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Lutein nanocrystals as antioxidant formulation for oral and dermal delivery

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ABSTRACT

Lutein is a well known antioxidant and anti-free radical used in cosmetic, nutraceutical industry with potential application in pharmaceutics as supportive antioxidant in treatments. As lipophilic molecule it is poorly soluble in water and has a low bioavailability. Lutein nanosuspension was prepared to enhance dissolution velocity, saturation solubility (C_s), which are major factors determining oral bioavailability and penetration into the skin. High pressure homogenization (HPH) was used to prepare lutein nanosuspension. Particle size was determined by photon correlation spectroscopy (PCS) and laser diffractometry (LD). The lowest PCS diameter obtained was about 429 nm, the LD diameter 90% of 1.2 μm. The zeta potential was about −40 mV in water and −17 mV in the original dispersion medium. The 3 month storage study at different temperatures (4 °C, 25 °C, 40 °C) confirmed physical stability despite the low zeta potential of −17 mV in original surfactant solution. A pronounced increase in saturation solubility by 26.3 fold was obtained for lutein nanocrystals compared to coarse powder. The lutein nanosuspension was converted into pellets and filled into hard gelatin capsules for nutraceutical use, showed a superior *in vitro* release (factor of 3–4). Lyophilized nanosuspension was prepared for subsequent incorporation into creams and gels. The lyophilized nanosuspension was very well re-dispersible (435 nm). Using cellulose nitrate membranes as *in vitro* model, permeation through this barrier was 14× higher for lutein nanocrystals compared to coarse powder. However, pig ear skin did not allow lutein to permeate but supported localization of the lutein in the skin where it should act anti-oxidatively.

1. Introduction

Lutein is a well-known antioxidant and free radical scavenger, able to protect human body from different types of dangerous free radicals (Roberts et al., 2009). Lutein is also able to filtrate the blue light which is known to induce photo-oxidative damage by generating reactive oxygen species (ROS). Thereby it provides protection for skin or eye from photo damage (Alves-Rodrigues and Shao, 2004). Free radicals affect negatively several body functions by attacking the cell membranes and producing harmful compounds known as lipofuscins. They also interfere with the ability of cells to reproduce themselves, disturb DNA and RNA synthesis, inhibit protein synthesis and destroy cellular enzymes, leading to an increased breakdown of collagen and elastin eventually causing wrinkles and skin aging (Dayan, 2008).

Many studies describe the benefits from lutein and its isomer "zeaxanthin" via oral administration or after topical application. They are able to reduce the risk of ocular diseases (Bartlett and Eperjesi, 2004; Landrum and Bone, 2001; Moeller et al., 2008) and provide protective effect against cardiovascular diseases and stroke (Asplund, 2002; Hak et al., 2004). Oral treatment with the carotenoids lutein and zeaxanthin leads to a carotenoid deposition in the skin (Lee et al., 2004; Wu et al., 2002). Palombo et al. (2007) reported that oral and topical application of lutein is able to increase the elasticity and hydration of the skin by reducing the peroxidation process of skin lipids and increasing the superficial skin lipids.

Lutein as other carotenoids is a lipophilic molecule and poorly soluble in water. Poorly soluble drugs/actives have delivery problems (Müller and Keck, 2004), i.e. low dissolution velocity causing low oral and dermal bioavailability (Aungst, 1993; Teeranachaideekul et al., 2008). Nanonization improves the solubility properties by reducing the drug particle size into the nano (sub-micron) range. This increases saturation solubility (C_s), dissolution rate (dc/dt) and subsequently bioavailability and surpasses many problems related to the formulation of poorly soluble drugs (Chingunpituk, 2007). High pressure homogenization and milling techniques are mainly used for production of nanosuspensions, they are generally applicable to most drugs/actives, yielding high concentrated suspensions and can be scaled up (Verma et al., 2009).

In this study, the feasibility of production of lutein nanosuspensions was investigated, using HPH. The aim of the nanocrystal

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production was to have formulations with increased dermal penetration and oral absorption. In this study, the nanosuspensions were converted into a dry product by incorporation into pellets. Lyophilized powder was produced for admixing to dermal formulations.

