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Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) for application of ascorbyl palmitate

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The aim of this study was to improve the chemical stability of ascorbyl palmitate (AP) in a colloidal lipid carrier for its topical use. For this purpose, AP-loaded solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and for comparison, a nanoemulsion (NE) were prepared employing the high pressure homogenization technique and stored at room temperature (RT), 4 °C and 40 °C. During 3 months, physical stability of these formulations compared to placebo formulations which were prepared by the same production method, was studied including recrystallization behaviour of the lipid with differential scanning calorimetry (DSC), particle size distribution and storage stability with photon correlation spectroscopy (PCS) and laser diffractometry (LD). After evaluating data indicating excellent physical stability, AP-loaded SLN, NLC and NE were incorporated into a hydrogel by the same production method as the next step. Degradation of AP by HPLC and physical stability in the same manner were investigated at the same storage temperatures during 3 months. As a result, AP was found most stable in both the NLC and SLN stored at 4 °C ($p > 0.05$) indicating the importance of storage temperature. Nondegraded AP content in NLC, SLN and NE was found to be 71.1% ± 1.4, 67.6% ± 2.9 and 55.2% ± 0.3 after 3 months, respectively. Highest degradation was observed with NE at all the storage temperatures indicating even importance of the carrier structure.

1. Introduction

Solid Lipid Nanoparticles (SLN) are an alternative colloidal drug carrier system to polymeric nanoparticles, liposomes and emulsions in various application routes due to their numerous advantages (Fundaro et al. 2000; Dubes et al. 2003; Cavalli et al. 2002). While controlled release of active ingredients over several weeks is possible with SLN, chemical stability against degradation can also be achieved (Venkatesvarlu and Manjunath 2004; Zur Mühlen and Mehnert 1998). During the last few years, SLN have also been studied with labile compounds such as vitamin E (Dingler et al. 1999), tocopherol acetate (Wissing and Müller 2001) and retinol (Jenning and Gohla 2001) for topical application. Therefore, it appeared as a promising drug carrier system, even for cosmetic applications (Shahgaldian et al. 2003). In a recent study, it was reported that placebo SLN prevented water evaporation from the skin, thus had occlusive properties. This point is very important, because penetration of drugs into skin depends strongly on skin hydration and can therefore be influenced by occlusive compounds (Mei et al. 2003; De Vringer and De Ronde 1995). Most of the lipids used in SLN production have an approved status, e.g. the GRAS status due to their low toxicity or are used as excipients in cosmetics or pharmaceutics (Anon., Code of Federal Regulations, Food and Drugs 2001). The scaling up feasibility of SLN production by high pressure homogenization technique was shown, whereas no satisfactory and accepted scaling up method exists for polymeric nanocapsules (Gohla and Dingler 2001).

In spite of all those advantages of SLN, their potential limitations such as limited drug loading capacity, drug expulsion during storage and high water content (70–95%) were also discussed (Müller et al. 2002a).

For avoiding or minimising all those problems, the new generation Nanostructured Lipid Carriers (NLC) have been introduced recently. NLC are produced by mixing solid lipids with spatially incompatible liquid lipids leading to special structures of the lipid matrix. Three types of NLC are described: (I) the imperfect structured type, (II) the structureless type and (III) the multiple type. All these types of NLC allow the production of highly concentrated particle dispersions (>30–95%) and increased drug content (Müller et al. 2002a, 2002b).

Vitamin C (L-ascorbic acid) is a natural antioxidant that combats the reactive oxygen species that can cause damage in cells endangering tissue integrity. It exhibits free radical scavenger activity, inhibitory effect on melanogenesis and anti-aging properties (Campos and Silva 2000). Due to its excellent reducing efficiency, it is extremely unstable especially under aerobic conditions, in contact with metal ions and under light. It is degraded irreversibly and quickly to the biologically inactive form (2,3-diketo-L-

Table 1: Melting points, melting enthalpies and recystallization indices of the solid lipid in the formulations after 3 months of storage at various temperatures

Formulation			Melting point (°C)	Melting enthalpy (J/g)	Recrystallization indice (%)
SLN		RT	41.2	29.6	24.9
	Placebo dispersion	4 °C	39.6	90.0	75.7
		40 °C	33.6	7.4	6.2
		RT	43.8	52.6	44.3
	AP-loaded dispersion	4 °C	39.2	86.3	72.5
		40 °C	33.6	6.4	5.4
		RT	42.3	130.0	109.3
	Hydrogel	4 °C	39.6	121.2	102.0
		40 °C	36.5	36.0	30.3
NLC		RT	39.0	17.1	14.4
	Placebo dispersion	4 °C	37.2	41.4	34.9
		40 °C	43.2	6.2	5.2
		RT	32.4	21.4	18.0
	AP-loaded dispersion	4 °C	38.6	31.0	39.2
		40 °C	35.5	11.4	9.6
		RT	42.4	66.7	56.1
	Hydrogel	4 °C	38.9	75.2	63.3
		40 °C	32.8	19.4	16.3

