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Skin photoprotection improvement: Synergistic interaction between lipid nanoparticles and organic UV filters

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ABSTRACT

A photoprotective formulation was developed with an increased sunprotection factor (SPF), compared to a conventional nanoemulsion, but having the same concentration of three molecular sunscreens, namely ethylhexyl triazone, bis-ethylhexyloxyphenol methoxyphenyl triazine, and ethylhexyl methoxycinnamate. The sunscreen mixture was incorporated into nanostructured lipid carriers (NLCs). The ability of nine different solid lipids to yield stable aqueous NLC suspensions was assessed. After the production by hot high pressure homogenization, the NLC were analyzed in terms of particle size, physical state, particle shape, ultraviolet absorbance and stability. The particle size for all NLC was around 200 nm after production. The NLC suspension with carnauba wax had superior UV absorbance, NLC from bees wax showed similar efficiency as the reference emulsion. The NLC formulations were incorporated into hydrogel formulations and the *in vitro* SPF was measured. This study demonstrated that approximately 45% higher SPF values could be obtained when the organic UV filters were incorporated into carnauba wax NLC, in comparison to the reference nanoemulsion and bees wax NLC. The data showed that the synergistic effect of NLC and incorporated sunscreens depends not only on the solid state of the lipid but also on its type.

1. Introduction

Ultraviolet radiation (UV) is responsible for a wide variety of different acute and chronic effects on the skin. Acute responses of human skin to UV radiation include some positive ones, like vitamin D synthesis, but also negative ones, like erythema. Chronic UV radiation effects include photoaging and photocarcinogenesis, which is considered to be induced by induction of immunosuppression and mutations (Ullrich and Schmitt, 2000; Ullrich, 2005; Walterscheid et al., 2002). Epidemiological studies show that more than 90% of epidermal squamous cell carcinomas and more than 50% of basal cell carcinomas display UV-induced mutations (Ziegler et al., 1994).

An increase in public awareness of the harmful effects caused by UV radiation on the skin has resulted in an increased interest in products denoted as sunscreens that can offer photoprotection. The sunscreen agents are classified in organic and inorganic, the latter group being restricted to zinc oxide and titanium dioxide. The organic UV filters are broadly divided into UVA, UVB or broad spectrum absorbers. Active ingredients in sunscreens, as well as the permitted maximum concentrations, differ considerably worldwide (Schneider, 2009). Regulatory agencies in Europe and elsewhere treat sunscreens as cosmetics, therefore the regulatory approval process is faster. In the USA, sunscreen active ingredients are treated like drugs and sunscreen formulations as over the counter (OTC) products.

The lipophilic nature of many UV filters may cause bioaccumulation in humans and animals. Adverse reactions from sunscreen ingredients reported include allergic and irritant contact dermatitis, phototoxic and photoallergic reactions, contact urticaria and isolated cases of severe anaphylactic reactions (Lautenschlager et al., 2007), although many of the UV filters that could cause this kind of reactions are in disuse nowadays. So far, the most controversial issue about sunscreen safety is their possible hormonal activity, possibly related also to neurodegenerative diseases (Weiss, 2007). Organic UV filters have been claimed to be able to elicit antiestrogenic (Kunz and Fent, 2006) or antiandrogenic (Nashev et al., 2010) activity, although some UV filters and their combinations activated estrogen receptors (Schlumpf et al., 2004a,b). Therefore, sunscreen formulations that may enhance the UV protection without increasing the amount of the organic UV filters are highly desirable.

Lipid nanoparticles, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are innovative carrier systems derived from o/w emulsions (Lucks and Müller, 1991; Müller et al., in press), where the liquid lipid was replaced by a solid

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lipid or a blend of solid and liquid lipids that is solid at room and body temperature. They have been shown to be able to enhance photoprotection by synergistically combining the advantages of organic and inorganic sun screening agents (Nesseem, 2011; Villalobos-Hernández and Müller-Goymann, 2006; Wissing and Müller, 2002a) and without addition of other chemical entities. The lipids used for the production of SLN/NLC can be chosen from those that are known to be physiologically well tolerated, such as mono-, di-, and triglycerides, both, solid or liquid, from natural or synthetic origin. Traditional sunscreen products are formulated as emulsions or hydro-alcoholic solutions. When immersed in water, during swimming, or by sweating, these formulations are partially or completely removed by water, due to the presence of high concentrations of surfactants or alcohol, respectively. In order to increase the skin residence time, sunscreens are also formulated as oils that leave a very unpleasant skin feel. The lipid nanoparticles show high adhesiveness to the surfaces (Attama et al., 2009), due to their size. This may increase the skin residence time of the sunscreens and decrease the number of applications for an optimal and claimed sun protection. Furthermore, SLN/NLC can be successfully incorporated into hydrogel formulations that are surfactant-free (Doktorovova and Souto, 2009) and which can provide an appropriate consistency for SLN/NLC based formulations.

The objective of this study was to develop and optimize sunscreen formulations based on lipid nanoparticles and to improve the sun protection factor (SPF) values, while maintaining constant the concentrations of the actives. Three common organic UV filters, namely bis-ethylhexyloxyphenol methoxyphenyl triazine, ethylhexyl triazone, and ethylhexyl methoxycinnamate, were chosen as model lipophylic UV filters. The synergistic effect of the lipid nanoparticles and organic UV filters may allow reducing the concentrations of the actives. To achieve this goal, nine solid lipids were screened for particle production and UV absorbance thereafter. Formulations optimized in terms of particle size, stability, thermal behaviour, and UV absorbing properties were further incorporated into semisolid formulations and *in vitro* SPF values were assessed.

