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# Solid Lipid Nanoparticles (SLN$^{TM}$) for topical drug delivery: incorporation of the lipophilic drugs *N,N*-diethyl-*m*-toluamide and vitamin K

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Solid lipid nanoparticles (SLN) for topical delivery were prepared by high pressure homogenization using solid lipids. The lipophilic agents DEET (*N,N*-diethyl-*m*-toluamide) and vitamin K were used as model drugs. These topical agents were incorporated into SLN which were characterized. Differential scanning calorimetry studies were performed in order to detect probable interactions in the SLN dispersions. Physical stability of SLN in aqueous dispersions and the effect of drug incorporation into SLN were investigated by photon correlation spectroscopy and zeta potential measurements. Characterization and short term stability studies showed that DEET and vitamin K are good candidates for topical SLN formulations.

## 1. Introduction

Solid lipid nanoparticles are recently being used for topical administration and have some advantages. First of all, their small particle size increases the penetration of the incorporated agent into the skin. As SLN provides sustained release, it is possible to applicate the active agents to the skin for a long period of time with reduced systemic absorption. Also the irritation potential of drugs could be reduced by incorporation into SLN lipid matrices (Müller et al. 2000; Jenning et al. 2000; Dingler et al. 1999).
*N,N*-Diethyl-*m*-toluamide (DEET) is a lipophilic organic compound which is used as an insect repellent (Couch and Johnson 1992). Topical administration of DEET is widespread throughout the world. In commercial products, DEET is mostly formulated in alcoholic solutions. For optimal protection, the insect repellent should remain on the surface of the skin and evaporate over several hours rather than be absorbed (Robbins and Chernak 1986). In many of the studies it is found that DEET penetrates through the skin to the blood circulation and causes adverse effects and also it has an irritation potential on skin (Reuveni and Yagupsky 1982; Libscomb et al. 1992). To reduce dermal and systemic side effects DEET is incorporated into the SLN which provides a carrier system for various drugs with controlled release characteristics.
The use of vitamin K in topical formulations is a new concept as it is usually taken by food to play a role as a cofactor in the synthesis of gammacarboxyglutamic acid concerning the blood coagulation factors (Jakob and Elmadfa 2000). Although the mechanism of action of topical vitamin K on clotting or clearing of purpura is unclear, topical vitamin K appeared to influence the disappearance of extravascular blood. Topically applied vitamin K is unlikely to alter systemic levels so it is thought to be effective due to peripheral effects (Shah et al. 2002). Vitamin K is being used topically for cosmetic purpose in order to promote the disappearance of bruises on the skin and also for the treatment of spider veins (Lewis and Gengler 1996; Lou et al. 1999). In this study SLN was used as a topical carrier system for the lipophilic drug vitamin K allowing prolonged efficacy and controlled release, as there are several examples of incorporation of vitamins into SLN dispersions (Jenning et al. 2000; Dingler et al. 1999; Wissing and Müller 2003a).
SLN formulations were developed using different lipids and lipid concentrations both for DEET and vitamin K. Particle size, zeta potential and differential scanning calorimetry (DSC) studies were performed in order to find suitable topical SLN formulations.

## 2. Investigations, results and discussion

### *2.1. Identification of lipid matrices for drug incorporation*

During preliminary studies, lipid screening was performed for the identification of matrices for DEET and vitamin K incorporation. According to the lipid screening studies for DEET, cetylpalmitate and Dynasan 116 were not good candidates as the drug was expelled from the matrices whereas, stearic acid and stearyl alcohol were found to be suitable for incorporation of minimum 10% of DEET. Tween 80 was used as a surfactant in all of the SLN dispersions. After preparation of SLN dispersions, Imwitor 900 and Compritol 888 ATO containing SLN dispersions showed large particle size distributions ($PI > 0.400$) and particle sizes were in the micrometer range when stored at room temperature. Also incorporation of 10% DEET caused immediate gelation within 1 day. This indicates

**Table: Compositions of the SLN formulations**

| SLN system | Lipid content (%) | Surfactant | Drug incorporated |
|---|---|---|---|
| Stearic acid-SLN | 10%, 20%, 30% | Tween 80 | DEET |
| Stearyl alcohol-SLN | 10%, 20% | Tween 80 | DEET |
| Apifil-SLN | 10%, 20%, 30% | Tween 20 | Vitamin K |
| Cera flava-SLN | 10% | Tween 20 | Vitamin K |

that the nature of the lipid matrix and compatibility with the drug are also important in drug loading of SLN dispersions.

