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Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions

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Abstract

Aqueous dispersions of solid lipid nanoparticles (SLN™) are basically stable for up to 3 years, however some systems show particle growth followed by gelation. To assess the destabilizing factors, a poloxamer 188 stabilized Compritol SLN formulation was prepared. Its stability was investigated as a function of storage temperature, light exposure and packing material (untreated and siliconized vials of glass quality I). In general, introduction of energy to the system (temperature, light) led to particle growth and subsequent gelation. This process was accompanied by a decrease in zeta potential from approximately -25 mV to -15 mV. The effect of the packing material was less pronounced. However, siliconization of the vials almost eliminated particle growth. By optimization of the storage conditions (8°C, in the dark, siliconized vials), a stability of the less stable aqueous Compritol SLN over 3 years was achieved.

Keywords: Solid lipid nanoparticles; Long-term stability; Zeta potential; Block copolymer

1. Introduction

Solid lipid nanoparticles (SLN™; Müller and Lucks, 1996) consist of toxicologically acceptable compounds which are biodegradable. e.g. nutritional glycerides. A full range of lipids and emulsifiers possessing the GRAS status (Generally Recognized As Save) are available (Food Drug Cosmetic Law Reports, 1994). SLN can be used as carrier system for hydrophobic and hydrophilic drugs (Weyhers, 1995). Drug-loaded SLN systems are suitable for i.v., oral or dermal application. Homogenization as production technique is a comparatively simple and cost-effective method. It can be used for a broad field of lipid/surfactant

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combinations. Mean particle sizes from 80 nm up to 1000 nm can be produced depending on the applied pressure, temperature and cycle number. Optimized SLN compositions possess a physical long-term stability of at least 3 years (zur Mühlen, 1996). Alternatively, the liquid can be converted into a dry product by spray drying or lyophilization to avoid occurring instabilities (Müller et al., 1995; Schwarz, 1995).

Aqueous dispersions of SLN are preferred with regard to the ease of handling (no reconstitution necessary) and for cost reasons (e.g. costs of freeze drying). Observed instabilities in aqueous dispersions such as flocculation can be circumvented by modifying the formulation using for example a co-emulsifier (i.e. sodium glycocholate (Siekmann and Westesen, 1992)). Stability determining factors are also storage conditions such as light, temperature and the packing material. With some less stable systems, a gelation has been reported which depends on the nature and composition of the surfactants but is expected to be affected also by the storage conditions and the packing. A study was performed investigating these factors to optimize the stability of aqueous SLN dispersions

An SLN dispersion was chosen consisting of Compritol stabilized with poloxamer 188. This formulation is ideal for investigating the destabilizing mechanisms. Due to its basically low stability even factors having no or little effect in optimized systems will lead to pronounced, easily detectable destabilizing effects in this formulation. Compritol 888 ATO (glycerolbehenate; US Pharmacopoeia, 1990) being a mixture of mono-, di- and triglycerides has a lot of lattice defects, a self emulsifying effect and a high recrystallization index (75–80% right after production). Therefore it is regarded as suitable for incorporating several drugs (Ford and Timmins, 1989; zur Mühlen and Mehnert, 1995; zur Mühlen, 1996). Poloxamer 188 is a frequently used emulsifier especially for i.v administration (Lucks, 1993). From this the Compritol/poloxamer formulation is attractive for drug delivery purposes when the destabilizing factors can be identified and avoided.

The poloxamer 188 stabilized Compritol SLN had simultaneously a tendency to gelation. Investigating this system should also contribute to the elucidation of the gelation mechanism. Knowledge of the triggering factors for the gelation process allows to avoid it and utilize even less stable SLN formulations without the need of using e.g. toxicologically less favorable co-emulsifiers or further formulation screening.

2. Materials and methods

Compritol 888 ATO was provided by Gattefossé (Weil a.R., Germany). It is glyceryl behenate with a fraction of 12–18% mono-, 52–54% di- and 28–32% triglycerides. The fatty acid fraction consists of greater than 87% behenic acid (docosan acid). The sufactant Pluronic F68 (poloxamer 188) was a gift from BASF AG (Ludwigshafen, Germany).

