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Lipid nanocarriers for dermal delivery of lutein: Preparation, characterization, stability and performance

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ABSTRACT

Topical application of lutein as an innovative antioxidant, anti-stress and blue light filter, which is able to protect skin from photo damage, has got a special cosmetic and pharmaceutical interest in the last decade. Lutein is poorly soluble, and was therefore incorporated into nanocarriers for dermal delivery: solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and a nanoemulsion (NE). Nanocarriers were produced by high pressure homogenization. The mean particle size was in the range of about 150 nm to maximum 350 nm, it decreased with increasing oil content of the carriers. The zeta potential in water was in the range −40 to −63 mV, being in agreement with the good short term stability at room temperature monitored for one month. *In vitro* release was studied using a membrane free model. Highest release in 24 h was observed for the nanoemulsion (19.5%), lowest release (0.4%) for the SLN. Release profiles were biphasic (lipid nanoparticles) or triphasic (NE). *In vitro* penetration study with a cellulose membrane showed in agreement highest values for the NE (60% in 24 h), distinctly lower values for the solid nanocarriers SLN and NLC (8–19%), lowest values for lutein powder (5%). Permeation studies with fresh pig ear skin showed that no (SLN, NLC) or very little lutein (0.4% after 24 h) permeated, that means the active remains in the skin and is not systemically absorbed. The nanocarriers were able to protect lutein against UV degradation. In SLN, only 0.06% degradation was observed after irradiation with 10 MED (Minimal Erythema Dose), in NLC 6–8%, compared to 14% in the NE, and to 50% as lutein powder suspended in corn oil. Based on size, stability and release/permeation data, and considering the chemical protection of the lutein prior to its absorption into the skin, the lipid nanoparticles are potential dermal nanocarriers for lutein.

1. Introduction

Lutein is one of the numerously known natural carotenoids, a member of the xanthophyll family, found as lipid soluble pigment in various vegetables (e.g. spinach, kale) and egg yolk (Landrum et al., 1997). Among hundreds of carotenoids found in nature, lutein (β,ε-carotene 3,3′-diol, $C_{40}H_{56}O_2$) (Jin et al., 2009) is one out 20 carotenoids found in the human body (Roberts et al., 2009) (Fig. 1).

Lutein and its isomer zeaxanthin are highly concentrated in the macula lutea of human eye (Landrum and Bone, 2001; Sommerburg et al., 1998), which is a small area of the retina responsible for central vision and high visual acuity. It is well known for its importance for improving vision (Bone et al., 2007; Rodriguez-Carmona et al., 2006; Zeimer et al., 2009) and in eye protection from harmful short wavelength blue light (Lakshminarayana et al., 2008). In addition, serum levels of lutein have been shown to be inversely related to the risk of ocular diseases, including age-related macular degeneration (AMD) (Eye Disease Case–Control Study Group, 1993; Mares-Perlman et al., 2001; Moeller et al., 2008). Lutein and zeaxanthin are found also naturally in the human skin as potentially important antioxidants (Palombo et al., 2007). They have been proposed to play an important role in skin via ingestion or topical application (Dreher and Maibach, 2001). They are able to quench singlet oxygen, a highly reactive free radical that can damage deoxyribonucleic acid (DNA) (Lockwood, 2007). Further, lutein plays a role in maintaining skin health by reducing UV-induced erythema and inflammation (Stahl and Sies, 2002). Given the established link between UV exposure and skin cancer, this in turn suggests that lutein may play a protective role against skin cancer, primarily as a filter of blue light, but also as a free-radical scavenger. This hypothesis was recently tested at Harvard University using the nude mouse model, and the results demonstrated that the mice consuming the lutein diet had significantly fewer tumors,

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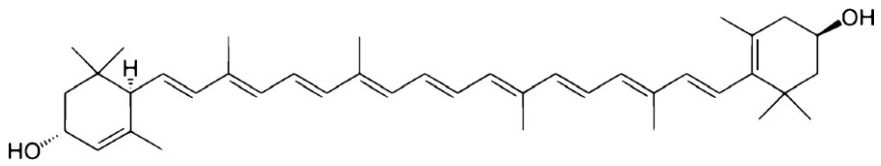

Fig. 1. Chemical structure of lutein containing nine conjugated double bonds in the polyene chain, characterized by the presence of two hydroxyl groups.

smaller tumor size, and longer survival time than those consuming the control diet (Granstein et al., 2001).

In 2006, a clinical trial was designed to study the efficacy of lutein and zeaxanthin on the skin. This study demonstrated that the topical treatment with lutein (50 ppm, 2 times per day) showed an immediate increase in superficial skin lipids, significant reduction in skin lipid peroxidation, increase in the photoprotective activity, skin elasticity and skin hydration. Even the combined oral and topical treatment of lutein exhibited the greatest efficacy in most previous cases (Palombo et al., 2007). As many other antioxidants, lutein is liposoluble and an unstable molecule. Its insolubility in water explains its moderate bioavailability presented in some research papers (Alves-Rodrigues and Shao, 2004). Moreover, it is an isoprenoid polymer containing many conjugated double bonds, which can be readily isomerized, oxidized and degraded (De Ritter and Purcell, 1981). To avoid these problems and to increase the lutein stability, the idea of inserting lutein in micro and nano carrier systems is born.

Delivery systems such as polymeric micro- or nanoparticles or liposomes have limitations regarding industrial applications, e.g. physical stability problems with liposomes, the lack of an accepted regulatory status of many polymers, and the lack of cost effective large scale production (e.g. polymeric nanoparticles) (De Ritter and Purcell, 1981). Therefore, solid lipid nanoparticles (SLN) and nanostructure lipid carriers (NLC) have been chosen as carrier systems for lutein, the NLC being already on the market in cosmetic dermal products.

SLN and NLC demonstrated potential for the topical route of application (Mehnert and Mäder, 2001; Müller et al., 2011). They are able to increase the stability of incorporated drugs/cosmetic actives and protect them against physical–chemical degradation (zur Mühlen et al., 1998), enabling controlled release of many active ingredients (Podio et al., 2000; Santos Maia et al., 2002; Yang et al., 1999), and increasing skin hydration and elasticity (Pardeike et al., 2009; Pardeike et al., 2010). In addition, NLCs and SLNs are organic solvent free, well tolerated, biodegradable and can be easily scaled up by using existing technologies (Lockwood, 2007; Shegokar et al., 2010). All that gave these systems special interest for the development of lutein formulations.

