could be observed.

Since no phase separation was observed, the drug might be incorporated in the crystal lattice of the lipid. These results from the melting and cooling behaviour indicated an increased interaction of the drug with the lipid with increasing amount of RMEZ98 in the mixture, leading to crystal order disturbance. Drug molecules might be localized in between the different lamella of the lipid or inside the lamella. However, a specific drug-lipid interaction cannot be postulated without X-ray diffraction analysis. It has to be mentioned that the isothermal storage for 1 h at 90 °C might not have been sufficient for complete transition processes, which has to be further observed during storage studies. In addition, high drug loading should be renounced to avoid possible inhomogenous drug distribution or massive disturbance of the recrystallization behaviour of the lipid. The drug RMEZ98 is highly potent, therefore a drug loading of 4% in this lipid (0.4% in the aqueous SLN dispersion with 9.6% lipid) results in a sufficiently high dose after administration of 1 ml drug-loaded SLN dispersion. This concentration of RMEZ98 showed only a negligible interaction with the lipid under investigation conditions, although detection limits of DSC measurements have to be taken into account.

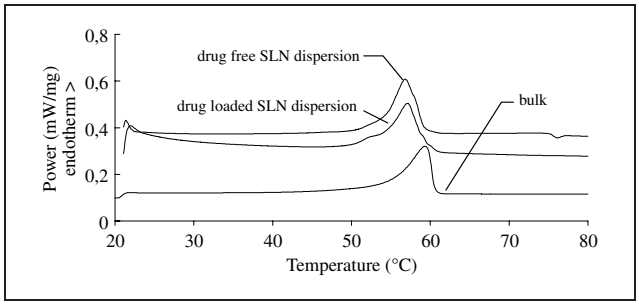

Fig. 3: DSC heating curves of drug-loaded (0.4% RMEZ98, regarding the total dispersion) and drug-free SLN dispersion (first heating curve) compared to the bulk Imwitor® 900 (second heating curve) at a heating rate of 5 K/min

Table 2 and Fig. 3 show, respectively, the obtained DSC parameters and melting curves of drug-free and drug-loaded SLN dispersions in comparison to bulk Imwitor® 900.

The maximum peak of the bulk Imwitor® 900 decreased from 61.3 °C in the first heating curve (simulating the production process of the SLN dispersion) to 58.9 °C in the second heating curve. This decline reveals a polymorphic transition of the lipid from the β'-modification to the α-modification, which has been confirmed by X-ray diffraction analysis (Runge 1998). However, it is not possible to quantify the crystallinity of the SLN by comparison of their melting enthalpies with the values obtained for the bulk Imwitor® 900, due to the difference of transition enthalpies for different polymorphs. With regard to drug-free formulation, a further decrease in melting temperature from 58.9 °C to 57.1 °C could be observed in comparison to the second heating curve of the bulk material (first heating process). The first heating of the SLN dispersion has to be compared with the second heating of the bulk (first heating and melting of the lipid in the SLN occurred during production). This decrease in peak maximum is due to several factors, such as the high specific surface area, the small particle size and the presence of water and emulsifiers. It can be described by the Thomson equation and refers to the Kelvin effect (Hunter 1986). Comparing the recrystallization phenomenon during the second cooling process, the bulk material crystallized in two peaks, whereas only one peak could be observed in the curve of the SLN dispersion. The recrystallization temperature of the main peak decreased from 56.3 °C (bulk Imwitor® 900) to 51.0 °C (aqueous SLN dispersion).

The decrease of the melting temperature, as well as of the recrystallization temperature may lead to supercooled melts. This phenomenon occurs especially in dispersions

Table 2: DSC data of drug-loaded (0.4% RMEZ98, regarding the total dispersion) and drug-free SLN dispersions compared with the second heating curve of the bulk Imwitor® 900 (heating rate of 5 K/min)