Many lipid matrices were tested for vitamin K and three of them, which were Apifil, Cera flava and Compritol HDS ATO were chosen for the SLN formulation studies. During preliminary studies, placebo SLN dispersions stabilized by 1.5% Tween 80, Tween 20, Pluronic F68 or sucrose ester 1670 were produced. According to the appearance and short term stability Apifil and Cera flava were chosen as lipid matrices where Tween 20 was the surfactant of both SLN dispersions. SLN formulations used in further studies are summarized in the Table.

DEET containing SLN dispersions that were prepared using stearic acid and stearyl alcohol showed better results as the dispersions were stable for 10% DEET. As the DEET concentration is 10% of the lipid matrix, in order to increase the DEET amount throughout the total formulation, solid lipid percentage was increased to 20% and 30%.

Vitamin K was incorporated into 10% Apifil and 10% Cera flava containing solid lipid particles. Although short term stability was good for both lipid matrices, SLN with Apifil gave a smaller particle size distribution so 20% and 30% Apifil containing formulations were also studied.

### *2.2. Differential Scanning Calorimetry (DSC) measurements*

The physical integrity of SLN can be examined by differential scanning calorimetry (DSC). The DSC diagram in Fig. 1a shows the melting peaks of 10% stearic acid-SLN placebo and DEET loaded dispersions. Heating the aqueous placebo SLN dispersion reveals a melting peak at 68.00 °C and a melting enthalpy of 203.2 J/g. Recyrstallization peak was observed at 43.63 °C with the cooling enthalpy of −202.9 J/g. In the DEET loaded SLN formulation, the melting peak was observed at 65.85 °C and a melting enthalpy was 203.2 J/g. During cooling the SLN dispersion recrystallization peak occured at 38.73 °C and the cooling enthalpy was −206.4 J/g. Existence of the peaks and no new peak detection point out there means no interaction or incompatibility in the medium. 10% stearyl alcohol-SLN dispersions were also investigated and melting peaks of the placebo formulation were found at 42.40/61.11 °C and the melting enthalpy was 214.8 J/g. The melting peak was at 42.66 °C and melting enthalpy was 86.2 J/g for the DEET loaded stearyl alcohol-SLN formulation (Fig. 1b). The melting enthalpy differed and also one of the melting peaks disappeared by the incorporation of DEET to the system which points out that there might be an interaction between the drug and the SLN dispersion. So differential scanning calorimetry studies show that stearic acid is a better lipid matrix for DEET than stearyl alcohol.

Vitamin K containing formulations were also examined by DSC. The melting peaks of the placebo and vitamin K loaded 10% Apifil-SLN dispersions were almost identical with temperatures of 61.97 °C and 60.96 °C respectively.

