International Journal of Pharmaceutics 396 (2010) 166–173

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Influence of nanostructured lipid carriers (NLC) on the physical properties of the Cutanova Nanorepair Q10 cream and the in vivo skin hydration effect

Jana Pardeike[a,b], Kay Schwabe[a,c], Rainer H. Müller[a,*]

[a] Department of Pharmaceutical Technology, Biopharmaceutics and Nutricosmetics, Freie Universität Berlin, Kelchstraße 31, 12169 Berlin, Germany
[b] Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, Karl-Franzens-Universität Graz, Universitätsplatz 1, 8010 Graz, Austria
[c] Dr. Rimpler GmbH, Neue Wiesen 10, 30900 Wedemark, Germany

ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 12 May 2010
Accepted 1 June 2010
Available online 9 June 2010

Keywords:
Nanostructured lipid carriers (NLC)
Dermal application
Coenzyme Q10
Cosmetic product
Skin hydration
Epicutaneous patch test

ABSTRACT

Cutanvoa Nanorepair Q10 cream, the first NLC containing cosmetical product introduced to the market in October 2005, was compared to an identical o/w cream without NLC with regards to particle size, melting behaviour, rheological properties and the in vivo effect on skin hydration. The consistency, the spreadability on the skin and the subjective feeling of increase in skin hydration were evaluated using a standardized questionnaire, and compared to hydration data measured. Furthermore, it was shown by epicutaneous patch test that Cutanova Nanorepair Q10 cream has no irritating effects on the skin. By laser diffraction (LD) and differential scanning calorimetry (DSC) measurements it could be shown that NLC are physically stable in Cutanova Nanorepair Q10 cream. After 7 days application of Cutanova Nanorepair Q10 cream and NLC negative control cream an increase in skin hydration could be objectively confirmed by measurements in vivo. From day 28 on the skin hydration measured in the test areas of Cutanova Nanorepair Q10 cream was significantly higher than the skin hydration in the test areas of the NLC negative control cream ($p = 0.05$). The subjective feeling of increase in skin hydration was also rated from the volunteers as superior for Cutanova Nanorepair Q10 cream. The rheological properties of Cutanova Nanorepair Q10 cream contributed to a better subjective impression of consistency and spreadability on the skin than found for NLC negative control cream.

1. Introduction

At present more than 300 ageing theories have been postulated (Medvedev, 1990). The most prominent ageing theory is the free radical theory postulated by Harman (1996) and Beckman and Ames (1998). Reactive oxygen species, such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical can cause oxidative damage to cellular macromolecules such as proteins, carbohydrates, lipids and nucleic acid and are thus cytotoxic. Exogenous agents like photochemical smog, ozone, pesticides, xenobiotics and ionizing radiation as well as a variety of endogenous processes such as mitochondrial respiration, cytochrome P-450 detoxification reactions, phagocytic oxidative burst and peroxisomal leakage can generate significant amounts of reactive oxygen species (Chakravarti and Chakravarti, 2006). Oxidative damage increases with age and contributes to the ageing phenotype as well as various diseases due to the fact that the activity of antioxidant enzymes and the level of non-enzymatic antioxidants decline with age, allowing oxidative damage to occur (Rabe et al., 2006; Kohen and Gati, 2000).

Coenzyme Q10 is a lipid soluble antioxidant composed of a tyrosine-derived quinone ring linked to a polyisoprenoid side chain consisting of 10 subunits, synthesized endogenously by the mevalonate pathway (Ernster and Dallner, 1995; Bentinger et al., 2007). Coenzyme Q10 is a cofactor in the mitochondrial respiratory chain, where it transfers free electrons from complex I and II to complex III during oxidative phosphorylation and ATP synthesis. Furthermore, the reduced form of coenzyme Q10 is a major chain-breaking antioxidant, decreasing oxidative damage caused by lipid peroxidation and thus decreasing the oxidative damage to lipids, proteins and DNA (Forsmark-Andree and Ernster, 1994; Beyer, 1988).

With increasing age coenzyme Q10 synthesis is reduced, leading to lower plasma levels and tissue concentrations in elderly individuals. The decreasing coenzyme Q10 concentration upon ageing is consistent with the free radical theory of ageing (Beyer et al., 1985).

In the skin coenzyme Q10 acts as an antioxidant with 10-fold higher levels in the epidermis than in the dermis (Shindo et al., 1994). The reduction in the efficiency of antioxidation systems has been proposed as a factor of skin ageing. Therefore, in the cosmetic industry the antioxidant coenzyme Q10 is widely used in anti-ageing products (Moore, 2002).

* Corresponding author. Tel.: +49 30 838 506 78; fax: +49 30 838 506 16.
E-mail address: rainer.mueller@fu-berlin.de (R.H. Müller).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.06.007

Table 1
Cosmetic and pharmaceutical actives loaded to lipid nanoparticles.

Active compound	Reference
Ascorbyl palmitate	Teeranachaideekul et al. (2007a), Teeranachaideekul (2008)
Betamethasone 17-valerate	Sivaramakrishnan et al. (2004)
Celecoxib	Joshi and Patravale (2008)
Clotrimazole	Souto et al. (2004b)
Coenzyme Q10	Dingler (1998), Pardeike and Müller (2007a), Teeranachaideekul et al. (2007b)
Cyproterone acetate	Stecova et al. (2007a)
DEET (N,N-diethyltoluamine)	Yaziksiz-Iscan et al. (2002)
Econazole nitrate	Sanna et al. (2007)
Flurbiprofen	Han et al. (2008)
Indomethacin	Ricci et al. (2005)
Ketoconazol	Souto and Müller (2005)
Ketoprofen	Puglia et al. (2008)
Kokoinut oil	Müller et al. (2007b)
Lidocaine	Pathak and Nagarsenker (2009)
Minoxidil	Silva et al. (2009)
Naproxen	Puglia et al. (2008)
Olive oil	Pardeike et al. (2008)
Parfumes	Müller et al. (2007a)
Podophyllotoxin	Chen et al. (2006)
Prednicarbate	Santos et al. (2000), Santos et al. (2002)
Psoralens 5-Methoxypsoralen 8-Methoxypsoralen 4,5,8-Trimethylpsoralen	Fang et al. (2008)
Triptolide	Mei et al. (2003)
UV blockers	Wissing and Müller (2001), Wissing and Müller (2002a), Song and Liu (2005)
Valdecoxib	Joshi and Patravale (2006)
Vitamin A derivates	Liu et al. (2007), Jenning and Gohla (2001), Jenning et al. (1999), Jenning et al. (2000), Jee et al. (2006), Shah et al. (2007)
Vitamin E derivates	Dingler et al. (1998)

Lipid nanoparticles, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), are innovative carrier systems that are derived from o/w emulsions. SLN are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or a blend of solid lipids (Lucks and Müller, 1991). In contrast to SLN, the particle matrix of NLC is composed of a blend of solid lipid and liquid lipid. The particle matrix of both lipid nanoparticles is solid at room and body temperature. The mean particle size of these carrier systems is in the submicron range, ranging from about 40 to 1000 nm (Lucks and Müller, 1991). SLN and NLC can be loaded with active ingredients.