Melting enthalpy and recrystallization indices were calculated according to the fact that the lipid contents in the formulations were standardized up to 100% lipid. Reference: Bulk lipid (Witepsol® E85) with 118.9 J/g melting enthalpy and 44.4 °C melting point

gulonic acid) by oxidation. Because of its low stability in aqueous solutions, its use in cosmetic formulations is very difficult. To overcome the stability problems, derivatives of vitamin C with improved chemical stability were synthesized. Ascorbyl palmitate (6-palmitoyl-L-ascorbic acid) (AP) which is a fatty acid ester with lipophilic properties is one of those derivatives. Nevertheless, it was reported that even though the stability of AP was much better than vitamin C, it still depended on the aerobic condition and structural properties of the carrier system and/or formulation, and the degradation was accelerated by metal ions and/or light. In the AP molecule, the fatty acid ester is in 6-position and the inorganic ester group is introduced in 2-position, involving the enediol system. Only the cyclic ring of the molecule is sensitive to oxidation (Austria et al. 1997; Spiclin et al. 2001; Buettner and Jurkiewicz 1996). This ascorbic acid derivative with favorable effects as an excellent skin antioxidant and lipophilic properties which possesses skin penetration, promises various advantages for skin applications.

Entrapment of AP in various carriers with the aim of improving its chemical stability was investigated by another research group. They found that SLN were among the two carriers which led the highest AP stability compared to microemulsions. They also investigated the effect of environmental oxygen on microemulsion formulation. The AP stability in microemulsions increased twice by de-airing (Kristl et al. 2003).

The aim of this study was to improve AP stability at the concentration of 1% (w/w) against chemical degradation in a lipid carrier including the new generation NLC in addition to SLN. A solid lipid (Witepsol® E85) different from that of the study mentioned above was used. Therefore, placebo and AP-loaded SLN and NLC dispersions as lipid carriers were produced by the high pressure homogenization technique. Physical stability of these formulations stored at various temperatures for a period of three months was compared to each other, placebo and AP-loaded NE which were prepared by the same manner. As the next step, topical hydrogel preparations including AP-loaded SLN, NLC and NE were produced. Taking into account the findings of Kristl et al., we filled them into the aluminium tubes under de-aired conditions. Chemical stability of the active ingredient as well as the physical stability was examined.

2. Investigations, results and discussion

2.1. Crystallinity of SLN and NLC

DSC experiments give information about melting and crystallization behaviour of crystalline materials like lipid nanoparticles. The breakdown and fusion of the crystal lattice by heating or cooling the sample yields inside information of polymorphism, crystal ordering, eutectic mixtures, glass transition processes and drug-lipid interactions (Ford and Timmins 1989).

Melting point and melting enthalpy values as degree of crystallinity were obtained from the measurements. Recrystallization indices (RIs) were calculated by taking enthalpy of the lipid content of Witepsol® E85 SLN (10% for placebo dispersion and 9% for AP-loaded dispersion and hydrogel formulation) and NLC (6.666%) formulations during 3 months of storage. For an example of RI calculation, melting enthalpy decreased compared to the bulk lipid from 118.9 to 2.96 J/g in case of the placebo SLN dispersion stored at RT. This results in a theoretical melting enthalpy and crystallinity of the placebo SLN dispersion of 29.6 J/g and 24.9%, respectively (Table 1) (Zur Mühlen et al. 1998).

We found a melting point of the bulk lipid to be 44.4 °C as reported earlier (Almeida et al 1997). Melting points of all the formulations decreased and polymorphic transition was found lower compared to bulk lipid. This phenomenon was supported by X-ray crystallization studies on Witepsol® E85 in an earlier study indicating that the polymorphic transition into the more stable β′-polymorph occurs faster in the bulk than in SLN (Westesen et al. 1997), and NLC as expected. It was reported that Witepsol® E85 was amorphous at RT (solid or lipid) or only partially recrystallized (Siekman and Westesen 1992).

The physical state of the particles is very important and stability problems that are related to the recrystallization process, e.g. the formation of gel-like systems or signifi-

cant particle growth due to inadequate stabilizer properties of the system, do not occur in dispersions of supercooled melts which essentially behave as emulsions. Although a considerable kinetic stability was observed for the dispersions of supercooled melts, gradual recrystallization upon long-term storage cannot be excluded. This may result in significantly changed properties of products upon storage (Westesen and Bunjes 1995).