2. Materials and methods

2.1. Materials

Lutein powder 90% was a gift sample from Rui Heng Industry Co. Limited (China), the emulsifier Plantacare® 2000 UP (Decyl Glycoside) from Cognis GmbH (Germany); Freshly prepared double distilled and ultra purified water was prepared with a Milli-Q system (Millipore GmbH, Germany); 0.9% sodium chloride solution was provided by B. Braun Melsungen AG (Germany). Buffer solution (pH = 1.2) was prepared by mixing 50 ml of 0.2 M potassium chloride + 85 ml of 0.2 M hydrochloric acid (Sigma–Aldrich, Germany). Tetrahydrofluran (analytical grade) was also obtained from Sigma–Aldrich. Trehalose and lactose were purchased from Fisher Scientific (NJ, USA). Hard gelatin capsules (size 1) were obtained as free sample from Capsugel Bornem, Belgium.

2.2. Methods

2.2.1. Preparation of lutein nanosuspension (Lu-HPH)

Lutein nanosuspensions were prepared by high pressure homogenization (HPH) in which particle size is reduced by cavitation, high shear forces and particle collision in and after leaving the homogenizing gap (=high energy technique) (Shegokar and Müller, 2010).

Lutein nanosuspension was produced by using a Micron LAB 40 (batch size 40 ml, APV Deutschland GmbH, Germany). The formulations contained 5.0% (w/w) lutein powder 90% as active ingredient and 1.0% (w/w) Plantacare® 2000 UP as stabilizer dispersed in Milli-Q water (MQW). The macrosuspension suspension was prepared by suspending the coarse lutein powder in the aqueous surfactant solution at 5 °C under moderate stirring and then using an Ultra Turrax T25 (Janke and Kunkel GmbH, Germany) for 1 min at 8000 rpm. The obtained coarse suspension was then subjected to pre-milling at low and moderate pressures (of 200, 500, 1000 bars, 2 cycles each). The homogenization at high pressure of 1500 bar was performed applying 25 homogenization cycles to obtain the final product. Particle size was analyzed after completion of the pre-milling and after 1, 5, 10, 15, 20 and 25 cycles.

2.2.2. Characterization of lutein nanosuspensions

The particle size analysis was performed by using dynamic light scattering and static light scattering techniques.

2.2.3. Photon correlation spectroscopy (PCS)

A Zetasizer Nano ZS (Malvern Instruments, UK) was used to measure the z-average (mean intensity-weighted particle size) and the polydispersity index (PdI) as a measure of the width of size distribution. A total of ten measurements were performed at 25 °C after diluting each sample in bidistilled water. The measuring range of a Zetasizer is approximately from 0.6 nm to 6 μm. Thus, to detect possible larger particles, laser diffraction (LD), with a measuring range up to 2000 μm was also employed as additional characterization method.

2.2.4. Laser diffraction (LD)

A Mastersizer 2000 (Malvern Instruments, UK) was used to measure the particle size in the micrometer range. The volume weighted diameters 50% $d(0.5)$ and 90% $d(0.9)$ were used as characterization parameters. The diameter values indicate the percentage of particles (50%, 90%) possessing a diameter equal or lower than the given value. The $d(0.9)$ is a sensitive parameter to quantify the presence of larger sized particles, like aggregates or large crystals. All parameters have been analyzed by using as optical parameters 1.52 for the real refractive index and 0.1 for the imaginary refractive index.

2.2.5. Zeta potential (ZP)

Zeta potential expresses the charge of the particles. In this study, the ZP was measured by using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) which measures the electrophoretic mobility of the particles in an electrical field, which is then converted into the zeta potential. Two different aqueous solutions were used to determine zeta potential: conductivity adjusted distilled water of 50 μS/cm using sodium chloride solution (0.9%, w/v) and the original dispersion medium containing 1.0% surfactant. Total 10 measurements were performed and the mean of them calculated.