Within the last 20-year lipid nanoparticles have been loaded with a number of active compounds for dermal application, using both cosmetic and pharmaceutical actives (Pardeike et al., 2008; Müller et al., 2007a,b). Table 1 provides an overview of cosmetic and pharmaceutical actives loaded to SLN and NLC. Lipid nanoparticles have proven to have many advantages as a carrier system for dermal application, e.g. composition of well tolerated biodegradable lipids (Müller et al., 1997; Scholer et al., 2001), occlusive properties without glossy skin appearance (Wissing et al., 2001; Teeranachaideekul et al., 2008), enhancement of the chemical stability of active compounds sensitive to light, oxidation or hydrolysis (Teeranachaideekul, 2008; Jenning and Gohla, 2001), controlled release profiles (Müller et al., 2000; Wissing and Müller, 2002a,b; Joshi and Patravale, 2006; Souto et al., 2004a,b), enhancement of penetration of active compounds into the skin (Pardeike and Müller, 2007a; Santos et al., 2000) as well as drug targeting within the skin or even substructures of the skin improving the benefit-risk ratio of topical drug therapy (Santos et al., 2002; Stecova et al., 2007a,b).

In this study the first NLC containing cream on the cosmetical market, Cutanova Nanorepair Q10 cream (Dr. Rimpler GmbH, Wedemark, Germany), was investigated with regards to its tolerability/irritancy on the skin. It was compared to an identical o/w cream without NLC with regards to physical properties, influence on skin hydration and skin applicability.

2. Materials and methods

2.1. Preparation of NLC

NLC composed of 4.80% (w/w) coenzyme Q10 (BIK Internationaler Handel, Horgen, Switzerland), 14.45% (w/w) cetylpalmitat (Cognis, Düsseldorf, Germany), 0.75% (w/w) Miglyol 812 (Caelo, Hilden, Germany), 1.80% (w/w) TegoCare 450 (Goldschmidt, Essen, Germany) and 78.20% (w/w) distilled water were produced by hot high pressure homogenization (2 cycles, 800 bar, 80 °C) using a Ekato Nanomix (Ekato Systems, Schopfheim, Germany).

2.2. Creams under investigations

Cutanova Nanorepair Q10 cream (Dr. Rimpler GmbH, Wedemark, Germany) with an NLC content of 10% and a NLC negative control cream, where the NLC were replaced by a conventional o/w emulsion, were used. Both test formulations had the same lipid content (approximately 40%) and the same content of coenzyme Q10 (0.48%). Table 2 summarizes the ingredients of the two test formulations.

2.3. Particle size measurements

The particle size of the creams and NLC was analyzed by laser diffraction (LD) using an LS 320 (Beckmann-Coulter, Krefeld, Germany). For LD data evaluation the Mie theory was used. Water with a refractive index of 1.33 was used as measurement medium. The real refractive index and the imaginary refractive index were set 1.456 and 0.01, respectively (Dingler, 1998; Teeranachaideekul et al., 2007a,b; Teeranachaideekul, 2008). Before LD measurements creams were diluted with distilled water to obtain a homogeneous distribution of the formulations in the measurement cell. Furthermore, the formulations were filtered through a 5.0 μm cellulose acetate filters (Sartorius, Göttingen, Germany) to increase the resolution for nanoparticles. In volume distributions the larger particle

Table 2
Summary of the ingredients of the two test formulations.

Cutanova Nanorepair Q10 Cream	NLC negative control cream
Aqua, cetearyl alcohol, octyldodecanol, glycerin, squalane, cannabis sativa, dimethicone, cyclopentasiloxane, arachidyl alcohol, cetyl palmitate, behenyl alcohol, cetearyl glucoside, ubiquinone, hibiscus sabdariffa flower extract, hydrolyzed wheat protein, hydrolyzed soy protein, zingiber officiale root extract, beta-carotene, zea mays, erythrulose, tocopherol, tripeptide-1, xanthan gum, tetrasodium edta, arachidyl glucoside, hydrogenated palm glycerides citrate, dimethicone crosspolymer, polyglyceryl-3 methylglucose distearate, caprylic/capric glycerides, phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben, parfum	Aqua, cetearyl alcohol, octyldodecanol, glycerin, squalane, cannabis sativa, dimethicone, cyclopentasiloxane, arachidyl alcohol, caprylic/capric triglyceride, cetearyl glucoside, behenyl alcohol, ubiquinone, hibiscus sabdariffa flower extract, zingiber officiale root extract, erythrulose, tocopherol, tetrasodium edta, hydrolyzed soy protein, xanthan gum, dimethicone crosspolymer, arachidyl glucoside, hydrolyzed wheat protein, tripeptide-1, hydrogenated palm glycerides citrate, zea mays, beta-carotene, phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben, parfum

fraction is pronounced over the smaller one, which might lead to misinterpretation if large particles are present.

2.4. Thermal analysis

Differential scanning calorimetry (DSC) was performed in order to determine the integrity of NLC after incorporation into the cream. Approximately 125 mg of the creams and 10 mg of the NLC dispersion, respectively, were analyzed in sealed aluminium pans heating the samples from 20 to 85 °C at a heating rate of 5 K/min under constant flushing with nitrogen (80 ml/min) using a Mettler DSC 821e (Mettler Toledo, Gießen, Germany).

2.5. Rheological investigations

Rheological measurements were performed using a rheometer Rheo Stress RS 100 (Haake, Karlsruhe, Germany) equipped with a cone-and-plate test geometry (plate diameter 20 mm, cone angle 4°). All measurements were carried out at 20 ± 0.1 °C. Continuous flow measurements were performed by increasing the shear rate from 0.00 to 100.00 s^{-1} over 250 s followed by decreasing the shear rate from 100 to 0.15 s^{-1} over 250 s. The yield points were determined by stress ramp with data sampling in log rate and performing two power law regression analyses. All measurements were performed in triplicate.