2. Materials and methods

2.1. Materials

Three organic UV filters were used: Parsol® MCX (ethylhexyl methoxycinnamate; ROCHE, Basel, Switzerland), Uvinul® T150 (ethylhexyl triazone; BASF, Ludwigshafen, Germany) and Tinosorb® S (bis-ethylhexyloxyphenol methoxyphenyl triazine; Ciba Specialty Chemicals Inc., Basel, Switzerland). PlantaCare® 2000 UP (decyl glucoside; Cognis, Düsseldorf, Germany), a non-ionic, unpreserved and dermatologically compatible surfactant, was used for the stabilizations of all the formulations. Lipids investigated included: Miglyol® 812 (caprylic/capric triglyceride; Caelo GmbH, Hilden, Germany), low odor carnauba wax (Kahl Wax, Trittau, Germany), bees wax (Caelo GmbH, Hilden, Germany), Dynasan® 118 (microcrystalline stearyl triglycerides; Sasol, Hamburg, Germany), Apifil® (PEG-8 bees wax; Gattefossé GmbH, Weil am Rhein, Germany), Syncrowax® ERLC (ethylene glycol di-ester of C18–C36 fatty acids; Croda GmbH, Nettetal Kaldenkirchen, Germany), Compritol® 888 ATO (glyceryl behenate; Gattefossé GmbH, Weil am Rhein, Germany), Softisan® 154 (hydrogenated palm oil; Sasol GmbH, Witten, Germany), Phytowax® Olive 16 L 55 (hydrogenated cetyl olive oil esters; Sophym, Peyruis, France) and Atowax (Gattefosse, Cedex, France). The ultra purified water used for all formulations was freshly prepared by a MilliQ® System (Millipore, Schwalbach, Germany). The optimized nanoparticles were incorporated into a hydrogel composed of 1% Carbopol® 940 (Caelo GmbH, Hilden, Germany), 10% glycerol (Glycerol 85%, Sigma–Aldrich, Deisenhofen, Germany), 0.3% Trizma® pre-set crystals ((tris(hydroxyl-methyl)aminomethane), Sigma–Aldrich, Deisenhofen, Germany) and preserved water ("konserviertes Wasser", according to the German Pharmacopoiea (DAB)).

2.2. Methods

2.2.1. Lipid screening

Prior to the NLC production a lipid screening was performed, in order to determine the suitability of the lipids, in terms of solubility and miscibility of the three UV filters with the selected lipids. This was done by heating the solid lipid 10° above its melting point and dissolving increasing amounts of the functional ingredients mixture (sunscreen mixture) therein. The ratio among the three actives was kept constant at 1:3:7 (Uvinul® T150:Tinosorb® S:Parsol® MCX). The obtained hot solutions were observed for transparency and phase separation, in order to assess complete solubilisation and mixing of the three UV filters with the lipids. After cooling down of the lipid–sunscreen mixtures to room temperature, the incorporation of the three UV filters was investigated by smearing a piece of the solid mixture (not in case of the mixture with Miglyol® 812) on a filter paper and observing if there were any oil spots on the filter paper that would be due to an incomplete miscibility of the liquid Parsol® MCX with the rest of the mixture, or by visually observing for the presence of crystalline actives forming a separate phase.

2.2.2. NLC production

The NLC were produced by hot high pressure homogenization, as described elsewhere (Müller and Lucks, 1996). Briefly, the lipid was heated to a temperature 5–10° higher than its melting point (in case of Mygliol® 812 to 80 °C) and the sunscreen mixture was dissolved therein, forming the lipid phase. The lipid phase was 20% of the formulation. The aqueous phase (80%), containing 2% of surfactant (PlantaCare® 2000 UP) was heated to the same temperature as the lipid phase. The hot lipid phase was dispersed in the hot surfactant solution, using an Ultra-Turrax T25 (Janke & Kunkel GmbH, Staufen, Germany) at 8000 rpm for 15 seconds, avoiding excessive foam formation. The obtained coarse emulsion was homogenized at a temperature of 5–10° higher than the melting point of the bulk lipid, using an APV Micron Lab 40 (APV Homogenizers, Unna, Germany) and applying a pressure of 800 bar and two homogenization cycles. The obtained product was transferred to silanized glass vials, sealed and let cool to room temperature, for solidification.

2.2.3. Particle size measurements

The particle size was analyzed by laser diffraction (LD) and photon correlation spectroscopy (PCS). The LD measurements were performed using a Coulter® LS 320 (Beckman-Coulter, Krefeld, Germany), equipped with polarization intensity differential scattering (PIDS) technology. The particle size was expressed as volume distribution, using the Mie theory for the raw data evaluation. The real and imaginary refractive indexes were set at 1.456 and 0.01, respectively. Water with a refractive index of 1.33 was used as measurement medium. The PCS measurements were performed by a Zetasizer Nano ZS (Malvern Instruments, Malvern, The United Kingdom). Prior to the measurements, samples were diluted with double distilled water to a weak opalescence. The average particle size (Z-ave) and polydispersity index (PI) are given as average of 3 measurements, each with 10 runs.

In case of semisolid formulations, prior to particle size measurements by LD, 1 g of each formulation was dispersed in 10 ml of double distilled water by sonication in an ultrasound bath Sonorex® Longlife RK 52 CH (Bandelin Electronic GmbH&Co. KG, Berlin, Germany).

2.2.4. NLC based semisolid formulation preparation

The NLC dispersions were added to the freshly prepared hydrogel, in a ratio 1:1, under continuous stirring at 1000 rpm for 3 min by a high speed stirrer (CitoUnguator, Konictzko, Bamberg, Germany).

2.2.5. Thermal analysis

Differential scanning calorimetry (DSC) analysis was performed in order to determine the melting temperatures of the bulk lipids, bulk lipid–actives mixtures and NLC, and to confirm the integrity of the particles in the semi-solid formulations. NLC dispersions or semi-solid formulations corresponding to approximately 2 mg of solid lipid content were accurately weighed and sealed in 40 μl or 160 μl aluminium pans and heated from 20 to 95 °C at a heating rate of 10 K/min under constant flushing with nitrogen (80 ml/min), using a Mettler DSC 821e (Mettler Toledo, Gießen, Germany).