A pre-emulsion consisting of 10% (w/w) Compritol 888, 1.2% (w/w) poloxamer 188 in distilled water was produced with an Ultra-Turrax K18 (Janke und Kunkel GmbH & Co KG, Germany) at 9500 rpm. In a second step high pressure homogenization (APV Micron Lab 40, APV Gaulin, Germany) of this pre-emulsion was performed applying three cycles at 500 bar and 90°C (Müller et al., 1995). The resulting SLN dispersion was characterized directly after production and during storage.

The particle size was determined by photon correlation spectroscopy (PCS; Zetassizer 4, Malvern Instr., UK), laser diffraction particle size analysis (LD; Mastersizer E, Malvern Instr.) and Coulter counter analysis (Multisizer II, Coulter Electronics, Germany). The LD data was evaluated using the volume distribution to detect even a few large particles.

The particle charge was quantified as zeta potential using a Zetassizer 4 (Malvern Instr., UK). Measurements were performed in distilled water adjusted with sodium chloride to a conductivity of 50 μS/cm (Müller, 1996).

The SLN dispersion was filled into glass vials and stored at varying conditions. Storage was performed at different temperatures (8°C, 20°C and 50°C) and light exposures (dark, daylight and artificial illumination). For artificial illumination

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

For each storage condition, 20 ml SLN dispersion were packed in 20 ml white and brown glass vials (glass quality I). Two batches of each were investigated (suppliers: Bünder Glas, Münnerstädter Glaswaren, Schmidt, all in Germany). Additionally siliconized vials were used to assess the influence of the glass surface being in contact with the SLN dispersion. The absorption spectra of white and brown glass vials were measured using a Lambda 9 UV/VIS/NIR Spectrophotometer (Perkin-Elmer, Germany) by placing a broken piece of the vial wall (wall thickness 1.2 mm) in the path of the light beam.

At certain storage times (1, 3, 5, 7 and 14 days, 1, 3, 6, 12, 24 and 36 months) characterization of the samples was performed with the methods mentioned before.

3. Results and discussion

3.1. Influence of light

To investigate the influence of light on the stability of Compritol SLN the samples were stored at 20°C but at different light exposures: in the dark, at day light and under artificial light (c.f. methods). In addition storage was performed in white and in brown glass vials differing in their absorption spectra of the light (Fig. 1).

Storage in white glass vials under artificial light (al) induced rapid gelation. The diameter 90% determined with LD (i.e. 90% of the particles evaluated by volume distribution) increased immediately after 1 day from 0.66 μm to 0.76 μm. At this time no changes could yet be detected macroscopically. At day 3 the samples were still optically homogenous but flocculated after dilution with water. At day 7 a steep increase in diameter 90% occurred (Fig. 2, line 'w/al') and the viscosity was visibly increased. After 14 days all samples were completely solid excluding further particle size determination.

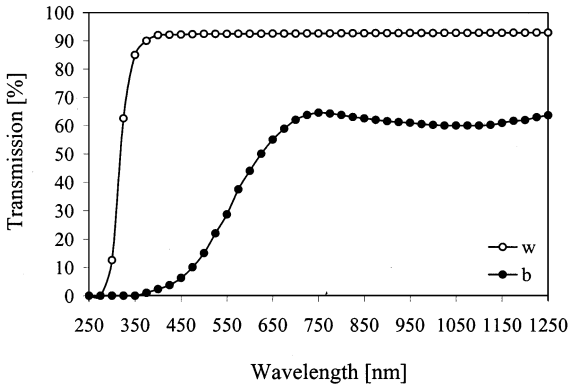

Fig. 1. Influence of light: Absorption spectra of the white (w) and brown (b) glass vials.

The gelation process is obviously slower under day light. Distinct particle size increase and flocculation of the system in water started after 1 month. The diameter 90% increased from 0.8 μm to 8 μm (Fig. 2, line 'w/day'). After 3 months the samples were ointment-like, merging into a firm state after 6 months.