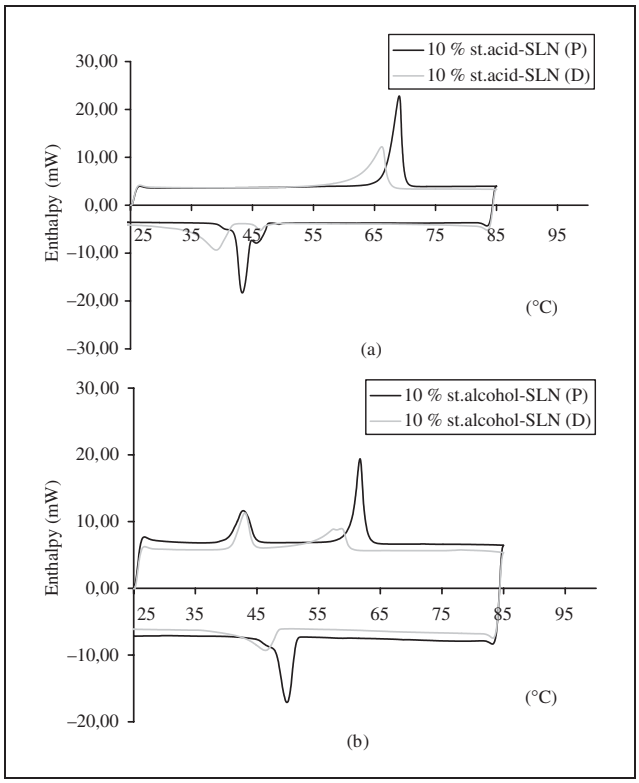


Fig. 1: Differential scanning calorimetry heating curves of placebo (P) and DEET (D) containing aqueous SLN dispersions. 10% stearic acid (a) and 10% stearyl alcohol (b) formulations after production

When cooling the molten formulations down to room temperature they recrystallized at 47.84 °C and 45.86 °C, values which were again close to each other (Fig. 2a). Incorporation of vitamin K did not affect the thermal characteristics of the system. The small shoulder of the cooling curve indicates the probability of an unstable modification existence (Freitas and Müller 1999). DSC thermograms of 10% Cera flava-SLN placebo and vitamin K containing dispersions are shown in Fig. 2b. Again similar results were observed showing that there were no interactions between vitamin K and SLN dispersion.

### *2.3. Particle size determination*

According to PCS measurements, particle sizes were all in the nanometer range and polidispersity index (PI) values indicate a narrow distribution of placebo SLN dispersions. Incorporation of DEET in stearic acid and stearyl alcohol-SLN led to an increase in the particle size ($p < 0.05$). The mean diameter of placebo formulations were approximately 300 nm and after drug loading the mean particle size became 500 nm. Especially in stearyl alcohol dispersions, incorporation of the drug increased the particle sizes to a great extent. Also distinct increases were observed in particle size distributions by the increase in the lipid concentrations ($p < 0.05$) (Fig. 3). The particle sizes given were measured right after production.

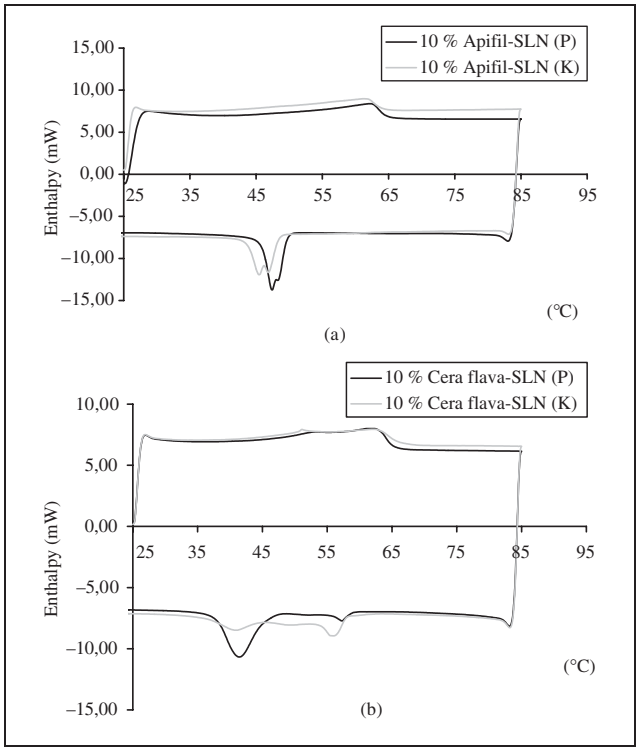


Fig. 2: Differential scanning calorimetry heating curves of placebo (P) and vitamin K (K) containing aqueous SLN dispersions. 10% Apifil (a) and 10% Cera flava (b) formulations after production