2.2.6. Short term stability study

A short term stability study was carried out to examine the physical stability of the nanosuspensions. Lutein nanosuspension was stored at three different temperatures (4 °C, room temperature (RT) and 40 °C) for 3 months. The particle size analysis and zeta potential measurements were performed on the day of production, followed by 1st,7th, 30th and 90th day.

Light microscopy (Ortophlan, Germany) was performed to analyze the presence of large crystals or aggregates at 600 fold magnification.

2.2.7. Determination of saturation solubility

One of the interesting features of nanosuspension is the increased solubility of poorly soluble drugs/actives (Keck and Müller, 2006). The saturation solubility was analyzed for coarse lutein powder (LP, 25 mg) and lutein nanosuspension (0.5 ml) at 25 °C. The pH-adjusted distilled water (pH 5.5) was added to the lutein nanosuspension to get a final concentration of 0.05% (w/w) of plantacare®2000 UP instead of initial 1% in the lutein nanosuspension (0.5 g nanosuspension + 9.5 g water). To study the saturation solubility of LP two different aqueous phases were used viz. distilled water (pH = 5.5) and distilled water containing surfactant solution (0.05% w/w Plantacare®2000, pH = 10.5), maintaining in each studied preparation the final weight of 10 g. The surfactant solution was used for the determination of the LP saturation solubility to assess an increase by the effect of the surfactant used in lutein nanosuspension during preparation (=solubilization). The vials were closed to avoid water evaporation and kept on a thermostatic shaker in the dark (Innova™ 4230, New Brunswick Scientific Co., Inc., USA) at 100 rpm. Sampling was performed after 1, 4 and 7 days.

The collected samples were subjected to centrifugation at 23,800 × g for 2 h, the supernatant was separated and again centrifuged using above conditions to get rid of any possible nanocrystals. In addition, the second supernatant was filtrated through cellulose acetate filter (0.2 μm) and the first drops were discarded. The lutein content in the sample was analyzed using a UV-spectrophotometer (UV-1700 PharmaSpec, Schimadzu, Japan) after dilution of the sample with a mixture of water and tetrahydrofuran (1:9 w/w). The test was performed in triplicate and the mean calculated.

2.2.8. In vitro permeation study

In vitro permeation of coarse lutein powder and nanosuspension through a synthetic cellulose nitrate membrane (0.1 μm) and through freshly obtained dermis of pig ear skin was studied using vertical Franz glass diffusion cells at 32 ± 2 °C. The diffusion area of the Franz cell was 0.636 cm^2 and the receptor chamber capacity of

6–6.1 ml. A mixture of water/ethanol (1:9) (v/v) was used as permeation medium. The composition of the receptor medium was chosen due to insufficient solubility of lutein in pure water and its solubility in this chosen receptor medium. The magnetic stirrer rotated at 500 rpm. About 300 μL of each preparation (coarse powder dispersed in surfactant solution and nanosuspension, both containing 5% lutein) was spread on the surface of the membrane or skin. Aliquots of 300 μL were taken from each receptor chamber and immediately replaced with fresh receptor medium at selected time intervals of 1, 2, 4, 6 and 24 h. The permeation studies were performed in triplicate for each type of membrane and collected samples were analyzed spectrophotometrically at 450 nm.

2.2.9. Conversion of lutein nanosuspension into dry dosage forms

In order to prepare formulations for oral administration, lutein nanosuspension was converted into dry forms viz. pellets and lyophilized powder which can be directly filled in capsules for nutraceutical use.

2.2.10. Preparation of pellets

A 2 g of lutein nanosuspension (5% w/w) or 2 g of coarse lutein powder suspension (5% w/w) was slowly admixed to lactose powder (10 g) using mortar and pestle. The formed paste was then passed through a mesh. The obtained granules were dried at 30 °C for 1 h and subjected to spheronization in a spheronizer (Caleva spheronizer model 120, England) to yield spherical pellets. The formed pellets were dried in an oven at 30 °C for 12 h. The pellets were sieved to remove any fine powder fraction and were filled into hard gelatin capsules. Particle size after redispersion of pellets was measured using PCS. An *in vitro* release study was carried out for capsules.