In the present work, aqueous lutein lipid dispersions (SLN, NLC) have been prepared and their particle size, zeta potential, physical stability and photo stability were evaluated. *In vitro* drug release and penetration were studied and compared with nanoemulsion and pure drug.

2. Materials and methods

2.1. Materials

Lutein corn oil 20% (DSM Nutritional Product Ltd, Basel, Switzerland). Lutein powder 90% was a gift sample from (Rui Heng Industry Co., Limited, China); Lipids: Cutina® CP (Cetyl Palmitate, Cognis, Germany); Dynasan®116 (Glyceryl tripalmitate, Contensio Chemicals GmbH, Germany); Carnauba Wax (Carl Roth GmbH, Germany); Miglyol® 812 (Caprylic/Capric Triglyceride, Sasol, Germany); emulsifier: Plantacare® 810 (Caprylyl/Capryl Glycoside, Cognis, Germany); Tween® 80 (polysorbate 80, Uniqema, Belgium). Freshly prepared double distilled and ultra purified water (Milli-Q, Millipore GmbH, Germany); 0.9% sodium chloride solution was purchased from B. Braun Melsungen AG (Germany). Solvents, tetra hydrofluran (THF) and ethanol (EtOH) were used of analytical grade.

2.2. Methods

2.2.1. Preparation of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)

All lipid nanoparticles were produced by high pressure homogenization (HPH) in freshly prepared Milli-Q water by using a Micron LAB 40 (40 mL, APV Deutschland GmbH, Germany). The formulations contain 1.0% (w/w) lutein corn oil (20% as active ingredient), 9% (w/w) of solid lipid or mixture of solid and liquid as lipid phase. Plantacare 810 at 1.5% (w/w) was used as stabilizer.

The hot emulsion was obtained by melting the lipid phase at around 80 °C, followed by addition of lutein corn oil. The hot aqueous surfactant solution was added to the melted lipid phase under stirring by ultra turrax T25 (Janke and Kunkel GmbH, Germany) for 1 min at 8000 rpm. The formed hot macroemulsion was homogenized by using the high pressure homogenizer for three cycles at 500 bar. The obtained hot o/w nanoemulsion was then immediately cooled in an ice bath to accelerate the recrystallization of lipid phase to form NLC and SLN. Samples for particle size characterization were collected after each homogenizing cycle. In similar manner, nanoemulsion (NE) was produced using 1.0% (w/w) lutein corn oil, 9% (w/w) Miglyol® 812 and 1.5% (w/w) of plantacare 810 (Table 1).

2.2.2. Characterization of lipid nanocarriers and nanoemulsion

2.2.2.1. Particle size analysis. Analysis of the particle size was performed by using dynamic light scattering and static light scattering techniques. In addition, light microscopy was employed to detect presence of aggregates or unencapsulated crystals.

Photon correlation spectroscopy (*PCS*). The mean particle size (*z*-average abbreviated as *z*-Avr.) and the polydispersity index (Pdi) were measured for all preparations by using a Zetasizer Nano ZS (Malvern Instruments, UK). Particle size measurements were performed on diluted lipid nanoparticles dispersed in bidistilled water at 25 °C. The measuring range of a Zetasizer is from approximately 0.6 nm to 6 μm. Thus, to observe the possible larger particles, static light scattering, also known as laser diffraction, with a measuring range up to 2000 μm was also employed as additional characterization method.

Laser diffraction (*LD*). Laser diffraction was performed by using a Mastersizer 2000 (Malvern Instruments, UK). The volume weighted diameters $d(0.5)$ and $d(0.9)$ were used as characterization parameters. The diameter values indicate the percentage of particles possessing a diameter equal to or lower than the given value. For example the $d(0.5)$ represents the size where 50% of the particles are below the given size. The $d(0.9)$ is sensitive parameters to quantify larger sized particles being present, like aggregates. All parameters have been analyzed by using the Mie characterization mode with the optical parameters 1.456 as the real refractive index and 0.01 for the imaginary refractive index.

Table 1
Various ratios of solid lipid and liquid lipid used in preparation of lutein SLN, NLC and NE formulations in %(w/w), made up with water to 100%.

Formulation	Solid lipid	%	Liquid lipid	%	Stabilizer	%	Active material	%
SLN.1	Cetyl palmitate	9	–	–	Plantacare® 810	1.5	Lutein corn oil	1
SLN.2	Glyceryl tripalmitate	9	–	–	Plantacare® 810	1.5	Lutein corn oil	1
SLN.3	Carnauba wax	9	–	–	Plantacare® 810	1.5	Lutein corn oil	1
NLC.1	Glyceryl tripalmitate	7	Miglyol® 812	2	Plantacare® 810	1.5	Lutein corn oil	1
NLC.2	Carnauba wax	7	Miglyol® 812	2	Plantacare® 810	1.5	Lutein corn oil	1
NE	–	–	Miglyol® 812	9	Plantacare® 810	1.5	Lutein corn oil	1

2.2.2.2. Light microscopy. The morphology of the particles was studied by light microscopy (Ortophlan, Leitz, Germany) by applying magnifications of 160-fold, 630-fold and 1000-fold. Light microscopy is recommended to get a fast indication of the presence of microparticles or aggregation in formulation (Müller, 1996). In addition, it is more sensitive than laser diffraction to detect a few large particles present.

2.2.2.3. Chemical stability. Chemical stability of lutein was also studied immediately after preparation to verify the validity of production process. The percentage of remaining lutein in each preparation was determined by UV spectrophotometer (UV-1700 PharmaSpec, Schimadzu, Japan).

2.2.2.4. Short term stability study. To examine the physical stability of the prepared batches, short term stability studies were carried out on the selected formulations. Samples were divided into three vials after the production, and stored at three different temperatures (4 °C, room temperature and 40 °C) for 30 days. Particle size analysis, zeta potential and pH determinations were carried out on the day of production, and on 1st, 7th, 14th and 30th day.

