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International Journal of Pharmaceutics 322 (2006) 161–170

INTERNATIONAL JOURNAL OF PHARMACEUTICS

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Pharmaceutical nanotechnology

Sun protection enhancement of titanium dioxide crystals by the use of carnauba wax nanoparticles: The synergistic interaction between organic and inorganic sunscreens at nanoscale

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Received 1 March 2006; received in revised form 17 May 2006; accepted 18 May 2006
Available online 2 June 2006

Abstract

Carnauba wax is partially composed of cinnamates. The rational combination of cinnamates and titanium dioxide has shown a synergistic effect to improve the sun protection factor (SPF) of cosmetic preparations. However, the mechanism of this interaction has not been fully understood. In this study, an ethanolic extract of the carnauba wax and an ethanolic solution of a typical cinnamate derivative, ethylcinnamate, were prepared and their UV absorption and SPF either alone or in the presence of titanium dioxide were compared. The titanium dioxide crystals and the cinnamates solutions were also distributed into a matrix composed of saturated fatty acids to emulate the structure of the crystallized carnauba wax. SPF, differential scanning calorimetry (DSC) and X-ray studies of these matrices were performed. Additionally, carnauba wax nanosuspensions containing titanium dioxide either in the lipid phase or in the aqueous phase were prepared to evaluate their SPFs and their physical structure. Strong UV absorption was observed in diluted suspensions of titanium dioxide after the addition of cinnamates. The saturated fatty acid matrices probably favored the adsorption of the cinnamates at the surface of titanium dioxide crystals, which was reflected by an increase in the SPF. No modification of the crystal structure of the fatty acid matrices was observed after the addition of cinnamates or titanium dioxide. The distribution of the titanium dioxide inside the lipid phase of the nanosuspensions was more effective to reach higher SPFs than that at the aqueous phase. The close contact between the carnauba wax and the titanium dioxide crystals after the high-pressure homogenization process was confirmed by transmission electron microscopy (TEM).

Keywords: Carnauba wax; Nanofine titanium dioxide; Cinnamates; Fatty acid matrices; Nanoparticles; Nanostructured lipid carriers; UV absorption; In vitro sun protection factor; TEM

1. Introduction

Solid lipid nanoparticles (SLN) are two-phase drug delivery systems consisting of a nanoscale drug-carrying solid phase composed of crystallized lipids at room temperature dispersed within an aqueous phase by means of high-pressure homogenization (Mehnert and Mäder, 2001; Müller et al., 2000). In the last years, SLNs and modifications thereof known as nanostructured lipid carriers (NLCs), in which the solid lipid phase of these nanoparticles has been loaded with liquid lipids, i.e. oils (Jenning and Gohla, 2000; Jenning et al., 2000), have attracted the attention as novel delivery systems for organic and inorganic sunscreens (Müller et al., 2002a; Wissing and Müller, 2002; Radkte, 2003). The reason resides not only in the intrinsic UV blocking properties attributed to their size, particularly in the size range between 200 and 400 nm (Wissing and Müller, 2001), but also in the possibility to integrate organic and inorganic filters inside a single particulated structure combining the UV absorption properties of both (Villalobos-Hernández and Müller-Goymann, 2005).

It has previously been reported that the combination of organic filters and inorganic substances offers many advantages in sunscreen formulations, for example, the diminution of the organic filter content to avoid the intrinsic irritation provoked by some of these compounds; the achievement of higher sun protection factors with simpler formulations and the reduction of the whitening effect caused by the use of large quantities of inorganic sunscreens, i.e. titanium dioxide (Anderson et al., 1997). In this context, the use of SLNs or NLCs can accomplish

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doi:10.1016/j.ijpharm.2006.05.037

this task because the entrapment of the organic sunscreen inside the lipid matrices may reduce their potential skin irritation and it simultaneously could contribute to optimize the organic UV filter–inorganic UV filter ratio avoiding the use of unnecessary quantities of inorganic pigments. Recently, it has been reported that those particles exhibited other interesting properties such as a homogeneous distribution of the actives within the aqueous phase and a very good long-term physical stability (Wissing and Müller, 2003; Müller et al., 2002b). Furthermore, some types of NLCs have demonstrated to improve the sun protection factor of sunscreens, i.e. titanium dioxide in aqueous media without the use of complex formulations, as reported by the use of carnauba wax (Villalobos-Hernández and Müller-Goymann, 2004). This phenomenon is probably based on the chemical composition of the carnauba wax and the UV absorption capacity of titanium dioxide. According to the literature, 2- or 3-hydroxy, 3,4-dihydroxy and 4-dihydroxy substituted cinnamic acids, *p*-methoxycinnamic aliphatic diesters and *p*-hydroxycinnamic aliphatic diesters can be found in the carnauba wax (Emås and Nyqvist, 2000; Wang et al., 2001). These substances possess commercial interest because of the many cinnamates being currently used as organic sunscreens (Jiménez et al., 2004; Faria de Freitas et al., 2005). The action mechanism of these compounds, like that of any other organic sunscreen, is based on the UV absorption attributed to the conjugated double bonds of their chemical structures as exemplified in Fig. 1 (Spruce, 1997; Wolf et al., 2001; Walters et al., 1997). In respect of the titanium dioxide, the UV absorption is explained by the semiconductor properties of its crystal lattice (Weller, 1993; Kutal, 1993), on which, a fully occupied valence band separated from empty conduction band by an energy gap are formed so that when they are irradiated by light of energy equal to or higher than that of the band gap, an absorption of this energy takes place favoring the promotion of electrons into the conduction bands as depicted in Fig. 2. Thus, considering the intrinsic properties of both materials, it could be expected that an increased UV absorption would take place if both substances were intentionally combined.

The aim of this paper was to present elements contributing to clarify the interaction between titanium dioxide crystals and the organic UV filters contained in the carnauba wax nanoparticles. To achieve this goal, the UV absorption and sunscreen properties of the carnauba wax and a derivative of the cinnamic acid, ethylcinnamate, distributed in different media were compared.

