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Research paper

Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase

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Abstract

Aqueous dispersions of solid lipid nanoparticles (SLN™) are usually physically stable for more than 3 years. However, in some systems gelation occurred leading to solid gels due to an unknown mechanism. To elucidate this mechanism, Compritol® SLN were stored at different temperatures, varying light exposure, in different packing materials and stressed by shear forces in short-term tests and a long-term study of 3 years. The SLN were analyzed by differential scanning calorimetry and sizing techniques. After production by hot homogenization of the melted lipid, the Compritol® SLN crystallize in a mixture of stable β' with unstable polymorphs (α, sub α). The destabilizing factors light, temperature and shear forces cause a distinct increase in the recrystallization index by transformation of the lipid to the β' modification being accompanied by gel formation. Physically stable SLN remain as a mixture of modifications, increase in crystallinity index during storage is slow and crystallization occurs mainly in unstable modifications. From this, stabilization of physically critical SLN dispersions seems possible by inhibition of the transformation of the lipid to the stable modification.

Keywords: Solid lipid nanoparticles; Long-term stability; Differential scanning calorimetry; Polymorphism

1. Introduction

Solid lipid nanoparticles (SLN^{TM}) [1] represent an alternative drug carrier system to emulsions and polymeric nanoparticles. At optimized composition of the SLN dispersions, physical stabilities of 3 years and more were reported [2]. However, it was observed that some formulations tended to form a gel after a certain period of storage time. This can be circumvented by changing the composition and concentration of the stabilizing surfactant mixture. Still, in some cases it might be desirable to use a specific formulation because of, e.g. toxicological considerations, good stabilization of incorporated drugs against chemical degradation or high loading capacity of the drug. For example, it was found that the surfactant mixture had a significant influence on the chemical stabilization of drugs which accumulate in the outer shell of SLN particles [3]. Therefore, in the first part of this paper the extent to which storage temperature, light exposure and packing material affect the gelation process was investigated [4]. The understanding of the destabilizing mechanisms can be used to rationally stabilize these formulations.

It was found that the gelation process was accelerated with increasing storage temperature and increasing light exposure. Any input of energy seems to destabilize physically critical SLN dispersions. It was noticed that simultaneously the zeta potential decreased to approx. minus15 mV being not high enough for a sufficient electrostatic stabilization. In addition, it was found that siliconization had an inhibiting effect on the gelation process. It has been reported that the recrystallization index has also an effect on the

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long-term stability of aqueous SLN dispersions [2]. In general, dispersions with a highly recrystallized lipid phase (high recrystallization index) showed an increased particle size growth. SLN are produced by high pressure homogenization of the melted lipid dispersed in an aqueous surfactant solution (hot homogenization technique). The oil in water nanoemulsion obtained is cooled down, recrystallizes and forms solid lipid nanoparticles. Depending on the nature of the lipid, this process can take place very quickly – within minutes. However, it has also been reported that the recrystallization of the lipid fraction can be retarded up to weeks or months [5]. In general, the recrystallization index of the SLN is below the crystallinity of the bulk material used for SLN production and increases with increasing storage time. To further elucidate the gelation mechanism the changes in the degree of crystallinity as a function of storage conditions (temperature, light exposure) was investigated in this second part. In addition, the effect of the kinetic energy of the particles (increased diffusion velocity at increased temperatures simulated by shaking) and the concentration of the lipid phase (2%, 5%, 10%) on crystallinity and resulting gelation was assessed.

2. Materials and methods

Compritol® 888 ATO which was obtained from Gattefossé (Weil a.R., Germany) is declared as glyceryl behenate with a melting point of 72ºC. It is a mixture of 12–18% mono-, 52–54% di- and 28–32% triglycerides. The fatty acid fraction consists of >87% behenic acid (docosan acid). The surfactant Pluronic® F68 (poloxamer 188) was a gift from BASF AG (Ludwigshafen, Germany).

A formulation consisting of 10% Compritol 888, 1.2% poloxamer 188 and water added up to 100% (all m/m%) was produced by high pressure homogenization (APV Micron Lab 40, APV Gaulin, Germany). The melted lipid was dispersed in the hot surfactant solution (90ºC), a pre-emulsion prepared by an Ultra-Turrax K18 (Janke und Kunkel GmbH and Co KG, Germany) at 9500 rev./min, and this emulsion homogenized (three cycles, 500 bar, 90ºC) [6]. Size distribution of the freshly prepared SLN and crystallinity of the dispersed lipid were analyzed.