2.2.2.5. Determination of zeta potential. The zeta potential (ZP) as quantification of the surface charge of the particles was measured by using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The Zetasizer Nano measures the electrophoretic mobility of the particles in an electric field, which then is converted into zeta potential by the Helmholtz–Smoluchowski equation built into the software. The measurements in water yield the Stern potential, which is directly related to the surface charge (Nernst potential). In water at very low electrolyte concentration, the diffuse layer is very thick, the potential decay in this layer is very slow. Thus, the potential decay between the Stern potential and the measured zeta potential at the slipping plane is negligible, the Stern and measured zeta potential can be set identical (Fig. 5). The zeta potential was determined in two different aqueous solutions: (I) conductivity adjusted distilled water to 50 μS/cm with sodium chloride solution (0.9%, w/v) and (II) the original dispersion medium (1.5% aqueous surfactant solution). The mean of 5 measurements is reported.

2.2.2.6. Differential scanning calorimetry (DSC). Thermal analysis of lipid nanoparticle formulations and pure drug was investigated using a Model DSC 821e Mettler-Toledo Differential Scanning Calorimetry (Mettler-Toledo GmbH Analytical, Gießen, Germany). The DSC thermograms for selected NLC formulations, lipid nanoparticles without drug (blank) and physical mixture was recorded by keeping total solid 1–2 mg into 40 μm pin-holed aluminium pans. DSC curves were recorded by heating the sample from 10 °C to 90 °C and cooling from 90 °C to 10 °C, respectively. A heating and cooling rate of 10 K/min was used for these studies. An empty pin-holed aluminum pan was used as reference. The DSC parameters, such as temperature onset, maximum peak and enthalpy were calculated using in build Mettler-Toledo STAREe software.

2.3. In vitro release study

2.3.1. Optimization of release medium

The release pattern of lutein-loaded nanocarriers was determined in an *in vitro* membrane free model. A screening for the most suitable release medium was performed. The release experiment was performed at 100 rpm and 32 °C in a temperature controlled assembly. The lutein NE (0.4 mL) was placed at the bottom of a Franz diffusion cell, 1.60 mL of an aqueous solution (release medium) was placed over the tested emulsion. Medium chain triglycerides (4.0 mL Miglyol® 812) were carefully placed on the top of the aqueous phase which served as acceptor medium. Following release mediums were selected for release studies ($n = 3$):

(i) Surfactant solution A: 3% (w/w) Tween 80 solution (pH = 5.5);
(ii) Surfactant solution B: 3% (w/w) Plantacare 810 solution (pH = 10.5);
(iii) Pure Milli-Q water (pH = 6.8);
(iv) pH adjusted Milli-Q water with hydrochloric acid (pH = 5.5).

Aliquots (250 μL) were taken from the oil phase at selected time intervals of 1, 2, 3, 18 and 24 h and replaced immediately with pure Miglyol® 812 to maintain sink condition. Samples were further diluted with 5 g of mixture A (9:THF + 1:H_2O). The absorbance was measured using a double-beam spectrophotometer. A solution of Miglyol® 812:Mixture A (0.250:5) (v/w) served as reference.

2.4. Release studies from lutein nanoparticles

Milli-Q water with adjusted pH to 5.5 has been chosen to carry out the release test from lipid nanoparticles (NE, SLN3, NLC1 and NLC2). Drug release was also determined for pure drug i.e. lutein suspended in corn oil at 20%. The assembly was prepared in similar manner as discussed under optimization of release medium. The release studies were performed in triplicate.

2.5. In vitro penetration study

This study was carried out by using vertical Franz glass diffusion cells for lutein-loaded NE, SLN3, NLC1, NLC2 and pure drug powder (i.e. lutein powder suspended in distilled water 0.2%). Franz glass diffusion cells consist of donor and receptor chambers between which a synthetic membrane or skin can be positioned. The area of diffusion was 0.636 cm^2 and the receptor chamber volume varied from 6.0 to 6.1 mL. Following barriers/membranes was mounted on diffusion cell:

(1) synthetic cellulose nitrate membrane (0.1 μm)
(2) fresh dermis of pig ear skin.

Freshly obtained pig ears were cleaned from hairs and excised with a scalpel, washed with water, dried with soft tissue paper

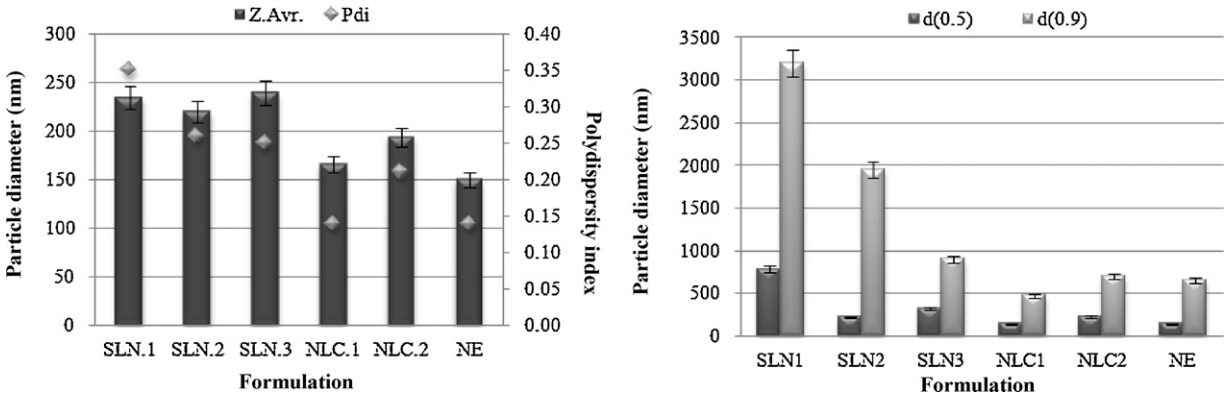

Fig. 2. Mean particle size (*z*-Avr.) and polydispersity index (Pdi) of various formulations measured by PCS (left) and volume weighed diameters [*d*(0.5) and *d*(0.9)] when measured by LD (right) on the day of preparation.

and used in the same day for the experiments. The temperature of the receptor chamber was maintained at 32 ± 2 °C. The receptor medium consisted of a solution of Miglyol® 812/ethanol (1:9) (v/v). The composition of the receptor medium was chosen because of the insufficient solubility of the studied lutein in aqueous media. Lutein is very well soluble in the chosen receptor medium. Each cell contained a magnetic stirrer rotated at 500 rpm. Selected formulations (300 μL) were evenly spread on the surface of the membrane or skin. Sampling was performed (300 μL) from each receptor chamber and replaced with fresh receptor medium at 1, 2, 4, 6 and 24 h. The penetration studies were performed in triplicate for each type of membranes and collected samples were analyzed by the spectrophotometer.