2.6. Human patch test

The epicutaneous patch test for the prediction of the skin irritation potential was performed with Cutanova Nanorepair Q10 cream by Derma Consult GmbH (Alfter, Germany). 50 volunteers with an age ranging from 22 to 57 years participated in this study. An aqueous 1% sodium dodecyl sulfate solution served as positive control. Water was used as negative control. Cutanova Nanorepair Q10 cream and the controls were applied in square test chambers (Haye's test chambers, Allergie, Düsseldorf, Germany) to the back of the volunteers for 48 h. The presence of irritation was evaluated visually 30 min after patch removal and 72 h after patch application. The degree of irritation was rated according to their potential to cause erythema, fissure and scaling with scores, i.e. 0 for no irritation, 1 for slight irritation, 2 for significant irritation, 3 for pronounced irritation and 4 for strong irritation.

2.7. Corneometer measurements

The skin surface hydration was measured using a Corneometer CM 825 (Courage and Khazaka, Cologne, Germany) connected to a Multi Probe Adapter MPA 5 (Courage and Khazaka, Cologne, Germany). The mean value of five single measurements at five different places within the test and control areas was evaluated. The obtained data were statistically analyzed using the Wilcoxon test ($p = 0.05$).

2.8. Design of the skin hydration study

The study was performed December 2005 to January 2006 at Freie Universität Berlin. 31 Caucasian female volunteers with healthy skin in the test and control areas participated in this study. The study was designed as a one-side-blind, placebo controlled study with intra-individual comparison of two test formulations and two untreated control areas. The test formulations were applied on the volar forearm twice daily form day 1 until day 41, followed by 1 week without treatment. The skin hydration was evaluated at day 1 before treatment, day 7, 28 and 42 12–16 h after the last application and at day 49 one week after the last application. 0.08–0.12 g of the test formulations were applied to the test areas each application. Each measurement was performed after 30 min acclimatisation at 20 ± 1 °C and 40–50% relative humidity.

2.9. Product evaluation

In a standardized questionnaire the volunteers were ask to rate the consistency, the spreadability on the skin and the subjective feeling of increase in skin hydration for both test formulations as very good, good, moderate or poor. Furthermore, the volunteers were asked which cream in direct comparison they found better with regards to the test parameters.

3. Results and discussion

If lipid nanoparticles are incorporated into creams instabilities, such as dissolution of the lipid particle matrix in the lipid phase of the cream or aggregation of lipid nanoparticles, might occur. Both instabilities would lead to a loss of the favourable characteristics of lipid nanoparticles in the cream formulation. However, the presence of the solid particle matrix of lipid nanoparticles in

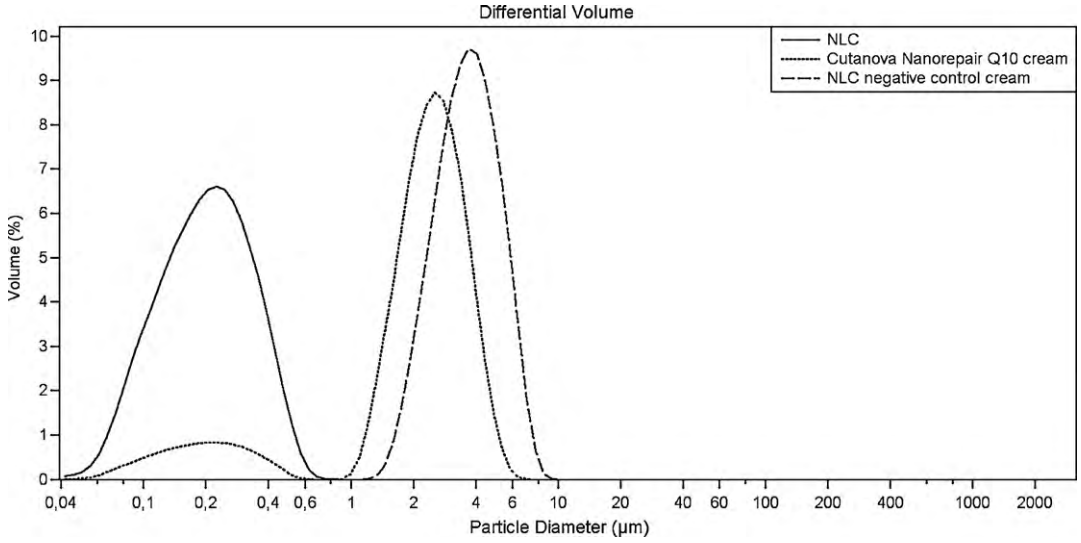

Fig. 1. Volume distribution measured by LD for NLC, Cutanova Nanorepair Q10 cream and NLC negative control cream.

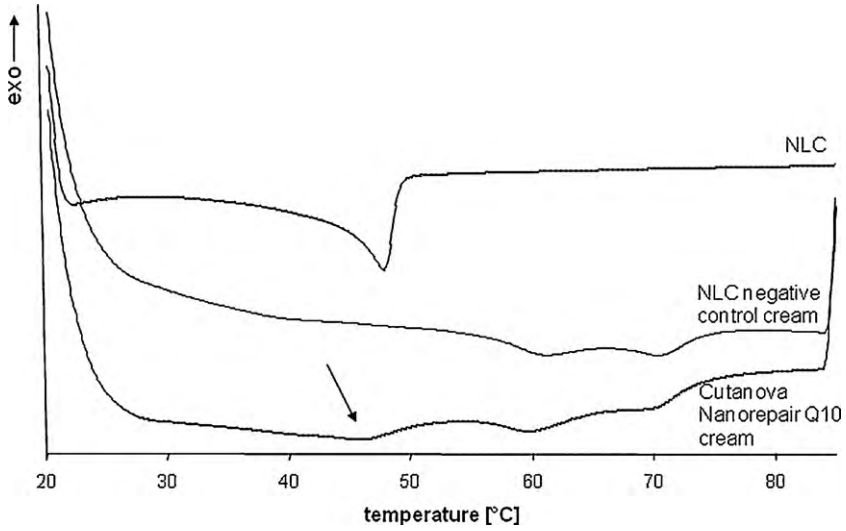

Fig. 2. DSC scan of NLC, Cutanova Nanorepair Q10 cream and NLC negative control cream. The melting peak of NLC in Cutanova Nanorepair Q10 cream is marked with an arrow.

a cream formulation can be proven using thermo analysis such as DSC (Müller and Dingler, 1998). Furthermore, nanoparticles besides microparticulate oil droplets can be detected by LD measurements (Pardeike and Müller, 2007b).