Exclusion of light lead to the best stability results (concerning storage at room temperature) but could not avoid the gelation process. Gelation started 1 month later compared to day light storage and the particle growth was slower (Fig. 2, line 'w/dark').

Comparing the storage data in white glass vials to those in brown vials a stability improve is

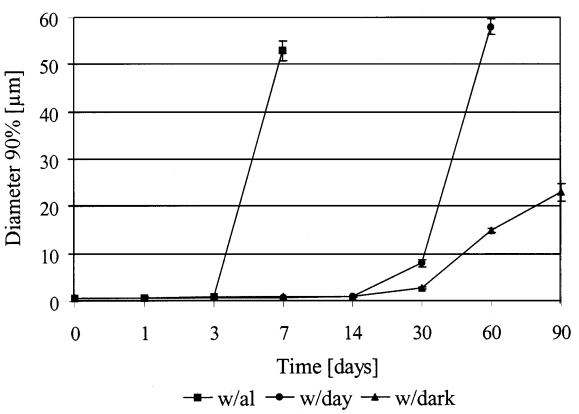

Fig. 2. Influence of light: Diameter 90% (LD data: volume distribution) of 10% Compritol SLN stored in white glass vials (w) in the dark, at day light (day) and under artificial light (al) over a period of 90 days.

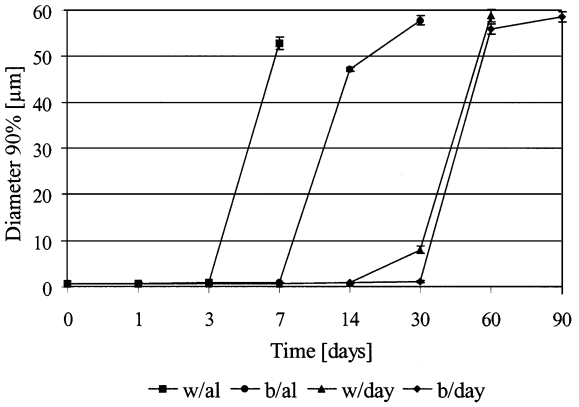

Fig. 3. Influence of light: Diameter 90% (LD data: volume distribution) of 10% Compritol SLN stored in white (w) and brown (b) glass vials at day light (day) and under artificial light (al) over a period of 90 days.

obvious. Brown glass delays pronounced particle growth (and subsequent gelation) under artificial light for 1 week and under day light for 1 month (Fig. 3, 'b/al' and 'b/day') compared to white glass storage ('w/al' and 'w/day'). Brown glass could not protect the SLN dispersion completely from all light. The gelation started earlier under daylight (after 1 month) than in the dark (after 3 months).

The data prove that light radiation has a destabilizing effect. Increase in the intensity of light radiation leads to accelerated particle growth and gelation. The brown glass showing higher stability of the SLN absorbs the light at short wave lengths (approx. 300–600 nm), that means the UV radiation (< 390 nm) and the higher energetic part of the day light spectrum (Fig. 1). High energetic radiation (UV, short wavelengths) leads to increased destabilization. Lipid dispersions such as emulsions for parenteral nutrition (e.g. Lipofundin™ and Intralipid™) are similar to SLN but show no destabilization by light. They are packed into white glass bottles. Physical stability has been reported for more than 3 years when stored at room temperature and day light (Müller et al., 1992).

3.2. Change of zeta potential at different light exposure

Additionally, zeta potential measurements were performed. The zeta potential of Compritol SLN right after production was -24.7 mV. When storing the SLN dispersions in white glass under artificial light the zeta potential dropped to -15.1 mV within 1 week (Fig. 4). At this point the system extremely started to gel as seen by the increase in diameter 90% (d90%) to 52 μm (Section 3.1).

The zeta potential of the sample under day light still was -19.5 mV after 7 days and remained on this level up to 1 month of storage. After 2 months when the dispersions started gelling (increase of d90% to 53 μm) a zeta potential of -15.7 mV was measured. In the dark the zeta potential values remained slightly higher at approximately -18 mV (Fig. 4).