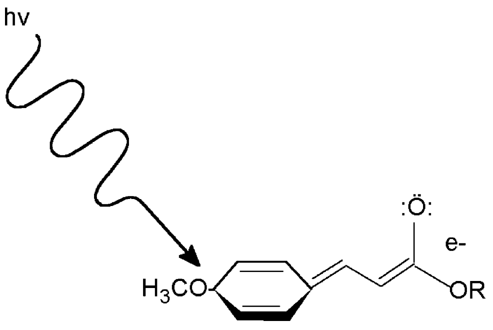

Fig. 1. Electron delocalization of an ester of the *p*-metoxycinnamic acid.

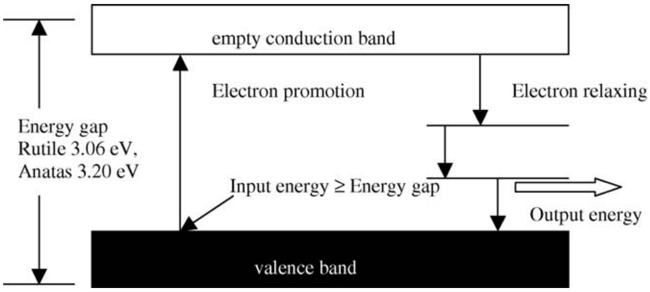

Fig. 2. Absorption of UV radiation by electronic transition in titanium dioxide (rutile or anatas modifications). The dark area is occupied by electrons (modified from Anderson et al., 1997).

For this purpose, an ethanolic extract of carnauba wax (CW-EE) was obtained by means of an ethanolic maceration. The CW-EE and an ethanolic solution of ethylcinnamate (EC-E) were added to diluted samples of high-pressure homogenized titanium dioxide crystals to observe their qualitative UV absorption spectra. Furthermore, the in vitro sun protection factors (SPFs) of both substances combined with titanium dioxide crystals either inside a liquid suspension or within a hard fat matrix were measured. Thereafter, DSC and wide angle X-ray diffraction diagrams of the carnauba wax matrices mixed with decyl oleate and titanium dioxide as well as the hard fat matrices loaded with ethanolic solutions of cinnamates and titanium dioxide were obtained to evaluate a possible modification of the crystals' structure of the solid lipids. These latter experiments with a hard fat were made to emulate the structure provided by the carnauba wax, which is mainly composed of long chain saturated fatty acids along with cinnamates (Basson and Reynhardt, 1988; Dorset, 1999). Additionally, to appreciate the performance of the nanosuspensions in terms of the SPF, two types of nanosuspensions containing carnauba wax (5 wt%) and titanium dioxide were prepared. In the first type, three different concentrations of the inorganic pigment, namely 2, 4 and 6 wt% were distributed in the aqueous phase of the nanosuspension, and in the second one, the same concentrations of titanium dioxide were distributed in the solid lipid phase. For comparative purposes, nanosuspensions containing particles of carnauba wax (5 wt.%) and nanosuspensions containing titanium dioxide crystals at the aforementioned ratios were also prepared and samples of all the nanosuspensions were removed to be freeze-fractured before being TEM-viewed.

2. Experimental materials and techniques

2.1. Materials

Alumina-stearic acid coated titanium dioxide with 100% rutile crystal structure having a primary particle size (PPS) of about 17 nm (Kemira OY, Finland), commercially refined carnauba wax Ph. Eur. (Caelo GmbH, Germany), decyl oleate (Caelo GmbH, Germany), polysorbate 80 (Atlas Chemie GmbH, Germany), simethicone antifoamer (Sigma–Aldrich GmbH, Germany), methylisothiazolinone (Brenntag GmbH, Germany) and double distilled and filtered water (0.2 μm) were used for the nanosuspensions. The hard fat commercially known as

Softisan® 378 (SASOL GmbH, Germany) and with the INCI denomination caprylic/capric/myristic/stearic trygliceride was used. Furthermore reagent grade ethylcinnamate (Fluka Chemie GmbH, Germany) and analytical graded toluene and ethanol (Sigma Aldrich GmbH, Germany) were also applied.

2.2. *Toluenic solution of carnauba wax and ethanolic extract of carnauba wax*

The toluenic solution of carnauba wax was obtained by dissolving 50 mg of carnauba wax Ph. Eur. in 50 mL toluene at a temperature of 30 °C in an ultrasonic bath. Further dilutions were made until a final concentration of 1 μg mL^{-1} was reached. The ethanolic extract of carnauba wax was prepared by macerating 100 g of carnauba wax in 400 mL of ethanol during 48 h at room temperature. The extract was separated from the wax flakes by glass fiber filters GF/B (Whatman Ltd., England) placed on a filtration unit (Millipore GmbH, Germany). No further purification was carried out.

2.3. *Hard fat matrices*

Two hundred, 400 and 600 mg of titanium dioxide were weighted and mixed with the hard fat to complete 10 g. The hard fat was molten at 60 °C and the crystals were dispersed by magnetic stirring during 15 min. After this time, 1 mL of the CW-EE without dilution or the EC-E were added separately in each case. The added ethylcinnamate solution was prepared in a concentration of 2 μg mL^{-1}.

2.4. *Nanosuspension formulations*

2.4.1. *Nanosuspension containing carnauba wax matrices (Formulation A)*

This formulation was produced by dispersing a lipid phase into an aqueous phase using high-pressure homogenization. The phases were initially pre-dispersed and subsequently high-pressure homogenized at 300 ± 10 bar within 30 passes using an homogenizer Niro Type II (Niro Soavi S.pA., Italy). This process was performed at a temperature of 75 ± 5 °C. After the homogenization, the product was cooled down at room temperature; the loss of water was compensated and the preservative was added. The chemical composition of the Formulations A–D is shown in Table 1.

2.4.2. *Nanosuspensions containing titanium dioxide without lipid phase (Formulations B)*

The titanium dioxide crystals were dispersed in the aqueous phase by high shear stirring at 26,000 rpm during 5 min. Subsequently, the dispersions were high-pressure homogenized applying 300 ± 10 bar at room temperature during 30 passes.

