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Effect of nanoparticle encapsulation on the photostability of the sunscreen agent, 2-ethylhexyl-*p*-methoxycinnamate

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Abstract

The aim of this study was to investigate the influence of nanoparticle-based systems on the light-induced decomposition of the sunscreen agent, *trans*-2-ethylhexyl-*p*-methoxycinnamate (*trans*-EHMC). Ethylcellulose (EC) and poly-D,L-lactide-co-glycolide (PLGA) were used as biocompatible polymers for the preparation of the particulate systems. The ''salting out'' method was used for nanoparticle preparation and several variables were evaluated in order to optimize product characteristics. The photodegradation of the sunscreen agent in emulsion vehicles was reduced by encapsulation into the PLGA nanoparticles (the extent of degradation was 35.3% for the sunscreen-loaded nanoparticles compared to 52.3% for free *trans*-EHMC) whereas the EC nanoparticle system had no significant effect. Therefore, PLGA nanoparticles loaded with *trans*-EHMC improve the photostability of the sunscreen agent.

Keywords: Nanoparticles; Sunscreen agent; 2-Ethylhexyl-*p*-methoxycinnamate; Photodegradation; Poly(lactide-co-glycolide); Ethylcellulose

1. Introduction

It is well recognized that the UV portion (290–400 nm) of the sunlight spectrum reaching the earth surface is responsible for skin photodamage (National Institute of Health, 1989). Adverse reactions to the sun's UV rays include short-term inflammatory responses (i.e. erythema, oedema) and long-term effects such as cutaneous photoageing, immunosuppression and skin cancers which are increasing throughout the world (National Institute of Health, 1989; Ziegler et al., 1994; Urbach, 1997; Hochberg and Enk, 1999; Tarras-Wahlberg et al., 1999).

The rising level of awareness of the harmful effects of sunlight has fuelled a growth in the use

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of sunscreens (National Institute of Health, 1989; Hayden et al., 1998; Green et al., 1999). Sunscreen preparations contain organic chemicals which lessen the amount of UV light reaching human skin by absorbing the radiation. The photoactivated sunscreen molecule disposes of the excitation energy in several ways: in the form of heat, by fluorescence, phosphorescence, interaction with neighbouring molecules or by undergoing photoinduced decompositions (Broadbent et al., 1996; Stokes and Diffey, 1999). The latter reactions not only decrease the sunscreen UV-protective capacity during usage (Berset et al., 1996; Stokes and Diffey, 1999; Tarras-Wahlberg et al., 1999) but can also produce allergic or toxic degradation products (Deflandre and Lang, 1988; Dromgoole and Maibach, 1990). Therefore a high photostability is a prerequisite for the effectiveness of sunscreen products.

trans-2-Ethylhexyl-*p*-methoxycinnamate (*trans*-EHMC; Fig. 1) represents the most widely used sunscreen compound (Broadbent et al., 1996; Hayden et al., 1998). It is approved by the regulatory agencies of Europe (EEC Directive, 1976), USA (US Food and Drug Administration, 1999), Japan and Australia (Hayden et al., 1998). *trans*-EHMC is classified as an UV-B filter in accordance to its higher absorption in the shorter wavelength region (290–320 nm) of the solar UV radiation. Several studies have demonstrated that *trans*-EHMC is unstable following irradiation both in solution (Berset et al., 1996; Broadbent et al., 1996; Tarras-Wahlberg et al., 1999) and in emulsion formulations (Deflandre and Lang, 1988). Moreover, various reports of photocontact sensitization induced by *trans*-EHMC have appeared in the literature (Kimura and Katoh, 1995; Schmidt et al., 1998). Consequently, in order to ensure adequate efficacy and safety for this sunscreen agent, there is a need for new carrier systems with enhanced *trans*-EHMC photostability.

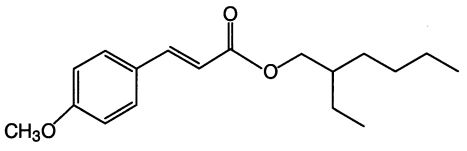


Fig. 1. Chemical structure of *trans*-EHMC.

Our interest is focused on nanospheres as biocompatible delivery systems for the prevention of the degradation of labile active ingredients. Nanospheres are solid polymeric spherical particles in which an active substance can be homogeneously dispersed in a polymeric matrix. Different kinds of polymers can be used to make nanospheres, depending on their final application (Soppimath et al., 2001). These systems are studied to enable the modified release of drugs and/or their stabilization and, in our opinion, they can also be evaluated as carriers for cosmetic substances such as sunscreens.

