



Encapsulation of retinoids in solid lipid nanoparticles (SLN[®])

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SLN have been suggested for a broad range of applications, such as intravenous injection, peroral, or dermal administration. The incorporation of the drug in the core of the SLN has to be ensured for these applications, but the inclusion of drugs in SLN is poorly understood. This study is a contribution to further describe the inclusion properties of colloidal lipids and to propose incorporation mechanisms. Besides the well known methods to investigate entrapment of actives in nanoparticles such as DSC or microscopy, the present study focussed on yet a different approach. Based on the different chemical stability of retinoids in water and in a lipid phase, a method to derive information on the distribution of the drug between SLN-lipid and the water phase was established. Comparing different lipids, glyceryl behenate gave superior entrapment compared to tripalmitate, cetyl palmitate and solid paraffin. Comparing three different drugs, entrapment increased with decreasing polarity of the molecule (tretinoin < retinol < retinyl palmitate). The encapsulation efficacy was successfully enhanced by formulating SLN from mixtures of liquid and solid lipids. These particles were solid and provided better protection of the sensitive drugs than an emulsion. X-ray investigations revealed that good encapsulation correlated with a low degree of crystallinity and lattice defects. With highly ordered crystals, as in the case of cetyl palmitate, drug expulsion from the carrier was more pronounced.

Keywords: Solid lipid nanoparticles, retinoids, encapsulation efficacy, drug leakage, chemical stability.

Introduction

SLN have been suggested for a broad range of applications. Among these, the parenteral (Domb 1993), peroral (Runge 1998) and dermal administration (Schütt *et al.* 1998) routes can be mentioned. Drug-free nanoparticles can be used in dermal preparations to improve occlusive properties (de Vringer 1992). In the case of drug loaded SLN, sustained release and stabilization of encapsulated agents are of importance. Sustained release of prednisolone in SLN over a period of several weeks, as well as the burst release with other drugs, were reported in Müller *et al.* (1994). Similar, SLN–drug combinations possess a loading capacity of up to 50% (related to the lipid phase) (Westesen *et al.* 1997). Yet other examples show only a limited encapsulation efficacy and drug expulsion from the carrier. The reasons and mechanisms why certain drugs are encapsulated very efficiently while others are not are still poorly understood. One hypothesis is based on the different

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melting points of the drugs (Müller *et al.* 1994). It is believed that drugs with higher melting points than the lipid precipitate first after the hot homogenization production process, and the drug, therefore, forms a core in the lipid phase. These systems (e.g. prednisolone) show sustained release and good encapsulation efficiency. In the case of drugs with lower melting points than the lipid, the lipid recrystallizes first and forms a lipid core, and the drug can only distribute to the surface. Thus, poor encapsulation and a burst release results. However, this hypothesis, derived from release studies, was not further investigated experimentally.

Preliminary experiments revealed a poor encapsulation efficiency of retinoids in SLN. Therefore, these compounds should be good test candidates to reveal mechanisms of encapsulation in the nanoparticles and drug expulsion from the carrier. For the present study, three different drugs were chosen with similarities but also distinct physicochemical differences. Retinol is the free vitamin A alcohol with a molecular weight of 286.4 and a melting point of 63°C. The second model drug is the ester of retinol with palmitic acid, retinyl palmitate, with a molecular weight of 468.8 and a melting temperature of 29°C. The third compound to be tested in this study is tretinoin (vitamin A acid), the oxidation product of retinol with a molecular weight of 300.3 and a melting point of 179°C. The aims of this study were to compare methods to evaluate drug encapsulation in SLN, the development of SLN with high payloads, and, finally, the investigation of possible mechanisms of drug encapsulation.