2.2.6. X-ray diffraction analysis

Wide angle X-ray diffraction patterns of selected NLC and semi-solid formulations were obtained using a Philips PW 1830 X-ray generator (Philips, Amedo, The Netherlands) with a copper anode and a PW18120 goniometer as a detector. Data of the scattered radiation were collected for 2θ values from 0.6° to 40°. For the analysis of NLC and the semi-solid formulations, the product to analyze was previously mixed with locust bean gum (1 g of formulation mixed with approximately 1 mg of gum), to obtain a thick paste for eliminating diffusion/movement of the particles.

2.2.7. Ultraviolet spectroscopy

Ultraviolet spectral analysis of the NLC and a reference o/w emulsion was performed using a spectrophotometer PharmaSpec UV-1700 (Shimadzu GmbH, Duisburg, Germany), in the range 200–400 nm. Before measuring, the samples were diluted with purified water. Particle size of the samples were comparable, therefore the results were not jeopardized by differences in light scattering.

2.2.8. Sun protection factor assessment

A Labsphere® UV-1000S ultraviolet transmittance analyzer (Labsphere Inc., North Sutton, NH, USA) was used to evaluate SPF *in vitro* of the semi-solid formulations. In accordance with the European Cosmetics Association (COLIPA) recommendation, 2 mg/cm^2 of the test formulation was applied on the Transpore™ tape (3 M Company, St. Paul, MN, USA) as substrate. The tape was placed on a free standing film holder. The transmittance was measured in 12 different points and the results are presented as average values. The quality of sample preparation, i.e. the uniformity of the product layer on the substrate, was evaluated analyzing the coefficient of variance (COV), considering acceptable just the SPF results with corresponding COV ≤ 5%.

2.2.9. Scanning electron microscopy

The morphology of the optimized NLC was investigated by scanning electron microscopy, using a Philips XL30 microscope (Philips Electron Optics, Eindhoven, The Netherlands). The samples were prepared by placing a droplet of the NLC suspension onto an aluminium specimen stub, let dry for 12 h and sputter coated with gold at 20 mA for 4 min, using an EMITECH K-550X sputter coater (Quorum Emitech, Ashford, Kent, The United Kingdom).

3. Results and discussion

A pre-requisite for a successful entrapment of a drug or a cosmetic active ingredient into an NLC formulation is its adequate solubility or miscibility with the lipid. Therefore the nine chosen lipids were screened in two different weight ratios to the UV filter mixture, namely 20:80 and 40:60 lipid to sunscreen mixture.

Table 1
Melting onset and melting peak temperatures for physical mixtures of the nine lipids and the actives, in two ratios 20:80 and 40:60 lipid to sunscreen mixture.

Lipid	Lipid/actives ratio	Melting onset (°C)	Melting peak (°C)
Carnauba wax	20:80	39.8	75.5
Carnauba wax	40:60	53.7	78.1
Bees wax	20:80	72.1	56.7
Bees wax	40:60	75.6	59.1
Softisan®	20:80	42.3	47.1
Softisan®	40:60	49.8	51.6
Atowax®	20:80	41.9	53.5
Atowax®	40:60	55.1	57.9
Compritol®	20:80	43.8	55.7
Compritol®	40:60	62.1	63.5
Apifil®	20:80	31.5	39.2
Apifil®	40:60	31.8	43.8
Dynasan®	20:80	53.9	61.2
Dynasan®	40:60	64.1	65.6
Phytowax®	20:80	44.5	47.8
Phytowax®	40:60	53.8	54.7
Syncrowax®	20:80	35.9	55.6
Syncrowax®	40:60	61.5	63.1

After a heating to temperatures 10° higher than the melting point of the lipid, all the hot solutions were clear, indicating full solubilization of Tinosorb® S and Uvinul® T150. After the solidification of the mixtures, no phase separation was detected. This indicated that the liquid component of the sunscreen mixture, i.e. Parsol® MCX, was fully miscible with the other components, in the given ratios. No crystals were detected, indicating that the other two sunscreens were dissolved in the solidified lipid blend.

A further step in pre-formulation was the determination of the melting point of the mixtures. The results of DSC scans for all the 18 blends are given in Table 1.

Based on melting point temperatures of the 18 blends, 16 NLC formulations were prepared. Apifil® was excluded from further studies, due to the melting temperatures of the blends with this lipid being below 40 °C. A melting point above body temperature is the general requisite for application of lipid nanoparticles, guarantying their solid nature at the body temperature. The melting onset of the Apifil was at about 32 °C, i.e. at the skin temperature. The NLC formulations were produced containing 20% of lipid phase. The rationale behind selecting such a high lipid concentration was the necessity of diluting the NLC formulations during further processing, i.e. admixing to a gel, to yield a semi-solid formulation. As it can be seen from Table 2, with the selected processing condi-

Table 2
Mean particle size (PCS, Z-ave) and polydispersity index (PI) of all the developed NLC formulations, one day and thirty days after the production. The formulations presenting phase separation in form of a cake were not analyzed for size (–).

Lipid	Lipid/actives ratio	Z-ave		PI	
		Day 1	Day 30	Day 1	Day 30
Atowax®	20:80	176.7	178.7	0.132	0.141
Atowax®	40:60	179.5	185.3	0.158	0.163
Compritol®	20:80	177.7	182.6	0.134	0.137
Compritol®	40:60	192.3	198.0	0.146	0.089
Softisan®	20:80	196.6	–	0.144	–
Softisan®	40:60	191.0	–	0.164	–
Dynasan®	20:80	205.9	–	0.168	–
Dynasan®	40:60	200.7	–	0.147	–
Phytowax®	20:80	205.4	–	0.202	–
Phytowax®	40:60	181.6	–	0.129	–
Syncrowax®	20:80	187.4	–	0.163	–
Syncrowax®	40:60	188.4	–	0.137	–
Bees wax	20:80	183.2	183.8	0.145	0.149
Bees wax	40:60	174.7	178.1	0.120	0.155
Carnauba wax	20:80	201.0	204.7	0.177	0.145
Carnauba wax	40:60	203.2	203.1	0.202	0.158