DSC parameters	Drug-free SLN	Drug-loaded SLN	Bulk Imwitor® 900	
	1st heating	1st heating	1st heating	2nd heating
Melting parameters:				
Onset (°C)	53.5	53.5	56.4	54.4
Peak maximum (°C)	56.6	56.8	61.3	58.9
Enthalpy (J/g)	104	104	178	121
Recrystallization parameters:				
Onset (°C)	52.8	52.3	56.9	56.9
Minimum (°C) 1st peak	51.1	51.0	56.3	56.3
2nd peak	–	–	53.6	53.5
Enthalpy (J/g)	−106	−112	−121	−121

with simple saturated triacylglycerols (Bunjes et al. 1998). For the SLN formulations developed with Imwitor® 900 and PVA and SDS as emulsifier/co-emulsifier, the obtained DSC results show the presence of a crystalline matrix. The recrystallization at room temperature can be attributed to the fact that Imwitor® 900 (glycerol monostearate 40%–50%) is a complex triacylglycerol mixture. The tendency of supercooling in emulsified triacylglycerols is much lower for mixed triacylglycerols (Bunjes et al. 1998), as well as for partial acylglycerols (especially monoacylglycerols) (Walstra and Beresteyn 1975). Longer chains in the lipid may act as nucleation centre and thereby induce the crystallization of the lipid. The type of emulsifying agent may also have an additional influence on supercooling phenomenon (Bunjes et al. 1998), although detailed mechanisms are not yet well understood. It also has to be considered that the melting point of the bulk material (61.3 °C) is relatively high, promoting recrystallization after production at room temperature (large difference between melting point of lipid and storage temperature).

Comparison of the drug-free SLN dispersion with the drug-loaded SLN formulation yielded only minor differences in the melting and recrystallization behaviour as shown by DSC measurements (Fig. 3, Table 2). Identical results were found for the onset temperature and the melting enthalpy of the heating curve. The peak maximum was slightly reduced by 0.2 °C in the drug-loaded SLN dispersion, a value that can be neglected. The differences in the recrystallization curves were also negligible (a slight decrease of 0.5 °C of the onset and a decrease of 0.1 °C of the peak minimum of the drug-loaded dispersion). The crystallization enthalpy of the two formulations can also be compared. Under the investigation conditions, it was not possible to distinguish clearly by DSC between the presence of the drug as amorphous clusters or as a solid dispersion due to detection limits. However, DSC results indicated that the used concentration of RMEZ98 had no essential influence on the melting or recrystallization behaviour of the SLN formulation.

2.2. ^{1}H NMR analysis

^{1}H NMR investigations were used to obtain information about the mobility and structure of both the nanoparticle system and the incorporated drug. The width of ^{1}H NMR lines is related to the phenomenon of relaxation (Harwood et al. 1986), thus decreased mobility of the molecules leads to a decrease in the relaxation time which results in considerable line broadening. ^{1}H NMR is therefore an appropriate tool to distinguish between mobile liquid and immobilized solid states. Fig. 4 shows the ^{1}H NMR spectra of the bulk Imwitor® 900.

The results obtained from DSC were confirmed by the ^{1}H NMR analysis. Only broad signals could be seen, indicating a highly restricted mobility of the acylglycerol molecules of the lipid, which has been shown by melting endotherms in the DSC heating curves. However, as already reported for certain triacylglycerols and hard fats (Bunjes et al. 1998; Westesen et al. 1997), the crystallization behaviour may be different in colloidal dispersed material. ^{1}H NMR investigations of the SLN formulations should therefore verify the information about the physical state of the lipid matrix after the production process.