Photon correlation spectroscopy (PCS) (Zetasizer 4, Malvern Instr., UK) and laser diffraction particle size analysis (LD) (Mastersizer E, Malvern Instr., UK) were employed to determine the particle size. The LD data was evaluated using volume distribution to detect even a few large particles.

The degree of crystallinity was analyzed by differential scanning calorimetry (DSC) (Mettler TA 3000 and DSC 821e, Mettler, Switzerland). The SLN dispersion or gel (amount containing 1 mg lipid) was weighed into standard aluminium pans using an empty pan as reference. A heating rate of 5 K/min was applied. The samples were heated from 20ºC to 90ºC and cooled from 90ºC to 20ºC under liquid nitrogen. The recrystallization indices (RI) were calculated as follows:

$$RI[\%] = \frac{\text{Enthalpy}_{\text{SLN dispersion}}[J/g^{-1}]}{\text{Enthalpy}_{\text{bulk material}}[J/g^{-1}] \cdot \text{Concentration}_{\text{lipid phase}}[\%]} \cdot 100$$

The 10% Compritol dispersion was exposed to stress conditions to induce gelation within a short time to determine its kinetics. The samples were either stored at 50ºC or to investigate the influence of shear forces at 20ºC in a shaking bath (frequency: 70/min, Köttermann, Germany). Storage was performed in the dark. To assess the effect of particle concentration samples were diluted with purified water to reduce the lipid concentration from 10% to 5% and 2%.

The viscosity of the systems was determined with a Rheo Stress RS 100 (Haake, Karlsruhe, Germany) equipped with a cone and plate fixture (diameter 20 mm, angle 4º).

Additionally, in a second separate long-term study samples were stored varying temperature and light influence:

- at 8°C, 20°C and 50°C (in the dark);
- under artificial illumination, under daylight and in the dark (at 20°C).

For artificial illumination the samples were placed between two fluorescent lamps (two 58 W tubes with the light spectrum of daylight and a light power of 96 lm/W (lumen per Watt)) in a defined distance to avoid warming up.

The SLN were packed in 20 ml white and brown glass vials (glass quality I, suppliers: Bünder Glas, Münnerstädter Glaswaren, Schmidt, Germany).

3. Results and discussion

3.1. Influence of shear forces and lipid concentration

It has been reported that shear forces (like pressing through the needle of a syringe) promote gelation of some suboptimal stabilized SLN formulations [7]. To induce and observe the process in a controlled way Compritol SLN were stored in a shaking bath at 20ºC and a frequency of 70/min. Particle size and degree of crystallinity were determined every day over a period of 2 weeks.

Concerning dispersions with a lipid concentration of 10%, the first aggregates could already be detected after 3 days. The LD diameter 90% increased from 0.77 ± 0.01 μm up to 23.34 ± 0.19 μm (Fig. 1, black bars). All samples gelled within 5 days. The crystallinity increased continuously during the observation time in the aqueous SLN dispersion and after solidification in the gel (Fig. 1, upper line).

Obviously, the formed gel became increasingly more solid with further storage. The DSC data agree well with

viscosity measurements showing an increase in gel viscosity with increasing storage time [8].

Reducing the lipid concentration from 10% to 5% and 2% improved the stability of the SLN dispersion. The 10% stock SLN formulation was diluted with purified water. Therefore, the ratio lipid versus surfactant did not change. Lower particle concentration means a lower probability of particle collision and subsequent reduced aggregation during the shaking process [9].

The 5% SLN dispersions did not gel within 2 weeks. Very distinct particle size increase started at day 5. After 7 days visually detectable particles had been formed. The enthalpy increase was lower (maximum value 14 J/g) compared with 10% lipid systems (maximum value 16 J/g) (Fig. 1).

The mean PCS particle size of the 2% dispersion remained unchanged (280.1 ± 5.0 nm at day 0 and 282.1 ± 3.9 nm at day 14; without figure). The LD diameter 90% changed only slightly, about 0.1 μm. The enthalpy also increased very little to a maximum of 13 J/g (Fig. 1).