RI values of the formulations after 3 months of storage at various temperatures are listed in Table 1. Partial recrystallization and retardation of crystallization in the supercooled melt were observed in the placebo SLN and NLC dispersions stored at RT, respectively. Additionally, the recrystallization process could not start from the beginning of 40 °C storage in all the SLN and NLC formulations. The melting point of the lipid in the nanoparticles decreased down to 33.6 °C followed by production with hot homogenization the same day. It did not improve during 3 months with the exception of the SLN in hydrogel at 40 °C displayed partial recrystallization. RI values for both the placebo and AP-loaded dispersions increased indicating that recrystallization improved at 4 °C.

In the case of AP-loaded SLN and NLC incorporated into hydrogel stored at RT and 4 °C, recrystallization got much higher. This could be attributed to entrapment of AP in the particles and addition of Carbopol® 940 as gelling agent to the aqueous phase. The polymorphic transition can also be accelerated by the presence of a drug in the carrier compared to non-loaded particles. These results were attributed even to high interactions between lipids and active ingredient (Heurtault et al. 2003; Zur Mühlen et al. 1998).

As an advantage in protection of the stability, crystallized lipid prevented the mobilization of AP molecules that its palmitic residue was orientated in the lipophilic phase. Subsequently, expulsion of AP surrounding the particle surface, even entrapped amount of AP in the structure of the lipid carrier did not occur (Kristl et al. 2003). When the hydrogel formulations were examined under a light microscope equipped with crossed polarizers and a λ-sheet after 3 months of storage, we did not observe any macroscopic drug crystals indicating the drug leakage. Chemical stability results given in the next section (2.3.) also support this conclusion.

Recrystallization in all the SLN formulations stored under all the storage conditions was found higher compared to the NLC formulations. The reason of lower recrystallization of NLC was attributed to mixing of Witepsol® E85 and Miglyol® 812 at certain ratios. Solid lipid contents of NLC formulations was 6.666%, whereas 10% in SLN (9% in case of AP-loaded SLN formulations) (see Section 3.2.). Incorporation of Miglyol® 812 into the lipid phase demonstrated lower recrystallization values in NLC rather than SLN as reported in an earlier study (Jenning et al. 2000).

2.2. Particle size and zeta potential measurements

The mean particle size did not change after incorporation of AP into SLN and NLC, according to the data obtained from PCS. The mean particle size and PI values of placebo SLN, NLC and NE were 228 nm (0.177 PI), 221 nm (0.135 PI) and 139 nm (0.165 PI). SLN, NLC and NE loaded with AP which can be assessed a nonsignificant change gave 238 nm (0.033 PI), 224 nm (0.211 PI) and 139 nm (0.251 PI), respectively (see the open bars of production date in Fig. 1). All those values increased slightly to 275 nm (0.244 PI), 262 nm (0.321 PI) and 207 nm (0.180 PI) with incorporation of AP-loaded SLN, NLC and NE into hydrogel (see the open bars of production date in Fig. 2) (Üner et al. 2004a). PI values up to 0.321 indicate narrow particle size distribution for the gel formulations. D_{50}, D_{90} and D_{99} values of those formulations obtained from LD prove absence of the particles in μm range and aggregation did not occur (Fig. 3), because 50% of the particles were below 250 nm.

All the formulations were further stored at RT, 4 °C and 40 °C to demonstrate their physical stability. Data obtained from PCS indicated constant particle size values for placebo and AP-loaded SLN, NLC dispersions and NE after 3 months of storage (Fig. 1). Mostly a 40 nm increase was detected for AP-loaded NE (RT). 50% of the particles in these formulations were still below 250 nm.

Constant particle size values were determined for the formulations in hydrogel except SLN stored at 40 °C according to the PCS measurements (Fig. 2). 40 °C already cannot be proclaimed to be a suitable storage temperature not only for SLN in hydrogel, but for all the formulations in this study (see Section 2.3.). Such consistency in particle size and PI up to 0.366 (excluded SLN stored at 40 °C) as well as D_{50}, D_{90} and D_{99} values obtained from LD indicated the high physical stability of the formulations.

Choice of TegoCare® 450 as the most suitable surfactant at 1.5% concentration for Witepsol E85 SLN (Üner et al. 2004b) and NLC formulations can be emphasized regarding the particle size data obtained from PCS and LD during 3 months of storage as any significant particle growth was not seen due to inadequate stabilizer (Figs. 1–3).