2.3. Dissolution test

The dissolution test was performed for lutein nanosuspension pellets and coarse LP filled in capsules using a USP II rotating paddle apparatus Pharmatest PTW SIII (Pharma Test, Germany) at 37 °C at rotating speed of 100 rpm in 500 ml of buffer (prepared by mixing 50 ml of 0.2 M KCl + 85 ml of 0.2 M HCl, pH = 1.2). The hard gelatin capsules containing lutein nanosuspension pellets or lutein powder pellets (corresponding to 15 mg pure lutein) were placed in the basket. The samples were withdrawn from the dissolution medium at selected time intervals of 5, 15, 30, 45, 60, 90, 120 and 180 min. Aliquots were filtered through 0.2 μm filter and assayed by UV-spectrophotometer as described above.

2.4. Lyophilization of lutein nanocrystals

Lutein nanosuspension was lyophilized using a Christ Gamma 2-20 lyophilizer (Gamma 2-20, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) in presence and absence of a cryoprotectant. Trehalose was selected as cryoprotectant at 3% (w/w) concentration. Primary drying was carried out at −25 °C and 0.250 mbar for 24 h and secondary drying for 8 h at −25 °C and pressure of 0.025. Particle size measurements were performed after redispersion (using ultrasound for 5 min) of the lyophilizate in distilled water using PCS.

3. Results and discussion

3.1. Preparation of lutein nanosuspension

In this study, HPH was used to produce lutein nanosuspensions. Fig. 1 (upper) shows the changes in the mean PCS particle size (Z-Avr.) and the polydispersity index (PdI) at increasing number of homogenizing cycles. After pre-milling, which is considered as an important step to crush the big particles and to avoid the blocking of the homogenizer gap (Mishra et al., 2009), the Z-Avr. was about 560 nm with a PdI of 0.84. Only a very small further decrease in the particle size to 429 nm with a PdI = 0.37 occurred up to 25 homogenizing cycles. This clear decrease in the PdI reflects an increase in sample homogeneity, i.e. a narrower size distribution. This was also reflected by the LD data (Fig. 1, lower). The diameter *d*(0.9), a very sensitive parameter toward larger particles showed also a clear decrease. After the pre-milling the diameter was 2.852 μm, it reduced to 1.240 μm after 25 cycles. The *d*(0.5), which generally is comparable to the PCS diameter characterizing the bulk of particles (Al Shaal et al., 2010), showed practically no change between the first homogenizing cycle at 1500 bar (222 nm) and the 25th cycle (205 nm). In summary, the maximum dispersivity was already reached after pre-milling; obviously the lutein is a relatively soft material. For many other nanocrystals, e.g. rutin and hesperetin, it took about 30 cycles at 1500 bar to get a similar size (Mauludin et al., 2009; Mishra et al., 2009). The applied 25 homogenizing cycles at 1500 bar mainly reduced the number of large particles (over 1 μm). There was still a further decrease in the diameter *d*(0.9) from cycle 20 to 25, but no noticeable effect on the small particles of the bulk population (below 1 μm), i.e. the diameter *d*(0.5).