2.4.3. *Nanosuspensions containing carnauba wax matrices with titanium dioxide distributed in the aqueous phase (Formulations C)*

A carnauba wax–decyl oleate dispersion was made by high-pressure homogenization and cooled down to room temperature as indicated in Section 2.4.1. Thereafter, titanium dioxide was added and pre-dispersed by stirring at 26,000 rpm during 5 min. Finally, the dispersion was high-pressure homogenized at 300 ± 10 bar within 30 passes at room temperature.

Table 1
The chemical composition and concentration of the tested formulations

Substances	wt%	Formulations			
		A	B	C	D
Decyl oleate	5	+	−	+	+
Carnauba wax	5	+	−	+	+
Titanium dioxide	2, 4, 6	−	+	+ (AP)	+ (LP)
Tween 80	1	+	+	+	+
Simethicone	0.01	+	+	+	+
Methylisothiazoline	0.028	+	+	+	+
Double distilled water	q.s. 100	+	+	+	+

Key: −, absent; +, present; LP, lipid phase; AP, aqueous phase.

2.4.4. *Nanosuspensions containing carnauba wax matrices with titanium dioxide distributed in the lipidphase (Formulations D)*

Particulate dispersions of lipid matrices made out of a binary mixture, carnauba wax–decyl oleate, containing inorganic pigments were prepared according to the amounts indicated in Table 1 by mixing the titanium dioxide crystals with the molten carnauba wax and the decyl oleate at 90 ± 5 °C and applying magnetic stirring during 30 min at a rate of 300 rpm, before combining this lipid phase with the aqueous phase. The aqueous phase was prepared by dispersing the components indicated in Table 1 at a speed rate of 300 rpm. The integration of both phases was done as indicated in Section 2.4.1.

2.5. *UV absorption studies*

Qualitative UV absorbance spectra from the substances and combinations thereof were obtained using an Spectrophotometer Spekol 1300 (Analytik Jena AG, Germany) in a wavelength range between 200 and 400 nm.

2.6. *Transmission electron microscopy (TEM)*

Samples of the nanosuspensions were freeze-fractured at −100 °C at a pressure of 5×10^{-6} bar in a BAF 400 device (Balzers, Germany). Thereafter they were shadowed with platinum/carbon (layer thickness 2 nm) at 45° and stabilized with carbon (layer thickness 20 nm) at 90°. Replicas were cleaned with sulfuric acid and viewed with a LEO 120 (Leo Inst., Germany) at 125 kV.

2.7. *X-ray diffraction*

Wide angle X-ray diffraction (WAXD) diagrams of bulk materials, wax and hard fat matrices obtained after melting and re-crystallization were taken using a Goniometer PW3050/6 (PANalytical B.V., The Netherlands) provided with a generator PW3040/60 XTert Pro. The samples were placed on stainless steel plates and the Bragg's angle was obtained from the diffraction pattern.

2.8. Differential scanning calorimetry (DSC)

Thermal behavior studies were carried out by means of a calorimeter DSC 220C (Seiko, Japan). For these tests, 5 mg of carnauba wax, hard fat and mixtures thereof were used. These samples were scanned in a range of temperature of 0–100 °C at a heating speed of 5 °C min^{-1}.

2.9. In vitro sun protection factor (SPF)

The sun protection factors of the preparations were measured by means of a SPF-290 computer operated analyzer (Optometrics Corp., UK). Samples (110 μL) were spread over Transpore® tapes (3 M GmbH, Germany) having an area of 55.5 cm^2 with a gloved finger. Radiation attenuation of the applied samples was performed in a wavelength range of 290–400 nm. The radiation was provided by a compact 125 W xenon arc lamp. Measurements of each formulation were done in duplicate. The in vitro SPF measured in this paper represents an indicator of the UVA/UVB protective property of the sunscreen product, calculated from the monochromatic protection factor, the solar irradiance and the erythemal constants according to Eq. (1)

$$\mathrm{SPF} = \frac{\sum_{290}^{400} E_\lambda B_\lambda}{\sum_{290}^{400} (E_\lambda B_\lambda / \overline{\mathrm{MPF}}_\lambda)} \tag{1}$$

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

Being the defined conditions for E_λ the midday midsummer sunlight at 40°N with a solar zenith angle of 20° (SPF Analyzer, 1997).

3. Results and discussion

3.1. UV absorption properties of the carnauba wax and its ethanolic extract

The qualitative UV absorption plot in a range of 200–400 nm of the refined carnauba wax solubilized in toluene (CW-T) and that of the ethanolic extract of carnauba wax being further diluted in ethanol at a ratio 1:50 (v/v) (CW-EE) are shown in Fig. 3. In the former case, it was observed that despite of the high dilution of carnauba wax (1 μg mL^{-1}), its toluenic solution presented a very high UV absorption along the scanned range having only one centered peak located at 308.2 nm, whereas in the latter case, two maximum peaks located at 205 and 310 nm in a range of 200–350 nm were detected. Since carnauba wax is known to contain hydroxycinnamic acid derivatives (Emås and Nyqvist, 2000; Wang et al., 2001) and some of those compounds could be solubilized in ethanol (Stafford, 1964; Baranowski and Nagel, 1981), it was suggested that some of the organic filters found in the toluenic solution (CW-T) might be present in the ethanolic extract of carnauba wax (CW-EE) as well. This could be appreciated in the same figure by the qualitative absorption of the extract in the range of 250–350 nm. However, based on the differences in polarity between ethanol and toluene, the presence of other more polar species in the ethanolic extract was also indicated because of the strong absorption between 200 and 250 nm presenting a peak at 205 nm.