2.6. Photostability studies

Literature reports have demonstrated an increasing stability of incorporated active components (drugs, cosmetic actives) when NLC and SLN are used as carrier system (zur Mühlen et al., 1998). Enhancement of lutein photostability was verified by comparing the percentage of degraded lutein when lutein-loaded NLCs, SLNs and NE were used, with that of lutein powder suspended in corn oil. Irradiation was done by using a solar simulator (Universal Arc Lamp Housing mode 66000 and Arc Lamp Power Supply model 68805, L.O.T. ORIEL ITALIA) equipped with a Xenon lamp calibrated with a radiometer (Goldilux Smart Meter model 70234 L.O.T. ORIEL ITALIA) provided with a UVB probe. The lamp was calibrated before each determination.About 10 mg of each tested preparation are deposited on a thin-layer cuvette with an optical path of 0.01 mm (Hellma) and irradiated by the solar simulator to 10 MED (Minimal Erythema Dose) corresponding to 300 mJ/cm^2. After irradiation each sample was recovered from the cuvette by a mixture of THF/H_2O (9/1), to a final volume of 5 mL and finally analyzed by spectrophotometry.

3. Results and discussion

3.1. Identification and characterization of lutein-loaded preparations

Lipophilic drugs mostly show high solubility in liquid lipids (oils) but exhibit limited solubility in solid lipids. In order to get stable lutein loaded lipid nanocarriers, three different solid lipids have been studied viz. cetyl palmitate, glyceryl tripalmitate and carnauba wax in combination with/without liquid lipid (Miglyol® 812). The concentration of the lipid phase, i.e. 10% including 1% of lutein corn oil, was kept constant. The various compositions of developed lipid carriers is shown in Table 1.

The selected pressure and number of homogenization cycles determined the particle size of lipid nanoparticles. Hence, initially drug free lipid nanoparticles were produced at constant pressure of 500 bar and varying number of homogenization cycles (1–5 cycles). This experiment revealed that increasing number of cycles till the third cycle leads to a decrease in mean particle size and narrowing of the polydispersity index (data not shown). Application of more than three cycles resulted in agglomeration of particles. Increasing the number of homogenization cycles often leads to an additional energy input which ultimately causes higher kinetic energy of the droplets as a result subsequent coalescence occur (Mehnert and Mäder, 2001). During production of lutein formulation, smaller size was obtained after 3 cycles at 500 bar, for additional homogenization cycles (after 3rd cycle) cycle, increase in particle size was obtained.

Therefore, production of drug lutein-loaded batches was carried out at homogenization pressure of 500 bar and for three cycles. The developed preparations were identified and characterized by: (I) measuring their particle size and polydispersity index by PCS and LD, (II) observation under light microscope and, (III) verifying the percentage of lutein.

3.1.1. Particle size analysis by PCS and LD

Fig. 2(left) shows that on the day of production for all preparations the mean particle size (*z*-Avr.) measured by PCS was in range of 150–240 nm with a Pdi under 0.35, and no evidence of any aggregations could be noticed. When LD was used (Fig. 2, right) the *d*(0.5) values for all preparations, except SLN1, were between 144 and 317 nm. This corresponds to the particle size diameters measured with PCS. Also the values of the *d*(0.9) were in the nanometer range (under 1000 nm) just for SLN3, NLC1, NLC2 and NE, but for SLN1 and SLN2 larger particle size diameters were found, values were between 2000 and 3200 nm and that means a limited fraction of the particles over the nano range.

Actually the effect of lipid matrix composition was very evident on the particle size. When carnauba wax was used alone as solid lipid in SLN3, it results in bigger particles (239 nm) compared to SLN2 prepared with glyceryl tripalmitate (220 nm). When 2% (w/w) of the solid lipid in SLN3 and SLN2 (239 and 220 nm) have been substituted with liquid lipid in case of NLC2 and NLC1, also smaller particle size have been obtained (194 and 166 nm, respectively). These effects of lipid composition on the particle size could be related to the interaction between the selected lipid and stabilizer, and crystallization degree of each lipid (Müller et al., 2000b). Increasing the oil content in the particle matrix reduces the viscosity of the melted droplets, thus easing dispersion resulting in smaller sizes. This is nicely confirmed by the nanoemulsion data, smallest sizes were found for the NE.

Table 2
Chemical stability of lutein in various compositions of SLN, NLC and NE analyzed by spectrophotometry at 451 nm (in terms of remaining lutein% of theoretical 100% value in the formulation) on the day preparation.

Formulation	Production temp. (°C)	Lutein (%) assayed
SLN.1	80	89.30
SLN.2	85	90.01
SLN.3	85	101.29
NLC.1	80	97.02
NLC.2	85	99.46
NE	65	100.54

3.1.2. Light microscopy

In parallel, to particle size measurement, light microscopy has been made for further observation of the formulations. Fig. 3 confirms the results obtained from PCS and LD that no big particles were present in the lutein-loaded SLN3, NLC1, NLC2 and NE. But very evident aggregations and some free lutein crystals were easily observed in SLN1 and SLN2.

3.1.3. Chemical stability of lutein

Lutein is a heat sensitive molecule and there are chances of degradation during the production process when high temperature is used like other carotenoid (Borsarelli and Mercadante, 2010). Taking this into consideration along with the melting point of each solid lipid, damage to lutein is possible. However, lutein prepared with carnauba wax (SLN3, NLC2) gave the highest thermo stability (101.29% and 100.54%, respectively) even when the preparation temperature of 85 °C was used (Table 2). Cetyl palmitate lutein (SLN1) gave the lowest thermo stability showing 89.30% even at reduced production temperature (80 °C). These results reflect the importance of lipid selection when sensitive material is used and confirm that the stabilization effect differs between the used lipids (Müller et al., 2002).

Depending on the particle size measurements, light microscopic observation and thermo stability results, only SLN3, NLC1, NLC2 and NE were the optimal formulations for dermal delivery (in nano particles range, almost no aggregations and no lutein degradation). Therefore, the other formulations SLN1 and SLN2 were excluded from further studies.

