Department of Pharmaceutical Technology[1], Faculty of Pharmacy, Istanbul University, Beyazit, Istanbul, Turkey; Department of Pharmaceutics, Biopharmaceutics and Biotechnology[2], Free University of Berlin; PharmaSol GmbH[3], Berlin, Germany

Skin moisturizing effect and skin penetration of ascorbyl palmitate entrapped in Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) incorporated into hydrogel

M. Üner[1], S. A. Wissing[2], G. Yener[1], R. H. Müller[3]

Received November 15, 2004, accepted November 29, 2004

Melike Üner Ph.D., Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Beyazit 34119 Istanbul, Turkey
melikeuner@yahoo.com

Pharmazie 60: 751–755 (2005)

This study was performed as a complimentary to our previous study regarding the chemical stability of ascorbyl palmitate (AP) in solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and for comparison, in nanoemulsion (NE) incorporated into a hydrogel produced by high pressure homogenization. AP is known as an effective antioxidant that protects tissue integrity similar to vitamin C. Recently, its moisturizing activity in conventional topical formulations was found to be high. The aim of the present study was to investigate the moisturizing potential of AP in SLN and NLC incorporated into hydrogel as colloidal carrier systems. It has been known that SLN and NLC have occlusive effects, but AP incorporation moisturized skin significantly better than placebo in short-term ($p < 0.001$) and long-term trials ($p < 0.01$) for both SLN and NLC. In the second part of the study, SLN and NLC were found to sustain the penetration of AP through excised human skin about 1/2 and 2/3 times compared to NE ($p < 0.001$ and $p < 0.01$), respectively, due to the solid state of Witepsol® E85 in the lipid phase.

1. Introduction

(6-Palmitoyl-L-ascorbic acid) (AP) which is a fatty acid ester with lipophilic properties is a vitamin C (L-ascorbic acid) derivative. It is an antioxidant that combats the reactive oxygene species that can cause damage in cells endangering tissue integrity similar to vitamin C. It exhibits free radical scavenger activity, inhibitory effects on melanogenesis and antiaging properties (Austria et al. 1997; Gallarate et al. 1999). In the molecule of this ascorbic acid derivative with favorable effects as an excellent skin antioxidant, the fatty acid ester moiety is located in 6-position and the inorganic ester group in 2-position, involving the enediol system (Reynolds 1996). This structural property promotes skin penetration and promises various advantages for skin applications. Its moisturizing potential compared to the hydrophilic compound calcium ascorbate, was reported to be considerably high in various topical formulations (Gönüllü et al. 2004).

Advantages of SLN and NLC over other carrier systems were reported many times including small particle size range with low content of microparticles, high drug payload for lipophilic drugs, non irritability and excellent tolerability (often GRAS status of excipients), biological compatibility, controlled drug delivery, improved bioavailability of poorly water-soluble drugs, low cost, ease of production and scale-up procedures in various application routes (Heiati et al. 1998; Müller et al. 1995; Patravale and Ambarkhane 2003). SLN also exhibits another advantage, the occlusive effect on the skin that leads to an improvement of skin permeation of drugs. It was reported that penetration of drugs into skin depended strongly on skin hydration and could thus be influenced by occlusive compounds (Jenning et al. 2000a). Its small particle size ensures adhesion to the stratum corneum and increases the amount of active substance penetrating into the viable skin (Mei et al. 2003; Santos Maia et al. 2000). Sustained drug release is also possible due to the solid matrix structure in topical application route (Jenning et al. 2000b).

Many drugs are chemically and biologically labile, therefore they need to be entrapped in a carrier system. Micelles (Hammad and Müller 1998), liposomes (Gregoriadis et al. 1993), micro- (Rossler et al. 1994) and nanoparticles (Cegnar et al. 2004) are studied with increasing attention in this respect. SLN is one of the most promising colloidal carrier systems (Dingler et al. 1999) including the second generation of oil-loaded SLN described as NLC (Jores et al. 2004; Müller et al. 2002). SLN and NLC produced by high pressure homogenization have been recently studied for the topical application of AP (Üner et al. 2004, 2005). We improved the chemical stability of the active ingredient in SLN and NLC incorporated into a hydrogel and physical stability of these formulations compared to data of the earlier studies (Kristl et al. 2003; Spiclin et al. 2001).