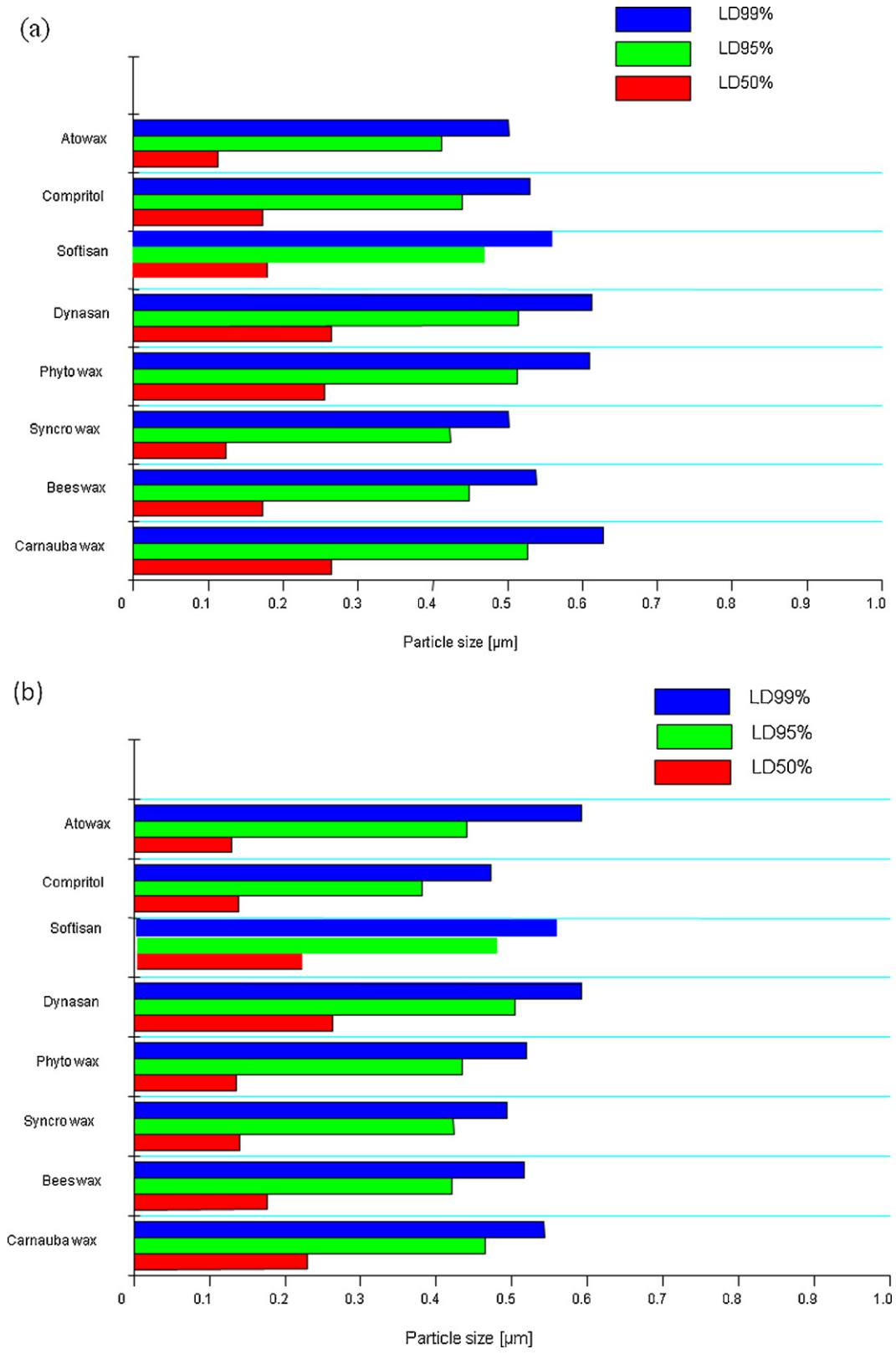

Fig. 1. Particle size (LD) of the NLC dispersions with lipid to actives ratio 20:80 (a) and 40:60 (b). The active is the sunscreen mixture.

tions particles with a mean diameter around 200 nm and a PI lower than 0.2 were obtained for all lipids, measured one day after the production.

The particle size was confirmed by laser diffractometry. The LD50% diameter was well below 0.3 μm and LD99% below 0.65 μm for all the formulations, as depicted in Fig. 1a and b, being well in agreement with the PCS data.

The particle size may change over shelf life. Therefore, the formulations were stored and analyzed again one month after the production. While the suspensions based on Atowax®, Compritol®,

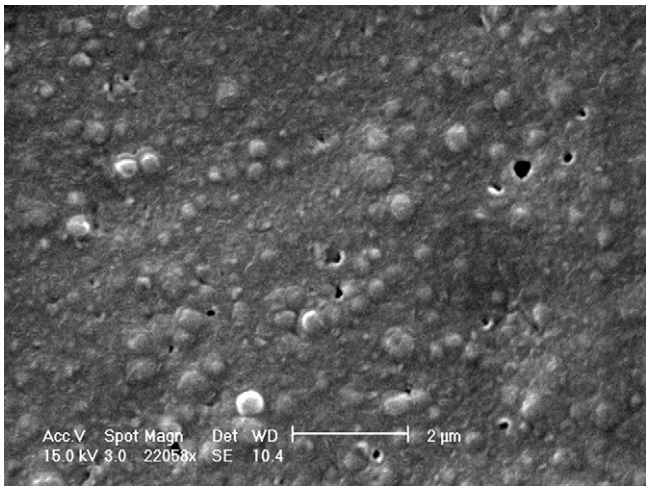

Fig. 2. Scanning electron micrograph of NLC formulation based on carnauba wax, lipid to actives ratio 40:60, six months after production.