An important advantage of nanospheres is their small size (below 1 μm) which facilitates the formulation of nanospheres in cosmetic systems and enables comfortable application to the skin. On the other hand, the presence of a polymeric envelop may exert a protective action towards the active ingredient.

In the present study, the preparation and characterization of ethylcellulose (EC) and poly-D,L-lactide-co-glycolide (PLGA) nanoparticles loaded with *trans*-EHMC are reported. In addition, the influence of the nanoparticle matrices on the photochemical stability of the sunscreen agent is also presented.

2. Materials and methods

2.1. Materials

trans-EHMC was supplied by Hoffmann-La Roche Ltd (Basel, Switzerland). Polylactide-co-glycolide copolymer Resomer RG 752 (D,L-lactide:glycolide, 75:25 molar ratio, viscosity 0.23 dl/g, 0.1% in chloroform) was purchased from Boehringer Ingelheim (Ingelheim, Germany); EC (viscosity 14 cP, 5% in toluene:ethyl alcohol, 80:20) was purchased from BDH Italia (Milano, Italy).

Polyvinyl alcohol (MW, 85 000–146 000) and $CaCl_2$ were purchased from Sigma (Milano, Italy). Methanol, acetonitrile, tetrahydrofuran and water were HPLC grade from Merck (Darmstadt, Ger-

many). All other chemicals were of analytical-reagent grade (Sigma).

2.2. High-performance liquid chromatography (HPLC)

The HPLC apparatus comprised a Model Lab-Flow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 20 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–Vis detector (Jasco, Tokyo, Japan) set at 307 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 80365 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Luna C_{18} column (150 × 4.6 mm i.d.; Phenomenex, Torrance, CA, USA) fitted with a guard column (5-μm particles, 4 × 2 mm i.d.) and eluted isocratically, at a flow-rate of 1.0 ml/min, with methanol–acetonitrile–tetrahydrofuran–water (65:10:10:15, v/v). The identity of *trans*-EHMC peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method.

2.3. Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) was performed with a GC 8060 gas chromatograph (CE Instruments, Milan, Italy) coupled with a MD 800 mass spectrometer (TermoQuest Italia, Milan, Italy) operating in the electron impact mode (70 eV) with transfer line and ion source temperature maintained at 250 °C. A SE-54 fused silica capillary column (25 m × 0.25 mm i.d.; CE Instruments) was used. The GC operating conditions were: injector temperature, 280 °C; column temperature, 100 °C for 2 min, then programmed at 10 °C/min to 270 °C; carrier gas (helium) inlet pressure, 70 kPa. The samples (1 μl) were introduced using split injection (split ratio 20:1). The GC-MS was controlled by the Mass Lab 1.12 software (TermoQuest Italia).

2.4. NMR spectroscopy

^{1}H-NMR spectra were recorded on a Bruker AC spectrometer (300 MHz). Samples were solubilized in $CDCl_3$. Typical parameters for the ^{1}H-NMR spectra were: 0.4 Hz/pt resolution, 16 scans, 18 s relaxation delay, 90° pulse.

2.5. Nanoparticle preparation

Nanoparticles were produced by a "salting out" method (Ibrahim et al., 1992). Several process parameters were investigated on blank nanoparticles made of PLGA or EC in order to prepare systems with suitable "in vitro" characteristics to encapsulate a sunscreen agent.

2.5.1. Blank nanoparticle preparation

The polymer (PLGA or EC) was dissolved in acetone (2.5% w/w); separately, a viscous aqueous solution was prepared dissolving polyvinyl alcohol in a concentrated $CaCl_2$ aqueous solution; addition of the aqueous solution to the organic phase under vigorous stirring at 13 500 rpm using an IKA Ultraturrax T25 equipped with a S25N dispersing tool gave a W/O emulsion that inverted to an O/W emulsion by further addition of the aqueous phase portion (final organic:aqueous solution, 1:2 weight ratio). Pure water was then dropped to the emulsion to induce diffusion of the organic solvent into the aqueous solution resulting in a nanoparticle dispersion. The nanoparticle purification was carried out by two different techniques as described below.

