Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin, Germany

Solid lipid nanoparticles (SLN) – a novel carrier for UV blockers

S. A. WISSING and R. H. MÜLLER

The formulation of safe sunscreen products is of high importance due to their increasing use because of the diminishing ozone layer. Solid lipid nanoparticles (SLN) are introduced as the new generation of carriers for cosmetics, especially for UV blockers for the use on human skin and/or hair and production thereof is described. The crystalline cetylpalmitate SLN particles have the ability of reflecting and scattering UV radiation on their own thus leading to photoprotection without the need for molecular sunscreens. An *in vitro* assay showed that a placebo cetyl palmitate SLN formulation is twice to three times as potent in absorbing UV radiation as a conventional emulsion. Incorporation of sunscreens into SLN lead to a synergistic photoprotection, i.e. higher than the additive effect of UV scattering caused by the SLN and UV absorption by the sunscreen. The photoprotective effect after incorporation of the molecular sunscreen 2-hydroxy-4-methoxybenzophenone (Eusolex 4360) into the SLN dispersion was observed to be increased threefold compared to a reference emulsion. Further, film formation on the skin was investigated by scanning electron microscopy, showing particle fusion due to water evaporation and formation of a dense film.

1. Introduction

Due to the worldwide decrease of the ozone layer thickness of 3% per decade at mid-latitudes [1] and the resulting increase of exposition of human skin to damaging UV radiation, the need for photoprotecting substances increases rapidly. On the one hand, UV-A radiation (320–400 nm) promotes tanning of the human epidermis, but on the other hand leads to loss of elasticity and further to premature aging including the appearance of wrinkles. UV-B radiation (290–320 nm) causes skin burns, erythema and induction of cancer [2]. The hazardous genetically damaging and immunosuppressive effects of UV radiation can be detected for example by the dramatic increase of skin cancer incidence in humans. Every five years, the number of patients with newly acquired malign melanoma is doubled [3]. Especially Australians are extremely endangered because of their geographical position coupled with clearer atmospheric conditions and a more significant ozone depletion [1]. Here, the UV radiation is 12 to 15 times higher than in comparable locations in the northern hemisphere [4], leading to a five-fold increase in incidents of malign melanoma compared to central Europe.

Since sunblockers are applied directly on the surface of human skin and is intended to remain there for hours [5], particular attention has to be paid regarding its effects, safety and efficiency. An ideal sunblocker should prevent UV induced sunburn and immunosuppression, photoageing and DNA damage by completely blocking UVA and UVB radiation without being physiologically reactive. It should have a high protective capacity even in low concentration. Since sunblockers are not intended to act systemically, penetration should be minimized and photostability provided. Further, they should not cause any side-effects such as photoallergies, phototoxicity, skin irritation.

In existing systems, sunblockers are incorporated mainly into creams, lotions or sprays. Yet, the name "sunblockers" for UV blockers in these systems is misleading because they do not block UV radiation completely; they merely decrease it to some extent, depending on their concentration and chemical structure.

There are two different mechanisms of action for UV blockers namely reflection and absorbance, as illustrated in Fig. 1. Chemical or molecular sunscreens (symbolized by ① in Fig. 1) are aromatic or linear molecules with conjugated double bounds and act as absorbers. The chromophor parts of the molecule contain π and n electrons which are excited by a distinctive radiation (long wavelengths or low energies) [6]. Depending on the electron orbital level, the energy required for transitions is determined and hence the absorption maxima for each molecule [5]. Incoming radiation is dissipated either as thermal energy or light (fluorescence).

However, chemical UV blockers possess some disadvantages that prevent their unlimited use: Percutaneous penetration is one of them, since many molecular UV blockers are lipophilic compounds, and decent solubility of a molecular UV blocker in the vehicle (oily phase in a cream or lotion) leads easily to penetration into the skin [7]. While penetration is desired for drugs, it is not for sunscreens because it leads to loss of activity and undesired side-effects in the skin.

Especially in people with sensitive skin, photoallergies and irritations are caused [3]. Some substances (salicylidenes) are strong irritants and cannot be used as topical compounds [8]. In order to lower these toxic risks, there is a desire for reducing the amount of molecular UV blockers in sunscreen formulations [9] while at the same time maintaining the sun protection factor.

Another disadvantage is photoinstability which has been observed for several compounds. Here, the UV blockers did not remain intact under irradiation; instead, they underwent significant photolysis either leading to inactive or even to toxic degradation products [10].