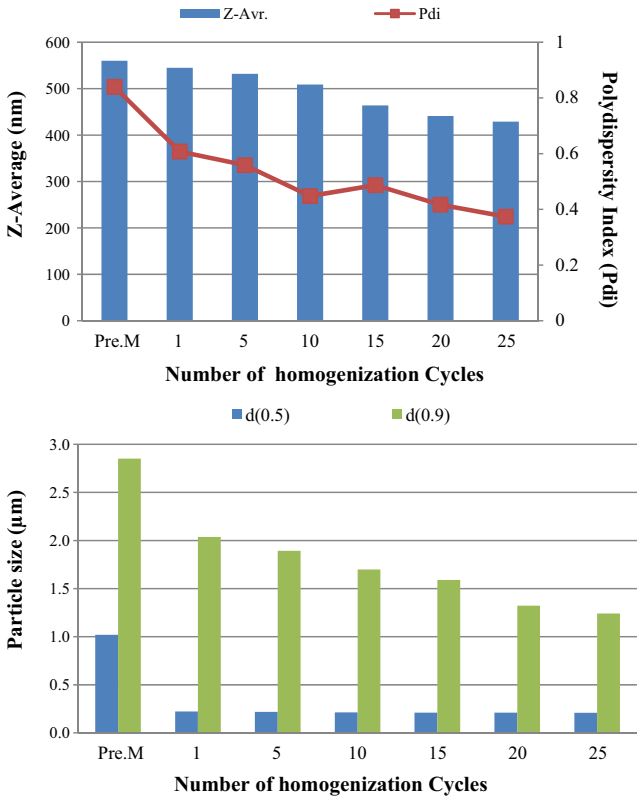

Fig. 1. Decrease in particle size of lutein as function of pre-milling (Pre. M) and number of homogenization cycles 1–25 measured using PCS (upper, Z-average and polydispersity index (PdI)) and LD (lower, diameters 50% (*d*(0.5)) and 90% (*d*(0.9)).

3.1.1. Characterization of lutein nanosuspension

3.1.1.1. Zeta potential (ZP) measurements. The measurement of the zeta potential of suspensions and emulsions gives a good estimation about formulation stability (Müller, 1996). The zeta potential was measured in both original dispersion medium and in distilled water (conductivity adjusted to 50 μS/cm) to fully describe the charge condition of the particles (Table 1). Measurement in conductivity adjusted water instead of distilled water avoids variations in ZP values due to slight changes in water conductivity from day to day.

Table 1
Zeta potential of lutein nanosuspension measured in conductivity adjusted water (50 μS/cm) and in the original dispersion medium on the day of production and during storage at room temperature.

Time (days)	Zeta potential (mV)	
	Water (50 μS/cm)	Original dispersion medium
0	−43.4 ± 1.07	−16.6 ± 0.30
1	−43.3 ± 0.78	−16.9 ± 0.44
7	−37.3 ± 0.81	−18.6 ± 1.32
30	−44.9 ± 0.95	−16.7 ± 0.41
90	−44.7 ± 3.70	−17.2 ± 0.95

In conductivity adjusted water, the nanosuspension exhibited a high ZP value of −43.4 ± 1.07 mV, which is equivalent to the Stern potential. Such a high Stern potential indicates a high surface charge (Nernst potential). As a rule of thumb suspension with absolute ZP values higher than 30 is considered stable (Kayes, 1977; Le Roy Boehm and Fessi, 2000). In the original dispersion medium, the measurement of the ZP is a measure for the thickness of the diffuse layer. Therefore lower ZP values are expected compared to the ZP measured in distilled water which reflects the Stern potential (Müller, 1996). Lutein nanosuspension showed a mean ZP value of −16.6 ± 0.30 mV indicating moderate stabilization. This would provide only short time stability in case of electrostatic stabilization only (Calcinari, 1970; Carstensen et al., 1972). However, despite low zeta potential value a good stability can be obtained due to the adsorption of the non-ionic Plantacare® 2000 UP. It is a steric stabilizer providing steric stabilization in addition to the electrostatic repulsion. The adsorption layer of steric stabilizers shifts the plane of shear in the ZP measurement, yielding lower measured "artificial" zeta potentials (Verma et al., 2009). In reality, the electrostatic repulsion contribution is indeed higher than being reflected by the measured ZP value. The ZP values stayed unchanged during storage (Table 1), as it can be expected from the theory if no change in the composition and thickness of the stabilizer layer, or to the surface charges occurs.