In Fig. 5 zeta potentials of samples stored in white and in brown glass vials under day light and under artificial light are compared. Throughout the whole observation time the zeta potentials of the dispersions in brown glass (less light exposure) always were higher.

The reduction in zeta potential is well in agreement with the reduction in physical stability. A minimum zeta potential of greater than -60 mV is required for excellent, of greater than -30 mV for a good physical stability (Riddick, 1968). A

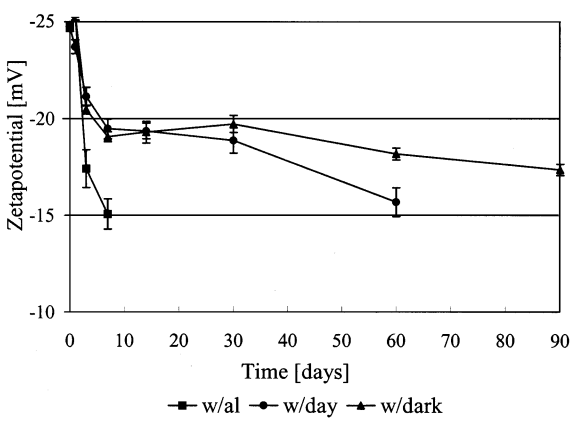

Fig. 4. Influence of light: Zeta potential of 10% Compritol SLN stored in white glass vials (w) in the dark, at day light (day) and under artificial light (al) over a period of 90 days.

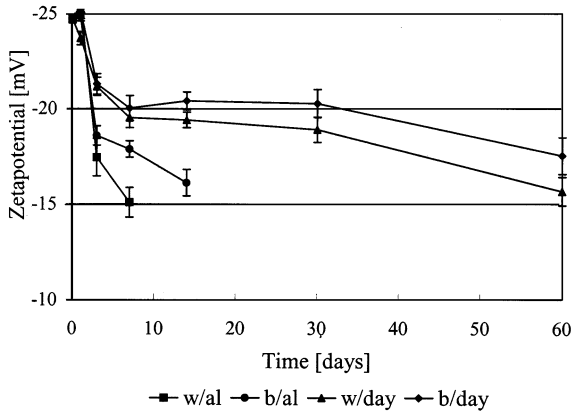

Fig. 5. Influence of light: Zeta potential of 10% Compritol SLN stored in white (w) and brown (b) glass vials at day light (day) and under artificial light (al) over a period of 90 days.

potential of -24.7 mV for freshly prepared Compritol SLN is just below the critical value. But it is in principle still sufficient for a stable system in combination with the sterically stabilizing effect of the poloxamer (Section 3.4). Values of approx. -15 mV were reported to lead to distinct coalescence of emulsion droplets in regimens for parenteral nutrition (Müller and Heinemann, 1994) thus explaining the destabilization of the SLN.

Obviously, the light exposure of the SLN causes changes in the system leading to a reduction of the zeta potential and consequently the physical stability. At a sufficient reduction of repulsive forces the particles can interact forming a gel network.

3.3. Influence of temperature

The effects of temperature on Compritol SLN were analyzed at 8°C, 20°C and 50°C under exclusion of light. Storage at 50°C induced rapid particle growth within 3 days. The LD diameter 90% (being 0.66 μm at day 0) increased to 13.4 μm (Fig. 6). All samples were solid after 7 days. Room temperature (20°C) improved the stability but led to the same solid status within 3 months.

A high film rigidity of the emulsifier (microviscosity) avoids fusion of the film layers after particle contact. Microviscosity is a temperature dependent factor. Temperature increase causes a microviscosity decrease leading to destabilization (Schuhmann, 1995).