It should be noted that the NE formulations which were prepared for comparison, also showed a good physical sta-

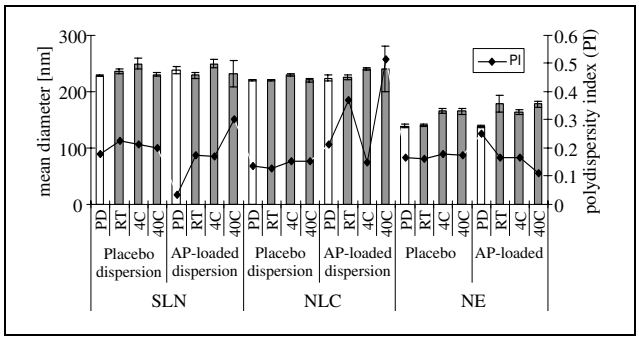

Fig. 1: Mean diameter (nm) and polydispersity index (PI) of the placebo and AP-loaded SLN, NLC dispersions and NE before (□) and after 3 months (■) of storage at various temperatures (PD: production date)

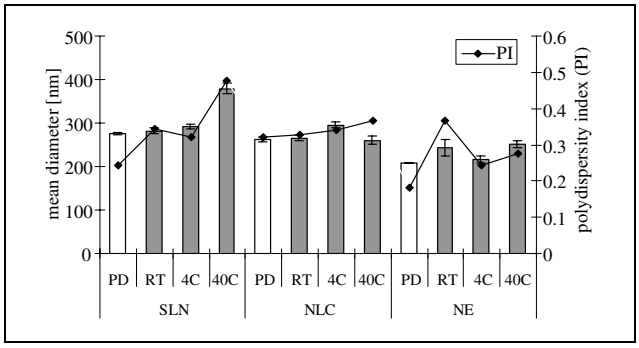

Fig. 2: Mean diameter (nm) and polydispersity index (PI) of the AP-loaded SLN, NLC and NE incorporated into hydrogel before (□) and after 3 months (■) of storage at various temperatures (PD: production date)

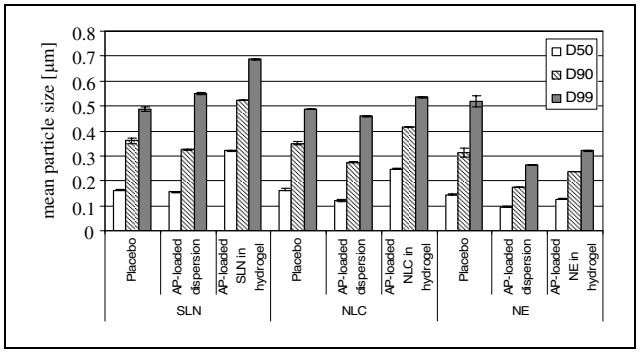

Fig. 3: D_{50}, D_{90} and D_{99} values of all the formulations before 3 months of storage (Laser Diffraction (LD) data: volume distribution)

bility. 1.5% TegoCare® 450 as surfactant exhibited a good stabilization.

2.3. Chemical stability

The calibration curve was found to be linear in a 10–50 µg/ml interval tested with three replicates ($r = 0.9990$) and AP was eluted within 5 min.

Table 2: Percentages of nondegraded AP in SLN, NLC and NE incorporated into hydrogel stored at various temperatures after 3 months

Formulation	Nondegraded AP ± SD
SLN (RT)	57.6 ± 1.8
SLN (4 °C)	67.6 ± 2.9
SLN (40 °C)	48.2 ± 1.2
NLC (RT)	60.9 ± 0.6
NLC (4 °C)	71.1 ± 1.4
NLC (40 °C)	59.2 ± 0.6
NE (RT)	53.6 ± 0.4
NE (4 °C)	55.2 ± 0.3
NE (40 °C)	50.2 ± 0.3

Table 3: Evaluation of the statistical data calculated from the results of chemical stability study

Formulation		q	P-value
SLN (4 °C) vs. NLC (4 °C)		–	ns
SLN (4 °C) vs.			
	SLN (RT)	4.151	$P < 0.05$
	SLN (40 °C)	8.707	$P < 0.001$
	NLC (RT)	–	ns
	NLC (40 °C)	5.712	$P < 0.01$
	NE (RT)	4.244	$P < 0.05$
	NE (4 °C)	5.120	$P < 0.01$
	NE (40 °C)	4.842	$P < 0.05$
NLC (4 °C) vs.			
	SLN (RT)	5.489	$P < 0.01$
	SLN (40 °C)	10.044	$P < 0.001$
	NLC (RT)	2.848	ns
	NLC (40 °C)	7.049	$P < 0.001$
	NE (RT)	5.581	$P < 0.01$
	NE (4 °C)	6.457	$P < 0.001$
	NE (40 °C)	6.179	$P < 0.001$