3.1.1.2. Short term stability study. Short term stability has been performed to assess the effect of different temperatures on the physical stability of the lutein nanosuspension. Both PCS and LD showed a comparable particle size profile during the storage time. A significant increase in the particle size of all preparation was observed after 1 day, at all storage temperatures. The size further increased until day 7 after production (Fig. 2). Respect to the original particle size on the day of production, the total increase was around 36% and 50% for the LD and PCS, respectively. Obviously, there was some ripening in the system occurring over 7 days, and then the

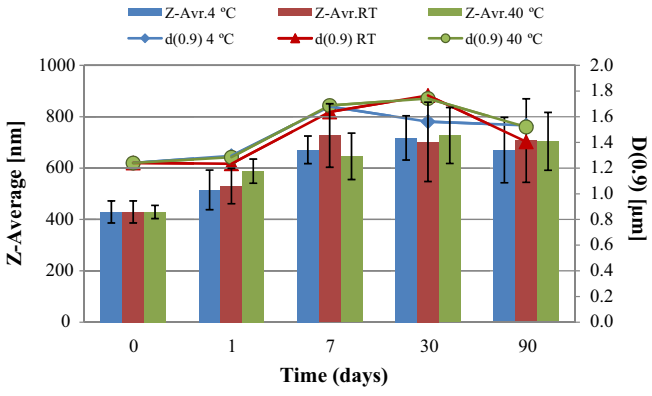

Fig. 2. Short term physical stability of lutein nanosuspension at three different storage temperatures (RT – room temperature) as function of storage time (days): PCS diameters (z-average) and LD diameter 90% *d*(0.9).

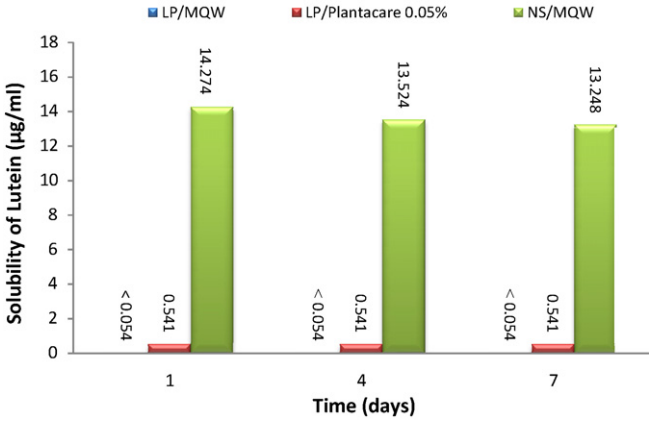

Fig. 3. Saturation solubility of coarse lutein powder (LP) in MilliQ water (MQW) and in water containing 0.05% Plantacare® 2000 UP (to check potential solubilization ability of Plantacare), and increased saturation solubility of lutein nanosuspension (NS) in MQW, as function of time at 25 °C.

nanosuspension stayed unchanged. The ripening might have been promoted by the high degree of supersaturation in the nanosuspension (see below). After day 7 until day 90 no size increase was observed, indicating good stability. In addition, zeta potential measurements were performed for formulations stored at room temperature. As outlined above, no change occurred up to 3 months (Table 1). Obviously due to the steric stabilization provided by Plantacare® 2000 UP, the ZP of around −17 mV was sufficient for the stabilization.

3.1.1.3. Saturation solubility. Fig. 3 shows the quantity of lutein dissolved in Milli-Q water from coarse lutein powder (LP, macrocrystals) and lutein nanosuspension (nanocrystals) and the quantity of lutein solubilized in an aqueous surfactant solution from LP. The maximum saturation solubility of lutein was reached after 1 day of shaking. The concentration of lutein dissolved in distilled water was below the concentration able to be detected in the spectrophotometer. For reasons of simplicity, the concentration was set to be lower than 0.054 μg/ml in the graph (=the minimum concentration that could be detected by spectrophotometry). The addition of 0.05% surfactant water to the lutein powder leads to a 10 fold increase in lutein solubility, reaching 0.54 μg/ml. Lutein nanocrystals possessed a very high increase in saturation solubility in distilled water when compared to lutein powder dissolved in distilled water (>264 folds) or solubilized in surfactant solution (>26.3 folds). This increase in kinetic lutein saturation solubility after nanonizing (up to 14.3 μg/ml) is due to an increase in dissolution pressure which shifts the equilibrium toward the dissolved molecules (Müller and Akkar, 2004).

