TECHNOLOGICAL STRATEGIES TO IMPROVE PHOTOSTABILITY OF A SUNSCREEN AGENT


*Department of Pharmaceutical Chemistry, University of Pavia, Pavia - Italy
*Department of Pharmaceutical Sciences, University of Ferrara, Ferrara - Italy

Received: April 2005. Presented at The VII ISCD World Congress "The New Frontiers of Dermo-Cosmetology: Efficacy, Stability and Safety", Rome, 4-6 November 2004

Key words: Suncare products; Sunscreen agent photodegradation; 2-ethylhexyl-p-methoxycinnamate; Nanoparticles.

Summary

Due to the reduction of the ozone layer, there is an increasing need of effective UV protection systems with minimised side-effects. Trans-2-ethylhexyl-p-methoxycinnamate (trans-EHMC) represents one of the most widely used sunscreen compound. Several studies demonstrated that trans-EHMC is unstable following UV irradiation both in solution and in emulsion formulations. Moreover, various reports of photocontact sensitization induced by trans-EHMC have appeared in the literature. Consequently, in order to ensure adequate efficacy and safety for this sunscreen agent, there is a need for new carrier systems to enhance trans-EHMC photostability.

In the present study the photostability of the filter in different formulation types (emulsion-gel, gel and emulsion) with various ingredients is evaluated.

In addition, nanoparticles based on poly-D,L-lactide-co-glycolide (PLGA) as carrier for trans-EHMC are investigated. The influence of nanoparticle matrix on the photochemical stability of the sunscreen agent is also presented.

The results obtained demonstrated that PLGA nanoparticles are effective in reducing the light-induced degradation of the sunscreen agent.

Moreover, the choice of formulation type and the excipients used play an important role in order to obtain a stable cosmetic product containing trans-EHMC.

Riassunto

A causa della continua riduzione dello strato di ozono risulta sempre più necessaria un’efficace protezione dagli effetti indesiderati di una continua esposizione alle radiazioni UV.
Il trans-2-etilesil-p-metossicinnamato (trans-EHMC) rappresenta uno dei filtri organici maggiormente utilizzati per la preparazione dei prodotti solari. Diversi studi hanno però dimostrato che il trans-EHMC risulta instabile all’irraggiamento UV sia quando è formulato in soluzione che in emulsione. Sono, inoltre, noti casi di dermatiti fotoallergiche associabili ad esso.

Al fine di assicurare adeguate condizioni di efficacia e sicurezza nasce quindi l’esigenza di uno studio accurato della fotodegradazione di questo filtro solare a seconda del tipo di formulazione utilizzata e dello sviluppo di nuovi sistemi di veicolazione in grado di aumentarne la fotostabilità.

In questo studio viene valutata la fotostabilità del trans-EHMC in differenti tipi di formulazione, quali emulsioni-gel, gel ed emulsioni.

Inoltre sono state studiati sistemi nanoparticellari a base di poli-D,L-lattide-co-glicolide per la veicolazione del filtro valutando in particolare l’influenza della matrice nanoparticellare sulla sua stabilità all’irraggiamento.

I risultati ottenuti dimostrano che le nanoparticelle di PLGA risultano efficaci nel ridurre la degradazione fotoindotta dell’agente solare. Inoltre, la scelta del tipo di formulazione e degli eccipienti utilizzati per le preparazioni contenenti il trans-EHMC è fondamentale al fine di ottenere un prodotto cosmetico stabile e quindi sicuro.
INTRODUCTION

Sunscreen preparations contain combinations of various organic chemicals which lessen the amount of UV light reaching human skin by absorbing radiations. 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 photo-induced decompositions (1, 2). The latter reactions not only decrease the sunscreen UV-protective capacity during usage (2-4) but can also produce allergic or toxic degradation products (5,6). Therefore a high photostability is a prerequisite for the effectiveness of sunscreen products.

Trans-2-ethylhexyl-p-methoxycinnamate (trans-EHMC) represents the most widely used UV-B filter used in sun protective formulations. Unfortunately, several studies have demonstrated that trans-EHMC is unstable following irradiation both in solution (1,3,4) and in emulsion formulations (5). Moreover, various reports of photocontact sensitization induced by trans-EHMC appeared in the literature (7,8).

Although it is known that formulation components can greatly influence the effectiveness and the safety of a sunscreen agent, at present there is little published data describing the influence of excipients and formulation type on trans-EHMC photostability (9-11).

In the present study the photostability of trans-EHMC in different formulations (emulsion-gel, gel and emulsion) with various ingredients was evaluated.

Furthermore, a new technological strategy to enhance trans-EHMC photostability is proposed. In particular, nanoparticulate delivery systems based on poly-D,L-lactide-co-glycolide copolymer as carrier for trans-EHMC are investigated. The structure of this polyester polymers, widely employed in the pharmaceutical field, is reported in Figure 1.