Comparison of AP-loaded SLN, NLC and NE incorporated into hydrogel stored at various temperatures to each other by Student's t-test
ns, non-significant

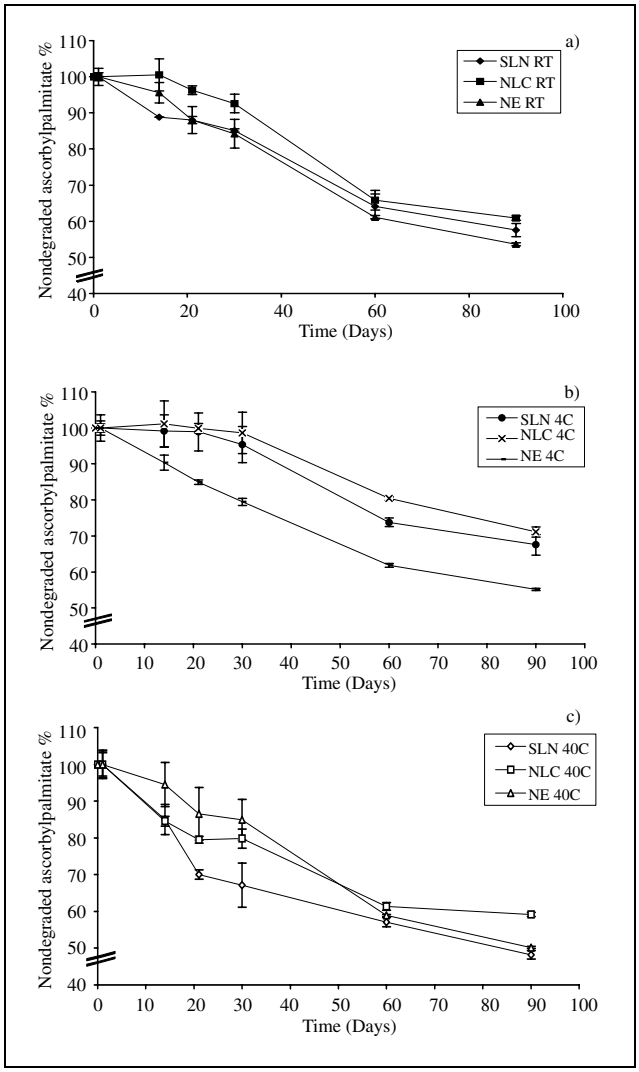

Fig. 4a, b, c: Degradation of AP in SLN, NLC and NE incorporated into hydrogel stored at various temperatures ($n = 3$)

The chemical stability study was carried out with AP-loaded SLN, NLC and NE incorporated into hydrogel stored at RT, 4 °C and 40 °C. Samples were periodically analyzed by HPLC, after proper dilution with methanol (see Section 3.6.2.). During the study, no interference peaks were detected in the chromatograms.

According to the data of 3 months, it was seen that AP was most stable at 4 °C for each formulation indicating the importance of the storage temperature and 40 °C was not found suitable (Table 2, Figs. 4a, b, c). Storage at low temperature reduces the rate of oxidation (Heurtault et al. 2003). All the data of 3 months obtained from the formulations stored at these temperatures were statistically compared to each other. q and p-values of the comparisons are listed in Table 3. NE formulations were most insufficient to protect AP against chemical degradation at all the storage temperatures in comparison to the other formulations. The highest nondegraded AP values were found in NLC and SLN stored at 4 °C with insignificant difference ($p > 0.05$) among them, followed by NLC (RT and 40 °C), SLN (RT), NE (4 °C, RT and 40 °C) and SLN (40 °C). There was not any significant difference between SLN (40 °C) and NE (RT and 40 °C) ($p > 0.05$). According to these data, SLN and NLC can be considered as suitable carriers compared to NE.

Due to the amphiphilic structure of AP with a partition coefficient (log P 7.19) confirming its hydrophilic-lipophilic character, it is envisaged as being located at the interface with palmitic residue in the lipophilic phase and the cyclic ring in the aqueous phase (Kristl et al. 2003). During the production by hot homogenization, drug partitions from the liquid oil phase to the aqueous water phase according to the drug-enriched shell model of drug entrapment. The amount of drug partitioning to the water phase increases with the solubility of the drug in the water phase with increasing temperature. The saturation solubility of the drug in the water phase is greater at higher temperatures. During the cooling of the produced o/w nanoemulsion, the solubility of the drug in the water phase decreases continuously with decreasing temperature of the water phase, that means a re-partitioning of the drug into the lipid phase occurs. When reaching the recrystallization temperature of the lipid, a solid lipid core starts to entrap the drug. Reducing the temperature of the dispersion further increases the pressure on the drug because of its reduced solubility in water to further re-partition into the lipid phase. The already crystallized core is not accessible anymore for the drug, consequently the drug concentrates in the still liquid outer shell of the SLN and/or on the surface of the particles (Müller et al. 2000). Lipophilicity of AP makes it an excellent candidate for entrapment in lipid particles, but its cyclic ring as the hydrophilic moiety which is orientated in the aqueous phase of an o/w emulsion or lipid nanoparticle dispersion, is still open to oxidation. However its stability is also related to the structure and solid lipid content of the carrier system.