In this present study, the moisturizing effect of AP entrapped in SLN, NLC and for comparison, NE incorporated into hydrogel was investigated. Additionally, the occlusive effect of the carriers on skin hydration was compared to placebo SLN, NLC and NE incorporated into hydrogel. Then, penetration of AP from these carriers through human skin was studied *in vitro*.

2. Investigations, results and discussion

2.1. *Effect of lipid crystallization on moisturization of skin*

Nonsignificant difference was found between AP-loaded SLN and NLC formulations ($p > 0.05$) compared to the loaded NE in both the short-term and long-term trials of moisturizing testing on skin. This similarity leads to the conclusion that the occlusive effect of the carriers improved the skin moisturizing potential of AP. This comparison can easily be made by considering the placebo formulations, as well (Table 1, Figs. 1 and 2a–c). According to the data, placebo SLN and NLC did not differ in skin hydration ($p > 0.05$) indicating the occlusive effect, but a significant difference was determined when NE was compared to them ($p < 0.001$). The results of the statistical evaluation for both the short-term and long-term trials can be seen in Table 2. Small particles of SLN and NLC possess a high specific surface area and therefore adhesive properties by leading occlusivity due to film formation after application on the skin. Film formation prevented water evaporation from skin and penetration of AP through human skin was influenced and improved. The small particle size of SLN and NLC in the nanometer size range caused adherence to the stratum corneum, subsequently increased AP penetration into viable skin. Particle size of the SLN, NLC and NE in hydrogel was similarly found to be 275 ± 2.9 nm (0.244 PI), 262 ± 4.8 nm (0.321 PI) and 207 ± 0.2 nm (0.180 PI). D_{50}, D_{90} and D_{99} values of these formulations obtained from LD prove ab-

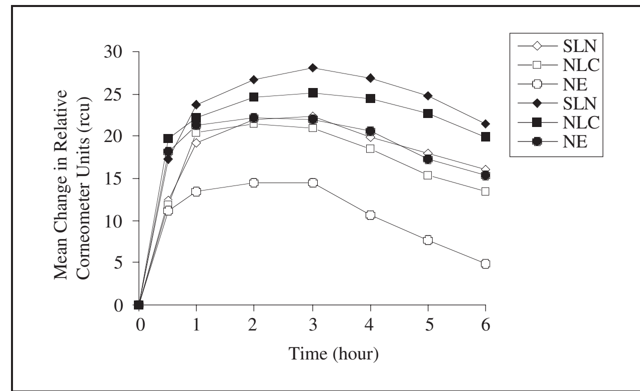

Fig. 1: Mean values of the changes in the skin hydration on inner forearms of 10 volunteers during the short-term trial of the formulations. Open and filled symbols are illustrating placebo and AP-loaded formulations, respectively

Table 1: Mean change in rcu values and standard deviations (SD) of the formulations in the short-term trial (rcu: relative corneometer unit)

Formulation	Mean ± SD						
	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Placebo							
SLN	12.3 ± 1.3	19.2 ± 1.3	21.9 ± 3.4	22.4 ± 2.1	19.9 ± 2.5	17.9 ± 1.7	16.0 ± 0.1
NLC	11.8 ± 1.2	20.4 ± 3.1	21.5 ± 3.0	21.0 ± 3.6	18.5 ± 2.8	15.3 ± 2.1	13.5 ± 0.7
NE	11.1 ± 0.8	13.5 ± 1.7	14.4 ± 2.5	14.4 ± 1.4	10.6 ± 2.7	7.7 ± 2.3	4.9 ± 0.9
AP-loaded							
SLN	17.3 ± 1.5	23.8 ± 1.0	26.7 ± 2.4	28.0 ± 3.2	26.9 ± 1.9	24.8 ± 1.0	21.5 ± 0.7
NLC	19.7 ± 1.3	22.2 ± 1.5	24.6 ± 0.8	25.1 ± 1.2	24.5 ± 1.4	22.6 ± 3.4	19.8 ± 0.2
NE	18.1 ± 1.9	21.3 ± 1.0	22.1 ± 1.2	21.9 ± 1.2	20.6 ± 1.9	17.3 ± 0.8	15.4 ± 0.5