2.5.1.1. Purification by centrifugation. The resulting dispersed nanoparticles were centrifuged at centrifugal forces ranging between 1000 and 30 000 *g*. Then nanoparticles were suspended in water and freeze-dried overnight at −40 °C and 40 mbar.

2.5.1.2. Purification by dialysis and centrifugation. The organic solvent and the excess of $CaCl_2$ were eliminated from the nanoparticle suspension by 2-h dialysis through 12–14 000 MWCO membranes followed by a 1-night dialysis through 300 000 MWCO membranes to eliminate PVA excess

(Spectra/Por, Spectrum Laboratories, Canada). The nanoparticles were recovered by centrifugation at 30 000 *g* and 1-night freeze-drying at −40°C and 40 mbar.

2.5.2. *trans*-EHMC loaded nanoparticle preparation

trans-EHMC loaded nanoparticles made of PLGA or EC were prepared by the "salting out" method described above, using the following conditions: sunscreen:polymer, 1:1 weight ratio; polyvinyl alcohol at 3% w/w; $CaCl_2$ at 40% w/w; purification of nanoparticles by dialysis and centrifugation as outlined above. All batches of nanoparticles were produced at least in triplicate.

2.6. Morphological characterization of nanoparticles

2.6.1. Scanning electron microscopy

Photographs of nanoparticles after freeze-drying were obtained by scanning electron microscopy (SEM) using a scanning electron microscope Jeol JX 840-A (Jeol Ltd, Tokyo, Japan); samples for SEM analysis were prepared by gold-sputtering the nanoparticles in an argon atmosphere.

2.6.2. Transmission electron microscopy (TEM)

Nanoparticles were analyzed on negative stain electron microscopy using a JEM 1200 EXII electron microscope (Jeol Ltd, Tokyo, Japan). A drop of the nanoparticle suspension was applied to carbon-coated grids, stained with saturated uranyl acetate aqueous solution and visualized at 80 kV.

2.6.3. Particle size analysis

Particle size analysis was performed by light diffraction method using a Coulter apparatus, model LS 230 (Coulter Corp., Hialeah, FL). This instrument works on laser diffraction optics and on another system, termed PIDS, based on polarized light of three wavelengths. The size range of the LS230 version is from 0.04 to 2000 μm. Nanoparticle samples were suspended in filtered water, sonicated for 30 s, and subsequently analyzed. Three analyses were performed for each sample.

2.7. Nanoparticle sunscreen content

The actual amount of *trans*-EHMC entrapped in the nanoparticles was determined by HPLC. The nanoparticles (20–25 mg) were dispersed in an organic solvent (methanol or acetonitrile) under sonication. The sample was diluted to volume (10 ml) and filtered through 0.45-μm membrane filters (Whatman, Clifton, NJ, USA). A portion (5 μl) of the resulting solution was assayed for *trans*-EHMC by HPLC, as outlined above.

2.8. Photodegradation studies

Photodecomposition experiments were carried out in lotion formulations (oil-in-water emulsion). The preparations contained *trans*-EHMC (0.15%, w/w) in conjunction with blank nanoparticles or an equivalent amount of sunscreen-loaded nanoparticles. The lotion excipients were: sorbitan monostearate, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, *p*-hydroxybenzoic acid methyl ester, isopropyl isostearate (Henkel Fino Mornasco, Italy), cetearyl isononanoate (Henkel), cetearyl alcohol (Henkel), D-sorbitol, dehydroacetic acid, EDTA, water. Blank or loaded nanoparticles were added in the cooling phase of the emulsion preparation at ca. 40 °C. A portion of the test sample (220–250 mg) containing the free or nanoparticle-loaded *trans*-EHMC, was transferred into a quartz cuvette (path-length, 2 mm) and then exposed to the solar simulator which consisted of a 200 W Xenon-Mercury lamp (Hanovia 901-B1) fitted with focusing lens, to centre the light on the sample, and with a WG 300 filter to cut off wavelengths shorter than 290 nm. The solar simulator emission was measured by a Goldlux radiometer (Oriel Corporation, USA) and was maintained at about 0.4 and 2.5 mW/cm^2 for UV-B and UV-A (320–400 nm), respectively. The samples were placed in front of the exit port of the solar simulator and air cooled during irradiation. After the appropriate exposure interval (4 h), the cuvette was removed and its content quantitatively transferred into a 10-ml calibrated flask, diluted to volume with methanol and filtered (0.45-μm membrane filter). A portion (5 μl) of the resulting solution was assayed by HPLC for