3.2. Short term stability study under stress conditions

3.2.1. Particle size

Stress conditions or accelerated stability tests can help to predict the shelf life of the product; Fig. 4 shows the changes in particle size (*z*-Avr.) and in Pdi during the storage time at different temperatures. At both 4 °C and room temperature, all batches remained stable. Only a small negligible increase in their particle size was noticed after one month (less than 40 nm), but their polydispersity index remained below 0.3 which reflects relatively homogeneous particles. At 40 °C significant changes were noticed for all preparations in both *z*-Avr. and Pdi, especially after one month. The most stable formulation was lutein-loaded NLC1 which exhibited (after 30 days) the smallest increase in particle size (from 166 nm to 350 nm) and Pdi (0.14–0.28). Lutein-loaded NLC2, SLN3 and NE exhibited clear particle aggregations/size increases after the same period. Actually, the high values of *z*-Avr. and Pdi at 40 °C were due to the appearance of new, second separated particle populations (aggregation) with very big size (over 3 μm). This could be because of insufficient quantity of stabilizer in the formulation (Feng and Huang, 2001; Snehalatha et al., 2008).

3.2.2. pH determination

pH values of the preparation were measured by simply plunging the electrode into the formulation. During one month, the pH values of all preparations remained stable at values between 6.5 and 7.0.

Table 3
Zeta potential data of different preparations; measured in conductivity adjusted water (50 μS/cm, pH 5.8) and in the original dispersion medium after 1 and 30 days of preparation.

Preparation	Day	Zeta potential (mV)	
		Water (50 μs/cm)	Original medium
NLC.1	1	−56.0 ± 2.48	−24.5 ± 0.93
	30	−52.1 ± 2.58	−23.0 ± 1.16
NLC.2	1	−60.8 ± 1.37	−48.8 ± 2.36
	30	−63.7 ± 4.55	−40.3 ± 0.14
SLN.3	1	−52.4 ± 2.70	−47.1 ± 1.45
	30	−51.0 ± 5.99	−39.9 ± 0.65
NE	1	−44.1 ± 3.82	−15.7 ± 3.79
	30	−44.9 ± 0.52	−16.7 ± 1.21

3.2.3. Zeta potential (ZP) analysis

The particle charge is one of the factors that determines the physical stability of emulsions and suspensions. The higher is the electrostatic repulsion between the particles the higher is the physical stability. Typically the particle charge is quantified as the so called zeta potential, which is measured e.g. via the electrophoretic mobility of the particles in an electric field (Mishra et al., 2009). The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. When conductivity adjusted water (50 μS/cm, pH 5.8) was used as dispersion medium, high values of ZP for all preparations are observed (Table 3). It was above −40 mV for the NE, and above −50 mV for all lipid nanoparticles. The obtained high values indicate relatively high surface charges, being a priori beneficial for the physical stability.

When the original medium was used as dispersed medium (aqueous solution with 1.5% of plantacare 810) lower values of ZP are obtained. The measurement of the ZP in the original dispersion medium is a measure for the thickness of the diffuse layer. The thinner the diffuse layer is, the stronger the potential decay, the lower the measured zeta potential at the slipping plane (Fig. 5). Particles with zeta potentials more negative than −30 mV are normally considered physically stable, and about 20 mV provide only a short-term stability (Mishra et al., 2009). If all the particles in suspension have a high negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together (Wissing and Müller, 2002). These "mV" values apply when the particles are solely electrostatically stabilized. In case of an additional steric stabilization – as in this case with Plantacare 810 also values around 20 mV are sufficient for stability. This was confirmed by the stability during storage study, only minor negligible increases in the PCS diameter. The instability under stress at 40 °C can be explained that other factors can occur at higher temperatures which have destabilizing effect, e.g. reduction of viscosity in stabilizer layers. One month after preparation no significant decrease in the ZP values were noticed and that indicated further good stability for these preparations. No change is in agreement with the theory, because changes should only occur in case of chemical alterations (e.g. decomposition of stabilizer, formation of charged molecules such as charged fatty acids from lecithin).

3.2.4. Differential scanning calorimetry (DSC)

DSC measurements were performed with the focus to compare SLN versus the respective NLC formulation i.e. both particles containing the same solid lipid, the NLC having 2/9 of the solid lipid replaced by the Miglyol oil. In unloaded lipid nanoparticles, SLN2 and NLC1 contain as solid lipid glyceryl palmitate. Admixing Miglyol in NLC1 lead to a slight decrease in the melting point from 60.44 °C (SLN2) to 57.91 °C (−2.53 °C). The melting point depression

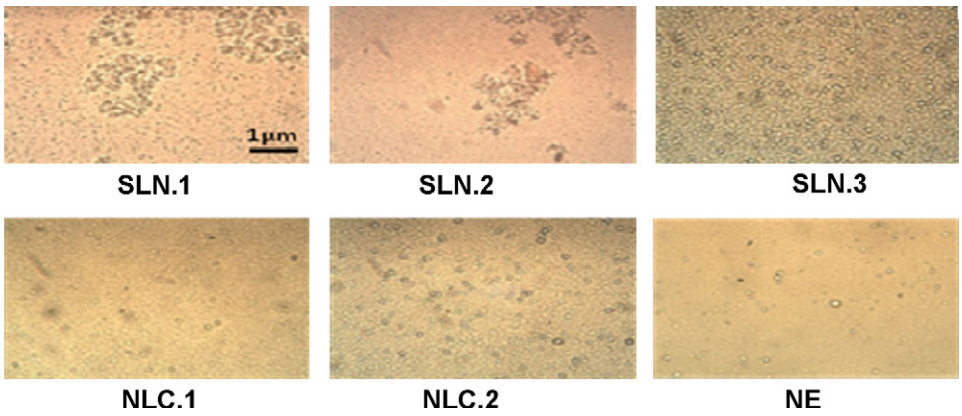

Fig. 3. Photomicrographs of SLN (above), NLC and NE (below) on the day of production (under 1000× magnification) show evident of particle aggregations in SLN1 and SLN2 and absence of large particles or aggregates in SLN3, NLC1, NLC2 and NE.

is in agreement with the theory after admixing a second component which dissolves in the first component. SLN3 and NLC2 contain both carnauba wax, here a melting point depression from 81.87 °C (SLN3) to 78.68 °C occurred (−3.19 °C). Simultaneously the onset temperature of both NLC was reduced compared to the respective SLN suspension. From these data, the oil is molecularly dispersed (=dissolved) in the solid lipid.