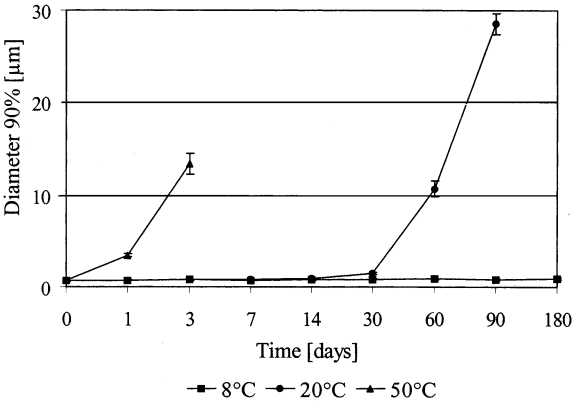

Fig. 6. Influence of temperature: Diameter 90% (LD data: volume distribution) of 10% Compritol SLN stored at 8°C, 20°C and 50°C over a period of 180 days.

Compritol SLN stored at 8°C in the dark were stable over the storage period of 3 years. The PCS mean diameter rose from 276 ± 2 nm to 297 ± 5 nm. The LD diameter 90% remained almost unchanged (increased by 0.08 μm, Fig. 7). Even the diameter 99% increased only by approx. 0.25 μm within 3 years of storage. The diameter 99% of the volume distribution is a very sensitive parameter for detection of even a few large particles. Additional Coulter counter measurements confirmed this data.

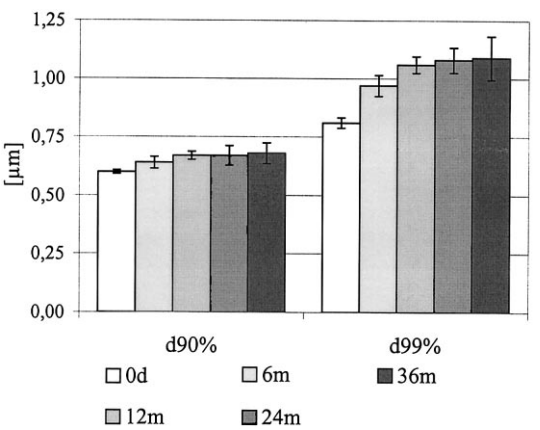

Fig. 7. Influence of temperature: Diameter 90% (d90%) and 99% (d99%) (LD data: volume distribution) of 10% Compritol SLN right after production (0 d) and after 6 (6 m), 12 (12 m), 24 (24 m) and 36 (36 m) months of storage at 8°C in the dark.

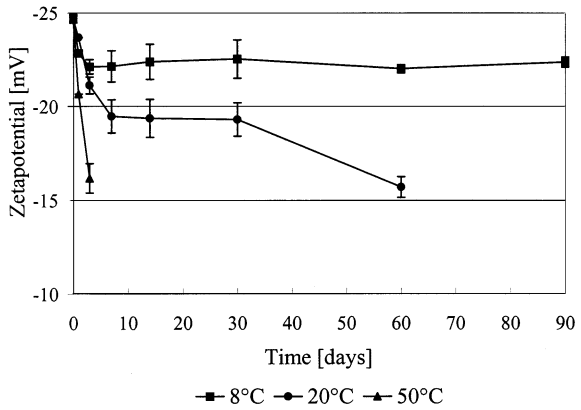

Fig. 8. Influence of temperature: Zeta potential of 10% Compritol SLN stored at 8°C, 20°C and 50°C over a period of 90 days.

3.4. Change of zeta potential at different temperatures

The results of zeta potential measurements are well in agreement with the observed instabilities and the stability of SLN at 8°C. Storage at higher temperatures led to a reduction of the zeta potential from −16 mV to −15 mV being fastest at 50°C (Fig. 8). As described above, the value of −15 mV seems to be critical and an indicator for the start of aggregation and gelation. Riddick defined the threshold of agglomeration in dispersions at a zeta potential range of −20 mV to −11 mV (Riddick, 1968). This data correspond to the values found for SLN just before solidification.

