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Note

In vitro erythemal UV-A protection factors of inorganic sunscreens distributed in aqueous media using carnauba wax–decyl oleate nanoparticles

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Abstract

This paper describes the *in vitro* photoprotection in the UV-A range, i.e. 320–400 nm obtained by the use of carnauba wax–decyl oleate nanoparticles either as encapsulation systems or as accompanying vehicles for inorganic sunscreens such as barium sulfate, strontium carbonate and titanium dioxide. Lipid-free inorganic sunscreen nanosuspensions, inorganic sunscreen-free wax–oil nanoparticle suspensions and wax–oil nanoparticle suspensions containing inorganic sunscreens dispersed either in their oil phase or their aqueous phase were prepared by high pressure homogenization. The *in vitro* erythemal UV-A protection factors (EUV-A PFs) of the nanosuspensions were calculated by means of a sun protection analyzer. EUV-A PFs being no higher than 4 were obtained by the encapsulation of barium sulfate and strontium carbonate, meanwhile by the distribution of titanium dioxide in presence of wax–oil nanoparticles, the EUV-A PFs varied between 2 and 19. The increase in the EUV-A PFs of the titanium dioxide obtained by the use of wax–oil nanoparticles demonstrated a better performance of the sun protection properties of this pigment in the UV-A region.

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1. Introduction

Modifications of solid lipid nanoparticles composed of binary mixtures of solid lipids and liquid lipids recently referred to as nanostructured lipid carriers (NLC) constitute suitable vehicles for the dermal delivery of different drugs [1]. These particles constitute not only intrinsic UV-blockers and scatters in virtue of their crystallinity and reduced particle size comprising a range between 200 and 400 nm [2], but also carriers capable to integrate organic and inorganic filters inside of a single structure to combine or enhance the UV-protection properties of both, particularly in the case of NLC. Experiments describing this enhancement have been already reported when inorganic sunscreens were enclosed inside or bonded with carnauba wax–decyl oleate nanoparticles [3]. The UV protection properties of the nanoparticles can be described in terms of the sun protection factor (SPF) like in the case of any other sunscreen product. However, the SPF mainly represents the protection against UV-B [4]. For this reason, the new developments in sunscreens have to provide a description of the protection against not only the UV-B radiation, but also the UV-A one [5,6]. The UV-A region also called near ultraviolet region is associated with the wavelengths between 320 and 400 nm. The UV-A radiation possesses lesser energy than UV-B (290–320 nm) or UV-C radiations (200–290 nm); i.e. it is of longer wavelength and because of this property it penetrates into the skin and reaches the dermis provoking several damages such as immediate and delayed tanning reactions, loss of collagen, diminution in the quantity of blood vessels, alteration

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of the connective tissue of the dermis and skin photosensitization upon others [7–9]. To describe the UV-A protection, many *in vivo* methods have been proposed [5,6]. Nevertheless, in order to avoid expensive and time-consuming *in vivo* UV-A protection testing, *in vitro* methods for UV-A protection assessment based on spectrophotometric analysis have been developed [10,11]. The *in vitro* erythemal UV-A protection factor is a parameter derived from the calculations of the SPF calculations and because of this reason, it is closely related to the general SPF performance. As described in the literature, the sun protection factor is a parameter indicating the performance of a sun protection product or sunscreen formulation [10]. It represents a number derived from the ratio of the time of exposure to a UV spectrum between 290 and 400 nm to produce erythema in human skin in the presence or absence of a sunscreen product applied in a specified dose. The *in vitro* SPF measured represents an indicator of the UV-A/UV-B protective property of a sunscreen product calculated from the mean monochromatic protection factor, the solar irradiance and the erythemal constants according to Eq. (1)

$$\mathrm{SPF} = \sum_{290}^{400} \mathrm{E}_\lambda \mathrm{B}_\lambda \Bigg/ \sum_{290}^{400} (\mathrm{E}_\lambda \mathrm{B}_\lambda / \overline{\mathrm{MPF}}_\lambda), \tag{1}$$

where E_λ is the spectral irradiance of terrestrial sun light under defined conditions; B_λ is the erythemal effectiveness; and $\overline{\mathrm{MPF}}_\lambda$ is the mean monochromatic protection factor.