To improve the AP stability in order to cut exposure of atmospheric oxygen, hydrogel formulations were filled under nitrogen flooding. But still dissolved amount of oxygen in the aqueous phase of the formulations caused limited oxidation. AP molecules surrounding colloidal particles of SLN and NLC in hydrogel firstly started to be oxidized. Entrapped amount of AP in the particles could not leave the structure of the SLN easily which had the higher solid lipid content. Solid lipid concentration decreased the mobilization of AP molecules located on the surface and entrapped in the particles. In case of NLC incorporated into hydrogel, addition of the liquid lipid in the lipophilic phase increased the AP solubility in the lipid nanoparticles and took part in homogenous AP distribution in the carrier structure, subsequently AP expulsion did not occur. 6.7% solid lipid content also prevented the mobilization of the drug inside nanoparticles.

In case of the NE incorporated into hydrogel, even though it showed a good physical stability according to the data, it was not found sufficient to protect AP against chemical degradation as much as NLC and SLN. This was due to the fact that oxidation rate increased with mobility of the active ingredient in the oil droplets of NE, i.e. due to the liquid state of the internal phase. Oxidation accelerated because of the mobility of AP molecules on the interface of the system. Additionally, AP degradation was faster since oxygen was considered to be an order of magnitude more soluble in oil than in water, and therefore it distributed preferentially into the oil phase which was the internal phase in our NE (i.e. o/w NE). But, high lipid content of both SLN and NLC did not let oxygen diffuse into the carrier easily and entrapped AP was protected in this manner, as well.

According to the data given above, this study demonstrates that AP stability is also related to the carrier system. In a recent study, the chemical stability of AP entrapped in a various colloidal carriers at a concentration of 1% (w/w) including SLN for 4 weeks was investigated for the first time and a degradative effect of environmental oxygen on AP in w/o and o/w microemulsions at 2% concentration was also reported in the same study. They found that non-hydrogenated soybean lecithin (Phospholipon 80) SLN which led the highest AP stability among the other carriers at an initial concentration of 1% (w/w) in the formulation without removal of atmospheric oxygen and the stability increased twice with the effect of de-airing (flooding with argon) in o/w and w/o emulsions at the same study. They indicated that the stability of AP also depended strongly on the structural properties of the drug carrier system and o/w microemulsion as a carrier was not enough to chemically protect the stability of AP as much as SLN with the same reasons and physicochemical interactions (Kristl et al. 2003).

Situation of all the formulations stored at 40 °C is assumed as catalyzing effect of storage temperature on oxidation as mentioned above.

Physical stability results obtained from DSC experiments and particle size measurements (PCS and LD) during 3 months of storage were in good agreement with the results of chemical stability study. As a conclusion, it could be stated that proper selection of the ingredients of the carrier system and appropriate storage conditions contribute to an increase in stability of AP.

Physical stability of the carrier which is in good agreement with the chemical stability of the entrapped drug can be maintained by choosing the suitable surfactant at a sufficient concentration (Mehnert and Mäder 2001). This is one of the most important factors influencing even the stability of vitamins. According to the physical stability studies on placebo SLN dispersions, TegoCare® 450 as surfactant was reported to be the most suitable stabilizer (Üner et al. 2004a, 2004b).

3. Experimental

3.1. Materials

Ascorbyl palmitate was obtained from Roche (Turkey). Witepsol® E85 (hard fat) was provided from Hüls AG (Germany). Miglyol® 812 (Beiersdorf, Germany), TegoCare® 450 (Goldschmidt, Germany) and Carbopol® 940 (Caelo GmbH, Germany) were provided as gifts. Glycerol was purchased from Sigma (Germany). MilliQ water was freshly prepared (FU Berlin, Germany). All the other chemicals were of analytical grade.