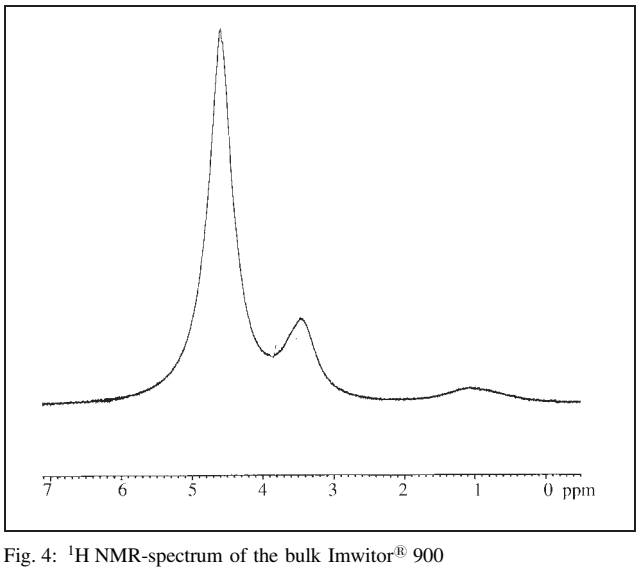

Fig. 4: ^{1}H NMR-spectrum of the bulk Imwitor® 900

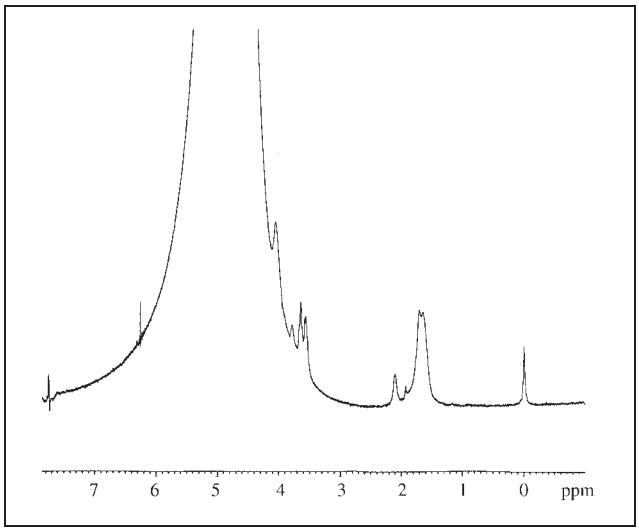

Fig. 5: ^{1}H NMR-spectrum of drug-free SLN dispersion (10% Imwitor® 900, 1% PVA and 0.1% SDS)

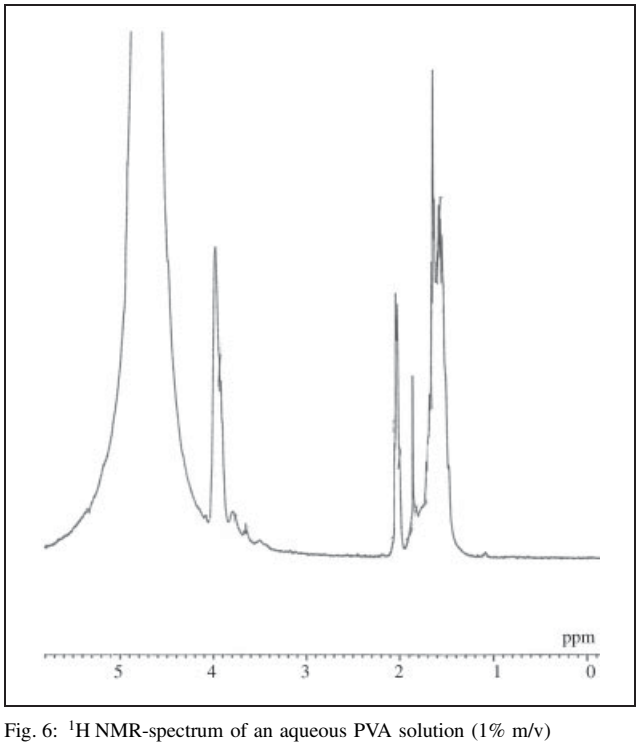

Fig. 6: ^{1}H NMR-spectrum of an aqueous PVA solution (1% m/v)

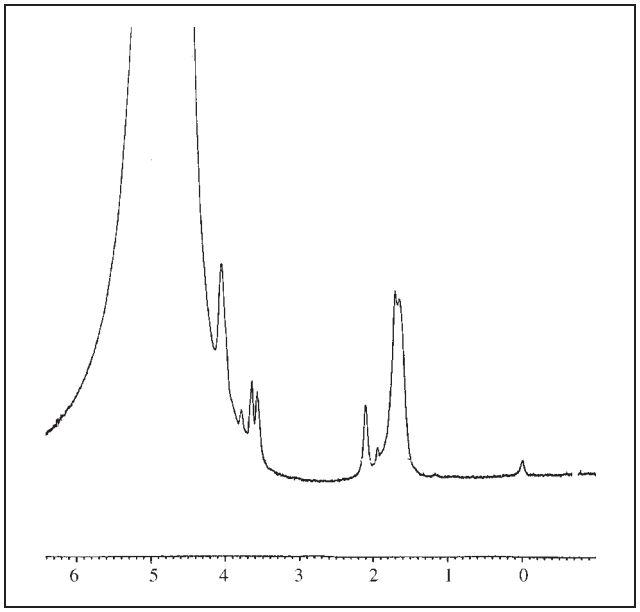

Fig. 7: ^{1}H NMR-spectrum of drug-loaded SLN dispersion (9.6% Imwitor® 900, 1% PVA, 0.1% SDS and 0.4% RMEZ98)