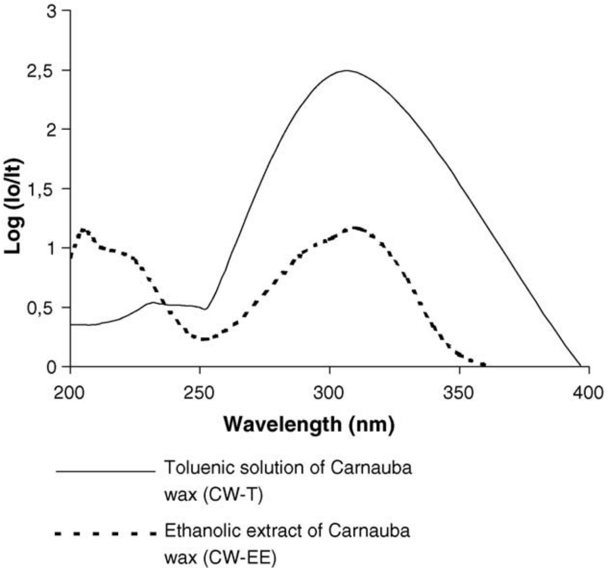

Fig. 3. Absorption spectra of the carnauba wax solubilized in toluene having a concentration of 1 μg mL^{-1} (CW-T) and a dilution of the ethanolic extract of carnauba wax in ethanol 1:50 (v/v) (CW-EE). The ethanolic extract was obtained by macerating 100 g of carnauba wax in 400 mL of ethanol. Solvent filled cuvettes of toluene and ethanol were used as baselines for the measurements correspondingly.

In a second experiment, a dilution of the nanosuspension containing titanium dioxide 2 wt% without lipid phase (Formulation II) (TD) and a dilution of the ethanolic extract of carnauba wax (CW-EE) were UV scanned in a range between 200 and 400 nm. The absorbances reported for the titanium dioxide suspensions were extremely low, meanwhile those of the ethanolic extract were found under 0.3 as observed in Fig. 4. According to literature reports, the titanium dioxide crystals of a primary particle size of 17 nm absorb UV light and this absorption presents a maximum located at approximately 300 nm (Kemira Pigments, 2004), but because of the high dilution of this nanosuspension (1 μM), only very low absorbances could be appreciated. However, when equal volumes of the titanium dioxide dispersion and the ethanolic extract were combined and diluted in water (CW-EE + TD), the UV absorption was remarkably increased. In this case, three absorption maximum peaks located at 224, 250 and 300 nm were detected and the absorption enhancement observed could be probably explained by the formation of one or more chemical complexes established between the compounds present in the extract and the titanium dioxide. Finally in Fig. 5, the same phenomenon, but at less extent, was observed. In this case an ethylcinnamate solution (EC) having a concentration of 2.54×10^{-10} M and showing absorbances under 0.5 in the range of 200–400 nm was added to the diluted nanosuspension of titanium dioxide (TD) and it was seen that the UV absorption in a range of 200–225 nm was enhanced meanwhile that of the range

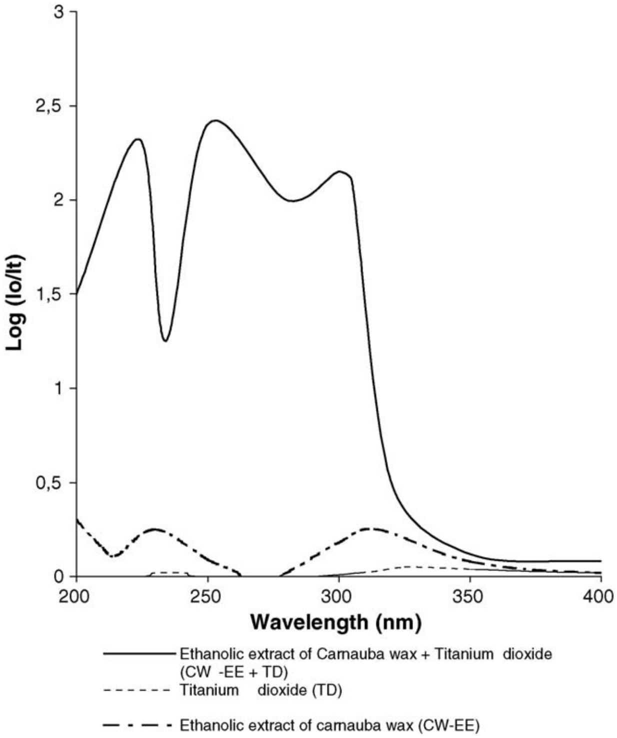

Fig. 4. Absorption spectra of the ethanolic extract of the carnauba wax at a ratio 1:50 (v/v) diluted in water 1:5 (v/v) (CW-EE), the nanosuspension containing titanium dioxide (2 wt%) diluted in water having a concentration of 1 μM (TD), and the mixture of one volume of the titanium dioxide nanosuspension 1 μM, one volume of the ethanolic extract of carnauba wax 1:50 (v/v) and three volumes of water (1:1:3) (CW-EE + TD). Double distilled water was used for the baseline measurements.

between 225 and 325 nm was only slightly modified (EC + TD). Considering these results, it could be speculated that different association phenomena at surface level established between the compounds of either the ethanolic extract or the ethylcinnamate and the titanium dioxide nanoparticles took place (Figs. 4 and 5) and that this possible bonding provoked an electron-excited state of the chemical species as suggested by some authors for substances forming coordination compounds (Tilley, 2000). Another indication of this phenomenon is the fact that the formation of organometallic compounds was always followed by an increased absorption of the formed species in the UV–vis range. This procedure was used in the past to identify trace metals by means of UV–vis spectrophotometry (Denney and Sinclair, 1987).

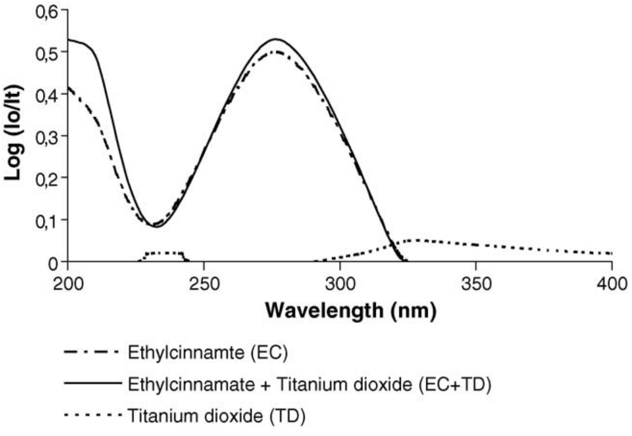

Fig. 5. Absorption spectra of the ethanolic solution of ethylcinnamate diluted in water (2.54×10^{-10} M) (EC), the nanosuspension containing titanium dioxide (2 wt%) diluted in water (1 μM) (TD), and the mixture of equal volumes of the nanosuspension and the ethanolic solution of the ethylcinnamate (2.54×10^{-10} M) diluted in three volumes water (1:1:3) (EC + TD). Double distilled water was used for the baseline measurements.