In case loaded lipid nanoparticles from glyceryl palmitate, the lutein was dispersed in the corn oil, not dissolved, due to its low solubility. An oil suspension was incorporated into the lipid nanoparticles. The melting peak of unloaded SLN 2 of 60.44 °C increased slightly after loading with lutein to 61.60 °C, the melting point of the corresponding NLC1 from 57.91 °C to 59.88 °C. In drug loaded lipid nanoparticles prepared using carnauba wax (SLN3, 82.09 °C) showed the melting peak practically identical to that of unloaded (SLN3, 81.87 ° C). The melting point of the corresponding NLC2 unloaded was 78.68 °C, after loading 79.67 °C. The incorporation of lutein did not lead to a reduction in melting point. The DSC data supports the assumption that the lutein is primarily dispersed in the solid particle matrices of both SLN and NLC, rather than dissolved. This is in agreement with the macroscopic observation that the hot lipid melts were turbid after addition of the lutein

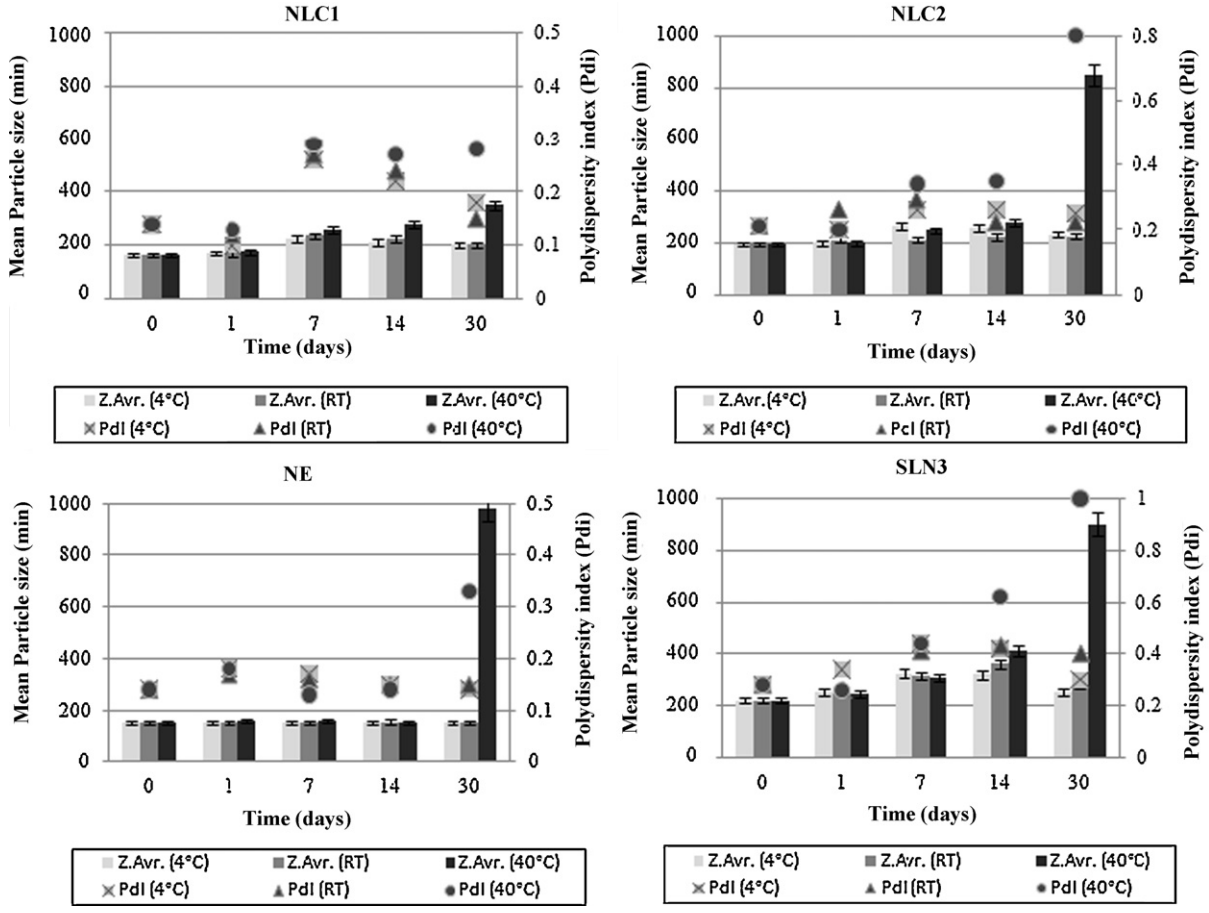

Fig. 4. Mean particle size (*z*-Avr.) and polydispersity index (Pdi) of lutein lipid carriers (NLC1, NLC2 and SLN3) and NE after storage at various stress conditions (please note different scale of secondary *y*-axis).

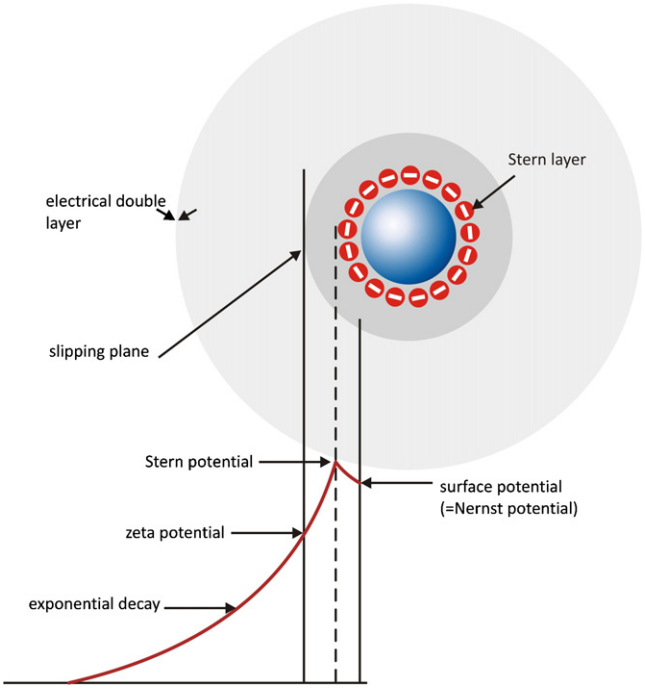

Fig. 5. Illustration of a particle, having a negative surface potential, a Stern layer composed of negative ions and the diffuse layer both surrounding the particle. The Stern potential is at the border between diffuse layer and Stern layer. The measured zeta potential is located at the slipping plane, the diffuse layer is shorn off at this plane during the zeta potential measurement. The diffuse layer is shorn off almost completely, but a rest remains between slipping plane and Stern plane. Exemplarily the course of the potential curve is plotted as a function of the distance from the particle surface.

corn oil suspension. In most cases, the solubility of compounds in lipids decreases when going from the liquid to the solid state, this further supports lutein being dispersed in the particle matrices.