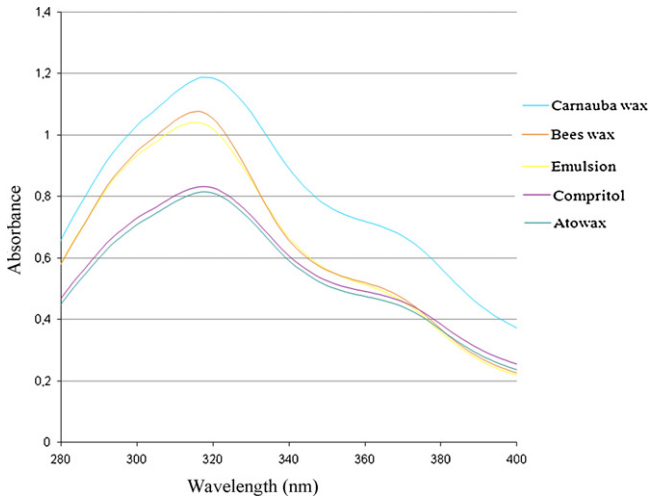

Fig. 3. Comparison of UV absorbance of four NLC formulations and a reference emulsion, lipid to actives ratio 40:60.

bees wax and carnauba wax remained stable, with almost unchanged sizes (Table 2), the other NLC suspensions showed phase separation, forming a compact cake. The lipid to actives ratio did not have any influence on stability, indicating that the type of lipid is crucial for long term stability of NLC. Different temperatures of storage (4 °C and 40 °C) had no influence on the formulation stability (data not shown). Furthermore, scanning electron microscopy was used to assess particle morphology of the NLC formulation based on carnauba wax, six months after the production. Due to the presence of the liquid component, i.e. Parsol® MCX, the preparation of the sample resulted in partial disruption of the particles. Nevertheless, spherical particles of diameter not larger than 600 nm are visible in Fig. 2, well in accordance with LD data.

It has been reported that the solid nature of the lipid nanoparticles may offer a synergistic effect in terms of UV absorbance (Villalobos-Hernández and Müller-Goymann, 2006; Wissing and Müller, 2002a,b), when compared to traditional sunscreen formulations, such as o/w emulsions. Therefore, the stable NLC formulations with lipid to actives ratio of 40:60 were further analyzed for UV absorbance in UVA and UVB range and compared with reference emulsions with analogue compositions, where the solid lipid was substituted by Miglyol® 812. The emulsions were prepared following the same procedure of production of the NLC, in order to minimize any differences due to the production process itself. The nanodroplets had PCS sizes comparable to the NLC. The reference emulsions were freshly prepared prior to the UV scans and their long term stability was not investigated.

In Fig. 3 significant differences in UV absorbance can be observed among the formulations. With the same concentration of the three UV filters and just changing the lipid, the highest absorbance was measured for the sample containing carnauba wax.

It is well in accordance with what was reported by Villalobos-Hernández and Müller-Goymann (2005, 2007) who detected a certain degree of intrinsic UV filtering properties of carnauba wax. The UV absorbance of the bees wax based formulation was comparable with the one of the reference emulsion, while Atowax® and Compritol® showed significantly lower absorbance compared to the rest of the formulations. These data indicate that the solid lipid influences the UV behaviour of the carrier system and that a lipid screening is necessary when optimizing an NLC based sunscreen formulation, not only in terms of formulation stability, but also in terms of UV absorbance. The differences observed with the four lipids may be also due to different distribution of the actives within the particles. This is described by Müller et al. (2002) as three models of incorporation of active ingredients into lipid nanoparticles. When cooling to room temperature, the lipid and the active ingredients blend can yield particles with the active enriched in the core, enriched in the shell or homogeneously distributed throughout the matrix (=3 models). The particles containing carnauba wax would correspond to a shell enriched structure and the interaction of the particulate carrier with the UV rays would be bivalent, i.e. UV filtering due to the presence of the organic UV filters and scattering of the UV rays due to the solid state of the particles, making them behave similarly to physical sunscreens. In case of nanoemulsions, the measured UV absorbance is only due to filtering of the UV radiation, as there is no solid component.

For the particles containing Atowax® and Compritol®, the core enriched model may be a plausible explanation. Most of the lipid may be concentrated in the outer part of the particle, preventing the contained organic UV filter form interacting with the UV rays. In this case, the UV absorbance would result mostly from scattering, rather than from filtering of the UV rays. For the bees wax based NLC, the portion of the organic filters entrapped in the deeper parts of the particles would be counterbalanced by the scattering, due to the solid nature of the carrier, yielding particles corresponding to the matrix model and absorbance comparable with the reference emulsion.

Conventional NLC dispersions contain up to 20% of lipid content and are liquid, although the preparation of semisolid nanoparticle dispersions with up to 60% of lipid has been described (Lippacher et al., 2000, 2001, 2002). The low viscosity of liquid NLC formulations, approximately 100 mPa s, and yield values of practically zero (Freitas, 1998) make them inappropriate for direct topical application. The advantage of using highly concentrated and rheologically acceptable nanoparticle dispersions is their one-step preparation by hot high pressure homogenization. However, in case of lipids with very high melting point, such as carnauba wax, the homogenization is tedious if formulating suspensions with more than 20% of lipid phase. Another important parameter in topical formulation development is also the skin-feel during application and whether the formulation can be easily and evenly spread on the skin. In case of sunscreen formulations the spreadability becomes of extreme importance. The claimed sun protection factor is correct only if the consumer can easily apply 2 mg of the formulation per 1 cm^2 of skin. Semi-solid formulations containing larger particles, such as microparticles, can be easily spread. The developed NLC formulations are characterized by very small particle size and,