both *trans*-EHMC and the photolytic degradant. All samples were stored in the dark after irradiation and preparation for HPLC analysis. The degree of photodegradation was measured by comparing the peak areas of *trans*-EHMC from the irradiated samples with those obtained by analysis of an equivalent amount of the non-exposed preparation. The percentage ratio of the photoproduct peak area to the *trans*-EHMC peak area was also used to evaluate the extent of photodecomposition. Statistical analysis of the results was carried out by Student's *t*-test. Significance was taken as $P < 0.05$.

3. Results and discussion

3.1. Nanoparticle preparation and characterization

The nanoparticle preparation method was set up using either PLGA or EC polymers. Several variables were investigated (Table 1) in order to obtain good yields of production of particles with a homogeneous size. For both polymers all PVA and $CaCl_2$ concentrations tested permitted to produce well-formed nanoparticles.

Table 2 reports d_{10}, d_{50}, d_{90} of blank PLGA nanoparticles at various PVA and $CaCl_2$ concentrations. The smallest nanoparticles ($d_{90} < 2.05$ μm) were produced with PVA at 3% w/w and $CaCl_2$ at 40% w/w. Similar results were obtained for EC nanoparticles (data not reported).

Centrifugation steps followed by freeze drying of each fraction permitted evaluation of the yield of production in relation to particle size and density. Centrifugal fields lower than 1000 *g* and within 1000 and 30 000 *g*, did not result in clear separations; however, two fractions of each batch were recovered by centrifuging exactly at 1000 and 30 000 *g*.

Table 1
Variables investigated in the nanoparticle production method

Variables	
Polymer	PLGA 752
	EC
PVA concentration	3% w/w
	6% w/w
$CaCl_2$ concentration	20% w/w
	40% w/w
Purification steps	Centrifugation
	Dialysis and centrifugation

SEM analyses indicated that particle density was related to particle size, as illustrated in Fig. 2a and b which show the micrographs of the first and the second fraction of batch PLGA/1, respectively. Fig. 3 shows the photomicrograph of the second fraction of batch PLGA/4.

Table 3 lists the actual mean yields of production of blank PLGA nanoparticles; the fractional amounts of nanoparticles recovered from the first and second centrifugation were calculated as the percentage of the actual yield of production. Good production yields were obtained using $CaCl_2$ at 40% (batches PLGA/1, PLGA/4); the PLGA/4 batch was the only one with the whole production yield corresponding to second fraction. The same trend was observed for blank EC nanoparticles (data not shown).

Dialysis steps through membranes with different cut-off permitted elimination of excess of reagents from nanoparticles, first of all the PVA. This result is highlighted by TEM analysis; Fig. 4 shows pictures of non dialyzed (Fig. 4a) and dialyzed through 300 000 MWCO (Fig. 4b) PLGA/4 batch.

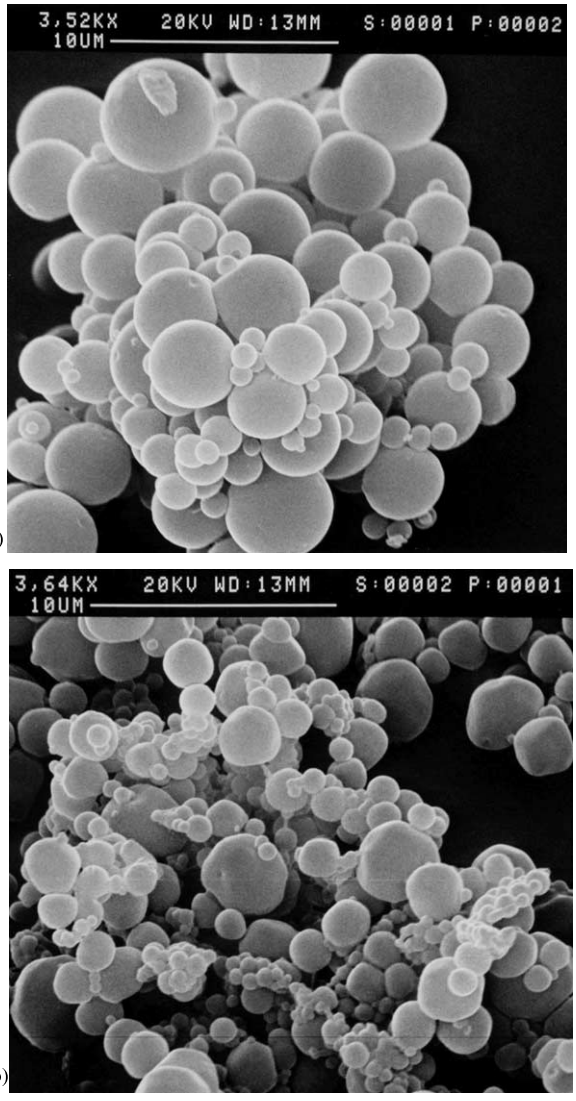
After this preliminary work on blank nanoparticles, *trans*-EHMC was encapsulated in PLGA and EC nanoparticles by the "salting out" method using the condition employed for the blank PLGA/4 batch. The purification of nanoparticles was carried out by dialysis followed by centrifugation at 30 000 *g*.