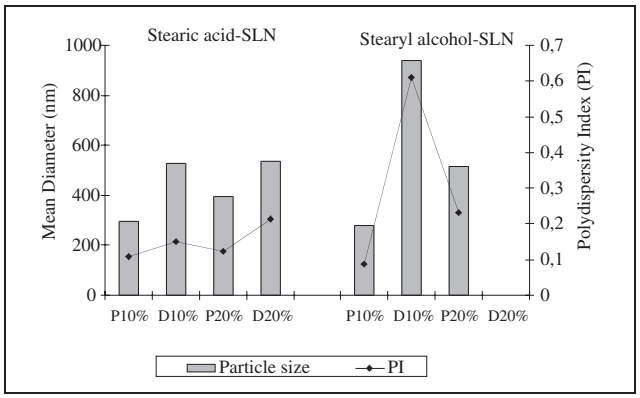


Fig. 3: Mean diameter (nm) and polydispersity index (PI) of DEET loaded stearic acid-SLN and stearyl alcohol-SLN containing 10% and 20% of lipid matrices both stabilized by Tween 80 (P: Placebo; D: DEET-loaded)
* 20% DEET loaded stearyl alcohol-SLN was not shown due to large particle size distribution

Short-term stability of placebo and DEET incorporated SLN dispersions containing 10–30% stearic acid were investigated using laser diffraction after storage of one week at room temperature. In 10% stearic acid-SLN dispersions both in placebo and DEET containing formulations mean particle sizes increased after one week storage ($p < 0.05$). In 20% stearic acid-SLN dispersions there was a decrease in the mean particle size of the placebo formulation where particle sizes increased in the drug containing formulation after one week ($p < 0.05$). In 30% stearic acid-SLN dispersions there was no difference in the placebo formulation ($p > 0.05$), but mean particle size increased to a great extent in the DEET containing SLN dispersion. In most of the formulations these small alterations in the mean particle sizes were found statistically important as the standard deviations of the measurements are too low (Fig. 4).

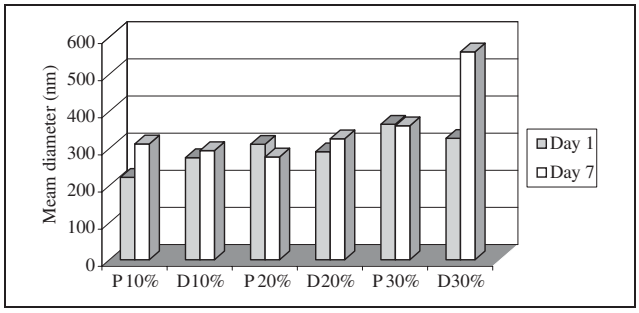


Fig. 4: Short-term stability of placebo (P) and DEET (D) incorporated SLN containing 10–30% stearic acid stored at room temperature (Drug contents are 10% of the lipid matrix)
* Measurements of laser diffraction (LD)

Storage conditions (4 °C, room temperature, 40 °C) effected the particle size distributions. When 10% stearic acid-SLN dispersions were stored at 4 °C, no change was observed in the particle size distributions of both placebo and drug containing formulations in comparison to the measurements that were taken on day one ($p > 0.05$) which indicates that storage at low temperatures does not affect the particle size distribution of the SLN dispersions. When both placebo and drug loaded formulations were stored at room temperature and especially at 40 °C, the mean particle sizes increased ($p < 0.05$). For 10% stearyl alcohol-SLN dispersions the mean particle size remained unchanged for the placebo formulation at 4 °C ($p > 0.05$), but a decrease was observed in the mean particle size of the drug loaded formulation after storage at 4 °C ($p < 0.05$). When the formulations were stored at room temperature and 40 °C mean particle sizes did not remain identical ($p < 0.05$), besides mean particle sizes were in the micrometer range at 40 °C (Fig. 5). The stearyl alcohol-SLN dispersions with 20% DEET increased in viscosity during storage, finally leading to a viscous gel after two weeks which could be the reason of recyrstallization properties of the lipid matrix (Westesen and Siekman 1997). On the other hand viscosity did not change in stearic acid-SLN dispersions with 20% DEET during two weeks storage.