3.2. Influence of the surface of the packing material

The effect of the contact between particles and the surface of the packing material was investigated by varying the ratio of volume to contact surface in the vials. Glass vials (20 ml) were filled up to the rim or only one-third of the vial volume with a 10% Compritol SLN dispersion. The volume to surface ratios were: 0.57 ml/cm^2 and 0.37 ml/cm^2. Again storage was performed in the shaking bath at 20ºC and 70/min. Gelation was accelerated and occurred 2 days earlier in the vials with a low volume to surface ratio (at day 3 instead of day 5) [8].

Aggregation and subsequent gelation of SLN is not only promoted by a particle to particle contact (c.f. Section 3.1) but also by a particle to vial surface contact. The influence of the packing material surface can be minimized by silicon-

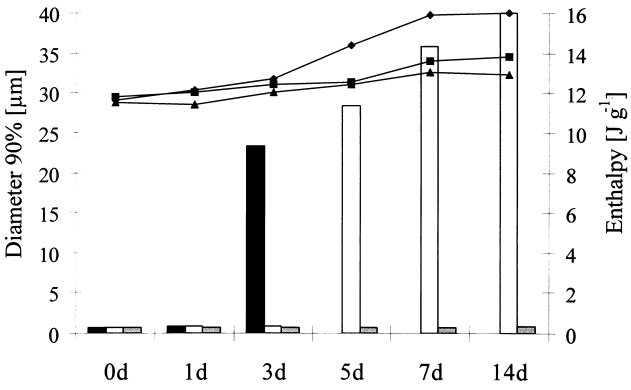

Fig. 1. Influence of shear forces and lipid concentration. Diameter 90% (bars, LD data) and enthalpy (lines, DSC data) of 10%, 5% and 2% Compritol SLN (Co 10%: black, (♦), Co 5%: white, (■), Co 2%: gray, (▲)) stored 20°C in a shaking bath over a period of 14 days (LD: standard deviation max ± 1.96 μm, DSC: standard deviation max ± 0.4 J/g; no size analysis of 10% Compritol SLN after day 5 because of gelation).

nization of the glass or packing in plastic containers [4,13]. It has been reported that emulsion droplets coalesce via adherence to the wall of the container [10]. This mechanism can be transferred to SLN due to the similarities between SLN and o/w emulsions (i.e. inner lipid phase stabilized by a surfactant). Of course some effect of the differences in the particle movements when shaking glasses fill to a different extents cannot be excluded.

3.3. Properties of the lipid: SLN versus bulk material

It could be shown in the first part of the paper that any energy input causes destabilization of the investigated Compritol SLN. A higher kinetic energy (e.g. elevated temperatures, light) promotes oscillation of the lipid particles leading to more frequent collisions [11]. Shear forces further increase the number of particle contacts. This could cause a partial ripping off or damaging of the surfactant film on the particle surface promoting aggregation. So far it has not been possible to elucidate completely the mechanism to explain the gelling phenomenon occurring in some aqueous SLN dispersions. Therefore, crystallinity measurements were performed.

Right after production the fat fraction of Compritol SLN is not completely solidified as indicated by a recrystallization index of about 75%. The recrystallization indices were calculated from the enthalpy (measured by DSC) of the SLN dispersion compared with the enthalpy of the physical mixture of the excipients. The enthalpy value of the physical mixture was set for 100% crystallinity (c.f. Section 2). During storage at conditions at which the Compritol dispersion is stable (8ºC, dark), the recrystallization index increased only slightly to a maximum value of about 87%.

Gelled systems had a much higher crystallinity of 100% to 130% indicating that the whole fat fraction was solid. Hence it would be possible that the observed crystallinity changes are attributed to the energetic changes. It was suggested that due to particle collision and partial destruction of the surfactant film liquid, lipid is set free which solidifies by 'bridging' the particles [2]. Comparable processes have been observed for cream and butter [12]. However, it should be pointed out that SLN although showing similarities to cream have a much lower lipid content and a less complex structure.

Recrystallization indices higher than 100% have been reported before with gelled formulations [13]. This reported increase was partially attributed to a less homogeneous sample drawing from the phase separated SLN dispersions, and partially to changes in the lipid structure. In the present study, the gels were homogeneous allowing a representative sampling. Therefore, the observed increases in crystallinity are attributed to energetic changes of the system.