Materials

Compritol 888 ATO (glyceryl behenate, tribehenin) is a mixture of mono-, di- and triglycerides of behenic acid (C22) and was a gift of Gattefossé (Weil, Germany). Fatty acids other than behenic acid, mainly of shorter chain length, account for less than 15%. Dynasan 116 (Hüls, Witten, Germany) is a tripalmitate (90% purity). Cutina CP (cetyl palmitate) came from Henkel (Düsseldorf, Germany) and solid paraffin from Caelo (Hilden, Germany). Tretinoin, retinol and retinyl palmitate were donated by BASF (Ludwigshafen, Germany). Miglyol 812 (caprylic/capric triglycerides) was provided by Hüls AG (Witten, Germany). All other chemicals were obtained from Sigma (Deisenhofen, Germany).

Methods

Preparation of SLN

Retinoid loaded SLN were prepared as described elsewhere (Müller and Lucks 1996). Briefly, the solid lipid was melted at 85°C and the respective drug was added. The hot lipid phase was dispersed in a surfactant solution and a pre-mix was formed using an Ultra Turrax mixer (IKA, Staufen, Germany). The pre-mix was passed through a Micron Lab 40 high pressure homogenizer (APV Gaulin, Lübeck, Germany). Three cycles at 500 bar and 85°C were performed.

Particle size analysis

Particle size analysis was performed by photon correlation spectroscopy, PCS (Coulter N4 Plus, Coulter Electronics, Krefeld, Germany). PCS yields the mean particle size and the polydispersity index (PI) as a measure of the width of the distribution.

HPLC analysis

HPLC (Kontron Instruments, Neufahrn, Germany) and a LiChrospher 60 RP select B column (Merck, Darmstadt, Germany) served to quantify tretinoin, retinol and retinyl palmitate concentrations. The mobile phases consisted of acetonitril/water (80:20) plus 0.1% phosphoric acid for tretinoin and retinol, and 100% acetonitril for retinyl palmitate, respectively. The retention times were 5.7 min for tretinoin, 6.1 min (retinol), and 4.2 min (retinyl palmitate). UV absorption was read at 325 nm and drugs were identified by their UV spectrum using a diode array detector. Reproducibility was 2.1%.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a Mettler DSC 821^e (Mettler Toledo, Gießen, Germany). Samples containing 14–16 mg dispersion were accurately weighted in 40 µl aluminium pans. DSC scans were recorded at a heating rate of 5°C/min. Melting points correspond to the maximum of the heating curve.

WAXS analysis

Wide angle X-ray scattering (WAXS) was performed on a Philips PW 1830 X-ray generator (Philips, NL-Amelo) with a copper anode (Cu-K α radiation, $\lambda = 0.15418$ nm). Scattering angles were transformed into short spacings using Bragg's equation. Liquid samples were thickened with 0.5% xanthan gum directly before the measurement. This procedure induced no changes in the diffraction pattern, as ensured by comparison with the diffraction pattern of liquid preparations.

Statistics

All data are presented as arithmetic mean values \pm standard deviation ($\bar{x} \pm SD$). Significance of differences was analysed using Shapiro-Wilk-, F -, and Student's t -test, $p \leq 0.05$ was considered significant.

Results

Methods for the evaluation of drug incorporation

Three different methods were employed to investigate the drug encapsulation in SLN. The first method is based on the decomposition kinetics of the drug in water and in an oil phase. Secondly, (polarized) light microscopy was employed to detect drug crystals in the suspension, as suggested by Westesen *et al.* (1997). In addition, the depression of melting point of the lipids due to the drug was recorded with DSC. In contrast to the second and third method, the first approach is new in

Table 1. Chemical stability of retinoids in different media. The drugs were added (a) to the surfactant solution used in the preparation of the SLN, (b) to drug free SLN after their production, and (c) to a submicron fat emulsion prepared of medium chain triglycerides.