The encapsulation of the sunscreen agent did not modify the morphological characteristics of nanoparticles. Fig. 5 shows, as an example, a picture of a batch made of EC and loaded with the sunscreen agent.

The encapsulation efficiency of *trans*-EHMC was higher for the PLGA nanoparticles (60.4% ± 4.8) as compared to the nanoparticles made of EC (13.3% ± 6.4). In addition, the latter system exhibited a large variability of the encapsulation yields from different batches with values as low as 8.3%.

Table 2
Particle size distribution of blank PLGA nanoparticles expressed as d_{10}-, d_{50}-, d_{90}- mean (S.D. < 0.3)

Sample	Nanoparticle composition		d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
	PVA (w/w)	$CaCl_2$ (w/w)			
PLGA/1	6	40	1.84	5.71	21.45
PLGA/2	6	20	1.79	5.85	16.16
PLGA/3	3	20	2.28	8.87	55.55
PLGA/4	3	40	0.48	0.68	2.05



(a)

(b)

Fig. 2. SEM photomicrographs of batch PLGA/1: (a) first fraction (1000 *g*); (b) second fraction (30 000 *g*).

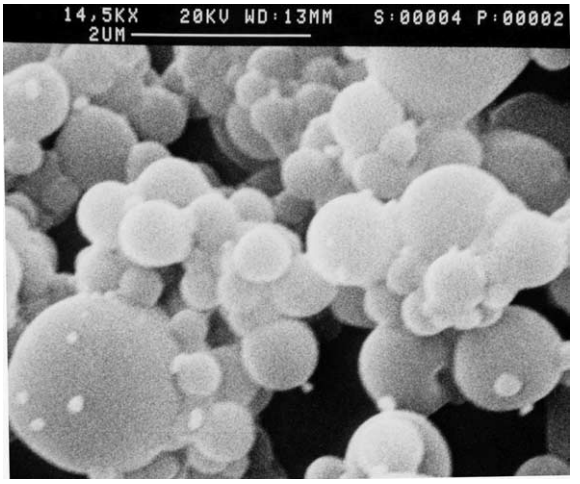


Fig. 3. SEM photomicrograph of batch PLGA/4.

3.2. Photodegradation studies

In order to study the effect of the polymeric particle carriers on the photochemical behaviour of *trans*-EHMC, the photolysis experiments were performed on a lotion (oil-in-water emulsion) as irradiation medium. This vehicle was selected as a model formulation since it represents the most common type of sunscreen preparation (Siemer, 1991) and hence simulates conditions of real use (Deflandre and Lang, 1988). *trans*-EHMC in combination with empty nanoparticles or the sunscreen-loaded nanoparticles were incorporated into the lotion and exposed for 4 h to the solar simulator. During the light-stability measurements, the applied UV-B energy corresponded to 20 minimal erythemal doses (MED) which is considered comparable to a daily solar emission (Tarras-Wahlberg et al., 1999). The degree of photodecomposition of the UV filter was mea-

Table 3
Yield of production of blank PLGA nanoparticles purified by centrifugation

Sample	PVA (w/w)	$CaCl_2$ (w/w)	Yield of production (%)		
			Actual	First fraction 1000 *g*	Second fraction 30 000 *g*
PLGA/1	6	40	74.28	46.15	53.85
PLGA/2	6	20	47.71	87.50	12.50
PLGA/3	3	20	51.43	77.78	22.22
PLGA/4	3	40	74.36	–	100