Another reason for solidification of Compritol SLN could be polymorphism of the lipid. Triglycerides are known to crystallize mainly in three polymorphic forms which transform monotrophically from α via β' to β [14]. For mixtures

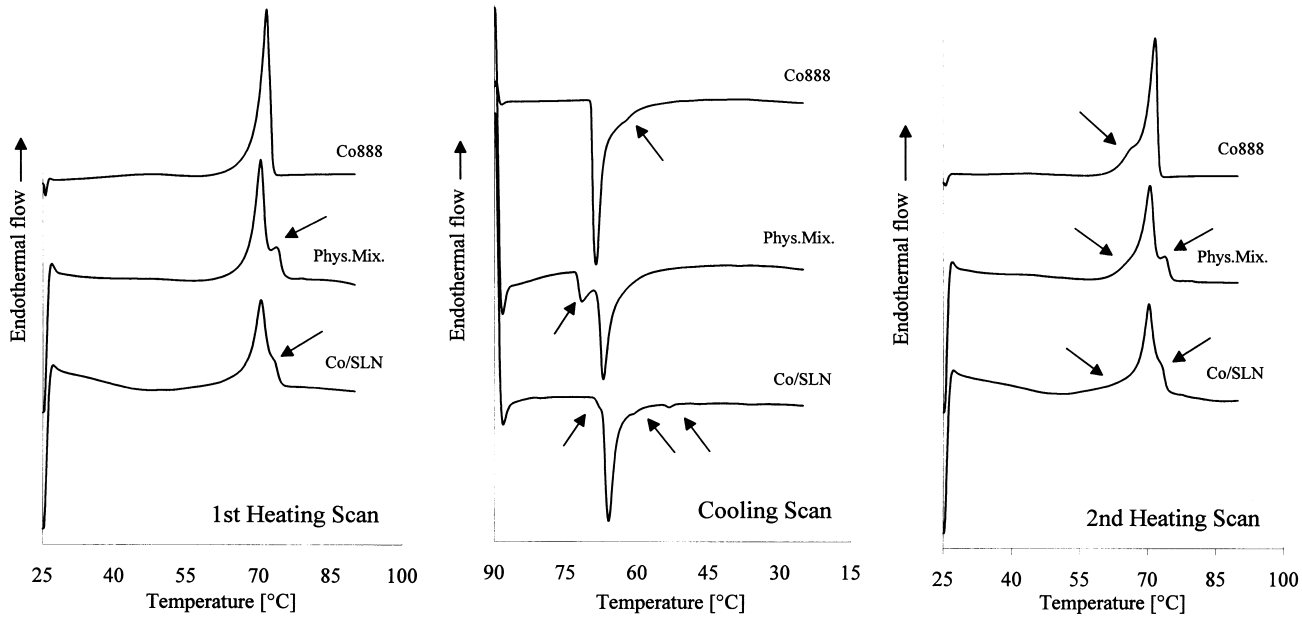

Fig. 2. Properties of the lipid: SLN versus bulk material. DSC thermograms of Compritol bulk material (Co888), of the physical mixture (Phys. Mix.) and of 10% Compritol SLN after production (SLN liq.). Left: first heating scan, middle: cooling scan, right: second heating scan (DSC 821e, Mettler).

of glycerides an intermediate form, β_i, between β' and β has been discussed [15]. In the USP Compritol is declared as glyceryl behenate [16] consisting of mono-, di- and triglycerides (c.f. Section 2). The bulk material melts between 63ºC and 77ºC with the melting point at 72.2ºC (Fig. 2, left, curve 'Co888'). When cooling the molten lipid down to room temperature it recrystallizes between 70ºC and 55ºC with a peak at 68.7ºC (Fig. 2, middle, curve 'Co888'). A shoulder of the cooling curve at lower temperatures indicates the existence of an unstable (α) modification. For pure tribehenate an α modification melting at approx. 69ºC has been reported [17]. Reheating of the lipid leads to an almost identical heating curve (Fig. 2, right, curve 'Co888'). Again a shoulder indicating lower melting modifications can be observed [2]. From the high content of diglycerides (>50%) it can be concluded that Compritol bulk material crystallizes in the β' modification [17].