On the other hand, physical sunscreens or pigments such as micronized titanium dioxide have a mechanism of action that is called reflection (symbolized by ② in Fig. 1). Because of the size of these insoluble particles, they are expected to remain on the skin surface where they scatter and reflect the entering UV radiation [10, 11]. Therefore, physical sunscreens have become increasingly important and frequently used during the last years.

Their traditional disadvantage (clearly visible whitening effect on the skin surface) has been overcome by using micronized particles [10, 12]. Yet, even for these pigments, undesired side-effects and a lack of protection against UVB induced immunosuppression have been found [12], and non-existing penetration cannot be taken for granted anymore [13]. For topical preparations, evidence for the penetration of drug crystals [14] and titanium dioxide particles [15] was published.

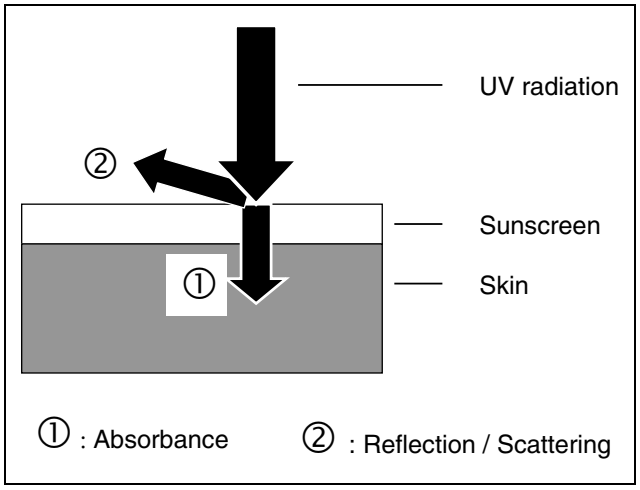

Fig. 1: Mechanism of action of chemical (①) and physical (②) sunscreens on the skin (after [24] with permission)

Concluding, it has to be remarked that due to the increasing UV radiation and the resulting increasing use of protective products, there is an immense need for more efficient and toxicologically safer sunscreen products, especially for extremely sensitive skin areas.
Solid lipid nanoparticles (SLN) [16–19] represent an innovative carrier system for active reagents in pharmaceutics and cosmetics. In this study, SLN were investigated regarding their use in UV-protecting formulations, especially as carriers for the incorporation of UV blockers. They show distinct advantages over conventional carrier systems because of their superior UV blocking effect which is due to increased reflection and scattering of radiation. Thus, concentration of UV blockers can be decreased, while the same sun protection factor is maintained. Molecular blockers can be incorporated into SLN and physical sunscreens (i.e. titanium dioxide) can be added to the formulation as well. Furthermore, antioxidants, skin-hydrating agents and skin care substances (to give some examples) can be added in order to form a creme, lotion or gel, so that there are various possibilities of developing a suitable formulation for different needs.

2. Investigations, results and discussion

2.1. UV absorbance of placebo SLN dispersions

Regarding the aim of this study, namely the development of a safer and more efficient carrier system for UV blockers, the basic idea was to decrease the amount of potentially hazardous chemical UV blockers while maintaining the protective effect against UV radiation. To minimize the redistribution of molecular sunscreens from the dispersed phase (i.e. oily phase in a lotion) into the continuous phase, the liquid lipids were replaced by solid lipids. The new system does not represent an emulsion anymore but a suspension. The UV blocking capacity of a placebo SLN formulation (consisting of 10% cetyl palmitate, 1.2% TegoCare 450 and water; without any UV blocker) was compared to a conventional placebo emulsion (consisting of 10% Miglyol 812, 1.2% TegoCare 450 and water, without any UV blocker). The particle size distribution in both formulations was similar, having a mean diameter of 220 ± 12 nm and a low polydispersity index of 0.12 ± 0.03.
Using the described *in vitro* photoprotection assay [21], it was observed that within the UV region, the absorbance