3.2. Production of the SLN, NLC and NE formulations

Placebo and AP-loaded SLN and NLC dispersions were produced by the high pressure homogenization technique at a temperature at least 5–10 °C above the melting point of the lipid as reported earlier (Wissing and Müller 2001). 10% Witepsol® E85 and 1.5% TegoCare® 450 for placebo SLN dispersion were used as solid lipid and surfactant (all w/w %), respectively. Melted lipid was dispersed in the hot aqueous surfactant solution (1% AP of total formulation was added into the melted lipid by reducing its fraction in case of loaded dispersion). A pre-emulsion was obtained by high speed stirring with an Ultra-Turrax T25 (Jahnke und Kunkel GmbH, Germany) at 8000 rpm for 1 min. This pre-emulsion was then passed through a high pressure homogenizer (Micron Lab 40, APV Gaulin GmbH, Germany) at 500 bar pressure and 75 °C. Miglyol® 812 as oil was used by reducing the fraction of Witepsol® E85 (1 : 2, w/w, respectively) for producing NLC dispersions (i.e. the total lipid content stayed unchanged). 1% AP of the total formulation was added by reducing the oil amount in the production of AP-loaded NLC dispersion. Placebo and AP loaded-NE were produced by completely replacing the solid lipid in SLN dispersions with Miglyol® 812 using the same production process for comparison. 10% and 9% oil content was used in the case of placebo and AP-loaded NE, respectively.

Production of AP-loaded SLN, NLC and NE incorporated into hydrogel: These formulations were produced in exactly the same manner as described above. The melted lipid (heated oil in case of NE) and drug mixture was dispersed in the hot aqueous surfactant solution containing 1%

Carbopol® 940 and 10% glycerol as gelling and hydrating agents, respectively.

3.3. Stability study

After production, the formulations were filled into clear siliconized glass vials (glass quality I, Bünder Glas, Münnerstädter Glaswaren, Schmidt, Germany), but the hydrogel formulations were given into aluminium tubes under nitrogen gas to eliminate the effect of atmospheric oxygen. All the formulations were stored at various temperatures: RT, 4 °C and 40 °C (all in the dark). Samples were taken from the formulations at the beginning of storage and subsequently on the 1st, 7th,14th, 28th, 60th and 90th day.

3.4. Crystallinity of SLN and NLC

The crystallinity degree of the lipid in the SLN and NLC formulations was determined using a Differential Scanning Calorimeter (DSC) (Mettler TA 3000 Controller and DSC821e, Mettler, Switzerland). The samples were weighed into 40 µl standard aluminium pans (amount containing 1–2 mg lipid) and heated from 25 °C to 85 °C with a heating rate of 5 K/min flushing with 80 ml N_2/min. Melting peaks and enthalpies were calculated using the Mettler Star software.

3.5. Particle size measurements

Before the measurements, all the formulations were diluted with water to eliminate the effect of viscosity caused by the ingredients. Particle size analysis was performed by photon correlation spectroscopy (PCS) (Zetasizer 4, Malvern Intruments, UK) for the particles in 3 nm–3 µm size range and laser diffraction (LD) (Coulter LS 230, Beckmann-Coulter Electronics, Krefeld, Germany) for the particles in the 100 nm–2000 µm size range. The diameters 50%, 90% and 95% (D_{50}, D_{90} and D_{95}) by volume were used to characterize the particles with LD. The polydispersity index (PI) value for each sample as measure of width of particle size distribution was also obtained from PCS.

3.6. Chemical stability

3.6.1. Determination of ascorbyl palmitate by HPLC

The formulations of AP-loaded SLN, NLC and NE incorporated into hydrogel were used to investigate the effect of the carrier on AP stability. Amount of nondegraded active ingredient in the samples was determined quantitatively at the considered time intervals using a Kontron HPLC instrument equipped with a pump autosampler 420 and UV detector 430. For this purpose a Lichrosorb NH_2 column (Li–NH_2 7 µm, 250 × 4 mm diameter) (Merck, Darmstadt, Germany) and methanol : acetonitrile : 0.02 M phosphate buffer pH 2.5 (85 : 5 : 10, v/v) were used as stationary and mobile phases, respectively. The flow rate was 1 ml/min and UV detection was carried out at 255 nm. Five standard solutions in methanol were prepared for the calibration curve. All the analyses were performed at room temperature.

3.6.2. Sample preparation for HPLC

Approximately 500 mg sample was accurately weighed and diluted with 10 ml methanol. This was kept in an ultrasonic bath at 50 °C for 10 min for liberation of AP from the lipid phase and extraction through methanol. After the suspension was cooled down to room temperature, it was centrifuged using a Biofuge 22R centrifuge (Heraeus, Germany) at 17000 rpm for 10 min. The further dilution from 1 ml supernatant with the same solvent up to 10 ml to gain a final dilution of 1 : 100 (w/v) and then injection into the chromatograph were performed.