Fig. 1 shows the volume distribution curve measured by LD for NLC, Cutanova Nanorepair Q10 cream and the NLC negative control cream. A monomodal particle size distribution of NLC is shown in Fig. 1, where 95% of the NLC calculated on the base of a volume distribution have a particle size below 0.441 μm. For the NLC negative control cream also a monomodal particle size distribution was found, indicating the presence of microparticulate oil droplets in the range of 1–10 μm. Cutanova Nanorepair Q10 cream showed a bimodal particle size distribution with one discrete peak in the nanometer range providing evidence for the presence of NLC and one discrete peak in the micrometer range due to the oil droplets of the cream.

In Fig. 2 the melting curves of NLC, Cutanova Nanorepair Q10 cream and NLC negative control cream are shown. NLC have a melting point of 47.5 °C with an onset at 43 °C. This proves the presence of a solid particles matrix at both room and body temperature. The melting enthalpy of the NLC dispersion was 22 J g^{-1}. The DSC scan of NLC negative control cream shows two endothermic events at 56 and 70 °C, respectively. These melting points are caused by the partly crystalline character of surfactants present in the cream, i.e. Montanov 202 (arachidyl alcohol, behenyl alcohol, arachidyl glycoside) and Montanov 68 (cetearyl alcohol, ceterayl glycoside). Cutanova Nanorepair Q10 cream showed the same melting peaks as the NLC negative control cream caused by the partly crystalline character of the present surfactants as well as an additional melting peak for the NLC. For NLC in Cutanova Nanorepair Q10 cream a melting enthalpy of 2.1 J g^{-1} was obtained. Due to the fact that 10% of the NLC dispersion were incorporated into the Cutanova Nanore-

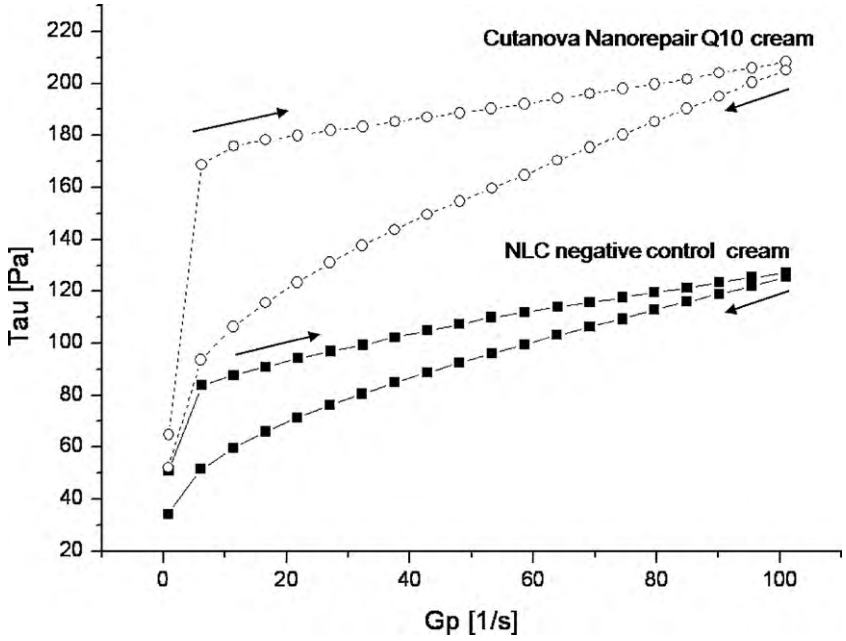

Fig. 3. Shear stress of Cutanova Nanorepair Q10 cream (–○–) and NLC negative control cream (–■–) as a function of shear rate at 20 °C.

pair Q10 cream, a melting enthalpy which correspondents to 1/10 of the melting enthalpy of NLC dispersion indicates 100% presence of NLC in the cream. The results of the DSC and LD measurements prove that NLC are still present to 100% in Cutanova Nanorepair Q10 cream and did not dissolve in the lipid phase of the cream. Furthermore, aggregation of NLC can be excluded with the LD results due to the presence of the peak in the nanometer range. In case aggregation would occur the peak in the nanometer range would disappear.

Fig. 3 shows the rheograms of Cutanova Nanorepair Q10 cream and NLC negative control cream. Plastic flow characteristics were found for both test formulations by continuous shear rheometry. The plastic flow of the two test formulations is superposed with thixotropic flow behaviour. The hysteresis loop of the Cutanova Nanorepair Q10 cream encloses a larger area than the one of the NLC negative control cream indicating a more distinct thixotropy of the Cutanova Nanorepair Q10 cream. At the same shear rate the Cutanova Nanorepair Q10 cream exhibits higher shear stress than the NLC negative control cream indicating a higher apparent viscosity of the Cutanova Nanorepair Q10 cream. Yield point measurements showed a yield point of 59.4 Pa for Cutanova Nanorepair Q10 cream and a yield point of 30.2 Pa for NLC negative control cream. These results indicate a more pronounced structural network in Cutanova Nanorepair Q10 cream. Cutanova Nanorepair Q10 cream contains 10% NLC dispersion with a particle content of 20%, i.e. 14.45%, cetyl palmitate, 0.75% Miglyol 812 and 4.80% coenzyme Q10. The lipid nanoparticles (cetyl palmitate) in Cutanova Nanorepair Q10 cream increases the yield point, apparent viscosity and thixotropy compared to NLC negative control cream (Pena et al., 1994).

The epicutaneous patch test allows the assessment of the skin irritation potential of cosmetic-finished products and raw materials. Skin irritation is defined as a locally arising, non-immunogenic, reversible reaction, which appears shortly after stimulation (Harvell et al., 1995). To cause skin irritation a substance must cross the stratum corneum and gain access to the viable epidermis. The human epicutaneous patch test has the advantage that irritation data are generated in the relevant species and are directly applicable to risk assessment. Furthermore, the influence of the vehicle on skin irritation can be investigated readily. However, a major limitation of this test is that user cover a broad spectrum of skin types (Kimber et al., 2001). Therefore, the epicutaneous patch test was performed on 23 volunteers with normal skin, 14 volunteers with eczema and 13 volunteers with sensitive skin. The age of the volunteers was between 22 and 57 years. Under the test conditions the positive control caused skin irritation in 13 subjects. The negative control showed no reaction. Also none of the volunteers showed any reaction to Cutanova Nanorepair Q10 cream. Therefore, Cutanova Nanorepair Q10 cream can be classified as harmless with regards to the possibility of skin irritation, i.e. well tolerated by the skin.