These results pointed out that stearic acid is a better candidate for DEET incorporation as the particle size distribu-

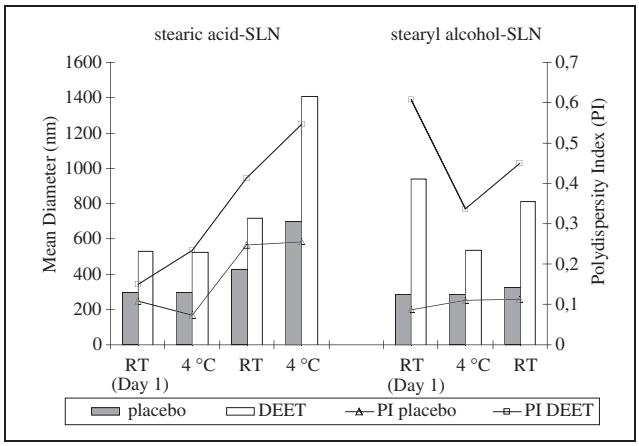


Fig. 5: PCS diameter (nm) and polydispersity index (PI) of 10% stearic acid-SLN and 10% stearyl alcohol-SLN placebo and DEET incorporated formulations and after 1 week of storage at 4 °C, room temperature (RT) and 40 °C. (Room temperature results on day 1 are also shown)
* Placebo and DEET loaded stearyl alcohol-SLN formulations stored at 40 °C were not shown due to large particle size distributions

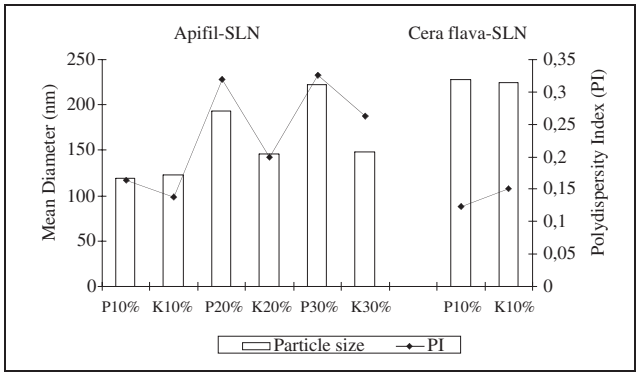


Fig. 6: Mean diameter (nm) and polydispersity index (PI) of vitamin K loaded Apfil-SLN and Cera flava-SLN containing 10%, 20% and 30% of lipid matrices for Apifil-SLN and 10% lipid matrix for Cera flava-SLN both stabilized by Tween 80 (P: Placebo; K: vitamin K-loaded)

tions increased to a great extent in stearyl alcohol-SLN both by drug loading and storage. Higher concentrations of the lipid phase caused an increase in the particle size distributions as well.

Incorporation of vitamin K decreased the mean particle sizes when compared to placebo formulations in both Apifil and Cera flava containing SLN dispersions ($p < 0.05$) except for 10% Apifil-SLN in which the particle size slightly increased after drug incorporation. In higher concentrations of Apifil (20% and 30%) vitamin K reduced the particle sizes when compared to placebo (Fig. 6). The reduction in the particle sizes due to vitamin K incorporation might be the reason of the liquid and lipophilic structure of the drug and the wax structure of lipid matrices. On the other hand, higher concentrations of the lipid led to an increase in mean particle sizes ($p < 0.05$). 10% Cera flava-SLN formulation has approximately 225 nm mean particle sizes where the particle sizes were around 120 nm for 10% Apifil-SLN. Although the mean particle size of 10% Cera flava-SLN was greater than 10% Apifil-SLN, polidispersity index value of the Cera flava-SLN was smaller than Apifil-SLN indicating a narrow particle size distribution.

Short-term stability of placebo and vitamin K incorporated SLN containing 10–30% Apifil were investigated using laser diffraction after storage for one week at room temperature. There was no change in 10% lipid containing dispersions but there were decreases ($p < 0.05$) in the mean particle sizes only in placebo SLN dispersions containing 20% and 30% lipid after 7 days. Cera flava-SLN showed a different behaviour, the particle sizes increased after one week for placebo formulation ($p < 0.05$) but again vitamin K incorporation did not change the mean particle size ($p > 0.05$) (Fig. 7).