According to the DLVO theory, a system can be regarded as stable if the electrostatic repulsion dominates the attractive van der Waals forces (Lagaly, 1984). The particles have to overcome an energy barrier of electrostatic repulsion to approach closely and form agglomerates. If their velocity or kinetic energy is high enough they will collide. Higher temperatures as well as light (Sections 3.1 and 3.3) increase the kinetic energy of a system, in combination with a reduced zeta potential this leads to SLN aggregation and gelation.

The potential of the samples stored at 8°C moved slightly from −25 mV to −22 mV within the first days and then remained stable (Fig. 8). After 3 years all samples stored at 8°C were still at approx. −22 mV to −24 mV. This proves that a zeta potential in the range of −25 mV (Section 3.2) in combination with steric stabilization is sufficient to physically stabilize SLN dispersions.

In conclusion—identical to increasing light exposure—increasing temperature load leads to a decrease in zeta potential. At no light exposure and low temperatures the zeta potentials remains practically unchanged and the SLN dispersion is stable.

The zeta potential decreases with increasing energy input (light and temperature). This energy input can lead to changes in the crystalline structure of the lipid. An increased formation of β modification was reported during the storage of tripalmitate SLN (Siekmann, 1994). Crystalline re-orientation can result in changes of the charges on the particle surface (Nernst potential) and subsequently the measured zeta potential. In addition, different sides of a crystal can possess a different charge density (e.g. aluminium silicates like Bentone™). During one-dimensional growth of a crystal (e.g. formation of long β crystals (Sato, 1988)) the surface ratio of differently charged crystal sides changes and consequently the measured zeta potential changes.

3.5. Influence of packing material

The packing materials has also an influence on SLN stability. For some SLN formulations with a gelling tendency a stability improvement has been reported when they were packed in plastic containers instead of glass vials (Siekmann, 1994). No long-term investigations had been performed in this case. It had been observed that the first visible flocculates of an unstable SLN dispersion always stuck to the wall of the glass vial. To assess whether siliconization of the glass surface had any effect on the stability, Compritol SLN were packed in siliconized and in untreated glass vials (glass quality I). They were stored at optimum conditions (i.e. at 8°C, in the dark) to exclude destabilizing effects from other sources.

After 1 year the LD diameter 99% of the samples in untreated glass was slightly higher compared to storage in siliconized glass vials

(1.16 ± 0.02 μm and 1.11 ± 0.04 μm, respectively). The size differences detected by LD were too small to give any evidence for the influence of the vial surface.

However, the zeta potentials of SLN in the siliconized vials were 3 mV higher than in the untreated vials (i.e. it remained at its initial value of -25 mV measured at day 0). Such a potentially small difference in repulsive charge can lead only to very small differences in particle aggregation behavior. Therefore the more sensitive Coulter counter analysis was performed additionally to LD. In Fig. 9, the absolute number of particles >5 μm, >7 μm and >10 μm/μl SLN dispersion as function of storage time is shown. During the whole monitored period all three fractions of particles were distinctly larger for SLN packed in non-siliconized glass vials (Fig. 9, left half of the figure).

Hence, it can be concluded that siliconization of the glass surface has a stabilizing effect. The mechanisms causing destabilization in emulsions, like coalescence of the lipid droplets, are well investigated. SLN show some similarities especially to o/w emulsions although the inner phase is solid. Both systems consist of an incoherent lipid phase with a high surface which has to be covered by surfactants. For emulsions it is reported that coalescence can take place via adherence to the wall of the container, e.g. in TPN regimens (Washington, 1990). Therefore, minimization of adherence of SLN to the surface of the vial by siliconization minimizes aggregation and enhances consequently the physical stability.

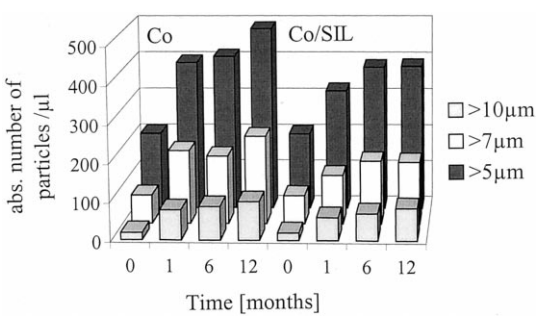

Fig. 9. Influence of packing material: Absolute number of particles per μl dispersion smaller than 10, 7 and 5 μm (Coulter counter data) of 10% Compritol SLN stored at 8°C in the dark over a period of 12 months. The SLN were packed either in untreated (Co, left half of figure) or in siliconized glass vials (Co/SIL, right half of figure).