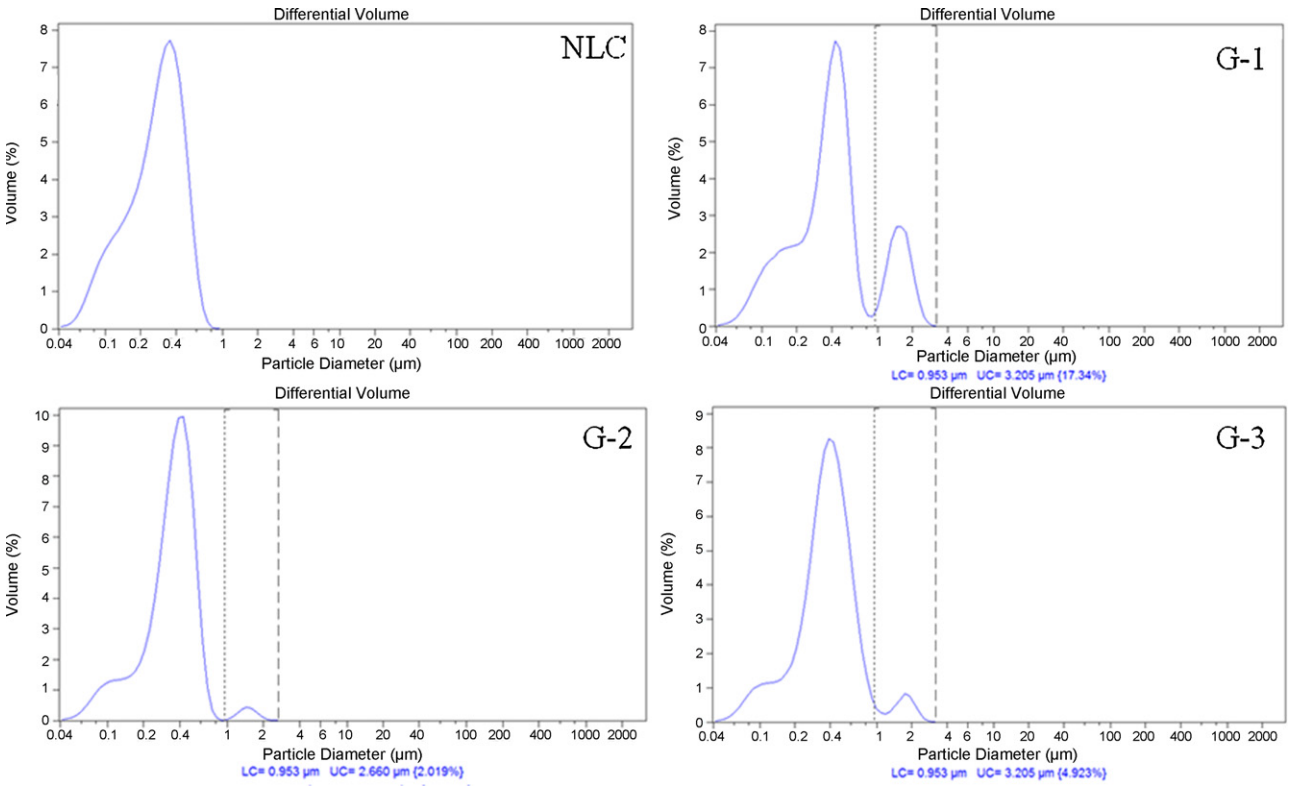

Fig. 4. Particle size (LD) distribution of the carnauba based NLC formulation (lipid to actives ratio 40:60) and after incorporation into the three semi-solid NLC based formulations G-1, G-2 and G-3, measured one day after production.

consequently, high adherence to the skin and potentially less even distribution on it.

To circumvent this problem, the NLC dispersions can be incorporated into an already established semi-solid formulation with optimal lubricity. This can be done by incorporating the NLC into a hydrogel. In general, hydrogels have a number of advantages, such as low or no toxicity, unique physical properties, biocompatibility, absence of surfactants and low price. In case of admixing NLC, special care has to be taken regarding the nature of the gelling agent. Some of them contain polar and sometimes charged groups, which can cause changes in particle's zeta potential and consequently influence negatively their stability (Souto et al., 2004). It has been reported that the particle size, their aggregation and polydispersity are strongly dependent on the gelling polymer used (Jenning et al., 2000). Among different gelling agents, carbomers (polyacrylate polymers) have a wide range of applicability in the pharmaceutical and cosmetic fields. For the purpose of this study, with the aim of assessing particle stability upon incorporation of NLC into hydrogels, the selected NLC formulation containing carnauba wax (lipid to actives ratio of 40:60, total lipid content in the NLC formulation 20%) was incorporated into freshly prepared gel based on Carbopol® 940, to yield three semi-solid formulations: G-1 (total NLC content 14.3%), G-2 (total NLC content 25.0%) and G-3 (total NLC content 33.3%). In order to exhibit gel forming properties, the carboxylic acid groups have to be neutralized. A stronger aggregation was reported (Jenning et al., 2000) when carbomer was neutralized with sodium hydroxide compared to tromethamine, used in this study.

One day after production and storage at room temperature, the gels were diluted with double distilled water to weak opalescence and the particle size was analyzed. The obtained LD size distribution graphs are given in Fig. 4.

LD measurement of the carnauba based NLC prior to incorporation into gel showed that all the particles were in submicron size range. Since the developed NLC based hydrogels contain besides NLC only water, glycerol and the gelling agent, laser diffraction signals can be attributed to NLC. The G-1, that contained the lowest amount of NLC surprisingly showed the highest percentage (17.3%) of particles larger than 1 µm. For the other two formulations, G-2 and G-3, the percentages were much lower, 2.0% and 4.9%, respectively. This increase in apparent particle size is more likely to be due to a stronger gel network in G-1, when compared to the other two formulations containing higher amounts of NLC. Therefore, we could speculate that the particles are strongly entrapped within the tridimensional gel structure, rather than agglomerated. For particles contained in a gel, in order to agglomerate, they would need to be able to move freely. This could happen if the gel structure broke down, so that the Brownian motions bring the particles into closer contact. If this were the case for G-1, the same would be observed for the other two formulations. On the contrary, higher amounts of NLC dispersion, added to the blank gel, caused less apparent increase in particle size. Analogue behaviour was observed 30 days after production. Again, the G-1 showed highest increase in apparent particle size, as shown in Fig. 5.