Storage conditions (4 °C, room temperature, 40 °C) had only little effect on mean particle sizes for 10% Apifil-SLN and 10% Cera flava-SLN (Fig. 8). In placebo formulation of 10% Apifil the mean particle sizes were too close to each other which were around 120 nm under all the storage conditions. As the standard deviations are low, in most of the formulations the difference between the particle size distributions were found statistically important. On the other hand the vitamin K containing 10% Apifil formulation remained unchanged ($p > 0.05$) under all the storage conditions. In 10% Cera flava-SLN formulations only storage at 4 °C did not affect the mean particle sizes ($p < 0.05$) both in placebo and vitamin K loaded formulations. In 20% and 30% Apifil-SLN dispersions sto-

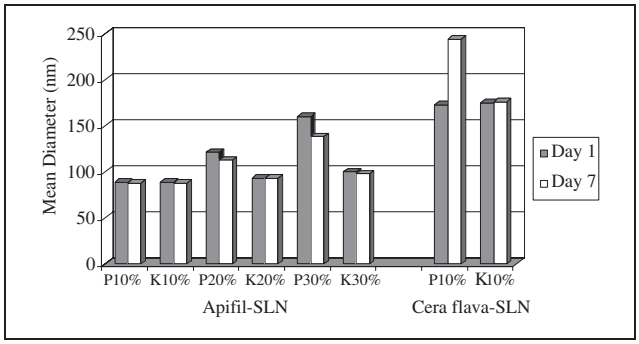


Fig. 7: Short-term stability of placebo (P) and vitamin K (K) incorporated SLN containing 10–30% Apifil and 10% Cera flava stored at room temperature (Drug contents are 10% of the lipid matrix)
* Measurements of laser diffraction (LD)

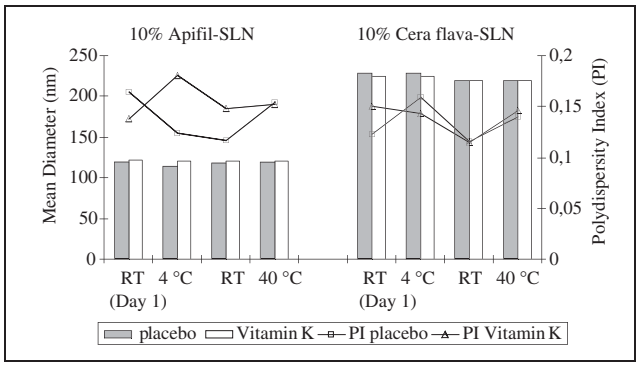


Fig. 8: Mean diameter (nm) and polydispersity index (PI) of 10% Apifil-SLN and 10% Cera flava-SLN placebo and Vitamin K incorporated formulations after 1 week of storage at 4 °C, room temperature (RT) and 40 °C. (Room temperature results on day 1 are also shown)

rage conditions were found to be effective on particle size ($p < 0.05$) except storage at 40 °C for placebo formulation and storage at room temperature for drug containing formulation of 10% Apifil-SLN.

According to the above data, Apifil was found to be a more suitable lipid matrix for vitamin K as the particle size distributions are smaller both for placebo and drug containing formulations. But increase in the lipid concentration in order to incorporate higher amounts of vitamin K caused larger particle size distributions.

### *2.4. Zeta potential determination*

Zeta potentials of the Tween 80 stabilized stearic acid-SLN and stearyl alcohol-SLN dispersions were approximately −30 mV. These zeta potential values are sufficiently high for electrostatic stabilization of the nanoparticle dispersions (Schwarz and Mehnert 1999). Incorporation of DEET did not affect the zeta potential values of 10% and 20% stearic acid containing SLN dispersions ($p > 0.05$), but short term storage influenced the zeta potential values of both placebo and drug loaded formulations which was observed like slight decrease or increase ($p < 0.05$). Zeta potential value of DEET containing 20% stearic acid-SLN was not affected by storage ($p > 0.05$). In stearyl alcohol containing SLN dispersions both drug incorporation and short term storage affected the zeta potential values in all of the formulations ($p < 0.05$).