The crystallization behavior of freshly prepared 10% Compritol SLN differs distinctly from the pure lipid. The peaks of the first and second DSC heating curves are broadened and the melting point is reduced to about 69ºC with a peak shoulder at approx. 73ºC (Fig. 2, left and right, curve 'Co/SLN'). This shoulder is caused by the presence of water and has also been found for a physical mixture of Compritol and water (Fig. 2, left and right, curve 'Phys. Mix.'). The broadening of the heating peak and the reduction of the melting point indicate an increased number of lattice defects [7]. The small particle size and therefore high surface being an energetically suboptimal state leads to a decrease of the crystallization point [18]. The cooling scan shows beside a main peak at approx. 65ºC two additional shoulders at 60ºC and at 53ºC. The main peak could be attributed to the β' modification and the peak at 60ºC to the α modification. The third peak at 53ºC could be attributed to the sub α modification caused by the fraction of monoglyceride in Compitol. A

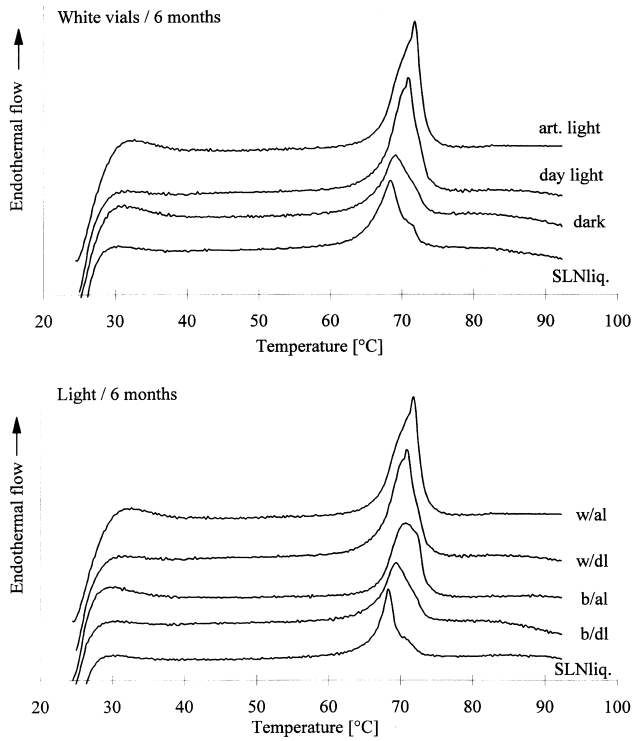

Fig. 3. Gelling tendency and light exposure. Upper: first heating scan of 10% Compritol SLN after production (SLN liq.) and after 6 months of storage in white glass vials in the dark, at daylight and under artificial (art.) light. Lower: first heating scan of 10% Compritol SLN after production (SLN liq.) and after 6 months of storage at 20ºC in white (w) and brown (b) glass vials at daylight (dl) and under artificial light (al) (TA 3000, Mettler).

sub α modification has been found for glyceryl monobehenate melting between 46ºC and 63ºC [17].

3.4. Gelling tendency and light exposure

Artificial light and daylight induced solidification of 10% Compritol SLN at 20ºC within 14 days and within 6 months, respectively [4]. The gelation velocity depended on the light intensity: the higher the intensity, the higher the velocity. To assess the influence of light on the status of the lipid phase DSC curves of SLN in white glass vials stored for 6 months in the dark, at daylight and under artificial light have been compared. At 6 months the batches exposed to light were both completely solidified (apparent viscosity approx. 1900 mPa/s), while the samples kept in the dark had formed soft gels (apparent viscosity approx. 500 mPa/s). The shape of the DSC curve of the latter gelled system changed little compared with the one of freshly prepared SLN (SLN liq.) (Fig. 3, upper). The melting point shifted from 68.8°C (SLN liq.) to 69.5ºC and the recrystallization index increased from 75% (SLN liq.) to 97%. The light exposure caused a further shift of the melting point to higher temperatures and a further increase in crystallinity (Table 1). The peaks of these samples are narrowed and the 'water shoulder' is not detectable anymore (Fig. 3, upper).

Packing in brown glass delayed gelation caused by light but could not protect the SLN dispersions completely from all energetic radiation. The protective effect of brown glass vials was supported by DCS data (Table 1, Fig. 3, lower). After 6 months brown glass samples stored in daylight (b/dl) had formed a soft gel while white glass samples (w/dl) already were solid. The curve of the 'b/dl' samples differs distinctly from the curve 'w/dl' but is quite similar to the curve named 'dark' in the upper half of Fig. 3. This is not only valid for the shape of the curve but also for the melting point (70.5ºC) and recrystallization index (103%). Melting point (70.6ºC) and recrystallization index (101%) of brown glass samples stored under artificial light (b/al) although being solid are almost the same compared to daylight storage. Nevertheless the melting peak is more blunt and the 'water shoulder' has almost disappeared. In general, avoidance of light keeps the degree of crystallinity and the crystallization point on a lower level.