3.6.3. Data treatment and statistics

Statistical evaluation of data obtained from the chemical stability study was performed by Student's t-test (GraphPad InStat).

References

Almeida AJ, Runge S, Müller RH (1997) Peptide-loaded solid lipid nanoparticles (SLN): influence of production parameters. Int J Pharm 149: 255–265.

Anon (2001) Code of Federal Regulations, Food and Drugs 21 (3): 170.

Austria R, Semenzato A, Bettero A (1997) Stability of vitamin C derivatives in solution and topical formulations. J Pharm Biomed Anal 15: 795–801.

Buettner GR, Jurkiewicz BA (1996) Catalytic metals, ascorbate and free radicals: combinations to avoid. Radiat Res 145: 532–541.

Campos PMM, Silva GM (2000) Ascorbic acid and its derivatives in cosmetic formulations. Allured's Cosm Toilet 115: 59–62.

Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF (2002) Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. Int J Pharm 238: 241–245.

De Vringer T, De Ronde HAG (1995) Preparation and structure of a water-in-oil cream containing lipid nanoparticles. J Pharm Sci 84: 466–472.

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.

Dubes A, Parrot-Lopez H, Abdelwahed W, Degobert G, Fessi H, Shahgaldian P, Coleman AW (2003) Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. Eur J Pharm Biopharm 55: 279–282.

Ford JL, Timmins P (1989) Pharmaceutical Thermal Analysis. Ellis Horwood, Chichester.

Fundaro A, Cavalli R, Bargoni A, Vighetto D, Zara GP, Gasco MR (2000) Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: Pharmacokinetics and tissue distribution after i.v. administration to rats. Pharm Res 42: 337–343.

Gohla SH, Dingler A (2001) Scaling up feasibilty of the production of solid lipid nanoparticles (SLN™). Pharmazie 56: 61–63.

Heurtault B, Saulnier P, Pech B, Proust JE, Benoit JP (2003) Physico-chemical stability of colloidal lipid particles, Biomaterials 24: 4283–4300.

Jenning V, Thünemann AF, Gohla SH (2000) Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int J Pharm 199: 167–177.

Jenning V, Gohla S (2001) Encapsulation of retinoids in solid lipid nanoparticles (SLN). J Microencapsul 18: 149–158.

Kristl J, Volk B, Gašperlin M, Šentjurc M, Jurkovič P (2003) Effect of colloidal carriers on ascorbyl palmitate stability. Eur J Pharm Sci 19: 181–189.

Mehnert W, Mäder K (2001) Solid lipid nanoparticles. Production, characterization and applications. Adv Drug Del Rev 47: 165–196.

Mei Z, Chen H, Weng T, Yang Y, Yang X (2003) Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur J Pharm Biopharm 56: 189–196.

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, Radtke M, Wissing SA (2002a) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 54 Suppl 1: S131–S155.

Müller RH, Radtke M, Wissing SA (2002b) Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 242: 121–128.

Shahgaldian P, Quattrocchi L, Gualbert J, Coleman AW, Goreloff P (2003) AFM imaging of calixarene based solid lipid nanoparticles in gel matrices. Eur J Pharm Biopharm 55: 107–113.

Siekmann B, Westesen K (1992) Submicron-sized parenteral carrier systems based on lipids. Pharm Pharmacol Lett 1: 123–126.

Spiclin P, Gasperlin M, Kmetec V (2001) Stability of ascorbyl palmitate in topical microemulsions Int J Pharm 222: 271–279.

Üner M, Wissing SA, Yener G, Müller RH (2004a) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) as carriers for ascorbyl palmitate: Physical stability. Poster presentation. 31st Annual Meeting and Exposition of the Controlled Release Society.

Üner M, Wissing SA, Yener G, Müller RH (2004b) Influence of surfactants on the physical stability of solid lipid nanoparticles (SLN) formulations. Pharmazie 59: 331–332.

Venkatesvarlu V, Manjunath K (2004) Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release 95: 627–638.

Westesen K, Bunjes H (1995) Do nanoparticles prepared from lipids solid at room temperature always possess a solid matrix? Int J Pharm 115: 129–131.

Westesen K, Bunjes H, Koch MHJ (1997) Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release 48: 223–236.

Wissing SA, Müller RH (2001) A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. Int J Cosm Sci 23: 233–243.

Zur Mühlen A, Mehnert W (1998) Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 53: 552–555.

Zur Mühlen A, Schwarz C, Mehnert W (1998) Solid lipid nanoparticles (SLN) for controlled drug delivery – Drug release and release mechanism. Eur J Pharm Biopharm 45: 149–155.