If the effect of a cosmetic product should be claimed on its package, the proof of this effect is required by law (Lebensmittel- und Futtermittelgesetzbuch, 2005; Kosmetik-Verordnung, 1977). One important criterion for the evaluation of cosmetic products is their effect on skin hydration. The aim of the study was to evaluate if Cutanova Nanorepair Q10 cream and a NLC negative control cream, with the same lipid and coenzyme Q10 content but without NLC, increase the skin hydration significantly after repetitive application. Furthermore, it should be evaluated if NLC containing Cutanova Nanorepair Q10 cream increases the skin hydration more than NLC negative control cream.

31 female volunteers with an average age of 25.5 years (median = 23 years; SD = 6.3) participated in this study. All volunteers were compliant. Approximately the same amount of Cutanova Nanorepair Q10 cream and NLC negative control cream were applied during this study. From Cutanova Nanorepair Q10 cream the volunteers used on average 10.14 g (median = 8.79 g; SD = 5.07). An average use of 10.74 g (median = 9.17 g; SD = 5.04) of the NLC negative control cream was found. The individual volunteers used approximately the same amounts of both test formulations.

Table 3
Mean, standard deviation (SD), median, minimum (min.) and maximum (max.) of the quotients of the test and control areas for Cutanova Nanorepair Q10 cream and NLC negative control cream.

Test formulation	Day	Mean	SD	Median	Min.	Max.
Cutanova Nanorepair Q10	1	0.80	0.12	0.78	0.64	1.26
NLC negative control cream		0.80	0.10	0.80	0.61	1.09
Cutanova Nanorepair Q10	7	0.99	0.11	0.97	0.77	1.15
NLC negative control cream		0.94	0.12	0.97	0.76	1.14
Cutanova Nanorepair Q10	28	1.00	0.11	1.02	0.77	1.16
NLC negative control cream		0.95	0.11	0.94	0.70	1.13
Cutanova Nanorepair Q10	42	1.01	0.12	1.04	0.74	1.25
NLC negative control cream		0.95	0.12	0.96	0.71	1.19
Cutanova Nanorepair Q10	49	0.85	0.11	0.84	0.68	1.04
NLC negative control cream		0.81	0.11	0.82	0.65	1.01

The skin hydration was measured in the test and control areas of each volunteer at each time point. From the obtained Corneometer measurements the quotients of the values measured in the test and control area were calculated and statistically evaluated. Table 3 provides a summary of the calculated mean values, standard deviations, medians, minima and maxima of the quotients of the test and control area for Cutanova Nanorepair Q10 cream and NLC negative control cream.

The average percentage change of skin hydration due to application of Cutanova Nanorepair Q10 cream and NLC negative control cream at each measurement point was calculated using the following equation:

$$\text{Change in skin hydration}\,(\%) = \left(\frac{\sum Q_{ti}}{\sum Q_{0i}} - 1\right) \times 100$$

t = time point; i = number of volunteer; Q_{ti} = quotient of measured value in the test area and the control area at the time point t for volunteer i; Q_{0i} = quotient of measured value in the test area and the control area at the begin of the study for volunteer i.

The average percentage changes of skin hydration for each measurement point are shown in Fig. 4.

At the beginning of the study (day 1) no significant difference with regards to skin hydration was present in the test areas for Cutanova Nanorepair Q10 cream and NLC negative control cream ($p = 0.05$). Both coenzyme Q10 containing creams were able to increase the skin hydration significantly compared to the base value within 7 days of application even in the young age of the volunteers ($p = 0.05$). After 28 days of application a significant difference in the skin hydrating effect of Cutanova Nanorepair Q10 cream and NLC negative control cream could be shown ($p = 0.05$). The NLC containing Cutanova Nanorepair Q10 cream increased the skin hydration significantly more than the NLC negative control cream ($p = 0.05$). After an application period of 6 weeks the Cutanova Nanorepair Q10 cream was still significantly superior with regards to skin hydration compared to the NLC negative control cream ($p = 0.05$). The increase in skin hydration obtained at day 42 with the NLC negative control cream was of the same order of magnitude as the increase in skin hydration obtained after 28 days of application of Cutanova Nanorepair Q10 cream. After an application free time of one week, the increase in skin hydration in the test area of the Cutanova Nanorepair Q10 cream was still significantly higher than the one in the test area of the NLC negative control cream ($p = 0.05$). However, the differences to the base values for both formulations