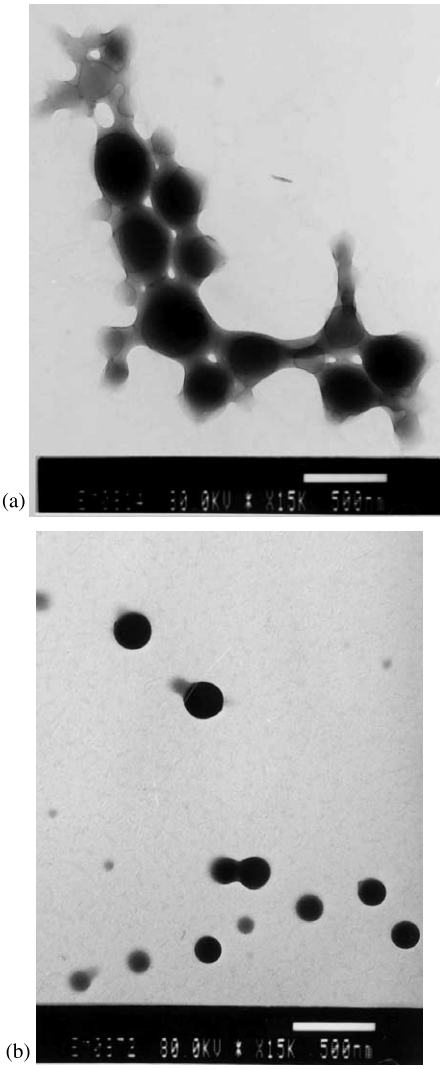


Fig. 4. TEM photomicrographs of batch PLGA/4: (a) non dialyzed; (b) dialyzed through 300 000 MWCO.

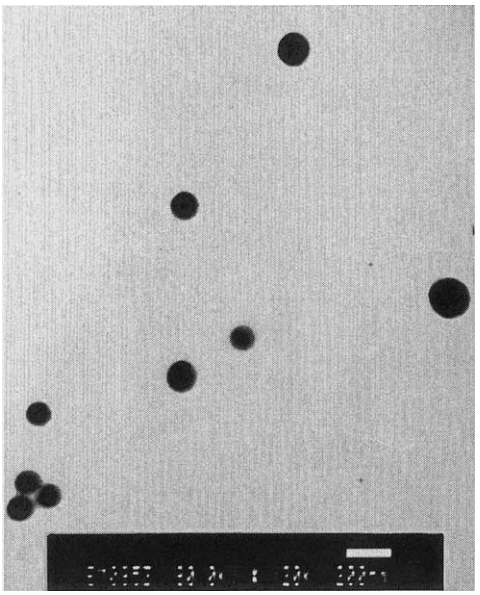


Fig. 5. TEM photomicrograph of sunscreen loaded nanoparticles made of EC.

sured by HPLC (Fig. 6). The only degradation product originated from the irradiation of *trans*-EHMC was identified by GC-MS and NMR as *cis*-EHMC, in accordance with previous studies (Deflandre and Lang, 1988; Berset et al., 1996; Tarras-Wahlberg et al., 1999). Since the *cis*-isomer absorbs the UV radiation less efficiently than *trans*-EHMC (Berset et al., 1996; Tarras-Wahlberg et al., 1999), the photo-induced isomerization of the sunscreen agent decreases its UV-protective capacity.

In the preparation containing *trans*-EHMC in combination with empty PLGA nanoparticles, 52.3% of the sunscreen content was lost following irradiation (Table 4). A statistically significant