Table 1

Melting points, enthalpies and recrystallization indices of 10% Compritol SLN stored for 6 months at 20ºC in the dark, at daylight (in brown and white glass vials) and under artificial (art.) light (in brown and white glass vials). Reference: freshly prepared SLN analyzed on day 1 (SLN liq.)

Storage condition	Melting point (ºC)	Enthalpy (J/g)	Recrystallization index (%)
SLN liq.	68.6	12.0	75
Dark	69.5	15.5	97
Daylight/brown vials	70.5	16.4	103
Daylight/white vials	70.9	17.3	108
Artificial light/brown vials	70.6	16.1	101
Artificial light/white vials	71.6	19.1	119

3.5. Gelling tendency and temperature

Storage at 50ºC in the dark induced rapid solidification within 1 week. Room temperature improved the stability up to 3 months. Ten percent Compritol SLN stored at 8ºC in the dark were still stable after 3 years [4].

The DSC data of Compritol SLN at different temperatures is well in agreement with the stability observations. After half a year melting point, recrystallization index and DSC curve of stable dispersions stored at 8ºC differ only slightly from those obtained right after production (Table 2, Fig. 4, 'SLN liq.'). The intermediate status of the system stored at 20ºC in the dark has already been explained in Fig. 3 (upper, curve 'dark'). A temperature of 50ºC leading to a completely solid system shows a narrow, sharp peak (quite similar to bulk material) in the DSC diagram with a maximum at 73.3ºC and an enthalpy of 19 J/g. This means a calculated recrystallization index of about 120% (Table 2).

3.6. Gelling tendency: temperature versus shear forces

The destabilization process and recrystallization behavior of Compritol SLN induced by temperature causing very firm, solid gels and by shear forces causing highly viscous, semi-solid gels are different. DSC measurements over a period of 14 days of 10% dispersions stored at 50°C show a continuous increase in melting point and crystal fraction (Fig. 5, upper). Still both values have not reached their maximum at day 7 (7d) although all samples were macroscopically solid. Also the shoulder at higher temperatures caused by incorporated water is detectable after 1 week. A melting point of 72.3ºC and a shape of the DSC curve similar to that in bulk material is reached after 2 weeks (14d).

The samples containing 10% Compritol stored at room temperature in a shaker (c.f. influence of shear forces) show a different crystallization behavior (Fig. 5, lower). The 'water shoulder' has already disappeared after 5 days. The enthalpy peaks do not narrow as much as at 50ºC storage and the melting point increases comparatively little (68.9°C at day 0 and 70.0°C at day 14).

Except for the missing 'water shoulder' the DSC curve of shaken 5% lipid dispersions is very similar to the one of the freshly prepared SLN (0d) until day 7. After 14 days the curve is more round and blunt and the melting point has shifted slightly to higher temperatures (about 1.2°C). The same observations were made for the 2% lipid systems.

3.7. Differences in the modification of stable and unstable SLN

The changes in the shape of the DSC heating curves during the gelation process indicate a change in modification of the lipid matrix. Unstable modifications being present in

Table 2

Melting points, enthalpies and recrystallization indices of 10% Compritol SLN stored for 6 months in the dark at 8ºC, 20ºC and 50ºC. Reference: freshly prepared SLN analyzed on day 1 (SLN liq.)

Storage condition	Melting point (ºC)	Enthalpy (J/g)	Recrystallization index (%)
SLN liq.	68.6	12.0	75
8ºC	68.7	13.3	83
20ºC	70.4	14.4	95
50ºC	73.3	19.4	121

freshly prepared SLN (α, sub α) could be transformed into more stable ones (β'). Due to the complex structure of Compritol it was not possible to detect clearly separated melting peaks for the different modifications from the obtained heating scans.