E_λ is defined by the midday midsummer sunlight at 40°N with a solar zenith angle of 20°. Considering the UV-A wavelength range, i.e. 320–400 nm and using the terms of Eq. (1), the in vitro erythemal UV-A PF can be calculated according to Eq. (2).

$$\text{Erythemal UV-A protection factor} = \sum_{320}^{400} \mathrm{E}_\lambda \mathrm{B}_\lambda \Bigg/ \sum_{320}^{400} (\mathrm{E}_\lambda \mathrm{B}_\lambda / \overline{\mathrm{MPF}}_\lambda). \tag{2}$$

The aim of this paper was to complete the studies on the UV protection properties of the nanosuspensions containing carnauba wax–decyl oleate nanoparticles used either as an encapsulation/bonding media or as accompanying vehicles for inorganic pigments, i.e. barium sulfate, strontium carbonate and titanium dioxide. The *in vitro* SPF of these nanosuspensions have been already reported in a former paper [3], for this reason, this manuscript was focused only on the description of the *in vitro* erythemal UV-A protection factors of those colloidal systems.

2. Materials and methods

2.1. Materials

The inorganic pigments used were alumina–stearic acid surface-treated nanofine titanium dioxide having a primary particle size (PPS) of about 17 nm (Kemira OY, Finland), nanofine barium sulfate (PPS ca. 30 nm) (Solvay GmbH, Germany) and strontium carbonate (PPS ca. 30 nm) (Solvay GmbH, Germany). The lipids applied were carnauba wax Ph. Eur. and decyl oleate (Caelo GmbH, Germany). Other excipients used were polysorbate 80 (Atlas Chemie GmbH, Germany), simethicone antifoamer (Sigma–Aldrich GmbH, Germany) and methylisothiazolinone (Brenntag GmbH, Germany). For all preparations bidistilled and filtered water (0.2 μm) was utilized.

2.2. Experimental methods

2.2.1. Manufacture of the nanosuspensions

The chemical compositions of the prepared nanosuspensions as well as the distribution of the pigments in the different phases; i.e., oil phase or aqueous phase, are shown in Table 1. For the manufacture of the nanosuspensions, a pre-dispersion of both phases using an Ultraturrax (IKA, Germany) followed by a high pressure homogenization performed in a Niro homogenizer (Niro Soavi S.p.A., Italy) was carried out. A detailed description of the preparation of these nanosuspensions can be found in a previous paper [3]. Four different types of nanosuspension were prepared. This includes: nanosuspensions of carnauba wax–decyl oleate matrices (Formulation I); lipid-free inorganic pigment nanosuspensions (Formulation II); nanosuspensions of carnauba wax–decyl oleate matrices loaded with inorganic pigments distributed in the lipid phase (Formulation III); nanosuspensions of carnauba wax–decyl oleate matrices loaded with titanium dioxide distributed in the aqueous phase (Formulation IV).

2.2.2. In vitro erythemal UV-A protection factor (EUV-A PF)

The *in vitro* EUV-A PF was measured by means of a SPF-290 computer operated analyzer (Optometrics Corp, UK). One hundred and ten microliters samples were spread over Transpore® tapes (3 M GmbH, Germany) having an area of 55.5 cm^2 with a gloved finger. Radiation attenua-

Table 1
The chemical composition and concentration of the tested formulations

Substances	wt (%)	Formulation			
		I	II	III	IV
Decyl oleate	5	+	–	+	+
Carnauba wax	5, 10	+	–	+	+
Pigment*	2, 4, 6	–	+	+ (LP)	+ (AP)
Tween 80	1	+	+	+	+
Simethicone	0.01	+	+	+	+
Methylisothiazoline	0.0285	+	+	+	+
Double dist. water	q.s. 100	+	+	+	+

Key: (*), barium sulfate, strontium carbonate or titanium dioxide; (–), absent; (+), present; LP, lipid phase and AP aqueous phase.

Formulation key: I, nanosuspensions of carnauba wax–decyl oleate matrices; II, lipid-free inorganic pigment nanosuspensions; III, nanosuspensions of carnauba-wax–decyl oleate matrices loaded with pigments in the lipid phase and IV, nanosuspensions of carnauba-wax–decyl oleate matrices loaded with titanium dioxide in the aqueous phase.