Table 2

The SPF ± S.D. of the nanosuspensions containing titanium dioxide without carnauba wax diluted (1:1) with ethanol, an ethanolic solution of ethylcinnamate and the concentrated ethanolic extract of carnauba wax

Mixture	Titanium dioxide content (%)		
	2	4	6
TiO_2 (nanosuspension)	1.51 ± 0.19	1.65 ± 0.13	2.10 ± 0.16
Ethanol + TiO_2 (1:1)	1.75 ± 0.21	1.85 ± 0.07	2.35 ± 0.21
EC-E + TiO_2 (1:1)	1.85 ± 0.07	2.35 ± 0.07	3.40 ± 0.14
CW-EE + TiO_2 (1:1)	1.75 ± 0.07	2.40 ± 0.28	2.80 ± 0.28

Hard fat (SPF = 1.05 ± 0.07); ethanol (SPF = 1.0 ± 0.10); CW-EE: non-diluted ethanolic extract of carnauba wax (SPF = 1.85 ± 0.07); EC-E: ethanolic solution of ethylcinnamate (SPF = 1.1 ± 0.05); hard fat + CW-EE (SPF = 1.0); hard fat + EC-E (SPF = 1.0).

3.2. Sun protection factor

3.2.1. Effect of the dispersion media on the sun protection factor

Another factor describing the effect of the UV light on the compounds present in the carnauba wax and on the titanium dioxide is the SPF (Walters et al., 1997). In a first series of experiments, mixtures 1:1 (v/v) of the nanosuspensions containing titanium dioxide (Formulations B) and the concentrated ethanolic extract of carnauba wax were prepared and their corresponding SPFs were measured as shown in Table 2. It was found that the SPFs presented only very slight increases after the addition of the concentrated extract of carnauba wax or the ethanolic solution of ethylcinnamate. These values were also compared with those corresponding to the addition of ethanol to discard solvent effects and no effect attributed to this latter could be supposed. The SPF of the ethanolic extract of carnauba was 1.85 and the highest SPF measured for this series was 2.80, whereas by the application of the ethanolic solution of ethylcinnamate the highest SPF was 3.40. However, using another dispersion media such as a hard fat, a closer contact between titanium dioxide crystals and the organic species of the CW-EE and the ethylcinnamate inside of a solid structure provided by the unsaturated fatty acids could be ensured and consequently high SPFs were measured as seen in Table 3. In this table, it was noticed that the distribution of titanium dioxide within the hard fat permitted to obtain higher SPFs than those of the non-diluted nanosuspensions described in Table 2. In these latter cases, the SPFs observed varied between 1.75 and 2.35, whereas those of the titanium dioxide dispersed in the hard fat oscillated between 2.70 and 7.50. Moreover, it has to be stated that no sunscreen properties could be attributed to the hard fat, because this product

Table 3
The SPF ± S.D. of the molten hard fat dispersions containing titanium dioxide either alone or combined with the concentrated ethanolic extract of carnauba wax

Dispersions	Titanium dioxide content (%)		
	2	4	6
Hard fat + TiO_2	2.70 ± 0.14	5.70 ± 1.41	7.50 ± 0.28
Hard fat + TiO_2 + EC-E	2.85 ± 0.21	6.75 ± 2.33	13.55 ± 0.35
Hard fat + TiO_2 + CW-EE	3.45 ± 0.07	8.25 ± 0.78	11.35 ± 1.48

Hard fat (SPF = 1.05 ± 0.07); ethanol (SPF = 1.0 ± 0.10); EC-E: ethanolic solution of ethylcinnamate (SPF = 1.1 ± 0.05); CW-EE: non-diluted ethanolic extract of carnauba wax (SPF = 1.85 ± 0.07); hard fat + CW-EE (SPF = 1.0); hard fat + EC-E (SPF = 1.0).

Table 4
The SPF ± S.D. of the fabricated nanosuspensions containing titanium dioxide

Description	Titanium dioxide content (%)		
	2	4	6
Formulations B	1.51 ± 0.19	1.65 ± 0.13	2.10 ± 0.16
Formulations C	3.50 ± 1.13	8.05 ± 1.79	22.93 ± 8.87
Formulations D	10.95 ± 2.05	22.38 ± 5.21	34.65 ± 11.5

Key: Formulation A—nanosuspension containing carnauba wax matrices (SPF = 2.2 ± 0.12); Formulations B—nanosuspensions containing titanium dioxide without carnauba wax; Formulations C—nanosuspensions containing carnauba wax matrices with titanium dioxide distributed in the aqueous phase; Formulations D—nanosuspensions containing carnauba wax matrices with titanium dioxide distributed in the lipid phase.

either alone or combined with the EC-E or the CW-EE exhibited SPFs from about 1.

Former research on the field of inorganic sunscreens demonstrated that the increase in the viscosity of the cosmetic preparations containing titanium dioxide was directly related to an increased SPF (Anderson et al., 1997) as confirmed by this paper when hard fat was used.

However, this explanation could be completed taking into account the semiconductor character of titanium dioxide. Accordingly, it may be supposed that as more titanium dioxide units came closer into a cluster, like in the case of the hard fat matrix, more continuous conduction bands probably formed (Fig. 2). Such bands contributed to reduce the energy necessary for the promotion of electrons into the empty conduction bands, which increased the UV absorption capacity of those systems. In the field of the photochemistry the aforementioned phenomenon is known as band theory (Nassau, 1983).