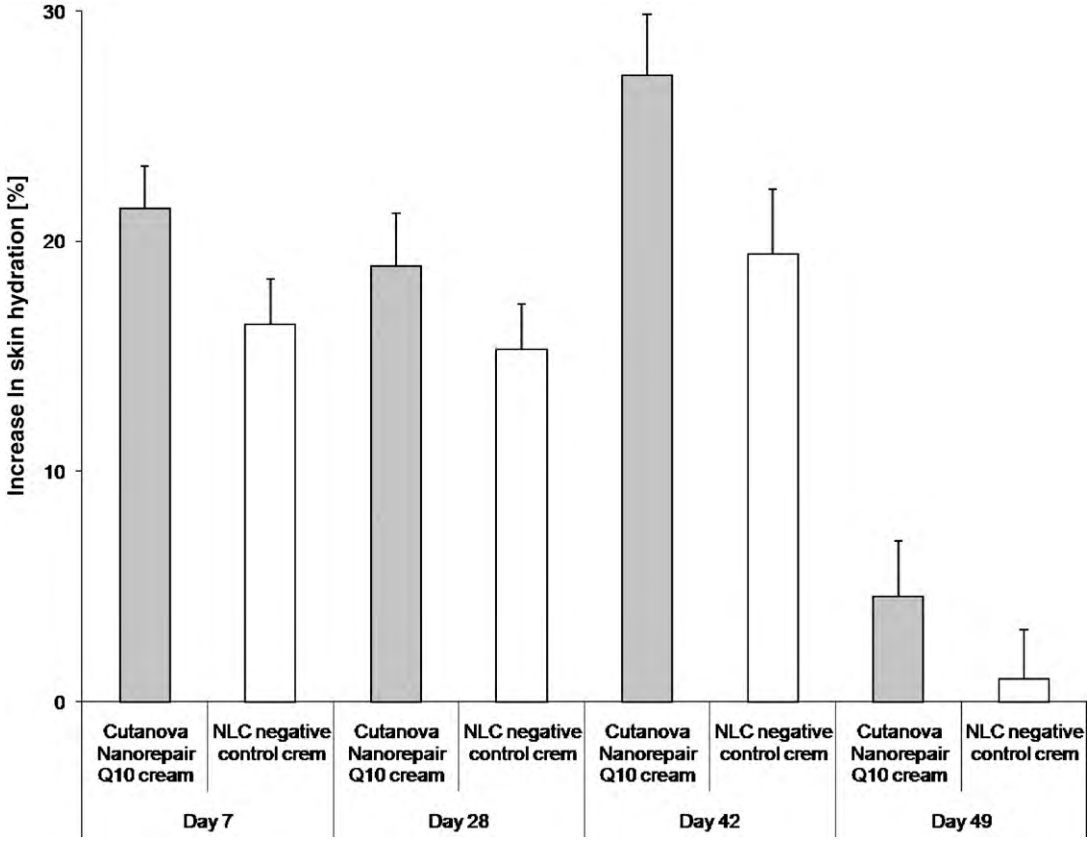

Fig. 4. Increase in skin hydration in percent after application of Cutanova Nanorepair Q10 cream and NLC negative control cream for 7, 28 and 42 days and at day 49 after 7 days application free time ($n = 31$; $\bar{x} \pm$ RSD).

were not significant ($p = 0.05$). Reasons for the increased effect on skin hydration of NLC containing Cutanova Nanorepair Q10 cream compared to NLC negative control cream are the distinctively occlusion effect of NLC as well as the enhanced penetration of coenzyme Q10 from NLC into the stratum corneum compared to a conventional formulation (Pardeike and Müller, 2007a; Teeranachaideekul et al., 2008; Souto et al., 2004a,b; Zhai and Maibach, 2001; Dingler, 1998). Wissing and Müller (2003) reported previously the ability of an SLN containing o/w cream to increase the skin hydration significantly more than a conventional o/w cream without SLN within 28 days. The results obtained by these authors for the first generation of lipid nanoparticles could be confirmed for the second generation of lipid nanoparticles in the present study.

Fig. 5 summarizes the results of the product evaluation with regards to consistency, spreadability on the skin and the subjective felling of increase in skin hydration for the two creams tested.

The consistency of the Cutanova Nanorepair Q10 cream was rated as very good or good by 77.7% of the volunteers. 22.3% found the consistency of this cream moderate. Only 55.5% rated the consistency of the NLC negative control cream as very good or good.

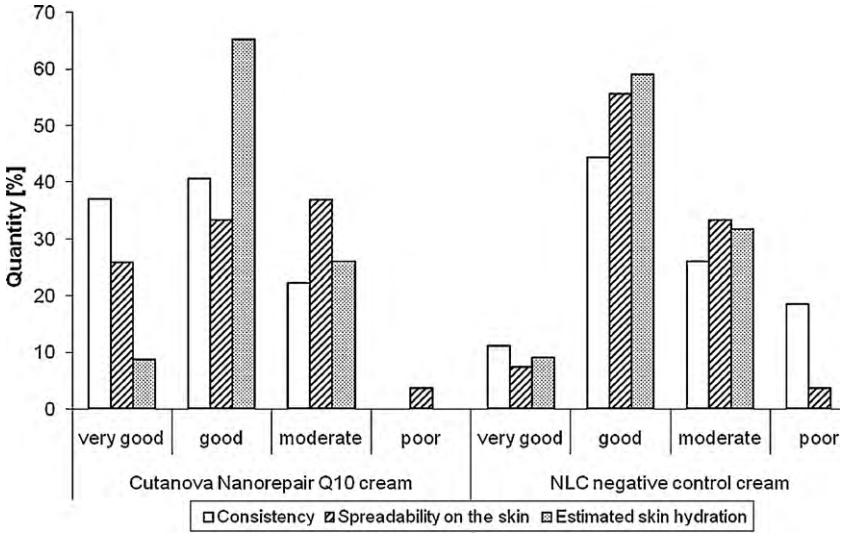

Fig. 5. Results of the product evaluation.

The consistency of the NLC negative control cream was judge as moderate or poor by 44.5% of the volunteers. That means in comparison the overall impression of the consistency of the Cutanova Nanorepair Q10 cream was better than the one of the NLC negative control cream. The spreadability on the skin of the Cutanova Nanorepair Q10 cream was estimated as very good by 25.9%, good by 33.3%, moderate by 37% and as poor by 3.7%. The spreadability on the skin of the NLC negative control cream was rated as very good by only 7.4% of the volunteers. 55.6% found the spreadability of NLC negative control cream good, 33.3% moderate and 3.7% poor. The differences found in the product evaluation performed by the volunteer with regards to consistency and spreadability on the skin can be explained by the different rheological properties of the two formulations. Due to the presence of NLC Cutanova Nanorepair Q10 cream had a higher apparent viscosity, a higher yield point and a more pronounced thixotropy than the NLC negative control cream, which contributed to a better subjective overall consistency and spreadability on the skin.

73.9% of the volunteers rated the subjective feeling of increase in skin hydration after application of Cutanova Nanorepair Q10 cream as very good or good. 26.1% found the subjective increase in skin hydration moderate. In case of NLC negative control cream 69.6% rated the increase in skin hydration as very good or good and 30.4% as moderate. No volunteer found the increase skin hydration of the test formulations poor. However, the subjective impression of the increase in skin hydration was found to be better for the Cutanova Nanorepair Q10 cream than for NLC negative control cream. Furthermore, the volunteers were asked which formulation in direct comparison they found better. Here 66.7% answered Cutanova Nanorepair Q10 cream and 33.3% NLC negative control cream, which clearly indicates a preference for the NLC containing cream.

4. Conclusion

In the present study it could be shown on the first NLC containing cosmetic product on the market, that lipid nanoparticles incorporated into this cream are physically stable. With the epicutaneous patch test it could be shown that Cutanova Nanorepair Q10 cream has no skin irritation potential, it is well tolerated. Furthermore, a significant higher increase in skin hydration was found after the application of Cutanova Nanorepair Q10 cream compared to a conventional cream having the same lipid and coenzyme Q10 content ($p = 0.05$). In a subjective product evaluation using a questionnaire the volunteers of the in vivo study estimated that the skin hydration effect of the Cutanova Nanorepair Q10 cream was better than the one of NLC-free cream. These results indicate that NLC containing creams are promising systems for cosmetics with a proven effect on skin hydration.