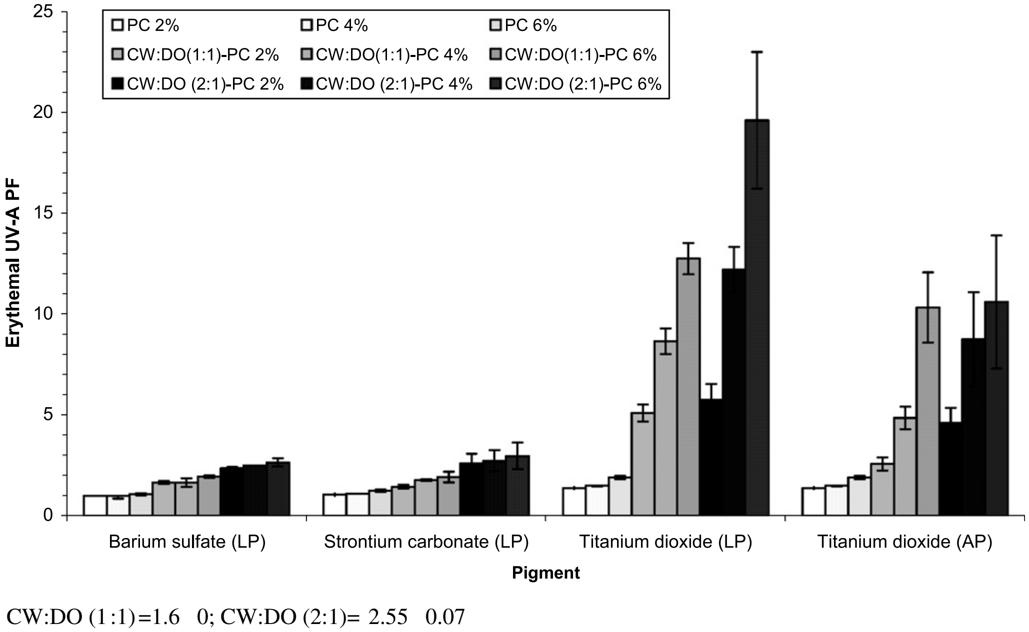

Fig. 1. *In vitro* erythemal UV-A protection factors of the different nanosuspensions as function of the pigment and its concentration, the carnauba-wax–decyl oleate ratio and the phase used for its distribution. Key: PC, pigment concentration; CW:DO, carnauba wax–decyl oleate ratio; LP, lipid phase and AP, aqueous phase.

tion of the applied samples was performed in a wavelength range of 320–400 nm. The radiation was provided by a compact 125 W xenon arc lamp. Measurements of each formulation were done in duplicate.

3. Results and discussion

3.1. Erythemal UV-A protection factor (EUV-A PF)

The EUV-A PF is a parameter analog to the SPF and represents a number derived from the ratio of the time of exposure to a UV spectrum between 320 and 400 nm to produce erythema in human skin in the presence or absence of a sunscreen product. The higher this value, the more UV-A protection a sunscreen offers. As shown in Fig. 1 (legend), the EUV-A PFs of the pigment-free wax–oil nanoparticles (CW:DO) prepared at ratios 1:1 and 2:1 were 1.6 ± 0.0 and 2.55 ± 0.07, respectively. Hence, from these results, the EUV-A PFs of the pigment-free wax–oil nanoparticles can be labelled as very low. On the other hand, the use of barium sulfate and strontium carbonate demonstrated to exhibit very low UV-A protection either in encapsulated or in non-encapsulated condition. Although, in these cases, slight increases probably attributed to higher pigment concentrations combined with different wax–oil ratios were observed, EUV-A PFs being no higher than 4 were detected. Conversely, in the case of titanium dioxide high EUV-A PFs were seen, particularly after the crystals were encapsulated with (LP) or dispersed within wax–oil nanoparticles (AP). These latter values varied between 2 and 19 although the original protection values of the corresponding lipid-free pigment suspensions were smaller than three. This indicated that the association with wax–oil particles also favoured the UV-A protection properties of the pigment and thus demonstrated that not only the UV-B protection character of the titanium dioxide could be enhanced by the encapsulation process of the pigments as reported before [3]. Therefore, a sunscreen exhibiting UV-A and UV-B protection could be referred to as a wide band sunscreen.

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