The same profile and comparable particle size was measured also for formulations stored at 4 °C and 40 °C (data not shown), indicating that the developed NLC-based semi-solid hydro gel formulations are stable when exposed to stress temperatures.

Further indication on physical particle stability can be obtained from X-ray diffraction studies. The aggregation of the particles is often accompanied by modifications in crystalline lattice structure (Jenning et al., 2000). These changes can affect not just the stability of the formulation, but also its release and skin penetration properties (Souto, 2005). Comparing the WAXS patterns of the pure NLC dispersion with the pattern obtained for NLC-based semi-solid formulations, depicted in Fig. 6, one month upon production, it is possible to conclude that no polymorphic transitions were present.

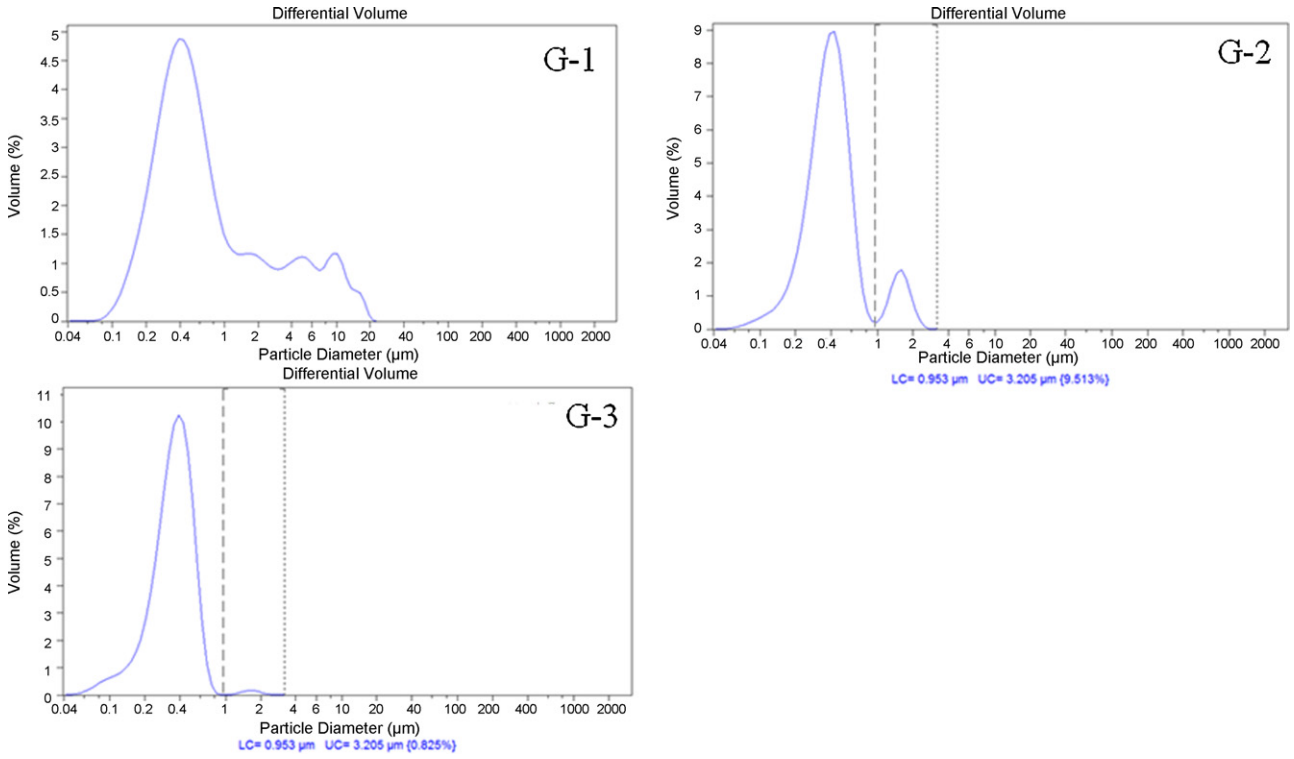

Fig. 5. Particle size (LD) distribution of the three semi-solid NLC based formulations from Fig. 4, measured thirty days after production.

These data are in accordance with what supposed for the size of NLC incorporated into hydrogels. As no polymorphic changes were detected, the increase in apparent particle size could be due to the strong gel network that keeps the particles close one to another, rather than to real agglomeration of the particles. The absence of the second peak in G-1 X-ray pattern is due to the insufficient signal collected (low NLC concentration), rather than absence thereof.

It is possible to differentiate between lipid nanoparticles and hydrogel network also by means of DSC analysis. The DSC parameters of the developed NLC-based semi-solid formulations obtained