Table 2: Evaluation of statistical data of the formulations as q and p-values compared to each other in short-term and long-term trials

Formulation	Short-term trial		Long-term trial	
	q	P-value	q	P-value
AP-loaded SLN vs. AP-loaded NLC	1.656	ns	2.510	ns
AP-loaded SLN vs.				
Placebo SLN	6.214	$p < 0.001$	6.523	$p < 0.001$
Placebo NLC	7.413	$p < 0.001$	9.690	$p < 0.001$
Placebo NE	14.573	$p < 0.001$	15.377	$p < 0.001$
AP-loaded NE	5.094	$p < 0.01$	6.742	$p < 0.001$
AP-loaded NLC vs.				
Placebo SLN	4.558	$p < 0.01$	4.013	$p < 0.01$
Placebo NLC	5.757	$p < 0.01$	7.179	$p < 0.001$
Placebo NE	12.917	$p < 0.001$	12.867	$p < 0.001$
AP-loaded NE	3.438	$p < 0.05$	4.231	$p < 0.05$
AP-loaded NE vs.				
Placebo SLN	–	ns	–	ns
Placebo NLC	2.318	ns	–	ns
Placebo NE	9.479	$p < 0.001$	8.636	$p < 0.001$
Placebo SLN vs. Placebo NLC	–	ns	3.166	ns
Placebo SLN vs. Placebo NE	8.359	$p < 0.001$	8.854	$p < 0.001$
Placebo NLC vs. Placebo NE	7.160	$p < 0.001$	5.688	$p < 0.001$