$t_{50\%}$ (d)	Retinol		Retinyl palmitate		Tretinoin	
	RT	40°C	RT	40°C	RT	40°C
Surfactant solution	111.4	61.4	25.5	21.3	49.1	32.3
Drug free SLN	92.7	69.0	—*	—*	56.8	21.3
Emulsion	231.8	143.2	630	204	1086	690

* The solubility of retinyl palmitate in the suspension of drug free SLN was below the detection limit. The degradation follows 1st order kinetics and is expressed as half life times ($t_{50\%}$).

the study of drug encapsulation in SLN. This approach is possible because retinoids are very unstable in water, whereas in lipids a much improved stability can be observed. Monitoring drug content of the suspension as a function of time can be used to determine the distribution of the drug between the water and the lipid phase of the SLN dispersion. High drug recovery rates after storage indicate that the drug is in the lipid phase. If only small amounts of retinoid are recovered, the retinoid was present in the water phase and, thus, subject to degradation. The different half-life times of the tested retinoids were determined in surfactant solution and in a dispersion of drug-free SLN (the drug was added to the suspension after the recrystallization of the nanoparticles) and in an emulsion. The latter describes stability in an oil droplet, whereas the first two show stability in micelles and adsorbed to particle surfaces (table 1). Because of the insolubility of the drugs in pure water, no direct measurement of stability in water was possible.

Table 1 shows that, in all three cases, significant differences in the $t_{50\%}$ values were observed for retinoids in water and for retinoids in the lipid phase. Thus, it is possible to differentiate between drug inside the lipid phase of the SLN dispersion and drug inside the water phase (in micelles or adsorbed to particle surfaces). A possible artefact of this kinetic method is direct chemical interaction of the lipid and the drug. Such chemical destabilization (e.g. due to radicals in the lipid) was excluded by monitoring the stability of the retinoids in the melted lipid phase. At 85°C, the retinoids dissolved in melted lipids or in the medium chain triglycerides displayed the same chemical stability. Therefore, a direct interaction of retinoids and lipids is unlikely.

Light microscopy is useful to detect crystals of expelled drug in the suspension. Tretinoin and retinol show characteristic shaped crystals of several micrometres and above, and can, therefore, be clearly distinguished from nanoparticles. In the case of retinyl palmitate, the melting point is low and, in general, no recrystallization occurs. In the case of retinyl palmitate being expelled from the SLN, small yellow oil droplets can be observed on the surface of the aqueous dispersion.

The inclusion of drug molecules in the crystal lattice is normally accompanied by a depression of the melting point (Müller 1986). A linear correlation between melting temperature and concentration of added drug is a good indicator of complete inclusion of the drug in the lattice. In contrast, if the thermal behaviour

of the lipid matrix is not altered in the presence of drug, most likely the drug is not incorporated in the lipid matrix. Therefore, from DSC measurements, valuable information on the interaction between lipid nanoparticle and drug can be derived.

Taking all three methods together, a comprehensive discussion of the inclusion properties of SLN is possible. All three methods do not directly yield a quantification of the encapsulation efficacy (e.g. a percentage). Quantitative methods are essentially based on the separation of the lipid and aqueous phase of the dispersion. These separations can be achieved by filtration (Müller *et al.* 1994), centrifugation (zur Mühlen 1996), gel permeation chromatography, or dialysis (Heiati *et al.* 1998). In the case of filtration (e.g. 0.1 µm membrane filters), expelled drug crystals are also removed by the procedure. Using the centrifugation method, both expelled retinyl palmitate and the SLN lipid will be in the supernatant. The dialysis method is restricted, because the diffusion of the drug through the membrane might be the rate limiting step. It is questionable whether this method can distinguish between adsorbed and incorporated drug.