3.6. Possible types of SLN gels

For hydrophilic o/w cremes stabilized with nonionic surfactants the existence of a coherent gel structure has been suggested (Nürnberg, 1985). This framework would consist of crystalline or liquid crystalline emulsifier which can incorporate water by swelling. The incoherent lipophilic phase would be mechanically immobilized. For block copolymers, like the Pluronics, the ability to form network structures has been reported (Harland and Peppas, 1993). Pluronic F68 itself has been used as plasticizer and gelling agent (Law et al., 1984; Frisbee and McGinity, 1994). Reduction in zeta potential can indicate thicker, more sticky films. Bridging effects appear also possible. Free water could be incorporated intralamellar (swelling) which would explain the hardening of the gel with time. However, the gelling phenomenon has been observed for SLN formulations with varying emulsifiers which do not all have gel forming properties.

Gelation of phospholipid stabilized tripalmitate dispersions was explained by the recrystallization of the spherical liquid droplets in polyedric platelets (Siekmann, 1994). New, unprotected interfaces are formed which have to be covered with emulsifier. Depending on the physico-chemical properties of the emulsifier a preferential adsorption on certain crystal surfaces (001) was observed. Particles can approach easily now from the other unprotected sides and aggregate. The measured reduction in zeta potential surely promotes particle approach. A subsequent sintering process leads to a coherent crystalline lipid network. Additionally, the extend of solidification seemed to be related to the amount of stable lipid modification.

Compritol SLN are of spherical shape although $\geq 75\%$ of the lipid phase are crystalline (zur Mühlen, 1996). Still, uncovered lipid surface can come into contact due to a partly damaged poloxamer film. Crystal growth between the particles could take place forming a solid, lipid network.

From this, possible reasons for the gel formation are structural changes occurring during storage of the SLN dispersions. The change in zeta potential proves the existence of such changes in the system, they are accelerated by factors such as light and temperature. Transformation of the polymorph forms and recrystallization of the lipid in platelets or rods promotes the formation of a network (e.g. similar to the network structure in Bentone™ gels). Reduction in zeta potential and subsequently in electrostatic repulsion eases aggregation of lipid crystals to build up the network. The use of Pluronic F68 being on its own a gelling agent can promote the gel formation, in addition polymer bridging is possible. At present, DSC measurements are being performed to investigate the crystalline changes in the Compritol formulation as a function of storage time.

4. Conclusion

The chosen SLN formulation consisting of 10% Compritol and 1.2% Pluronic F68 represented a highly sensitive system towards several destabilizing factors. Particle growth and subsequent gelation could be induced by any influence of kinetic energy. It was accompanied by a decrease in zeta potential promoting the approach of particles and formation of a gel network. A superimposition of different factors is likely (i.e. particle charge, film rigidity and sterical effects).

Possible mechanisms of the gel formation are structural changes of the lipid phase leading to zeta potential reduction and particle growth. The large lipid particles can approach each other due to the loss of electrostatic repulsion and form a network, possibly promoted by the stabilizer (gel formation properties of poloxamer, bridging). The change in zeta potential indicates structural changes. Therefore further investigations concerning especially the structural properties of the lipid phase are being performed to confirm the suggested mechanism of gel formation.

Nevertheless by developing optimal storage conditions (i.e. 8°C, dark, siliconized glass vials) a Compritol SLN formulation which had been regarded as unstable did not alter in physical stability over a period of 3 years. This opens the perspective of using basically less stable but from the drug delivery site or toxicological aspects desirable formulations by optimizing storage and packing conditions.

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