ns, non-significant

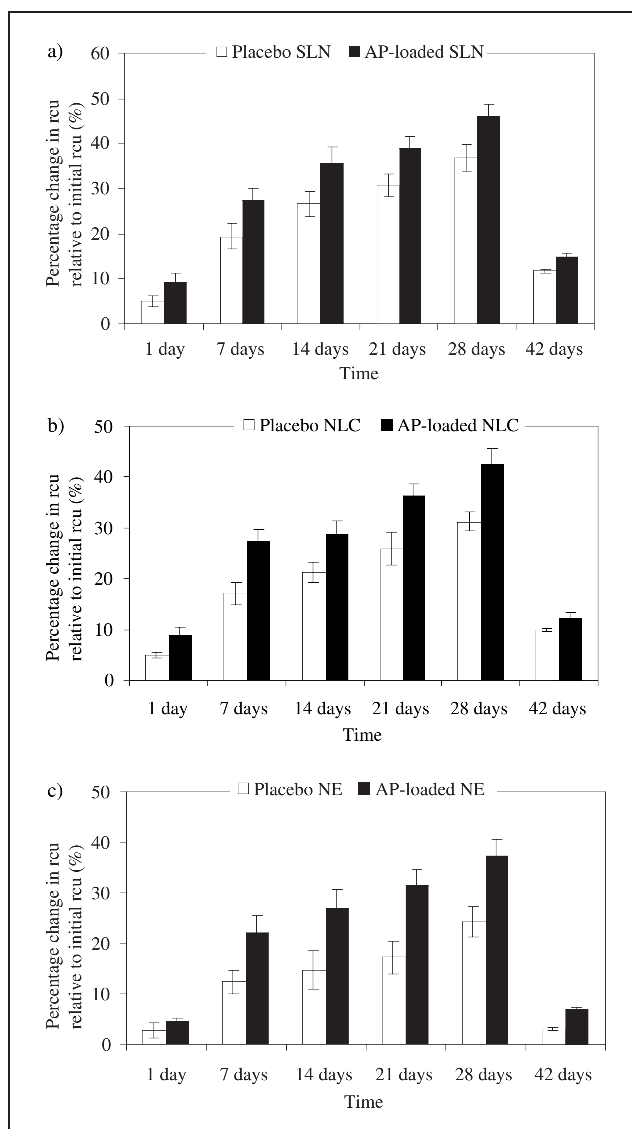

Fig. 2: a, b, c: Percentage change in mean values of skin hydration on inner forearms of 10 volunteers during twice daily 28 days of application and 42nd day values of the placebo and AP-loaded SLN (a), NLC (b) and NE (c) incorporated into hydrogel in long-term trial. Mean values of the changes were standardized by subtracting the percentage change for the control (rcu: relative corneometer unit)

sence of particles in the μm range and aggregation did not occur (Fig. 3). 50% of the particles were below 250 nm. Due to the similarity of size, effects caused by size differences were excluded (Üner et al. 2005).

The extent of occlusivity of the SLN and NLC dispersions correlates not only with particle size, but also with lipid concentration and degree of crystallinity of the lipid matrix.

Table 3: Melting points, melting enthalpies (calculated according to the fact that the lipid contents in the formulations were standardized up to 100% lipid) and recystallization indices (RIs) of the solid lipid in the AP-loaded formulations

Formulation	Melting point (°C)	Melting enthalpy (J/g)	RI (%)
SLN	42.3	130.0	109.3
NLC	42.4	66.7	56.1

(Ref. Üner et al. 2005)
Reference: Bulk lipid (Witepsol® E85) with 118.9 J/g melting enthalpy and 44.4 °C melting point

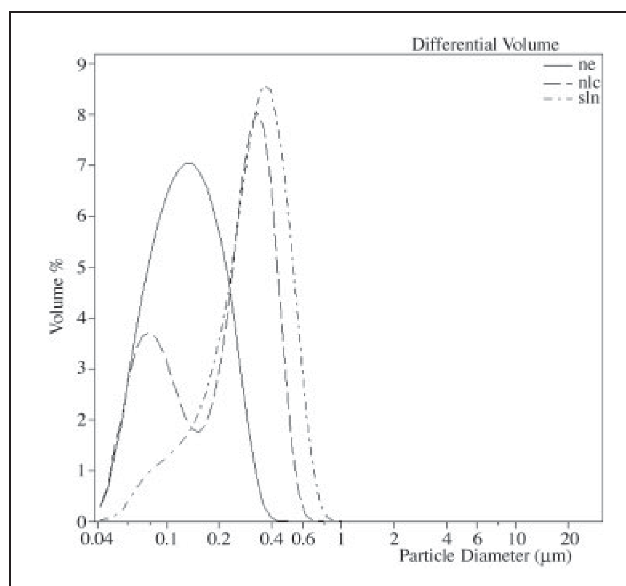

Fig. 3: Particle size distribution of AP-loaded SLN, NLC and NE incorporated into hydrogel. (Laser Diffraction (LD) data: volume distribution)

Solid state of SLN and NLC, and liquid state of the inner phase of NE created this difference. Thermodynamic and crystallization behaviour of Witepsol® E85 in SLN and NLC incorporated into hydrogel was investigated by DSC and it was found that recrystallization was higher in SLN rather than NLC. The reasons behind this situation were explained extensively (Üner et al. 2005). Data on thermodynamic behaviour of the lipid in the AP-loaded SLN and NLC incorporated into hydrogel can be seen in Table 3. All these data demonstrate that occlusivity of lipid nanoparticles correlates to lipid concentration and the degree of crystallinity (Jenning et al. 2000a; Wissing et al. 2002).