References

1977 Kosmetik-Verordnung in der Fassung der Bekanntmachung vom 7. Oktober 1997 (BGBl. I S. 2410), zuletzt geändert durch Artikel 1 der Verordnung vom 4. Juli 2008 (BGBl. I S. 1226).

2005 Lebensmittel-und Futtermittelgesetzbuch in der Fassung der Bekanntmachung vom 26. April 2006 (BGBl. I S. 945), zuletzt geändert durch Artikel 12 des Gesetzes vom 26. Februar 2008 (BGBl. I S. 215).

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Bentinger, M., Brismar, K., Dallner, G., 2007. The antioxidant role of coenzyme Q. Mitochondrion, S41–S50.

Beyer, R.E., 1988. Inhibition by coenzyme Q of ethanol- and carbon tetrachloride-stimulated lipid peroxidation in vivo and catalyzed by microsomal and mitochondrial systems. Free Radic. Biol. Med. 5, 297–303.

Beyer, R.E., Burnett, B.A., Cartwright, K.J., Edington, D.W., Falzon, M.J., Kreitman, K.R., Kuhn, T.W., Ramp, B.J., Rhee, S.Y., Rosenwasser, M.J., 1985. Tissue coenzyme Q (ubiquinone) and protein concentrations over the life span of the laboratory rat. Mech. Ageing Dev. 32, 267–281.

Chakravarti, B., Chakravarti, D.N., 2006. Oxidative modification of proteins: age-related changes. Gerontology 53, 128–139.

Chen, H., Chang, X., Du, D., Liu, W., Liu, J., Weng, T., Yang, Y., Xu, H., Yang, X., 2006. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J. Control. Rel. 110, 296–306.

Dingler, A., 1998. Feste Lipid-Nanopartikel als kolloidale Wirkstoffträgersysteme zur dermalen Applikation. Freie Universität Berlin, Berlin.

Dingler, A., Hildebrand, G., Niehus, H., Müller, R.H., 1998. Cosmetic anti-aging formulation based on vitamin E-loaded solid lipid nanoparticles. Controlled Release Society.

Ernster, L., Dallner, G., 1995. Biochemical, physiological and medical aspects of ubiquinone function. Biochim. Biophys. Acta 1271, 195–204.

Fang, J.Y., Fang, C.L., Liu, C.H., Su, Y.H., 2008. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur. J. Pharm. Biopharm. 70, 633–640.

Forsmark-Andree, P., Ernster, L., 1994. Evidence for a protective effect of endogenous ubiquinol against oxidative damage to mitochondrial protein and DNA during lipid peroxidation. Mol. Asp. Med., s73–s81.

Han, F., Li, S., Yin, R., Shi, X., Jia, Q., 2008. Investigation of nanostructured lipid carriers for transdermal delivery of flurbiprofen. Drug Dev. Ind. Pharm. 34, 453–458.

Harman, D., 1996. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.

Harvell, J.D., Lammintausta, K., Maibach, H.I., 1995. Irritant contact dermatitis. In: Guin, J.D. (Ed.), Practical Contact Dermatitis. McGraw-Hill, New York.

Jee, J.P., Lim, S.J., Park, J.S., Kim, C.K., 2006. Stabilization of all-trans retinol by loading lipophilic antioxidants in solid lipid nanoparticles. Eur. J. Pharm. Biopharm. 63, 134–139.

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

Jenning, V., Gysler, A., Schäfer-Korting, M., Gohla, S.H., 1999. 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., Schäfer-Korting, M., Gohla, S., 2000. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J. Control. Rel. 66, 115–126.

Joshi, M., Patravale, V., 2006. Formulation and evaluation of nanostructured lipid carrier (NLC)-based gel of valdecoxib. Drug Dev. Ind. Pharm. 32, 911–918.

Joshi, M., Patravale, V., 2008. Nanostructured lipid carrier (NLC) based gel of celecoxib. Int. J. Pharm. 346, 124–132.

Kimber, I., Basketter, D.A., Berthold, K., Butler, M., Garrigue, J.L., Lea, L., Newsome, C., Roggeband, R., Steiling, W., Stropp, G., Waterman, S., Wiemann, C., 2001. Skin sensitization testing in potency and risk assessment. Toxicol. Sci. 59, 198–208.

Kohen, R., Gati, I., 2000. Skin low molecular weight antioxidants and their role in aging and in oxidative stress. Toxicology 148, 149–157.

Liu, J., Hu, W., Chen, H., Ni, Q., Xu, H., Yang, X., 2007. Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. Int. J. Pharm. 328, 191–195.

Lucks, J.S., Müller, R.H. 1991. Medication vehicles made of solid lipid particles (solid lipid nanospheres SLN) EP0000605497.

Medvedev, Z.A., 1990. An attempt at a rational classification of theories of ageing. Biol. Rev. Camb. Philos. Soc. 65, 375–398.

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.

Moore, A., 2002. The biochemistry of beauty. The science and pseudo-science of beautiful skin. EMBO Rep. 3, 714–717.

Müller, R.H., Dingler, A., 1998. The next generation after liposomes: solid lipid nanoparticles (SLN Lipopearls) as dermal carrier in cosmetics. Eurocosmetics 7/8, 19–26.

Müller, R.H., Immig, H., Hommoss, A., 2007a. Prolonged release of parfumes by nano lipid carriers (NLC) technology. Eurocosmetics 11, 12.

Müller, R.H., Petersen, R.D., Hommoss, A., Pardeike, J., 2007b. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv. Drug Deliv. Rev. 59, 522–530.

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

Müller, R.H., Ruhl, D., Runge, S., Schulze-Forster, K., Mehnert, W., 1997. Cytotoxicity of solid lipid nanoparticles as a function of the lipid matrix and the surfactant. Pharm. Res. 14, 458–462.

Pardeike, J., Hommoss, A., Müller, R.H., 2008. Lipid nanoparticles (SLN NLC) in cosmetic and pharmaceutical dermal products. Int. J. Pharm. 366, 170–184.

Pardeike, J., Müller, R.H., 2007a. Coenzyme Q10 loaded NLCs: preparation, occlusive properties and penetration enhancement. Pharm. Technol. Eur. 19, 46–49.