Encapsulation of retinoids in different lipid matrices

Chemical stability of retinol in different SLN formulations is shown in figure 1. All SLN formulations displayed an inferior stability compared to the emulsion. Glyceryl behenate SLN showed best encapsulation at room temperature and 40°C, while solid paraffin SLN revealed the fastest degradation of the active agent. In all cases, the 5% retinol load (related to the lipid) was too high and

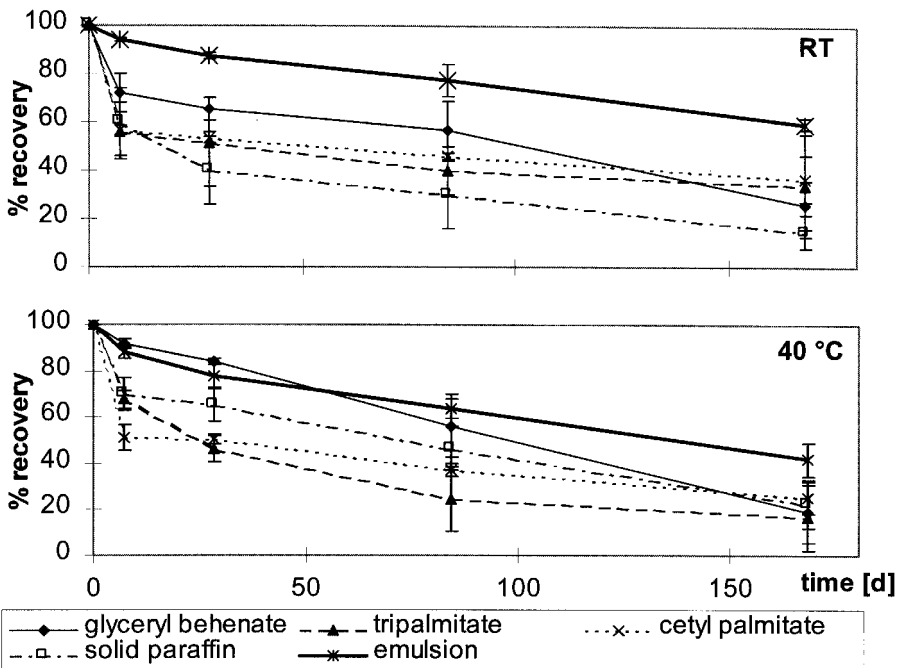


Figure 1. Stability of retinol in different SLN formulations at room temperature (RT, top) and 40°C (bottom), $n = 3$.

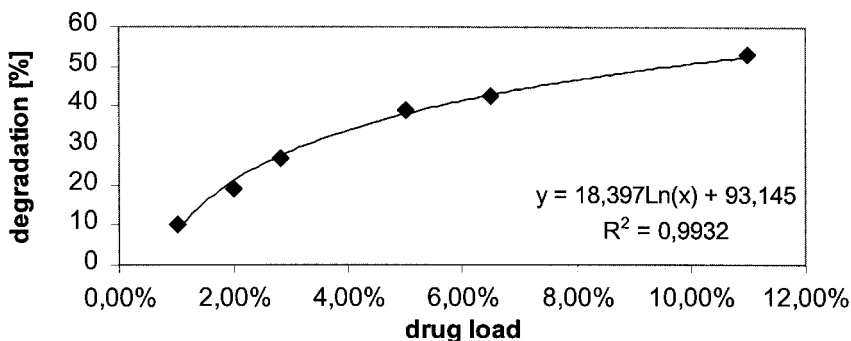


Figure 2. Relation between initial (first week) retinol degradation and drug load. A reasonable fit can be gained using a logarithmic equation.

the drug was expelled from the particles. These results were confirmed by light microscopy showing drug crystals in these suspensions. Comparing room temperature and 40°C, rather similar degradation rates are observed in the case of SLN. This can be explained by the temperature-dependent solubility of the drug. At the higher temperature, solubility increases and drug expulsion from the carrier is less pronounced. This behaviour counteracts the well known temperature-dependent increase in degradation according to the Arrhenius equation.