Fig. 5 and 6 show the ^{1}H NMR-spectra of the drug-free SLN dispersion and of an aqueous solution containing 1% (m/v) PVA, respectively. The spectrum of the drug-free SLN dispersion confirmed the assumption of immobilized molecules of the dispersed lipid phase. The spectrum was dominated by the signals of the emulsifer PVA (1.6–2.1 ppm, 3.6–4.1 ppm, Fig. 6) and the huge water signal at 4.7 ppm. The lack of lipid signals indicated the restricted mobility of the lipid molecules. These results excluded formation of supercooled melts of the colloidal dispersed lipid after the production process.

Fig. 7 shows the ^{1}H NMR-spectrum of the drug-loaded SLN dispersion. Comparing the drug-loaded SLN formulation with the drug-free colloidal dispersion, no essential difference could be observed. Again, the spectrum was dominated by the water and the surfactant signals. No signals of Imwitor® 900 could be detected. The incorporated drug did not disturb the recrystallization of the dispersed lipid after preparation of the nanoparticles as shown by the absence of narrow peaks. Furthermore, the spectrum did not show any signals which could be assigned to the incorporated drug. RMEZ98 gives rise to signals at about 2.5 ppm which are clearly separated to those of the carrier dispersion. Although the concentration of the drug in the SLN dispersion was very low (0.4%), high mobile drug molecules would have been possibly detected due to the separated chemical shift of the exhibited signals. It could be concluded, that the drug did not exist in a high mobile state in the aqueous phase. RMEZ98 was somehow associated to the colloidal carrier. However, the question whether the drug was incorporated into the lipid matrix or was bound to a semi-solid film of emulsifier at the surface of the carrier could not be answered by this NMR sequence. This problem has to be investigated by the technique of solid state NMR of by atomic force microscopy (Dingler 1998).

2.3. Particle size analysis

Particle size analysis is important for the characterization of the physical stability of colloidal SLN dispersions. PCS and LD measurements were performed to investigate the influence of drug incorporation on the SLN dispersion. All formulations – drug-free and drug-loaded – were practically identical in particle size, also important for the interpretation of the DSC results (effect of size on melting). Drug-free formulation yielded a mean particle size of 170 nm while drug-loaded formulation yielded a mean value of 160 nm. The drug-loaded formulation showed a slightly increased PI of 0.258, compared with a PI of 0.199 for the drug-free SLN dispersion, indicating a slightly broader distribution of the particle size. An increase in PI of SLN after drug incorporation is a general phenomenon observed with many drugs. LD measurements were in agreement with the PCS results (Fig. 8). For the drug-free formulation the LD50% and LD99% were, respectively, 96 nm and 295 nm. Concerning drug-loaded formulations there parameters decreased to 92 nm and 292 nm, respectively. The incorporation of 0.4% (m/m) RMEZ98 in the SLN formulation did not have an essential influence on particle size parameters.

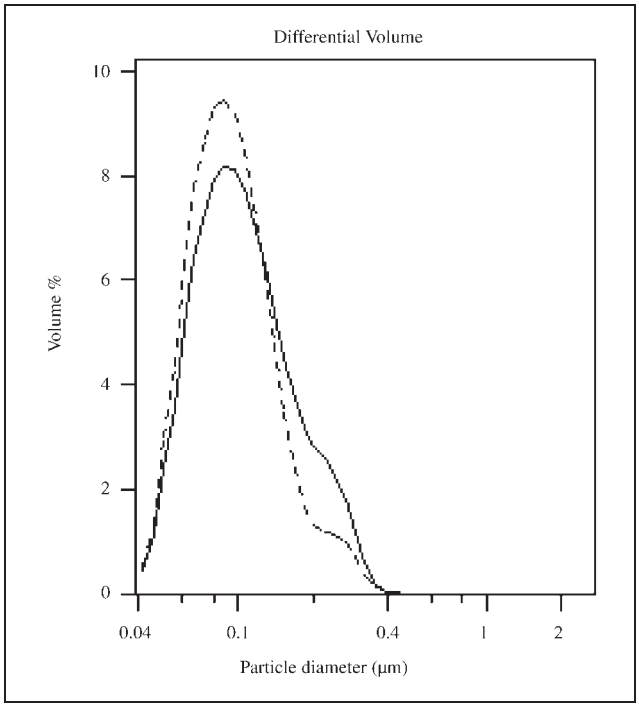

Fig. 8: LD measurements of drug-free (——) and drug-loaded (----) SLN dispersions (% volume distribution)