3.3. Drug release study

3.3.1. Method optimization: release from nanoemulsion

An *in vitro* membrane-free model was developed for studying lutein release from prepared formulation independent of membranes. It generally gives lower drug release values compared to release studies performed using Franz diffusion cells where organic or buffered solutions are used (Mares-Perlman et al., 2001). In this study, the diffusion of the active ingredient lutein from the formulation to a lipophilic receptor medium was determined. Fig. 6 shows that the highest percentage of released lutein was obtained in pH 5.5 water (19.54% in 24 h). In case of aqueous surfactant solutions (3% Plantacare 810 or 3% Tween 80) a lower percentage was obtained (below 5% in 24 h). Reason might be that a certain percentage of lutein was solubilized in the aqueous surfactant solution and did not reach the lipophilic receptor [data not published yet]. Based on the data obtained, Milli-Q water at pH 5.5 was selected for the release studies from lipid nanoparticles. The NE showed a triphasic release pattern in pH-adjusted water (Fig. 7). In the first hour, a fast release was noticed around 40% was released quantity of lutein. In the next 5 h another 40% are released, that means in the first 6 h 80% of total released lutein was noticed and during the last 18 h just 20% was released.

3.3.2. Release study from lipid nanoparticles

Lutein release from the prepared NLC and SLN was carried out using Milli-Q water (pH 5.5) which has pH similar to skin. Fig. 7 shows the release profile of lutein from lutein dispersed

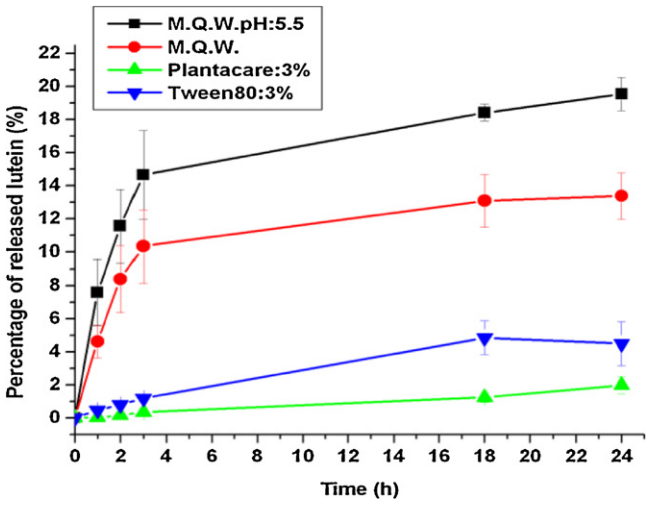

Fig. 6. Percent of released lutein from nanoemulsion as function of time using the *in vitro* membrane-free model in pH adjusted and surfactant containing aqueous media (Milli-Q water is referred as M.Q.W).

in corn oil, NE, NLC1, NLC2 and SLN3. Lutein released from the NE gave the highest release profile after 24 h i.e. 19.54%. The droplets have a liquid character and lutein is incorporated in the oil less tightly compared to solid lipid of NLC and SLN (Wissing and Müller, 2002). On the other hand, both NLC1 (12.12%) and NLC2 (7.38%) released lutein after 24 h in percentage higher than that one released from SLN3 (0.42% respectively). The release profiles from these systems are described to be depending on the lipid matrix composition, the active load and the surfactant type (Hommoss et al., 2007; Pardeike et al., 2009). The very low percentage of released lutein from SLN might be explainable by the mode of lutein incorporation in the particle matrix. If the active is enriched in the particle core (drug core–shell model), release is prolonged, as described for prednisolone (zur Mühlen et al., 1998). It is also worth to note that lutein-loaded NLC1 and NLC2 gave biphasic release profile different from that of lutein-loaded nanoemulsion. In the first hour, a fast release was noticed (30% of total released lutein), then for the next 23 h almost a stable increasing release was observed.

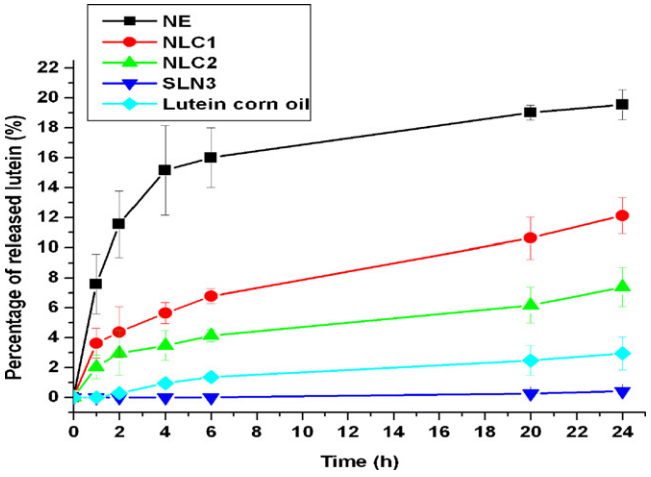

Fig. 7. Release profiles of lutein from lipid nanoparticles (SLN, NLC) in comparison with NE and pure lutein (suspended in corn oil) in Milli-Q water (pH adjusted to 5.5) as release medium using the *in vitro* membrane-free model.

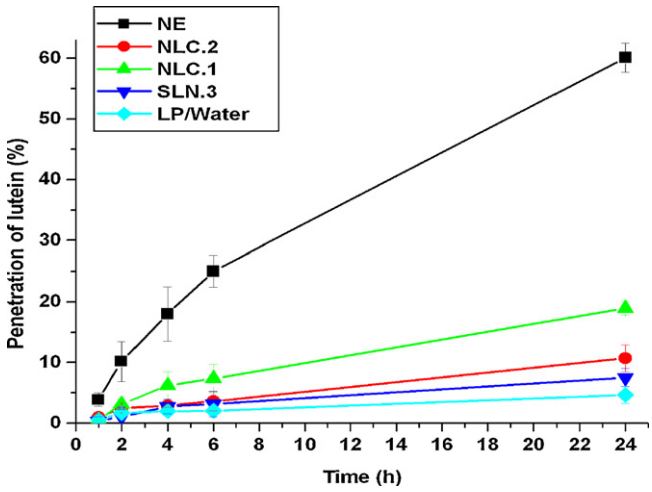

Fig. 8. *In vitro* penetration profile of lutein-encapsulated lipid nanocarriers (SLN3, NLC1, NLC2) and nanoemulsion (NE) through 0.1 μm cellulose nitrate membrane in comparison with coarse lutein powder (LP).