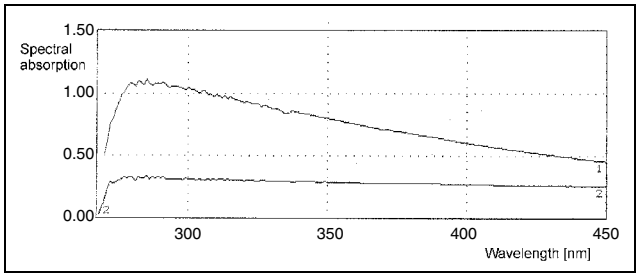

Fig. 2: Absorption spectra (280–450 nm) of SLN (1) and emulsion (2) formulation containing 10% cetyl palmitate, 1.2% TegoCare 450 and water (Uvikon 940 spectrophotometer, Kontron Instruments, Germany)

caused by the SLN is between twice and three times higher (Fig. 2) than the absorbance caused by the emulsion.
This is due to the special physical character of the SLN system. Spectrophotometrically, the term absorbance generally refers to solutions. Yet, in this assay, dispersions were examined so that reflecting and scattering effects have to be taken into account as well. In order to determine the nature of this effect, organic solutions of the lipid and aqueous solutions of the surfactant were prepared and screened. No absorbance was found in the screened wavelength area. Thus, SLN whose components do not absorb UV radiation when dissolved, decrease spectral transmission completely by reflection and scattering. Since the components of the emulsion do not absorb UV radiation in solution either, it can be stated that the solid lipids in SLN reflect UV radiation to a greater extent than droplets of liquid oil. Thus, less harmful radiation can pass the formulation and damage the skin.

2.2. Protective effect of UV blocker loaded SLN dispersions

In order to determine the efficacy of sunscreens in different carrier systems, the lipophilic molecular UV absorber (2-hydroxy-4-methoxybenzophenone = Eusolex 4360) was incorporated into the solid particles of a SLN dispersion and into the oil droplets of a reference emulsion (formulations: 10% cetylpalmitate or Miglyol 812, 1.2% TegoCare 450, 1% Eusolex 4360, water) and the absorbance in the UV region was measured using the assay described above. Photon correlation spectroscopy determined a mean particle size of 206 ± 3 nm and a polydispersity index of 0.116 ± 0.01 for the SLN dispersion (216 ± 4 nm, PI $= 0.099 \pm 0.01$). The SLN formulation showed an absorbance which was about three times as high as the absorbance caused by the emulsion (Fig. 3). This figure shows the typical absorption pattern of this particular UV blocker with two peaks (at about 340 and 290 nm).

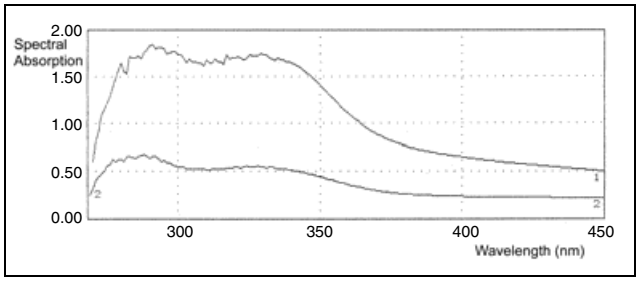

Fig. 3: Absorption spectra (280–450 nm) of SLN (1) and emulsion (2) formulation containing 10% cetyl palmitate, 1.2% TegoCare 450, 1% Eusolex 4360 and water (Uvikon 940 spectrophotometer, Kontron Instruments, Germany)

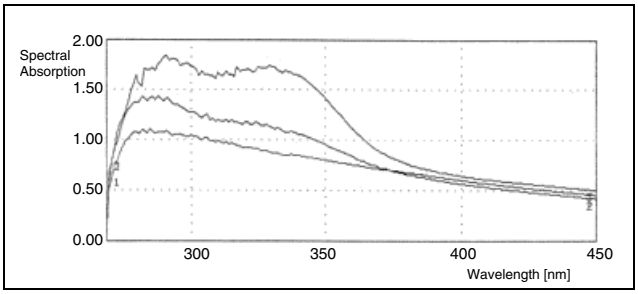

Fig. 4: Absorption spectra (280–450 nm) of placebo SLN formulation (bottom), SLN formulation containing 1% Eusolex (top) and theoretical absorption profile of SLN formulation containing 1% Eusolex 4360 (middle) (Uvikon 940 spectrophotometer, Kontron Instruments, Germany)

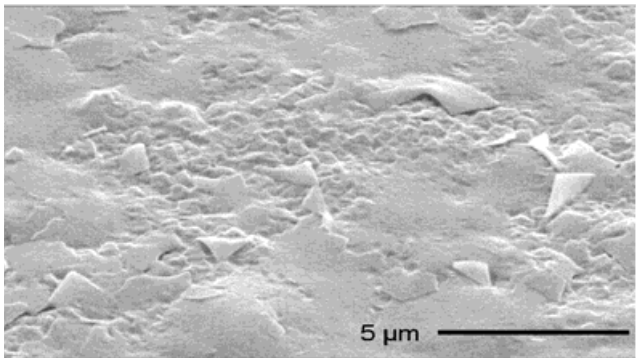

Fig. 5: Electron scanning micrograph of a dried SLN film (Scanning electron microscope: S 360, Cambridge Instruments, England); (after [24] with permission)

Since the same concentration of sunscreen was incorporated into both formulations, it can be stated that again, the SLN formulation is more effective *in vitro* and shows improved photoprotection. Here, we find a combination of the reflecting properties of the solid particles and the absorbing characteristics of the molecular UV blocker.