Zeta potentials of Tween 20 stabilized Apifil-SLN dispersions were also around −30 mV. 10% Cera flava-SLN dispersions have −45 mV zeta potential values that are sufficiently high for optimum stabilization of the SLN

dispersions. Although Cera flava-SLN dispersions seem to be promising due to their high zeta potential values, Apifil-SLN with the smaller size distributions were chosen for the preparation of high lipid concentrations of 20% and 30%. Zeta potentials of 10% Apifil containing formulations were slightly decreased by drug loading ($p < 0.05$), short term storage also caused a decrease which is around −12mV ($p < 0.05$). In 20% Apifil-SLN, incorporation of vitamin K had no effect on zeta potential values and zeta potential values of vitamin K loaded SLN dispersions were not affected during short term storage ($p > 0.05$). On the other hand, 30% Apifil-SLN were affected both by drug loading and storage ($p < 0.05$). Zeta potential values of the 10% Cera flava-SLN dispersions were also decreased by incorporation of vitamin K and storage ($p < 0.05$).

Previous studies showed that solid lipid nanoparticles are suitable systems for the incorporation of active ingredients for dermal delivery (Jenning et al. 2000; Dingler et al. 1999; Lou et al. 1999; Müller et al. 2002; Wissing and Müller 2003b). In this study, both highly lipophilic model drugs were incorporated into SLN. Different solid lipid matrices were found suitable for each drug showing that the nature of the lipid matrix is also important in drug loading. Examination of the crystalline status of the SLN formulations indicated the suitability of the lipid and the drug. The lipid phase concentration of the SLN dispersions were increased in order to apply higher amounts of drug to the skin but high lipid concentrations unfortunately caused an increase in the particle size distributions. Drug incorporation did not or even slightly influence particle size and zeta potential. Short-term stability studies revealed promising results especially for vitamin K. Data suggests that these topical drugs could be incorporated into SLN for controlled drug delivery.

## 3. Experimental

### *3.1. Materials*

DEET was purchased from Fluka Chemika (Buchs, Switzerland), Vitamin K, stearic acid and Tween 20 were obtained from Sigma (Steinheim, Germany), stearyl alcohol and cetylpalmitate were provided by Caelo GmbH (Hilden, Germany), Tween 80 was obtained from Uniqema (Everberg, Belgium), Apifil (PEG-8 beeswax), Compritol ATO 888 (glycerol behenate) and Compritol HDS ATO (PEG-8 behenate, tribehenin) were provided by Gattéfosse (Priest, France), Cera flava (beeswax) was obtained from ComPharma (Hamburg, Germany). Dynasan 116 (glycerol tripalmitate) and Imwitor 900 (glyceryl monostearate) were provided by Hüls AG (Germany), Pluronic F68 (poloxamer 188) was donated by BASF AG (Ludwigshafen, Germany)

### *3.2. Preliminary studies on lipid matrices*

Preliminary studies were performed in order to find out the suitable lipid matrices for DEET and vitamin K. Different solid lipids were tested for each lipophilic drug. Drug solubility was examined by mixing the solid lipid and the drug in different ratios. Drug-solid lipid mixtures were prepared using a water bath at 85 °C, investigated when hot and after cooling down to room temperature and also after 24 h.

### *3.3. Preparation of SLN*

Solid lipid particles were prepared by high pressure homogenization. All of the formulations were prepared identically where the drug was mixed in the melted lipid and the hot lipid-drug solution was dispersed in a heated aqueous surfactant solution. The mixture was stirred using an Ultra Turrax K18 (Janke und Kunkel, Staufen, Germany) at 8000 rpm for 1 min at 75–85 °C, then this pre-emulsion was homogenized using APV Micron LAB 40 (APV Gaulin, Lübeck, Germany) at 85 °C, 500 bar, applying three homogenization cycles. The homogenized hot nanoemulsion (o/w) recrystallizes upon cooling and forming solid lipid nanoparticles (Müller et al. 1995). The final SLN dispersions had a lipid content of 10–30% (w/w). The drug incorporated was between 1–3% as the drug contents was 10% of the lipid matrix in all the formulations. SLN dispersions were stored at 4 °C, room temperature and 40 °C in a small glass vials over a period of 15 days.