Therefore, heating, cooling and reheating scans of stable and of gelled SLN systems were performed (Fig. 6). The samples had been stored over three years in the dark at 8ºC (stable) and at 50ºC (gelled). The heating scans obtained after 3 years (Fig. 6, left) are similar to those obtained after 6 months (c.f. gelling tendency and temperature, Fig. 4). Cooling was performed from 90ºC to 20ºC (Fig. 6, middle). The two additional peaks at 60°C (α) and at 53ºC (sub α) found for freshly prepared SLN (SLN liq. 1d) were even more pronounced with the 3 years old stable SLN dispersion (SLN liq. 3y). The peak minima shifted slightly to higher temperatures indicating a healing of lattice defects during storage (α: from 60°C after 1 day to 62°C after 3 years, sub α: from 53°C after 1 day to 56°C after 3 years) [7]. Especially the fraction of the sub α polymorph has increased during storage time as indicated by the increased peak at approx. 56ºC. This means that the liquid lipid of stable SLN recrystallizes mainly as unstable modifications. Generally molten triglycerides crystallize at first in the unstable (α) form due to the lower activating energy. The transformation α to β is retarded in the presence of emulsifier [19]. Poloxamer 188 has been used as emulsifier in the investigated Compritol formulation. A retardation of the transformation

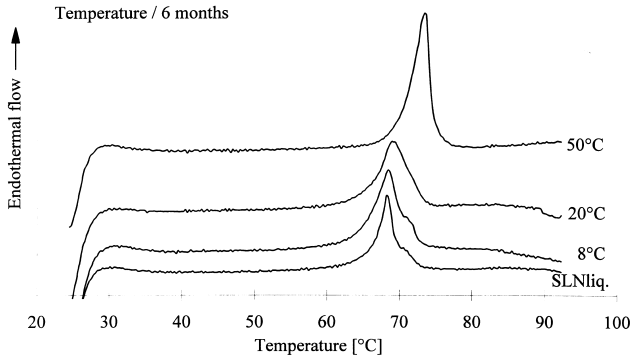

Fig. 4. Gelling tendency and temperature. First heating scan of 10% Compritol SLN after production (SLN liq.) and after 6 months of storage in the dark at 8ºC, 20ºC and 50ºC (TA 3000, Mettler).

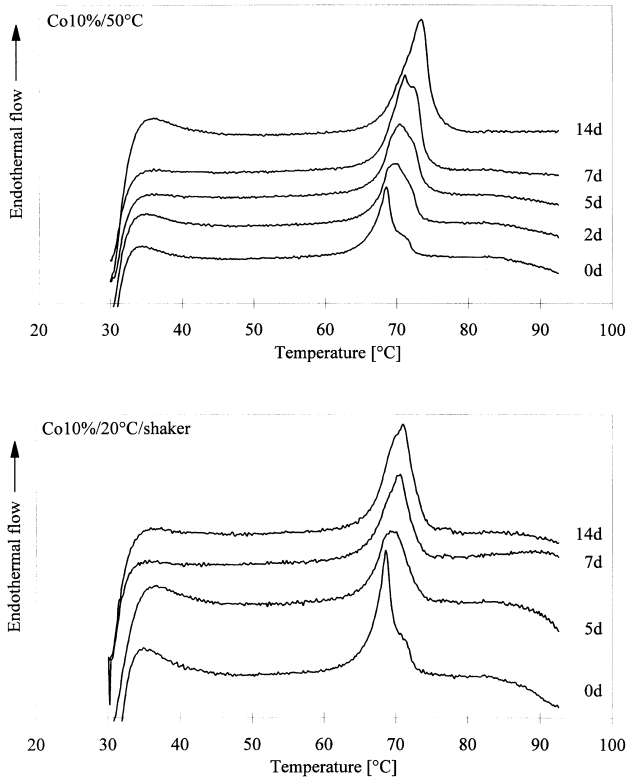

Fig. 5. Gelling tendency: temperature versus shear forces: Upper: first heating scan of 10% Compritol SLN after production (0d) and after 2 (2d), 5 (5d), 7 (7d) and 14 (14d) days of storage at 50°C. Lower: first heating scan of 10% Compritol SLN after production (0d) and after 5 (5d), 7 (7d) and 14 (14d) days of storage at 20ºC in a shaking bath (TA 3000, Mettler).

of lipid modifications has also been reported for other SLN systems stabilized with poloxamer [5].

No additional recrystallization peaks were detectable with the gelled system ('SLN gel 3y'). Even the shoulder at approx. 60ºC found for pure Compritol had disappeared (see Fig. 1, middle, curve 'Co888'). The gel recrystallized in the stable β' modification.

The peak shoulder at approx. 73ºC caused by the water present in the formulation disappeared with progressing gelation (see above). It can be assumed that the gel has incorporated the water by swelling. In the second heating scan also the gel shows a slight water shoulder (Fig. 6, right, upper curve). The gel network had not been fully restored within the short recrystallization phase after the first melting.