2.2. *Effects of carrier type on AP penetration through excised human skin*

Among the formulations tested, NE showed the highest penetration profile followed by NLC and SLN ($p < 0.05$) (Fig. 4). Release profile of AP was statistically identical in SLN and NLC (Table 4) displaying a steady state release up to the 6th hour. The difference between the penetration rates of the formulations at 6th hour which is the last hour of steady state, and the 9th hour helps to understand the background of the release of AP from the formulations and penetration through skin. In the case of SLN and NLC, AP was entrapped by the carrier, according to the drug-enriched shell model. Due to the partition coefficient (log P 7.19) of AP which explains its hydrophilic-lipophilic character, it is envisaged as being highly located at the interface with palmitic residue in the lipophilic phase and the cyclic ring in the aqueous phase (Üner et al. 2005).

Table 4: Evaluation of statistical data of the AP-loaded formulations as q and p-values compared to each other in skin penetration study

Formulation	q	P-value
SLN vs. NLC	2.019	ns
SLN vs. NE	6.568	$P < 0.001$
NLC vs. NE	4.550	$P < 0.01$

ns, non-significant

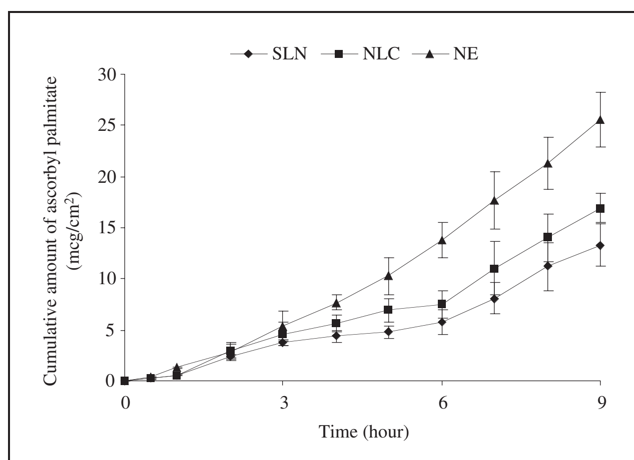

Fig. 4: AP penetration through human skin from the hydrogel formulations (n = 3)

Shoulders in the penetration profiles of SLN and NLC indicate a steady-state release from the carriers up to the 6th hour with penetration rates of 0.951 ± 0.207 μg/cm²/h and 1.250 ± 0.217 μg/cm²/h followed by 1.477 ± 0.232 μg/cm²/h and 1.872 ± 0.157 μg/cm²/h at the 9th hour, respectively (Fig. 4). While the values increased at the 9th hour in case of SLN and NLC, no change was observed with NE (2.845 ± 0.296 μg/cm²/h). This means that AP localized on the surface of the particles was liberated first and then from the outer shell in case of SLN and NLC. A faster permeation profile without showing steady state release was seen in case of NE due to the liquid state of its internal phase and subsequently, the high mobility of AP molecules on the interface of the system. Diffusion of the active substance took place more quickly from NE droplets than from the solid crystalline lipid of SLN and NLC. Solid state of SLN and NLC immobilized AP molecules.

As a conclusion, penetration of AP was sustained and decreased by being hosted in SLN followed by NLC and then by NE. The results of statistical treatment and comparison of the formulations are given in Table 4.

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 formulations

Placebo and AP-loaded SLN, NLC incorporated into hydrogel formulations were produced by the high pressure homogenization technique at a temperature of at least 5–10 °C above the melting point of the lipid as described previously (Üner et al. 2004, 2005). Witepsol® E85 and TegoCare® 450 for SLN and NLC in hydrogels were used as solid lipid and surfactant, respectively. Melted lipid was dispersed in the hot aqueous surfactant solution containing Carbopol® 940 and glycerol as gelling and hydrating agents, respectively. 1% AP of total formulation was added into the melted lipid. 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 the high pressure homogenizer (Micron Lab 40, APV Gaulin GmbH, Germany) at 500 bar pressure and 75 °C. Miglyol® 812 as oil was used to replace 1/3 of the fraction of Witepsol® E85 for producing the NLC formulation. The total lipid content stayed unchanged being 10% (i.e. 10% Witepsol® E85 in SLN, 6.7% Witepsol® E85 and 3.3% Miglyol® 812 in NLC for placebo formulations; the AP-loaded SLN content 9% Witepsol® E85, the loaded NLC 6.7% Witepsol® E85 and 2.3% Miglyol® 812). Placebo and AP-loaded NE in hydrogel were produced by completely replacing the solid lipid with Miglyol® 812 using the same production process, for comparison. After production, the formulations were filled into aluminium tubes under nitrogene gas to eliminate oxygen effects (Üner et al. 2005).