Comparing the four different lipids, glyceryl behenate was the best matrix material for retinol. The loading capacity of this material was, therefore, further investigated. As shown in figure 1, most of the degradation of retinol at room temperature occurs within the first 2 weeks of storage. The partly expelled drug probably degrades within this time period. A reduced or absent decomposition of retinol within the first week is, therefore, an indication of the absence of expelled drug in the water phase. In figure 2, the degradation of retinol within the first week as a function of drug load is plotted. The data can be fitted by a logarithmic equation, shown in figure 2. From this equation, a maximum retinol load of 0.63% can be calculated (for $y = 0$). Using microscopic evaluation, SLN dispersions with 1% and below show no drug crystals. Again, microscopic and kinetic data agree.

The encapsulation of tretinoin and retinyl palmitate in SLN was further investigated. Retinyl palmitate showed a $t_{50\%}$ values of 204 d in the emulsion at 40°C (table 1). Glyceryl behenate SLN formulations with 5 or 2.5% drug load revealed $t_{50\%}$ values of 84 and 186 d, respectively. Obviously, up to 2.5% retinyl palmitate can be encapsulated in the matrix. However, the formulation with 5% revealed no retinyl palmitate droplets on the surface, which were observed with 10% drug.

Tretinoin SLN, even in a low concentration of 1%, possessed numerous drug crystals. This material was even more difficult to encapsulate than retinol, and was, therefore, precluded from further investigation.

These data demonstrate that retinoids are difficult to encapsulate in conventional SLN formulations. Only the retinyl palmitate SLN with drug loads of 2.5–5% seem to offer sufficient drug loads.

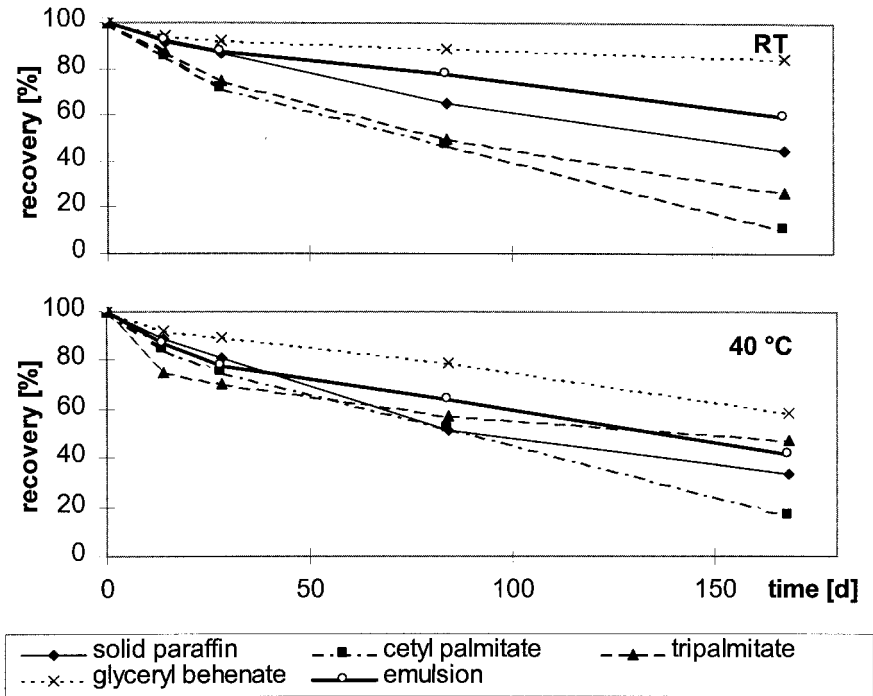


Figure 3. Stability of retinol in different oil loaded SLN formulations at room temperature (RT, top) and 40°C (bottom), $n = 3$. The formulations comprise 10% solid lipid, 5% medium chain triglycerides, and 0.5% retinol.