4. Conclusion

From the DSC analysis a correlation between physical stability of Compritol SLN dispersions and modification of the lipid could be shown. Liquid, stable systems still are not fully recrystallized after 3 years of storage, besides the stable β' also the α and sub α modification seem to be present. Only in solid gels the lipid phase was found to be

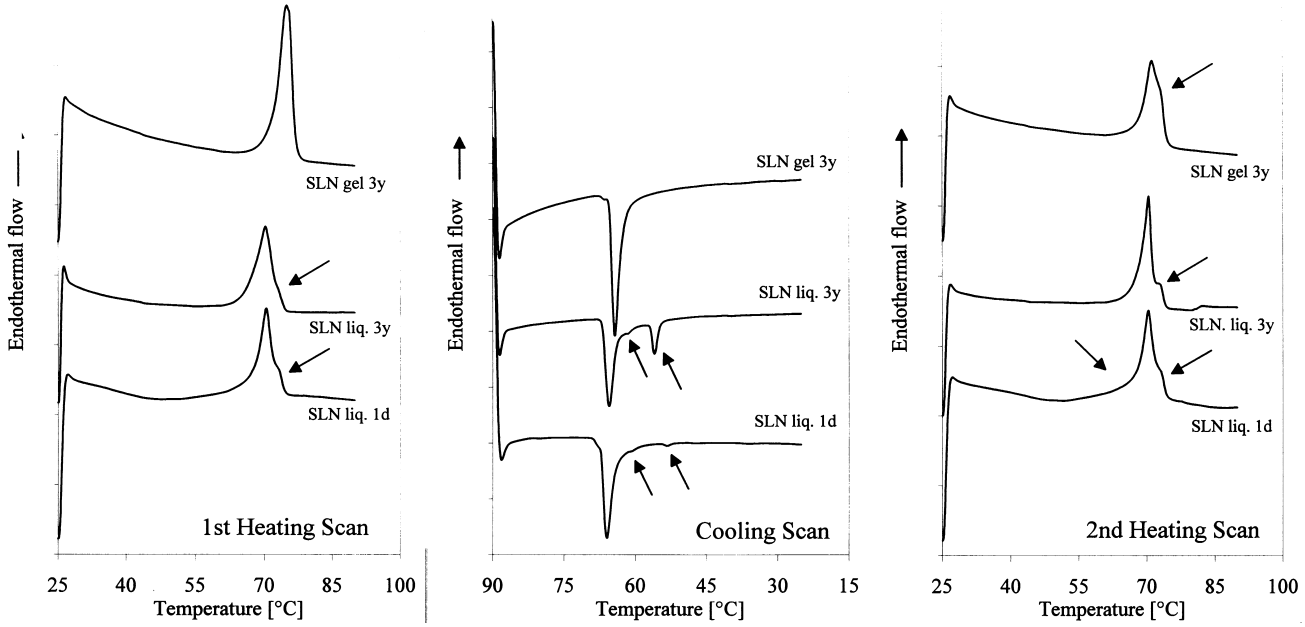

Fig. 6. Differences in modification of stable and unstable SLN. DSC thermograms of 10% Compritol SLN 1 day after production (SLN liq. 1d), after storage for 3 years in the dark at 8°C (SLN liq. 3y) and after storage for 3 years in the dark at 50°C (SLN gel 3y). Left: first heating scan, middle: cooling scan, right: second heating scan (DSC 821e, Mettler).

entirely crystalline, i.e. the lipid recrystallized completely in the β' modification.

The increase in solidification was accompanied by a reduction in the number of lattice defects, as indicated by the reduced melting peak width. Additionally, the amount of β' modification increased at simultaneous decrease in the fraction of unstable modifications. The enthalpy and melting point values both became similar to those of physical mixtures of the bulk material. Also the shape of the DSC curve is more congruent to the one of the bulk material the further gelation has developed.

In the liquid SLN and semi-solid remaining systems the slope or shoulder of the curves at lower temperatures can be interpreted as presence of unstable modifications. Cooling and reheating curves of these systems support this, showing two separate peaks instead of a shoulder. These peaks decrease to a slight shoulder and finally disappear the further the gelation process has progressed.

Based on these data, SLN dispersions with a gelling tendency can be stabilized by inhibiting the transitions in lipid modification, e.g. by addition of inhibitors to the lipid matrix.

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