3.3. Crystallinity of SLN and NLC

Thermodynamic behaviour of the lipid was determined using a Differential Scanning Calorimeter (DSC) (Mettler TA 3000 Controller and DSC821^e, 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 and then recrystallization indices (RIs) were calculated.

3.4. 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 were performed by photon correlation spectroscopy (PCS) (Zetasizer 4, Malvern Intruments, UK) for the particles in the 3 nm–3 μm size range. The polydispersity index (PI) value as a measure of width of particle size distribution was also for each sample from PCS. Laser diffraction (LD) (Coulter LS 230, Beckmann-Coulter, Krefeld, Germany) was employed 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.

3.5. Moisturization testing on skin

This study was performed in two orders as short and long-term trial. Prior to both of these trials, baseline values of 10 female Caucasian volunteers (31.2 ± 6.8 years) with no signs of skin disorders were taken using 2 cm × 2 cm 3 test areas 2 cm apart from each other on each inner forearms. The study was performed as one-sided blind, placebo controlled study of 3 AP-loaded formulations. The basic values were obtained on three subsequent days prior to the applications. Two weeks after the short-term study, the long-term study was performed on the same volunteers. Each designated area was then treated with 0.02 ml of 6 hydrogel formulations. Five skin hydration readings of each test side were recorded by a corneometer at 22 °C and 60% relative humidity. Capacitance changes were detected as corneometer units.

A Corneometer CM 825 (Courage Khazaka, Germany) was used. The corneometer method is based on the physical principle of a common capacitor. A complex of two metal plates is electrically insulated by a medium that acts as a dielectric. An excess of electrons is built up on one plate (negative charge) and an electron deficiency (positive charge) on the other plate. The quantity of this electric charge stored is called the capacity. The capacitor shows changes of capacitance according to the moisture content of the skin. The probe is connected to a computer which processes and displays the current reading. The capacity of skin is directly correlated to the amount of water in the skin. The corneometer is very sensitive for measurements at low hydration and less sensitive in the range of very high hydration values (Gönüllü et al. 2004; Wiechers 1999).

3.5.1. Short-term trial

Placebo and AP-loaded SLN, NLC and NE incorporated into hydrogel were applied once at the beginning of the measurement day. Skin hydration readings were taken 0.5, 1, 2, 3, 4, 5 and 6 h after applying the formulations. In the calculations, basal values were substracted from read-outs for each volunteer (Gönüllü et al. 2004).

3.5.2. Long-term trial

The same formulations were applied twice everyday for 28 days and the readings were taken on the 1st, 7th, 14th, 21th, 28th and 42nd day. The results are calculated with eq (1)

$$\text{Parameter changes } (\%) = (\Sigma Q_{ti}/\Sigma Q_{0i} - 1)100, \qquad (1)$$

where Q_{ti} is the quotient after application time t up to the 28th and the 42nd day for each volunteer i, and Q_{0i} is the quotient before application time for each volunteer i (Wissing and Müller 2003).