3.1.1.4. Permeation study. Apart from an oral nutraceutical and supportive pharmaceutical application, lutein as antioxidant is also of high interest for dermal application. Therefore a comparative permeation study was performed. The enhancement in the permeation of lutein nanocrystals compared to lutein powder has been studied. The use of ethanol as receptor medium in this study even promotes the permeation of lutein through skin because of its ability to dissolve the active and solubilize the skin lipids. The usage of ethanol evaluates therefore the maximum possible permeation of lutein.

Fig. 4 shows the lutein penetration profiles through cellulose nitrate membranes. After 1 h of placing the preparation on the membrane, just 0.57% of lutein could reach the receptor medium in case of lutein powder, while about 14 times more, i.e. 8.16% in case of the lutein nanocrystals. This means an improvement of about

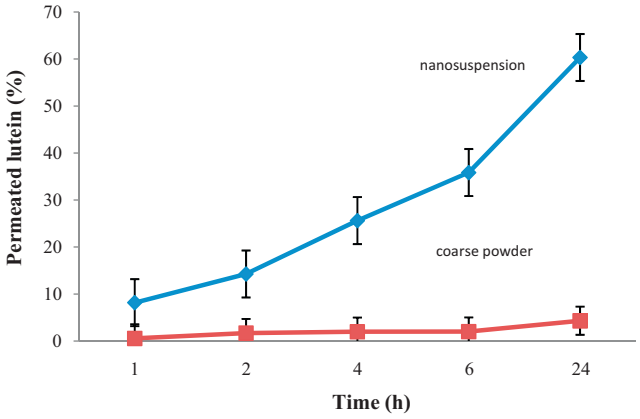

Fig. 4. *In vitro* penetration profiles of lutein nanosuspension and coarse lutein powder through a 0.1 μm cellulose nitrate membrane.

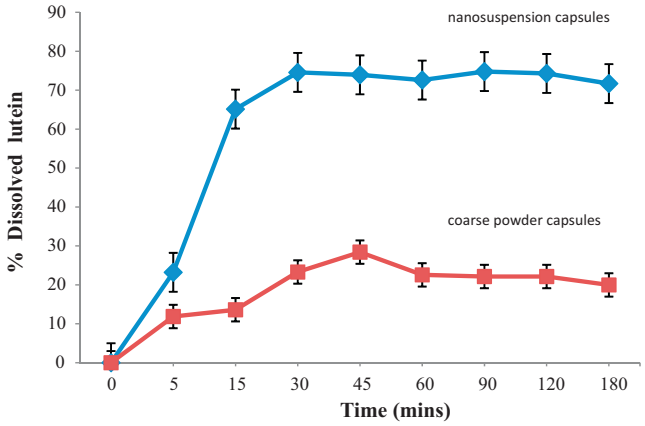

Fig. 6. Dissolution profiles of lutein from nanosuspension loaded pellets and from coarse lutein powder pellets, both filled into hard gelatin capsule.

1400% has been achieved. During time a very rapid increase in the permeated lutein from the nanocrystals occurred to about 14%, 25% and 36% after 2, 4 and 6 h, respectively. In contrast, lutein powder showed a very slow increase in lutein permeation, i.e. 1.7%, 2.0% and 2.0% at 2, 4 and 6 h, respectively. After 24 h 60% of lutein from the nanocrystals had permeated through the membrane compared to just 4% of lutein from the powder. This is due to the increases in the saturation solubility and dissolution velocity of the nanocrystals. Literature confirms that nanocrystals in topical formulations enhance the diffusion of the drug into the skin (Mishra et al., 2011; Müller et al., 1999; Shim et al., 2004).

There is high enhancement in lutein nanocrystals permeation through the synthetic membrane, but in the development of dermal cosmetic and many pharmaceutical products it is important that the applied formulation shows a local effect without reaching the blood circulation (Müller et al., 2002). Therefore, a permeation study using the dermis of pig ear was done. The results of this study does not show any traces of lutein in the receptor medium, which could indicate that the active penetrates into the skin, but not permeate through the skin.