### *3.4. Characterization of SLN*

The crystalline status was determined using the Differential Scanning Calorimetry (TA 8500, Mettler Toledo GmbH, Gieβen, Germany). Approximately 15 mg of the samples were weighed in small aluminum pans and heated from 25 °C to 85 °C with a scan rate of 5 K/min. Particle sizes were analyzed by photon correlation spectroscopy (PCS) with a Zetasizer 4 (Malvern Instruments, UK) and laser diffraction (LD) with a Mastersizer E (Malvern Instruments, UK). PCS yields the mean particle size and the polydispersity index (PI) as a measure of the width of the distribution. The LD data were evaluated using volume distribution. Zeta potential measurements were also performed using a Zetasizer 4 and prior to the measurement; all the samples were diluted in distilled water that was adjusted to 50 μS/cm.

**References**


Couch P, Johnson CE (1992) Prevention of the lyme disease. AJHP 49: 1164–1173.

Dingler A, Blum RP, Niehus H, Müller RH, Gohla S (1999) Solid lipid nanoparticles (SLN™/Lipopearls™)-a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. J Microencapsul 16: 751–767.

Freitas C, Müller RH (1999) Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase. Eur J Pharm Biopharm 47: 125–132.

Jakob E, Elmadfa I (2000) Rapid and simple HPLC analysis of vitamin K in food, tissues and blood. Food Chem 68: 219–221.

Jenning V, Schäfer-Korting M, Gohla S (2000) Vitamin A-loaded solid lipid particles for topical use: drug release properties. J Control Rel 66: 115–126.

Lewis AB, Gengler EC (1996) Resurfacing with topical agents. Semin Cutan Med Surg 15: 139–144.

Lipscomb JW, Kramer JE, Leikin JB (1992) Seizure following brief exposure to the insect repellent *N*,*N*-diethyl-*m*-toluamide. Ann Emerg Med 21: 315–317.

Lou WW, Quintana AT, Geronemus RG, Grossman MC (1999) Effects of topical vitamin K and retinol on laser-induced purpura on nonlesional skin. Dermatol Surg 25: 942–944.

Müller RH, Mäder K, Gohla S (2000) Solid lipid nanoparticles (SLN)- for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm 50: 161–177.

Müller RH, Mehnert W, Lucks JS, Schwarz C, zur Mühlen A, Weyhers H, Freitas C, Rühl D (1995) Solid lipid nanoparticles (SLN)- An alternative colloidal carrier system for controlled drug delivery. Eur J Pharm Biopharm 41: 62–69.

Müller RH, Radtke M, Wissing SA (2002) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Del Rev 54: S131–S155.

Reuveni H, Yagupsky P (1982) Diethyltoluamide containing insect repellent. Adverse effects in worldwide use. Arch Dermatol 118: 582–583.

Robbins P, Chernak MG (1986) Review of the biodistribution and toxicity of the insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET). J Toxicol Environ Health 18: 503–525.

Schwarz C, Mehnert W (1999) Solid lipid nanoparticles (SLN) for controlled drug delivery II. Drug incorporation and physicochemical characterization. J Microencapsul 16: 205–213.

Shah NS, Lazarus MC, Bugdodel R, Hsia SL, He J, Duncan R, Baumann L (2002) The effects of topical vitamin K on bruising after laser treatment. J Am Acad Dermatol 47: 241–244.

Westesen K, Siekmann B (1997) Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. Int J Pharm 151: 35–45.

Wissing SA, Müller RH (2003) Cosmetic applications for solid lipid nanoparticles (SLN). Int J Pharm 254: 65–68.

Wissing SA, Müller RH (2003) The influence of solid lipid nanoparticles on skin hydration and viscoelasticity-in vivo study. Eur J Pharm Biopharm 56: 67–72.