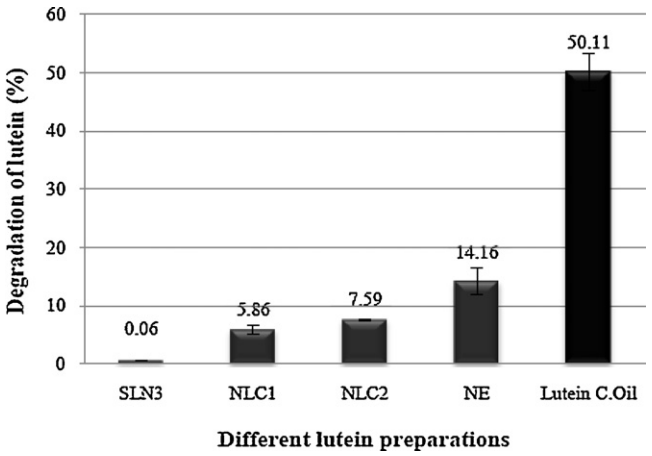

Fig. 9. Photostability of lutein in lipid nanoparticles (SLN3, NLC1, NLC2) and nanoemulsion (NE) in comparison with pure lutein (suspended in corn oil) after irradiation to 10 MED.

3.4. *In vitro penetration study*

3.4.1. *Cellulose nitrate membrane (0.1 μm)*

A cellulose nitrate membrane was chosen (thickness 105–140 μm) for *in vitro* penetration studies as it has similar thickness to that of human epidermis skin. Fig. 8 shows the penetration profiles from coarse lutein powder suspended in water (0.2%), lutein-loaded NLC, SLN and NE. A very high quantity of lutein (60%) reached the receptor medium in case of the NE as compared to lutein encapsulated into lipid nanoparticles. That can be correlated to the fast release of lutein from liquid lipid of the NE and the smaller particle size (150 nm) compared to the other preparations. Lipid nanoparticles have slower release times and larger particle sizes (Müller et al., 2002). It is also noticeable that the improvement in penetration after 24 h of application between lutein powder suspended in water (LP), which gave as expected the lowest penetration result (4.67%), and lutein-loaded NLC1 (19%), NLC2 (11%) and SLN3 (7.85%).

3.4.2. *Dermis pig ear skin*

Fresh hairless pig ear dermis with thickness between 1 and 2 mm was used to estimate the probable quantity of lutein which could cross biological barrier. This information was very important to check if the developed lutein nanoparticles enter blood circulation or not, or deliver lutein systemically. Results obtained have shown no penetration for all selected preparations even after 24 h of application, except lutein-loaded nanoemulsion. The nanoemulsion showed penetration of 0.37% after 24 h which is almost negligible.

It was very advantageous that these lutein-loaded nanocarriers formulations stay in the skin when used for topical and cosmetic products, penetrate sufficiently deep but not that deep which lead to systemic availability (Müller et al., 2002).

3.5. *Photostability test*

Improvement in lutein photostability was verified by studying the percentage of degraded lutein in lutein-loaded NLCs, SLNs, NE comparison with coarse lutein powder suspended in corn oil after irradiation to 10 MED. All prepared lutein-loaded nanocarriers demonstrated very good photostability apart from free lutein suspended in corn oil. Pure lutein (suspended in corn oil) degraded 50% of its initial concentration (Fig. 9), while SLN3, NLC2 and NLC1 improved the lutein photostability 10 times more than the free lutein. NE also exhibited a good enhancement in the lutein photostability (14.16) but less than the lipid nanoparticles. This can be explained by the absence of solid lipid which incorporate and protect the lutein. In addition, lipid nanoparticles are described as a UV protection system for the skin (Bennat and Müller-Goymann, 2000; Müller et al., 2000a; Wissing and Müller, 2001). The crystalline lipid reflects UV rays. In addition, certain compounds in the lipid acts as a molecular sunscreen, e.g. in carnauba wax. This surely contributes to the excellent photo stabilizing effect.

The most remarkable thing was the outstanding stabilizing effect of the carnauba wax SLN3. Firstly, they are made from a very well UV protecting lipid, the carnauba wax. Secondly, the high stability further supports the assumption made from the release profiles, that the lutein is primarily localized (enriched) in the core of the particles. The explanation for this is very simple. Lutein has a low solubility in the melted solid lipid, this solubility further decreases when the temperature decreases after the homogenization, when the hot nanoemulsion is cooled. In the droplet, the saturation solubility of the lutein is exceeded, it starts precipitating. During the ongoing cooling process, finally the lipid solidifies and forms a lipid shell around the formed lutein nanocrystal. This is in agreement with the enriched core–shell model described by Mehnert and co-workers (zur Mühlen et al., 1998).

4. Conclusion

Middle-chain triglyceride mixed with glyceryl tripalmitate (NLC1) or with carnauba wax (NLC2), carnauba wax alone (SLN3) and middle-chain triglyceride alone (NE) formed stable lutein-loaded lipid nanocarriers. Particle size was affected by the lipid type, but the mean particle diameter was between 150 and 240 nm with Pdi values below 0.3. The short term stability test indicated good stability of the aqueous dispersions, slight instability at 40 °C. However, when the particles are incorporated in a viscous dermal formulation, diffusion velocity of the particles is reduced and even 40 °C should be no problem anymore for the stability.

The particles provided different release profile, which gives option to choose for a final dermal formulation. They showed good *in vitro* penetration, from relatively fast (nanoemulsion) to slowest with the SLN system. From the pig ear studies, systemic uptake can be excluded, which is good for a dermal formulation. The active remains in the skin. Remarkable is the photoprotection of the labile lutein achieved by the nanocarriers, being highest for SLN (0.06% compared to 50% of lutein powder in oil). Based on these data, a dermal formulation can now be designed for delivery of stabilized

lutein not only to skin to the skin, cosmetic but also for pharmaceutical or nutraceutical use. *In vivo* studies need to show, which of the nanocarriers systems finally has to be preferred.

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