3.1.1.5. Conversion of lutein nanosuspension into pellets. An aqueous nanosuspension of lutein was directly used as granulation fluid for preparing pellets by extrusion. Lactose was used as only excipient as pellet matrix. By avoiding water insoluble excipients, the produced pellets can be dissolved in water to measure to the size of the nanocrystals after redispersion (=proof of lack of aggregation during pelletization). There are no disturbing particles from insoluble excipients. The lutein pellets were disintegrated in water and particle size was determined by the PCS. An increase in the particle size was noticed from 530 nm (*z*-average value of nanosuspension after 1 day of preparation) to 679 nm, PdI = 0.46. That could be due to some aggregations created during the pelletization process. Further optimization should be able to reduce this, but this optimization was not the focus of the present study. The nanocrystals are still in the nano size range, and should have a clear superior performance in dissolution compared to the powder. The formed pellets were filled into hard gelatine capsules (Fig. 5).

3.1.1.6. In vitro dissolution study. The lutein nanocrystal pellets and the coarse lutein powder filled in capsules were subjected to an *in vitro* dissolution study. Fig. 6 shows the dissolution profiles of capsules containing pellets loaded with nanosuspension and with lutein powder. Within the first 5 min almost double the quantity of lutein was dissolved when nanocrystal loaded pellets were used (23.2%) compared to lutein powder loaded pellets (11.8%). After 30 min more than 3 times was the enhancement in lutein dissolution for the nanocrystals (74.6%) compared to the lutein powder (23.31%). Taking in consideration that the particle size of the nanocrystals (530 nm, measured after pellet dissolution) is very small compared to the coarse powder ($d50\% = 8.5$ μm, $d90\% = 35$ μm), the obtained results are in line with the Noyes–Whitney equation describing the dissolution velocity dc/dt. According to the Noyes–Whitney an enlargement of the surface area (i.e. decrease in size) in combination with increased saturation solubility leads to an increase in the dissolution velocity (List et al., 1982; Müller et al., 2001).

Fig. 5. Photograph of golden colored lutein nanosuspension loaded spherical lactose pellets (left) and pellets filled in capsules (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.1.1.7. Lyophilization of lutein nanosuspension. Lutein nanocrystals lyophilized in the presence of cryoprotectant showed after redispersion a mean particle size of 435 nm, with a PdI of 0.34, a slightly larger size of 532 nm (PdI = 0.453) without cryoprotectant. No major increase in particle size occurred during lyophilisation compared to the original preparation (429 nm). Converting aqueous nanosuspension into dry forms can enhance chemical and physical stability of the active ingredient (Teeranachaideekul et al., 2008). In addition, dry forms of lutein nanosuspension give the possibility to formulate solid dosage form like tablets, capsules and some other nutraceutical dosage forms (e.g. sachets). Furthermore, it also facilitates the formulation of cosmetic products by a simple admixing of dry powder to pre-formulated creams or gels.

4. Conclusion

Lutein nanocrystals could be produced by high pressure homogenization with a size as low as about 400 nm just applying the pre-milling procedure. This opens the perspective of a fast production of cosmetic and pharmaceutical lutein nanocrystals, in contrast to many drugs requiring additionally 20 cycles of high pressure homogenization. Compared to the poorly water soluble powder, the lutein nanocrystals had manifold higher saturation solubility. This increases release from oral dosage forms, as shown for pellet-filled capsules, and will promote oral uptake of class II compounds of the biopharmaceutical classification system (BCS). Lutein nanocrystals are therefore a promising oral antioxidant formulation.

Higher solubility and increased surface area of the small nanocrystals accelerate also release from dermal formulations, promoting penetration of lutein as a lipophilic compound into the skin, being supported by the observed increased permeation through synthetic membranes, used as model for a penetration barrier. No permeation through pig ear skin occurred, which supports that the lutein rather penetrates into the skin and remains there where it should act as antioxidant. Before the dermal usage, further studies in skin need to investigate an extent of penetration and measure *in vivo* the antioxidative effect.

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