Further *in vitro* studies were performed in order to investigate the extent of UV blocking effects after incorporation of molecular sunscreens into SLN. In the literature, synergistic effects have been described for the combination of molecular and physical sunscreens [22]. Our studies showed that incorporation of sunscreens into SLN lead to more than additive protection levels as well. However, there was no need for the addition of potentially harmful physical sunscreens since the native lipid serves as such on its own. For this set of experiments, the *in vitro* photoprotection assay mentioned above [21] was used. The values of the absorption profile of a solution containing 1% Eusolex 4360 were added to the values of the placebo cetyl palmitate SLN dispersion leading to a theoretical absorption profile of an Eusolex 4360 – containing cetyl palmitate SLN dispersion. Fig. 4 shows that synergism was detectable throughout the scanned wavelength area since the actual absorption values of a SLN dispersion with Eusolex 4360 remained constantly above the theoretical values. Due to this synergistic effect, the concentration of potentially harmful molecular UV blocker can be decreased while maintaining the desired UV protective effect without further need of an additional physical sunscreen.

2.3. Film forming properties of SLN

Previously published studies assumed that SLN form a fine film on the skin, i.e. a film with very narrow interspaces between the nanoparticles [23]. Because of this film formation, water evaporation through the skin is decreased and thus skin hydration is increased. A proposed reason for this occlusivity is the size of the particles in the tightly packed film. If the particles are extremely small, the spaces (capillaries) between the particles become smaller, are hydrodynamically unfavorable and limit water evaporation [23]. However, in this study, different observations concerning the structure of this film were made. Fig. 5 shows a scanning electron microscopy picture of a dried SLN dispersion (formulation contains 10% cetyl palmitate, 1.2% TegoCare 450, water).

Clearly visible, there is a complete film formation. Individual particles cannot be distinguished anymore. After water evaporation, the lipid particles seem to fuse, thus forming the film. The formation of this film formation depends e.g. on the thickness of the sample layer and on the nature and concentration of the lipid(s) used. Although further studies still have to be done, it can be stated that this effect presumably supports the skin barrier, especially if the natural lipid film on the stratum corneum is damaged. The influence of the occlusivity is clearly detectable [23] and encourages physiological rehydratation and thus regeneration of the skin.

In conclusion, these first studies show that the incorporation of molecular UV blockers into solid lipid nanoparticles offers an inventive, safe and efficient new carrier system for sunscreen products. As presented in this paper, SLN show a superior reflection of UV radiation compared to traditional emulsions. Incorporation of molecular sunscreens has not only an additive but a synergistic effect on absorbing capacity and the mechanism of complete film formation accounts further to the great efficiency of this system.

3. Experimental

3.1. Materials

Cetylpalmitate (Gattefossé, France), Miglyol® 812 (Beiersdorf, Germany) and TegoCare® 450 (Goldschmidt, Germany) were kindly provided as gifts. Eusolex® 4360 was purchased from Merck (Germany) and Transpore™ tape was obtained from 3M (USA).

3.2. Methods

3.2.1. Production of SLN

Regarding the production of SLN, there are two simple and effective techniques of high pressure homogenization which can be used depending on the nature of the compound [20]:

– hot homogenization technique
– cold homogenization technique

The hot homogenization technique is intended mainly for lipophilic, thermostable drugs. Here, the lipid is melted approximately 5 °C above its melting point, the active ingredient dissolved or finely dispersed in the melt and then the hot surfactant solution is added and mixed using a high-speed stirrer. The obtained pre-emulsion is homogenized applying a pressure between 200–500 bar and 2–3 homogenization cycles.