2.4. Conclusions

This study investigated the structure of the lipid matrix before and after preparation of SLN dispersion and the influence of the incorporated poorly water soluble drug RMEZ98. Physicochemical characterization was undertaken by DSC and ^{1}H NMR measurements. The combination of both analytical methods proved very suitable to obtain information about the structure of the lipid matrix in the dispersed colloidal systems. The results showed that Imwitor® 900 seems to be a suitable lipid matrix for the incorporation of the drug RMEZ98; however, high drug loading should be avoided. The investigations proved the solidification of the dispersed lipid after the production of the SLN dispersions, while stored at room temperature. The phenomenon of supercooled melts could be excluded. The drug showed a certain association to the lipid matrix, but the incorporation of the drug in the concentration of

4% (m/m, with regard to the lipid matrix) did not have an essential influence on the melting and recrystallization behaviour of the lipid phase or on the particle size distribution of the colloidal dispersion. In conclusion, a suitable SLN formulation with the lipid Imwitor® 900 could be developed for the drug RMEZ98.

3. Experimental

3.1. Materials

Imwitor® 900 (glycerol monostearate, 40–50%) was a gift of Condea (Witten, Germany). Polyvinylalcohol (PVA), av. molecular weight 30,000–70,000, and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (Deisenhofen, Germany). The drug RMEZ98 (macrolide with immunosuppressive properties exhibiting a triene and ester structure) was provided by Novartis (Basel, Switzerland).

3.2. Preparation of aqueous SLN dispersions

Imwitor® 900 (10%, m/v) was melted at 80 °C and added to an aqueous solution containing 1% (m/v) PVA and 0.1% (m/v) SDS of the same temperature. The mixture was stirred with a T 25 ultra-turrax (IKA, Staufen, Germany) for 1 min at 8000 rpm. The obtained pre-emulsion was homogenized at 80 °C using the high pressure homogenizer Micro LAB 40 (APV Deutschland GmbH, Lübeck) at 500 bar for 3 homogenization cycles. The samples were stored at room temperature (20 °C). For the preparation of drug-loaded SLN dispersions, the lipid was partially replaced by the drug RMEZ98 (4% with regard to the lipid phase, i.e. 0.4% referring to the total dispersion). The drug was dissolved in the melted lipid prior to the production of the pre-emulsion. Details in the production method are given elsewhere (Müller et al. 2000).

3.3. Differential scanning calorimetry (DSC)

DSC analysis was performed using a Mettler DSC 821^e (Mettler Toledo, Gießen, Germany). DSC scans were recorded at a heating and cooling rate of 5 K/min. The samples were heated up to 80 °C, kept for 15 min at 80 °C, cooled down to 20 °C, kept at 20 °C for 16 min, reheated again up to 80 °C and cooled down again to 20 °C. Melting and recrystallization points correspond to the maximum and minimum of the DSC curves, respectively.

3.4. Nuclear magnetic resonance (^{1}H NMR)

^{1}H NMR spectra were obtained on an Avance 400 DPX spectrometer (Brucker, Rheinstätten, Germany) operating at 400 MHz at 20 °C. Deuterium oxide was added to the samples prior to the measurements. Tetramethylsilane (TMS) was used as an internal standard for 0 ppm in the SLN formulations.

3.5. Particle size analysis

Laser diffractometry (LD) was performed using the Coulter LS 230 (Coulter Electronics, Kiel, Germany) yielding the volume distribution of the particles. Mie analysis of the raw data was applied. Characterization parameters were the diameters LD50% and LD99%. A diameter LD50% of 1 μm means that 50% of all particles possess a diameter of 1 μm or less.

Photon correlation spectroscopy (PCS) was carried out using a Malvern Zetasizer IV (Malvern Instruments, UK). The system was used in the auto measuring mode. PCS yields the diameter of the bulk population (z-average) and the polydispersity index (PI) characterizes the width of the distribution.

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