Table 3 also showed that the addition of CW-EE and EC-E to titanium dioxide crystals increased the original SPFs of the titanium dioxide dispersed in the hard fat, but not that of the hard fat alone. In theses cases, the increases were better appreciated when the highest concentration of titanium dioxide, i.e. 6 wt%, was used. According to this, for the addition of EC-E and CW-EE, SPFs of 13.55 and 11.35 were reported, respectively. To understand this increase, it is necessary to consider the chemical properties of the substances within these matrices. In respect of these, the ethylcinnamate and very probably the cinnamate derivatives contained in the ethanolic extract of carnauba wax possessed delocalized π orbitals leading to high electronic conductivity (Tilley, 2004). These compounds could be seen as organic molecules adsorbed at the surface of the stearic acid-aluminum coated titanium dioxide crystals dispersed into wax or hard fat matrices so that when they were UV irradiated, the delocalized electrons probably might be transferred into the conduction bands of the titanium dioxide. This phenomenon could be reflected by an increased UV absorption and thus by an increased SPF. Former studies carried out by Smestad (1998) and Cherepy et al. (1997) in the field of solar conversion demonstrated the occurrence of chelation, complexation and molecular self-assembly phenomena during the attachment of the dye molecules such as cyanines or chlorophyll, i.e. molecules with π delocalized systems, to the surface of titanium dioxide crystals deposited over a glass plate (Nishimura et al., 2003; Smestad and Gräzel, 1998). Considering the above described information, it could be expected that the contact between organic molecules and the coated titanium dioxide within the fatty acid matrix may induce the formation of UV absorbing chemical species at a surface level inside the matrices formed.

3.2.2. *The sun protection factor of the nanosuspensions*

The SPFs of the Formulations A–D can be appreciated in Table 4. According to these results, the nanosuspension containing carnauba wax particles (Formulation A) presented a SPF of 2.2, whereas those nanosuspensions containing titanium dioxide without carnauba wax (Formulations B) showed SPFs no higher than 2.10. Considering the SPF values of Formulations A and B, it could be expected a simple additive effect would give a SPF of about 4. However, when the titanium dioxide was distributed in the aqueous phase (Formulations C), increases between 2.33 and 10.9 times were observed and when the titanium dioxide crystals were added to the lipid phase of nanosuspensions (Formulations D), the SPFs presented increases varying from 7.25 to 16.5 times in comparison to those exhibited by the Formulations B. Taking into account those results, it was supposed that the close contact of the molecular species integrating the carnauba wax with the titanium dioxide crystals (Formulations C and D) induced their adsorption at the surface of those stearic acid-aluminum coated titanium dioxide crystals enhancing the UV absorption of the system as demonstrated by the higher SPFs observed, particularly, when the titanium dioxide crystals were dispersed into the lipid phase. Furthermore, considering the previous section of this paper, it was demonstrated that the SPF of titanium dioxide crystals dispersed into a matrix composed of saturated fatty acids, like that of carnauba wax, was increased by the addition of organic molecules possessing delocalized electron systems. The obtained SPFs demonstrated the advantages of the use of mixed particles composed of liquid and solid lipids (NLCs) as carrier systems to integrate organic and inorganic UV filters in a single ordered structure, by which a rational optimization of a sunscreen formulation containing both species would be affordable. Although it was demonstrated that in those carriers, carnauba wax was directly responsible for the UV absorption, the use of decyl oleate was justified in virtue of its low viscosity enabling a good distribution of the titanium dioxide crystals inside the carnauba wax matrices before being hot pressure homogenized. Otherwise the very fast crystallization of this wax inducing high

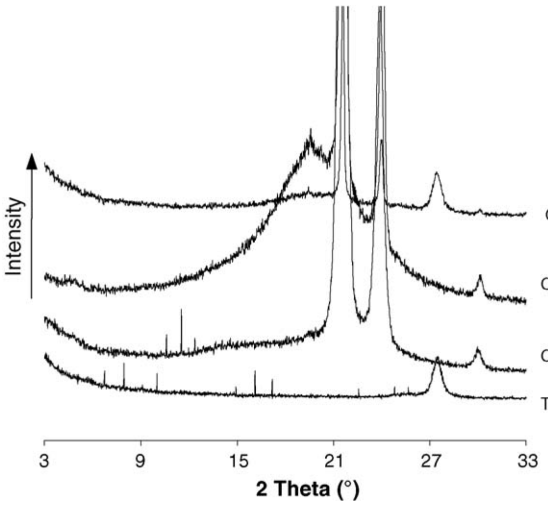

Fig. 6. WAXD patterns of the carnauba wax and mixtures thereof. The curves have been displaced vertically for better visualization.

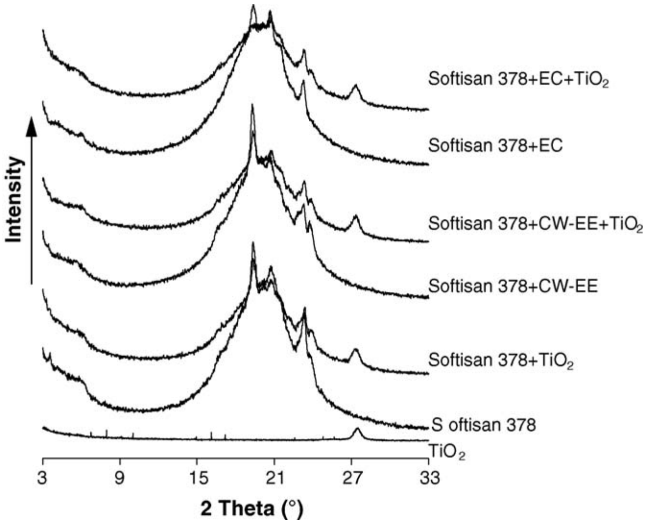

Fig. 7. WAXD patterns of the hard fat (Softisan® 378) and mixtures thereof. The curves have been displaced vertically for better visualization.

viscosity would exhibit severe technical difficulties to integrate both species into single particles.