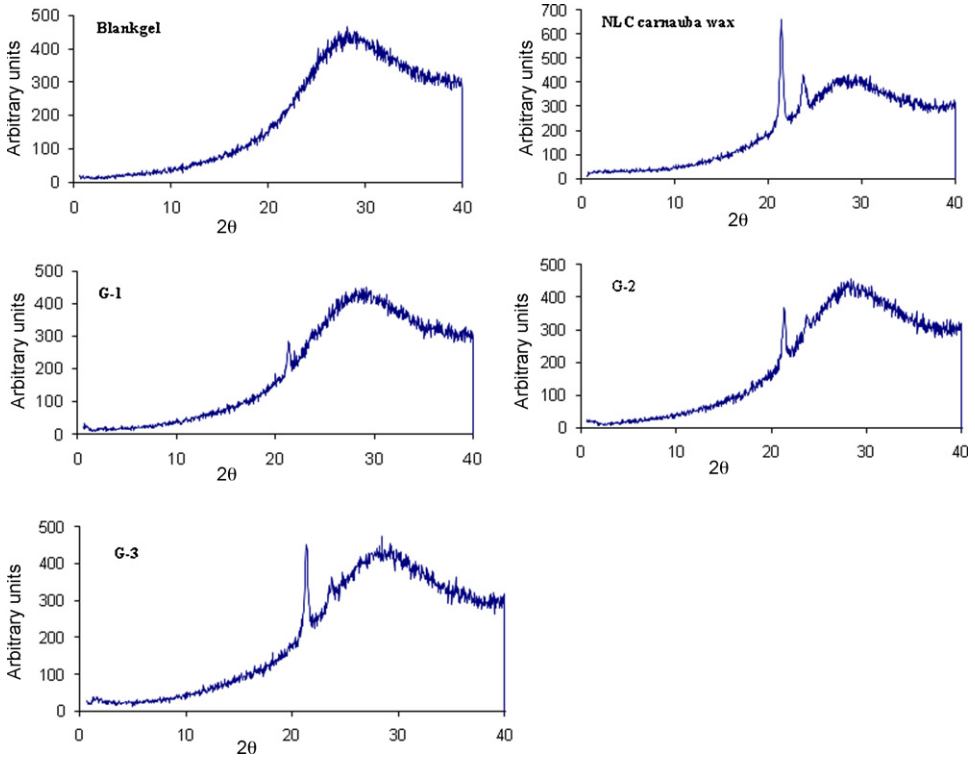

Fig. 6. X-ray diffraction patterns of blank carbopol gel (upper left), of an NLC dispersion based on carnauba wax (lipid to actives ratio 40:60, upper right) and of three hydrogels G-1, G-2 and G-3 with incorporated carnauba wax NLC.

Table 3
Melting onset and melting peak temperatures of the endothermic event at the day of production (stored at room temperature until measurement) and thirty days after the production (stored at 4 °C, 25 °C and 40 °C) for the developed semi-solid formulations based on NLC containing carnauba wax (lipid to actives ratio 40:60).

Formulation	Day 0		Day 30					
	Melting point (°C)	Onset (°C)	Melting point (°C)			Onset (°C)		
			4°	25°	40°	4°	25°	40°
G-1	**83.57**	74.43	**80.34**	**78.22**	**80.03**	68.85	69.12	68.00
G-2	**83.95**	76.23	**80.59**	**80.98**	**80.17**	67.55	68.50	65.87
G-3	**82.48**	74.32	**80.26**	**80.91**	**80.01**	66.43	67.61	65.91

at the day of production and after thirty days of storage at three different temperatures are presented in Table 3.

After thirty days, the melting point slightly decreased, from approx. 83 °C to approx. 80 °C, for all the three tested formulations. The quantity of solid lipid in the formulation did not have any influence on melting point modification over time, showing, again, that the particles are stable in the semi-solid medium. Together, WAXS and DSC data demonstrate that the NLC particles dispersed in a hydrogel remain in their crystalline and solid state, independently from storage temperature and time. The crystalline modifications, due to either storage period or storage temperature, are often accompanied by drastic change in particle size and polydispersity (Rosenblatt and Bunjes, 2009). The developed NLC particles maintained their colloidal size after incorporation into the semi-solid formulations, confirmed by the overall picture of size, WAXS and DSC data.

There are different reports on the quantity of a sunscreen formulation that needs to be applied in order to reach a good correlation between *in vivo* and *in vitro* results regarding SPF (Bimczok et al., 2007; Ferrero et al., 2002; Garoli et al., 2008, 2009; Heinrich et al., 2004). Besides the applied quantity, the measured SPF depends also on the formulation type (Heinrich et al., 2004). It has been reported that novel cosmetic carriers such as NLC can offer enhanced sun protection due to the particulate nature of the carrier (Müller et al., 2002; Villalobos-Hernández and Müller-Goymann, 2005, 2006).

The SPF values cannot be predicted from simple UV spectroscopic analysis. Therefore, a set of semi-solid formulations was prepared, containing 10% of lipid phase (as particles or droplets, in case of NLC or reference emulsion, respectively), and the *in vitro* SPF was measured.

The gel containing carnauba wax based NLC (G–C) showed significantly higher *in vitro* SPF (SPF = 20.19 ± 1.03), when compared with G–B (SPF = 14.13 ± 1.33) and G–M (SPF = 13.76 ± 1.44), containing bees wax and Miglyol® 812, respectively, confirming the results from UV scans. With just 6% of UV filter mixture an SPF higher than 20 was achieved when the lipid used for NLC production was carnauba wax. In the same experiment, the other two formulations had very similar SPF values, but much lower than the SPF of G–C. Although the solid state of the particles was confirmed by DSC, the synergistic effect due to the particulate state of the particles could not be observed in case of G–B. The simple solid state of the particles was not the reason for the synergistic effect, but the type of lipid itself. i.e. its chemical and probably crystalline structure.

4. Conclusion

In the present study it was demonstrated that a synergistic sun screening effect of the solid nature of the particles and the presence of organic UV filters can be achieved by choosing an appropriate solid lipid. For the mixture of the three UV filters used in this experiment, carnauba wax NLC showed the best results, increasing SPF values by more than 45% at the same concentrations of organic UV filters, compared to bees wax and a reference emulsion. It was further demonstrated that the particles incorporated into hydro gels remained stable.

An interesting finding is that not only the physical state (=solid) but also the chemical nature of the solid lipid plays a key role for a synergistic effect. Some solid lipids with sunscreens provide even lower SPF values than the reference nanoemulsion. The structure of the NLC particle matrix might also play a role (enriched core versus enriched shell model) which needs further investigation.

The SPF enhancing effect opens the perspective in producing photoprotective formulations with less organic sunscreen, thus being better tolerable and having less side effects. Formulations with SPF 50+ are however not feasible, because for them higher concentrations of molecular sunscreens, often in combination with titanium dioxide are required. These amounts cannot be incorporated in lipid nanoparticles and simultaneously maintaining a good spreadability of the formulation on the skin. Feasible appear formulations with SPF up to 30. Due to the costs of the system, they will not be a mass sun protection product. Such formulations are rather suited in exclusive skin care products for the face, the most important part of the body for which one is prepared to pay more for an effective and simultaneously safer photoprotective formulation.

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