Pardeike, J., Müller, R.H., 2007b. Physical stability of nanostructured lipid carriers (NLC) in an o/w urea cream. In: Annual Meeting of the Controlled Release Society (CRS). Controlled Release Society, Long Beach, USA.

Pathak, P., Nagarsenker, M., 2009. Formulation and evaluation of lidocaine lipid nanosystems for dermal delivery. AAPS PharmSciTech 10, 985–992.

Pena, L.E., Lee, B.L., Stearns, J.F., 1994. Secondary structural rheology of a model cream. J. Soc. Cosmet. Chem. 45, 77–84.

Puglia, C., Blasi, P., Rizza, L., Schoubben, A., Bonina, F., Rossi, C., Ricci, M., 2008. Lipid nanoparticles for prolonged topical delivery: an in vitro and in vivo investigation. Int. J. Pharm. 357, 295–304.

Rabe, J.H., Mamelak, A.J., Mcelgunn, P.J., Morison, W.L., Sauder, D.N., 2006. Photoaging: mechanisms and repair. J. Am. Acad. Dermatol. 55, 1–19.

Ricci, M., Puglia, C., Bonina, F., Di Giovanni, C., Giovagnoli, S., Rossi, C., 2005. Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies. J. Pharm. Sci. 94, 1149–1159.

Sanna, V., Gavini, E., Cossu, M., Rassu, G., Giunchedi, P., 2007. Solid lipid nanoparticles (SLN) as carriers for the topical delivery of econazole nitrate: in-vitro characterization, ex-vivo and in-vivo studies. J. Pharm. Pharmacol. 59, 1057–1064.

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

Santos, M.C., Mehnert, W., Schaller, M., Korting, H.C., Gysler, A., Haberland, A., Schäfer-Korting, M., 2002. Drug targeting by solid lipid nanoparticles for dermal use. J. Drug Target. 10, 489–495.

Scholer, N., Olbrich, C., Tabatt, K., Muller, R.H., Hahn, H., Liesenfeld, O., 2001. Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages. Int. J. Pharm. 221, 57–67.

Shah, K.A., Date, A.A., Joshi, M.D., Patravale, V.B., 2007. Solid lipid nanoparticles (SLN) of tretinoin: potential in topical delivery. Int. J. Pharm. 345, 163–171.

Shindo, Y., Witt, E., Han, D., Epstein, W., Packer, L., 1994. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. J. Invest. Dermatol. 102, 122–124.

Silva, A.C., Santos, D., Ferreira, D.C., Souto, E.B., 2009. Minoxidil-loaded nanostructured lipid carriers (NLC): characterization and rheological behaviour of topical formulations. Pharmazie 64, 177–182.

Sivaramakrishnan, R., Nakamura, C., Mehnert, W., Korting, H.C., Kramer, K.D., Schafer-Korting, M., 2004. Glucocorticoid entrapment into lipid carriers—characterisation by parelectric spectroscopy and influence on dermal uptake. J. Control. Rel. 97, 493–502.

Song, C., Liu, S., 2005. A new healthy sunscreen system for human: solid lipid nanoparticles as carrier for 3,4,5-trimethoxybenzoylchitin and the improvement by adding vitamin E. Int. J. Biol. Macromol. 36, 116–119.

Souto, E., Müller, R.H., 2005. SLN and NLC for topical delivery of ketoconazole. J. Microencapsul. 22, 501–510.

Souto, E., Wissing, S.A., Barbosa, C.M., Müller, R.H., 2004a. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm. 278, 71–77.

Souto, E.B., Wissing, S.A., Barbosa, C.M., Muller, R.H., 2004b. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int. J. Pharm. 278, 71–77.

Stecova, J., Mehnert, W., Blaschke, T., Kleuser, B., Sivaramakrishnan, R., Zouboulis, C.C., Seltmann, H., Korting, H.C., Kramer, K.D., Schafer-Korting, M., 2007a. Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. Pharm. Res. 24, 991–1000.

Stecova, J., Mehnert, W., Blaschke, T., Kleuser, B., Sivaramakrishnan, R., Zouboulis, C.C., Seltmann, H., Korting, H.C., Kramer, K.D., Schäfer-Korting, M., 2007b. Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. Pharm. Res. 24, 991–1000.

Teeranachaideekul, V., 2008. Nanostructured Lipid Carriers (NLC)—Stability Improvement and Release Modification of Ascorbyl Palmitate and Coenzyme Q10. Mahidol University, Bankok.

Teeranachaideekul, V., Boonme, P., Souto, E.B., Müller, R.H., Junyaprasert, V.B., 2008. Influence of oil content on physicochemical properties and skin distribution of Nile red-loaded NLC. J. Control. Rel. 128, 134–141.

Teeranachaideekul, V., Müller, R.H., Junyaprasert, V.B., 2007a. Encapsulation of ascorbyl palmitate in nanostructured lipid carriers (NLC)—effects of formulation parameters on physicochemical stability. Int. J. Pharm. 340, 198–206.

Teeranachaideekul, V., Souto, E.B., Junyaprasert, V.B., Müller, R.H., 2007b. Cetyl palmitate-based NLC for topical delivery of Coenzyme Q(10)—development, physicochemical characterization and in vitro release studies. Eur. J. Pharm. Biopharm. 67, 141–148.

Wissing, S.A., Lippacher, A., Müller, R.H., 2001. Investigations on the occlusive properties of solid lipid nanoparticles (SLN). J. Cosmet. Sci. 52, 313–324.

Wissing, S.A., Müller, R.H., 2001. A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. Int. J. Cosmet. Sci. 23, 233–243.

Wissing, S.A., Müller, R.H., 2002a. The development of an improved carrier system for sunscreen formulations based on crystalline lipid nanoparticles. Int. J. Pharm. 242, 373–375.

Wissing, S.A., Müller, R.H., 2002b. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J. Control. Rel. 81, 225–233.

Wissing, S.A., Müller, R.H., 2003. The influence of solid lipid nanoparticles on skin hydration and viscoelasticity—in vivo study. Eur. J. Pharm. Biopharm. 56, 67–72.

Yaziksiz-Iscan, Y., Wissing, S.A., Müller, R.H., Hekimoglu, S., 2002. Different production methods for solid lipid nanoparticles (SLN) containing the insect repellent DEET. In: 4th World Meeting ADRITELF/APGI/APV, Florence.

Zhai, H., Maibach, H.I., 2001. Effects of skin occlusion on percutaneous absorption: an overview. Skin Pharmacol. Appl. Skin Physiol. 14, 1–10.