3.3. X-ray diffraction (WAXD) studies and differential scanning calorimetry of fatty acid matrices

Possible changes in the crystal structure of the fatty acid matrices of carnauba wax and hard fat attributed to the incorporation of decyl oleate and titanium dioxide in the first case and cinnamates and titanium dioxide in the second one were evaluated by means of X-ray diffraction and differential scanning calorimetry. The results are shown in Figs. 6 and 7 and Table 5, respectively. In Fig. 6, it can be observed that carnauba wax possessed three main diffraction bands located at 4.1×10^{-1} nm ($2\theta = 21.6°$), 3.7×10^{-1} nm ($2\theta = 23.8°$) and 2.9×10^{-1} nm ($2\theta = 29.9°$) each. By the incorporation of decyl oleate, an additional band located at 4.5×10^{-1} nm ($2\theta = 19.5°$) appeared, whereas the rest of the diffraction bands remained unchanged. This change in the crystal structure could be interpreted as crystal disordering, which was confirmed by the diminution of the melting point from 83.5 to 78.5 °C as shown in Table 5. However, by the addition of titanium dioxide to the wax–oil mixture, a new band located at 3.2×10^{-1} nm ($2\theta = 27.5°$) appeared, meanwhile the band at 2.9×10^{-1} nm ($2\theta = 29.9°$) diminished in its intensity. The rest of the diffraction bands of the matrix composed of carnauba wax and decyl oleate remained unchanged and the melting point was only slightly modified from 78.5 to 76.9 °C. According to reports describing the advantages of the use of NLCs, the crystal disordering of the lipid matrices obtained by the addition of liquid lipids seemed to favor the long-term loading capacity of these systems (Souto et al., 2004). This is supposed because the presence of liquid lipids avoids the complete reordering of the crystal structure of the solid lipids to create new spaces, which can be eventually occupied by drugs or other substances (Jores et al., 2004). For this reason, the incorporation of decyl oleate may be considered as advantageous for the constitution of drug loaded carnauba wax nanoparticles.

In respect of the crystal structure of the hard fat matrix, diffraction bands with different intensities at 5.3×10^{-1} nm ($2\theta = 16.7°$), 4.6×10^{-1} nm ($2\theta = 19.4°$), 4.28×10^{-1} nm ($2\theta = 20.7°$), 3.81×10^{-1} nm ($2\theta = 23.3°$) and 3.71×10^{-1} nm ($2\theta = 23.9°$) were detected as seen in Fig. 7. In the same figure, it can be appreciated that after the addition of the ethanolic extract of carnauba wax (CW-EE) and the ethanolic solution of ethylcinnamate (EC-E) to the hard fat, the main diffraction bands of the hard fat could be observed again and no new bands were detected. The same behavior was observed when titanium dioxide was incorporated to the mixtures containing cinnamates. Nevertheless, in these cases, a constant diffraction band located at 3.2×10^{-1} nm ($2\theta = 27.5°$) attributed to the addition of titanium dioxide crystals was found. On the other hand, the results of the DSC (Table 5) showed that the melting points of the hard fat were only slightly changed when cinnamates and

Table 5
The melting points (T_m) in °C measured by DSC of the carnauba wax, the hard fat and mixtures thereof

	CW	CW:DO (1:1)	CW:DO:TiO$_2$ (1:1:0.38)	TiO$_2$
T_m	83.5	78.5	76.9	–

	SFT	SFT:CW-EE (1:0.1)	SFT:CW-EE:TiO$_2$ (1:0.1:0.4)	SFT:EC-E (1:0.1)	SFT: EC-E:TiO$_2$ (1:0.1:0.4)	SFT:TiO$_2$ (1:0.4)	TiO$_2$
T_m	32.4	32.6	33.5	33.1	35.6	33.8	–

Key: CW, carnauba wax; DO, decyl oleate; SFT, Softisan® 378; CW-EE, ethanolic extract of carnauba wax; EC-E, ethanolic solution of ethylcinnamate.

Fig. 8. Transmission electron micrograph of the formulation containing titanium dioxide-free lipid nanoparticles.

titanium dioxide crystals were added. The obtained values for this parameter varied between 32.4 and 35.6 °C. Finally, since no crystal modification by means of WAXD and DSC was demonstrated, no formation of new species could be supposed, which indicated that the contact between organic filters and titanium dioxide was attributed to the surface adsorption of the organic molecules deposited over the titanium dioxide crystals.

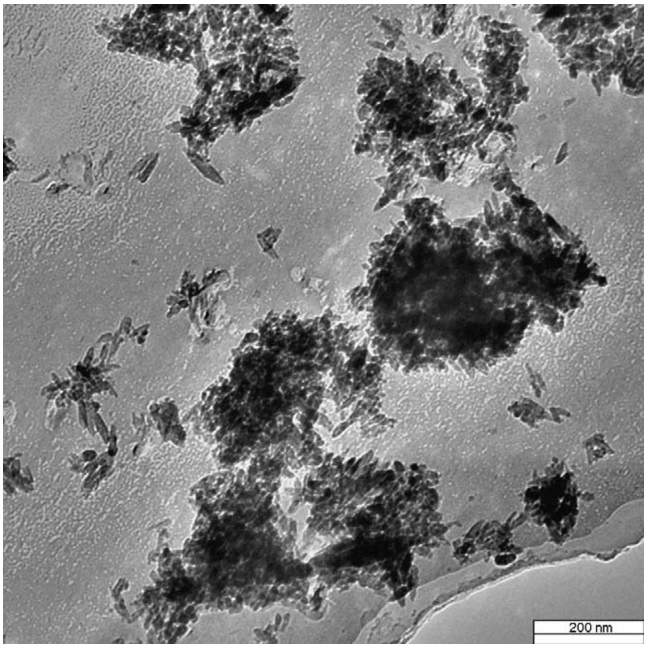

Fig. 9. Transmission electron micrograph of the titanium dioxide dispersion after the homogenization process.

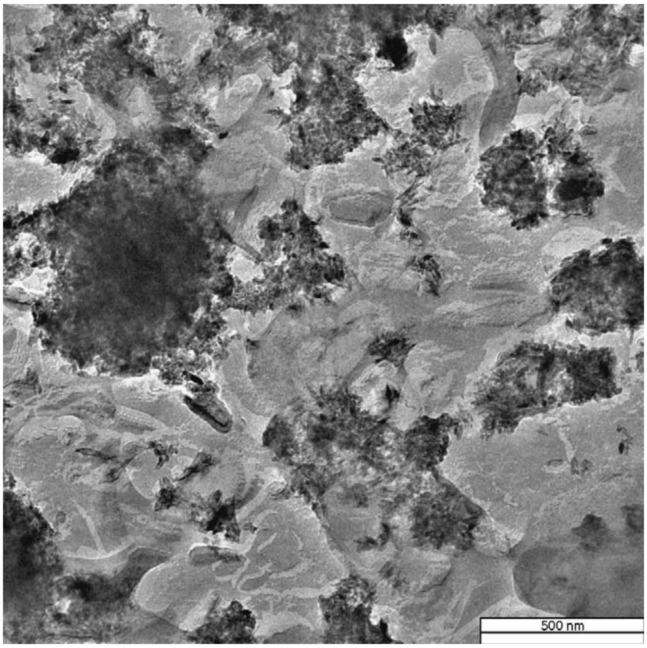

Fig. 10. Transmission electron micrograph of the formulation containing titanium dioxide agglomerates dispersed in the presence of the lipid matrices.