SLN based on mixtures of liquid and solid lipids

Encapsulation efficacy of retinoids was improved by blending small amounts of liquid oils with solid lipids whose crystal lattice lacks sufficient incorporation of retinoids. In contrast, they are very soluble in oils such as medium chain triglycerides (Miglyol 812). For example, the solubility of retinol in Miglyol 812 is 26%. It was attempted to improve the encapsulation efficacy of SLN by mixing medium chain triglycerides and glyceryl behenate. A comprehensive physico-chemical characterization of this carrier system is currently in preparation. In figure 3, the stability of retinol in SLN prepared of 5% medium chain triglycerides and 10% lipid is demonstrated. The payload was 3.3% (related to the lipid). The kinetic data indicate an improved encapsulation efficiency as a result of the presence of the liquid oil. In the case of glyceryl behenate, the stability of retinol is even better than in the emulsion, demonstrating protective effects of the solid matrix. By means of NMR, DSC, WAXS and SAXS analysis, one could preclude for these glyceryl behenate SLN the coexistence of oil droplets and solid nanoparticles. Instead, the nanoparticles contain solid and liquid domains *within* one particle (data not shown). However, in the case of tripalmitate and cetyl palmitate, drug leakage from the carrier occurred, despite the admixture of the oil.

The mixture of medium chain triglyceride and glyceryl behenate was further investigated with respect to the possible payloads of these systems. Table 2

Table 2. Effect of different oil and retinol concentrations (related to the lipid phase) on the inclusion properties of glyceryl behenate SLN. Chemical stability is compared to an emulsion formulation: + : better stability, = : equal stability, - : worse stability.

Oil concentration/ drug concentrations (%/%)	Microscopy	Chemical stability	Melting peak (DSC)
16.5%/1.65%	No crystals	-**	68.9°C
33.0%/3.30%	No crystals	+	64.4°C
50%/5%	No crystals	=	65.6°C
33%/1.65%	No crystals	+	65.0°C
33%/6.6%	+*	+	63.1°C

* After 3 months of storage at 4°C.

** Not significant.

summarizes the results from the stability study, microscopic investigation and DSC measurements. It can be seen that the oil concentration and retinol concentration can be varied within a broad range without signs of drug leakage from the system. The melting point of glyceryl behenate is dependent on the concentration of retinol in the carrier. Within the tested range of 0–6.6% retinol, the relation is linear ($R^2 = 0.997$), indicating a complete inclusion in the particles. Similarly, the kinetic data show similar or even improved retinol stability compared to an emulsion. Drug crystals are absent. Only the sample with 6.6% retinol stored at refrigerated temperatures for 3 months shows the beginning formation of drug needles in the suspension. The maximum loading capacity is, therefore, ~5% with this oil containing-SLN system. Comparing conventional SLN and SLN based on mixtures of liquid and solid lipids, the encapsulation efficacy improved 5-fold.

For tretinoin, the manipulation of the SLN by admixing the oil component did not sufficiently increase the loading capacity. Samples containing 2.5 or 1% tretinoin revealed typical tretinoin crystals. Only for 0.5% tretinoin were these crystals absent. For this concentration, the chemical stability was dependent on the SLN formulation. Conventional tripalmitate showed the worst tretinoin stability, which increased by using conventional glyceryl behenate SLN. Best stability and, thus, best encapsulation was observed for glyceryl behenate SLN containing small amounts of medium chain triglycerides. The same order was measured for SLN loaded with 5% retinyl palmitate: tripalmitate SLN < glyceryl behenate SLN < glyceryl behenate + oil SLN. In the case of retinyl palmitate, the comparable good encapsulation in conventional glyceryl behenate SLN was further improved by using oil loaded SLN.