Cooling after homogenization leads to recrystallization of the lipid within the nanoemulsion and thus formation of solid lipid nanoparticles (SLN) (Fig. 6). Using the hot homogenization technique, the nanoparticles are extremely small and a narrow distribution is obtained. SLN and emulsion systems in this study were high pressure homogenized using a Lab 40 high pressure homogenizer (APV Deutschland GmbH, Germany), applying a pressure of 500 bar for 3 cycles while maintaining a temperature of 70 °C.

The cold homogenization technique is applied mainly for thermosensitive or hydrophilic drugs which are intended to be incorporated into the lipid. It has to be pointed out that the thermal stress that is applied to a sample only has very little impact on chemical stability because of the short duration. Here, lipid and active ingredient are melted together and then rapidly cooled and ground using a mortar mill. The obtained lipid microparticles are dispersed in a cold aqueous surfactant solution using a high speed stirrer. Then, the obtained pre-suspension is homogenized at room temperature or below by cooling the high pressure homogenizer, applying a pressure of at least 1000–1500 bar and passing the homogenizing gap at least 5 times.

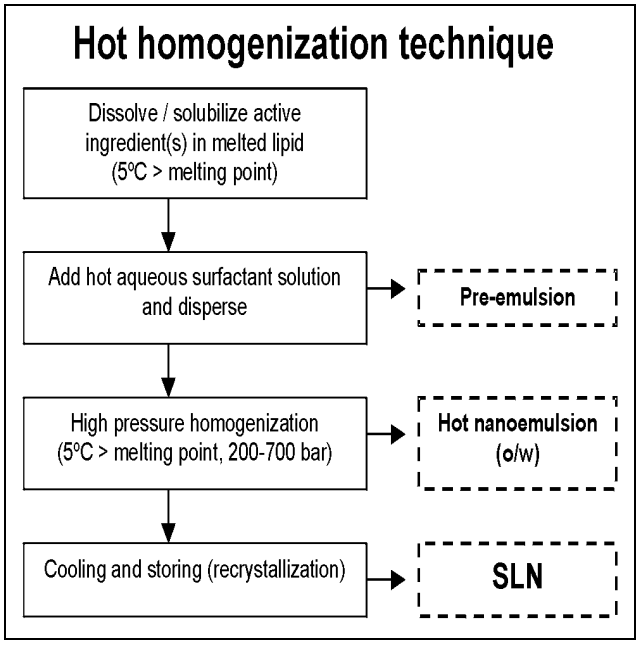

Fig. 6: Scheme for the production of SLN by the hot homogenization technique

3.2.2. Particle size examination

The particle size was analyzed by photon correlation spectroscopy (PCS) using a Zetasizer 4 (Malvern Instruments, UK). PCS gives information about the mean diameter of the bulk population and about the width of the distribution via the polydispersity index (PI).

3.2.3. In vitro photoprotection assay

The UV blocking effect was determined using a recently developed assay [21] whose assembly is shown in Fig. 7. In this assay, the absorbance of ultraviolet radiation through a sample of Transpore tape is measured and has been shown to be comparable to *in vivo* data to a certain extent [21]. 50 µl samples (e.g. emulsion, SLN) are spread evenly onto 4.5 cm^2 of Transpore tape which is placed on a quarz cuvette and then screened from 450–280 nm using a Uvikon 940 spectrophotometer, Kontron Instruments, Germany.

3.2.4. Film formation investigations

Film formation was investigated using a scanning electron microscope (S 360 Cambridge Instruments, England). Films were prepared by evenly spreading the samples on a flat glass surface and letting them dry at 32 °C for 24 h.

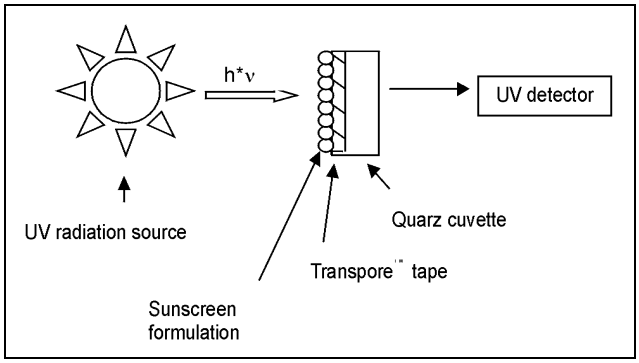

Fig. 7: *In vitro* assay set up for the determination of UV blocking effects

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Prof. Dr. R. H. Müller
Institut für Pharmazeutische Technologie
Freie Universität
Kelchstr. 31
D-12169 Berlin
mpharma@zedat.fu-berlin.de