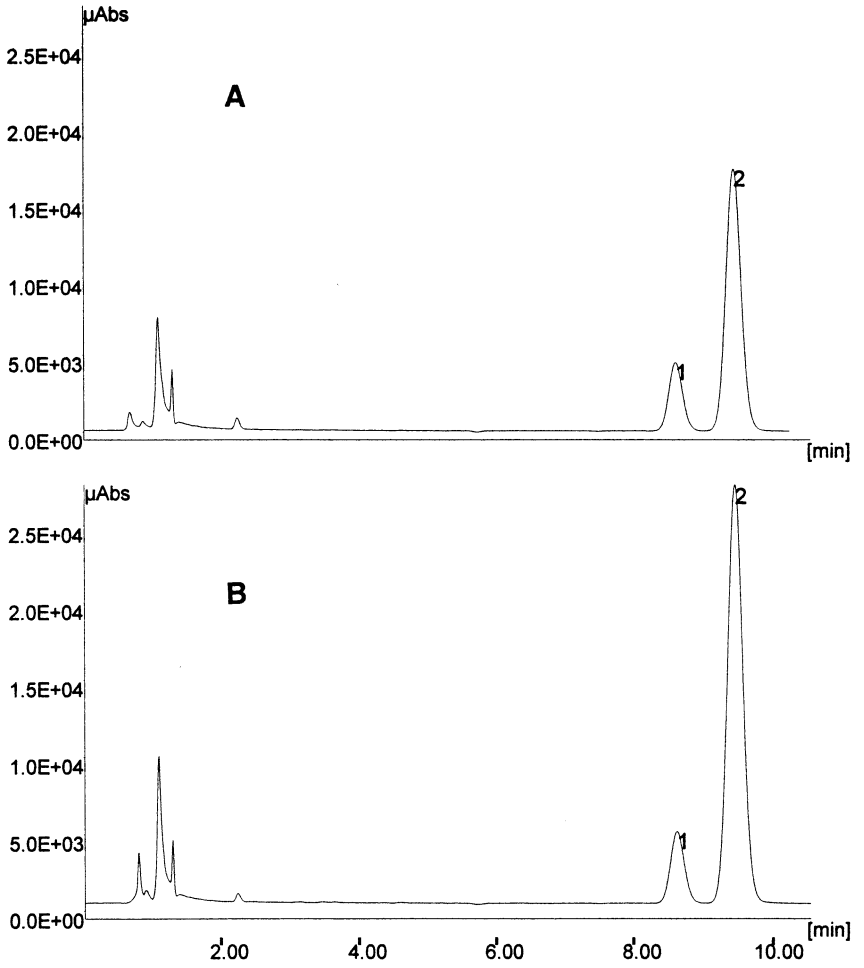


Fig. 6. HPLC chromatograms of lotions containing free *trans*-EHMC (A) or *trans*-EHMC loaded PLGA nanoparticles (B), after 4 h irradiation with the solar simulator. Peaks: 1 = *cis*-EHMC, 2 = *trans*-EHMC. Operating conditions as described under Section 2.

reduction of the extent of degradation to 38.3% (Table 4) was obtained in the lotion containing the sunscreen-loaded PLGA nanoparticles. This result was corroborated by the *cis*- to *trans*-isomer peak area ratio which was smaller for the formulation incorporating the nanoparticle-entrapped UV filter (13.3 ± 1.5%) as compared to the lotion containing free *trans*-EHMC with the unloaded nanoparticles (19.4 ± 1.4%). In contrast with the data obtained with the PLGA nanoparticulate system, the irradiation-induced decomposition of the sunscreen was not significantly affected by encapsulation into the EC nanoparticles (Table 4). In addition, for the latter system a higher dispersion of the photodegradation data was observed (Table 4). Hence, the protective effects of the examined particulate systems correlate with the nanoparticle encapsulation efficiencies.

4. Conclusions

Although the application of micro- and nanoparticles as carrier of sunscreen agents has been reported before (Fairhurst and Mitchnick, 1995; Lahmani and Simoneau-Agopian, 1995), the methodologies and polymers used were patented and hence the information available is insufficient

Table 4
Comparative photodegradation values for free and nanoparticle-encapsulated *trans*-EHMC after 4 h irradiation with the solar simulator

Formulation	% Sunscreen loss[a]	
	trans-EHMC	*trans*-EHMC loaded nanoparticles
Lotion containing PLGA nanoparticles	52.3±2.7	38.3±2.1[b]
Lotion containing EC nanoparticles	49.1±6.9	52.4±5.9[c]

[a] Each value represents the mean±SD of six determinations.
[b] $P < 0.01$ compared to free *trans*-EHMC.
[c] $P > 0.4$ compared to free *trans*-EHMC.

to allow a comparison with the technique described here. Moreover, these studies did not examine the influence of the polymeric particles on the sunscreen photostability, which is of fundamental relevance for the effectiveness of the system.

The results obtained demonstrated that the preparation based on PLGA nanoparticles loaded with *trans*-EHMC was effective in reducing the light-induced degradation of the sunscreen agent.

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