Possible mechanisms of drug incorporation

As suggested in the literature (Müller *et al.* 1994), drugs with a melting point below the melting point of the lipid matrix preferentially distribute to the surface of the particles. This applies to retinol and retinyl palmitate, because their melting point is below those of glyceryl behenate and tripalmitate. Glyceryl behenate shows a much better entrapment efficacy than tripalmitate. A possible explanation could be that these particles differ in their size and, thus, surface area. The better entrapment of glyceryl behenate might be the result of smaller particles which

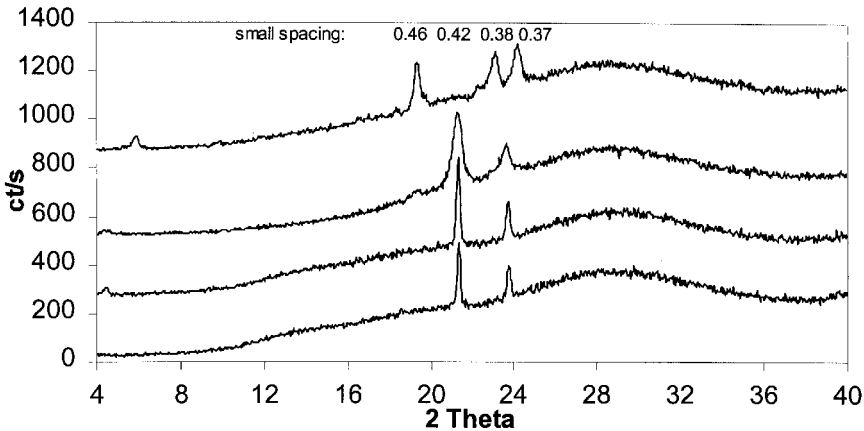


Figure 4. WAXS diffraction pattern of different SLN formulations (10% solid lipid, 5% medium chain triglycerides, and 0.5% retinol). From top: tripalmitate SLN, glyceryl behenate SLN, solid paraffin SLN, cetyl palmitate SLN.

Table 3. PCS mean diameter and polydispersity of different SLN formulations measured 1 day after production.

	Diameter	PI
Glyceryl behenate	224 ± 9 nm	0.205 ± 0.012
Tripalmitate	265 ± 15 nm	0.140 ± 0.024
Cetyl palmitate	210 ± 19 nm	0.102 ± 0.018
Solid paraffin	242 ± 13 nm	0.095 ± 0.011

provide a higher surface area. In table 3, the PCS mean diameter and polydispersity of the different particles are listed. From table 3, no basic differences in particle sizes and, thus, surface areas can be seen. All preparations are between 210–260 nm.

The crystalline order of the nanoparticles was investigated by wide angle X-ray scattering (WAXS). The scattering pattern of the nanoparticles is demonstrated in figure 4. Cetyl palmitate and solid paraffin can be characterized by their sharp reflections, compared to the broad reflections of the glycerides. This is an indication of a high crystal order in this colloidal particles. The X-ray lines are at 0.38 and 0.42 nm, which is typical for an orthorhombic subcell. Glyceryl behenate revealed broad lines at 0.38 and 0.42 nm. According to Larsson (1966), this is typical for the orthorhombic β' polymorph of triglycerides. This β' form is a metastable polymorph which can be characterized by numerous lattice defects (Precht 1988). In contrast, tripalmitate SLN show the reflection pattern of the stable β form. The crystal order in this β form is higher than in the β' (Hernqvist 1988). The WAXS investigations suggest that good entrapment efficacy in solid particles can be achieved with lipids of low crystalline order and metastable polymorphs. High crystallinity or the formation of the stable polymorph can be correlated with drug expulsion from the carrier. The correlation between poly-

morphic transition from the metastable to the (more) stable polymorph and drug expulsion was also observed in drug release studies (Jenning *et al.* 1999).

Conclusions

The obtained encapsulation of retinoids with conventional SLN formulations was unsatisfactory. The admixture of small amounts of an oil to the glyceryl behenate matrix improved the inclusion of the active, in the case of retinol even 5-fold. Good inclusion in the core of SLN could not be correlated with particle sizes and, thus, surface area. Instead, a good correlation between the degree of crystallinity and drug inclusion was demonstrated. Successful entrapment is favoured by lipids with low crystallinity in the colloidal state. Metastable polymorphs with numerous lattice defects further improve the inclusion of retinoids.

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