3.4. TEM characterization

By the use of TEM, morphological differences in the species integrating the nanosuspensions could be established and the distribution of the pigments in the different phases of the formulations was confirmed. These viewings are shown in Figs. 8–11. After the hot high-pressure homogenization process, the cooling down of the nanoparticulate system occurred and with this, the re-crystallization of the carnauba wax. The structures obtained

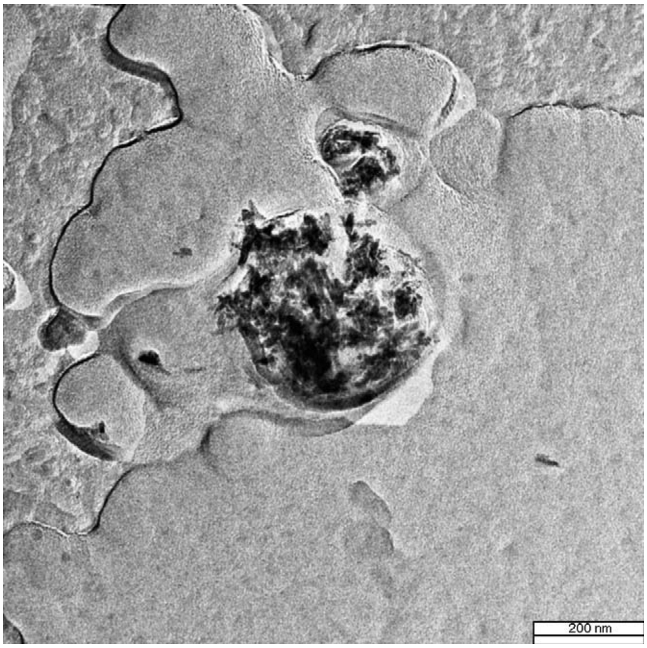

Fig. 11. Transmission electron micrograph of the formulation containing titanium dioxide agglomerates bonded by lipid matrices.

from this process, i.e. pigment-free carnauba wax–decyl oleate nanoparticles (Formulation A) are depicted in Fig. 8. On the other hand, in Fig. 9 a sample of nanofine titanium dioxide crystal formulations (Formulation B) is shown. As observed, crystals forming rounded agglomerates and small crystal groups separated from these agglomerates were found. When these titanium dioxide crystals were distributed into the aqueous phase of the nanosuspension (Formulation C), wax nanoparticles surrounded by titanium dioxide crystals were detected as seen in Fig. 10. It could be supposed that by the use of high pressure (300 bar) some crystals could be incrusted in or attached to surface of the wax particles. Finally, when the titanium dioxide was dispersed in the lipid phase (Formulation D), crystals being incorporated into the matrix of carnauba wax nanoparticles were detected as depicted in Fig. 11. In this figure, a more organized structure showing the distribution of the pigments inside the nanoparticle and thus demonstrating the induced absorption of organic molecules by the crystals of titanium dioxide due to high-pressure homogenization was found.

4. Conclusion

The surface contact between organic molecules possessing double conjugated systems such as the cinnamates and hydrophobic titanium dioxide demonstrated to increase the UV absorption capacity of both species. This adsorption was particularly favored by the use of a solid matrix composed of saturated fatty acids, which provided a medium for the fixation of the organic molecules onto the surface of the titanium dioxide crystals. Furthermore, the crystalline structure of this matrix seemed not to be modified neither by the incorporation of diluted amounts of organic molecules nor by the dispersion of titanium dioxide crystals reinforcing the hypothesis of the adsorption. Nevertheless, further studies have to be performed taking into account not only the complexation at the surface but also the nature of the coatings conventionally applied to titanium dioxide crystals to preserve its photostability. On the other hand, it was shown that by the use of high-pressure homogenization, the solid lipid matrix could be divided into nanoparticles, which remained dispersed into the aqueous and low viscous medium. The submicron size ensured the close contact, i.e. adsorption, of the organic molecules with the titanium dioxide crystals within the crystallized wax. Considering the aforementioned facts, the synergistic interaction between carnauba wax and titanium dioxide provoking the enhancement of SPF of those formulations combining both materials at nanoscale could be probably explained in virtue of a mechanism of electron transfer taking place from the π delocalized organic compounds, i.e. cinnamates to the empty conduction bands of the titanium dioxide aggregates, being this electron transfer more effective when the titanium dioxide crystals were encapsulated or bonded by the carnauba wax and not when these crystals were attached to the surface of the wax particles. Although it is known that the electron transfer establishes the formation of new chemical species such as chelates, complexes or molecular self-assemblies, the existence of those species has to be confirmed in a later study. Considering the exposed background, this study demonstrated the possibility to fabricate aqueous-based sunscreen products without the use of organometallic synthesis or "sol–gel" procedures. This process implicates the rational integration of semiconductors and nanosize crystallized lipid matrices previously loaded with UV absorbing compounds by means of high-pressure homogenization or other high efficient nanodispersion techniques to obtain single units establishing chelates or other complexes at surface level being capable to exhibit high UV filtering properties based on not only the UV absorption, but also on their intrinsic UV blocking properties attributed to their small size.

Acknowledgments

Mr. Villalobos is recipient of a fellowship granted by the DAAD. Kemira Chemicals GmbH and Brenntag GmbH, are thanked for the donation of substances. The availability of the TEM at the Institute of Physics of the Condensed Matter of the Technical University of Braunschweig and the technical assistance of Mr. Hanno Dierke are gratefully acknowledged.

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