3.6. Penetration study through excised human skin

Female full-thickness skin samples were obtained from the hospital following aesthetic abdominal surgery. The underlying fatty tissue was removed by blunt dissection. 1 g of the AP-loaded SLN, NLC and NE incorporated into hydrogel formulations were applied on top of the skin mounted on the donor chamber of the diffusion cells. Franz diffusion cells (Çalışkan Cam Teknik, Turkey) with 1.76 cm² cross-sectional area and 10 ml receptor volume were used for the penetration studies. A propylene glycol : water : ethanol (45 : 30 : 25 v/v) mixture was used as receptor phase

and the whole assembly was placed in a water bath at 37 ± 1 °C. Three replicates were conducted for each experiment. Samples were taken over a 9 h period and AP concentrations were determined by HPLC.

A ThermoFinnigan SpectraSystem HPLC instrument equipped with an UV1000 detector, AS3000 autosampler and P1000 pump, and a Lichrosorb NH_2 column (Li-NH_2 7 μm, 250 × 4 mm diameter) (Merck, Darmstadt, Germany) were used for the analysis. A mobile phase consisting of methanol, acetonitrile, 0.02 M phosphate buffer pH 2.5 (85 : 5 : 10, v/v) was used. The flow rate was 1 ml/min and injection volume was 20 μl. UV detection was carried out at 255 nm. AP was eluted within 5 min (Üner et al. 2005).

3.7. Data treatment and statistics

Statistical evaluation of the formulations for both the skin hydration studies and penetration study through human skin were performed by Student's t-test (GraphPad InStat).

Acknowledgement: This work was supported by the Research Fund of the Istanbul University. Project number: UDP-288/06052004.

References

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

Cegnar M, Kos J, Kristl J (2004) Cystatin incorporated in poly(lactide-co-glycolide) nanoparticles: development and fundamental studies on preservation of its activity. Eur J Pharm Sci 22: 357–364.

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.

Gallarate M, Carlotti ME, Trotta M, Bovo S (1999) On the stability of ascorbic acid in emulsified systems for topical and cosmetic use. Int J Pharm 188: 233–241.

Gönüllü Ü, Yener G, Üner M, Incegül T (2004) Moisturizing potentials of ascorbyl palmitate and calcium ascorbate in various topical formulations. Int J Cosm Sci 26: 31–36.

Gregoriadis G, Florence AT, Patel HM (1993) Liposomes in Drug Delivery, Harwood Academic, Amsterdam, 1993.

Hammad MA, Müller BW (1998) Solubility and stability of clonazepam in mixed micelles. Int J Pharm 169: 55–64.

Heiati H, Tawashi R, Phillips NC (1998) Drug retention and stability of solid lipid nanoparticles containing azidothymidine palmitate after autoclaving, storage and lyophilization. J Microencapsul 15: 173–184.

Jenning V, Gysler A, Schäfer-Korting M, Gohla SH (2000a) Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur J Pharm Biopharm 49: 211–218.

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

Jores K, Mehnert W, Drechsler M, Bunjes H, Johann C, Mäder K (2004) Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. J Control Release 95: 217–227.

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.

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, 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 Deliv Rev 54 Suppl 1: S131–S155.

Patravale VB, Ambarkhane AV (2003) Study of solid lipid nanoparticles with respect to particle size distribution and drug loading. Pharmazie 58: 392–395.

Reynolds JEF (ed.) (1996) Martindale, The Extra Pharmacopoeia, 31st ed., Royal Pharmaceutical Society, London, p. 1117.

Rossler B, Kreuter J, Ross G (1994) Effect of collagen microparticles on the stability of retinol and its absorption into hairless mouse skin *in vitro*. Pharmazie 49: 175–179.

Santos Maia C, Mehnert W, Schäfer-Korting M (2000) Solid lipid nanoparticles as drug carriers for topical glucocorticoids. Int J Pharm 196: 165–167.

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 (2004) 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 (2005) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for application of ascorbyl palmitate. Pharmazie 60: 577–582.

Wiechers JW (1999) Comparing instrumental and sensory measurements of skin moisturization. Cosm Toil 114: 29–34.

Wissing SA, Müller RH (2002) The influence of the crystallinity of lipid nanoparticles on their occlusive properties. Int J Pharm 242: 377–379.

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.