Nanoparticles are solid structures in the nanometric size range made of a polymeric matrix inside which an active substance is dispersed. In the pharmaceutical field these systems are studied to enable the modified release of drugs and/or their stabilization (12). In our opinion, they can be evaluate as carriers of cosmetic substances such as sunscreens, in order to improve their photostability. The use of nanoparticles for sunscreen vehiculation can be very advantageous. At first, thanks to their particle size it is possible to concentrate the sunscreen at the upper layers of the skin. In fact, it is well known from literature that particles smaller than 3 μm were randomly distributed in the hair follicles and stratum corneum without penetration into the deeper skin tissue (13, 14). This effect is propitious in the case of sunscreen agent because this agent has to act on the surface of the skin. Furthermore, nanoparticle powders dispersed in cosmetic formulations can give the opportunity to obtain the physical scattering effect, improving sun protection factor and thereby reducing sunscreen concentration in the final product.

The aim of this work was, therefore, to look at the properties of nanoparticulate systems with regard to their possible photostabilizing effect on trans-EHMC. For this purpose various cosmetic formulations, such as gel, emulsion-gel, and O/W emulsion, representative of many personal care products, have been examined. The photostability of trans-EHMC was evaluated in these cosmetic preparations both as free filter and as sunscreen loaded nanoparticles.

MATERIALS AND METHODS

Materials

Trans-EHMC was supplied by Hoffmann-La
Roche Ltd (Basel, Switzerland). Poly(lactic-co-glycolic) acid copolymer Resomer RG 752 (D,L-lactide:glycolide 75:25 molar ratio), inherent viscosity 0.23 dl/g, 0.1% in chloroform was purchased by Boehringer Ingelheim (Ingelheim, G.); Isopropylmyristate, Sorbitan stearate and Polysorbate 60, were purchased by Fluka, Sigma-Aldrich S.r.l. (Milan, I.); Lanolin, was obtained by Variati S.p.A. (Milan, I.); Cetearyl octanoate, polyacrylamide, C13-14 isoparaffin, laureth-7, phenoxyethanol, methylparaben, ethylparaben, propylparaben and butylparaben were supplied by Seppic Italia S.r.l. (Milan, I.). Methanol, acetonitrile, tetrahydrofuran and water HPLC grade were from Merck (Darmstadt, G.). All other chemicals were of analytical-reagent grade (Sigma, Milan, I.).

Fig. 1 Structure of PLGA polyester used in nanoparticle preparation.

**PLGA** R=H

**Set up of cosmetic formulations**

A gel, two emulsion-gels and an O/W emulsion, representative of many personal care formulations, were chosen and tested in this work. Their compositions are reported in the Table 1. Emulsion-gels were prepared at room temperature by dispersion of the polimeric gelifiant agent in the oil phase followed by addition of the aqueous solution. Gels were obtained by direct solubilization of polymer into water. The sunscreen agent was dispersed into the polymer before the addition of the aqueous phase.

The O/W emulsion was prepared by a procedure referred to as the inversion phase process. The oil- and aqueous-soluble components were separately heated to approximately 60 °C and the aqueous phase was slowly added to the oil phase while stirring with a homogenizer Silverson model SL2T at 3,500 rpm. Mild agitation was continued until the emulsion cooled at room temperature. Trans-EHMC were added in the cooling phase of the production process at about 40 °C.

**Nanoparticle preparation and characterization**

Trans-EHMC loaded nanoparticles were prepared by the “salting out” method (15). Briefly, polymer and sunscreen (1:1 weight ratio) were dissolved in acetone as organic solvent; separately, a viscous aqueous solution was prepared dissolving polyvinyl alcohol (3% w/w) in a concentrated CaCl2 aqueous solution (40% w/w); 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 phase: aqueous phase, 1:2 weight ratio). Pure water was then dropped into the emulsion to induce diffusion of the organic solvent from the polymer organic solution into the aqueous solution resulting in a nanoparticle dispersion.

The purification of nanoparticles was performed by 2 hours dialysis through 12-14,000 MWCO membranes followed by one night dialysis through 300,000 MWCO membranes (Spectra/Per Por, Spectrum Laboratories, Canada); then nanoparticles were recovered by centrifugation at 30,000 g and one night freeze-drying at
- 40°C and 40 mbar. All batches of nanoparticles were produced at least in triplicate. Nanoparticles were characterized for particle size distribution and sunscreen content. Particle size analysis was performed by light diffraction method using a Malvern Mastersizer 2000 (Malvern Ltd, Malvern, UK); this instrument works on laser diffraction optics. The size range of this version is from 0.02 μm to 2000 μm. Nanoparticle samples were suspended in filtered water, sonicated for 30 seconds, and subsequently analyzed. Three analyses were performed for each sample. The actual amount of trans-EHMC entrapped in the nanoparticles was determined by HPLC method, as previously set up (16). The HPLC apparatus comprised a Model LabFlow 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 set at 307 nm (Jasco, Tokyo, Japan). Separations were performed on a 5-μm Zorbax CN column (150x4.6 mm i.d.; Agilent Technologies, Waldbronn, Germany) fitted with a guard column (5-μm particles, 4x2 mm i.d.) and eluted isocratically, at a flow-rate of 1.0 ml/min, with methanol-acetonitrile-water (40:25:35, v/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. Nanoparticles (20-25 mg) were dispersed in acetonitrile under sonication, diluted to volume (10 ml) with the solvent and assayed for trans-EHMC after filtration through 0.45-μm membranes. Trans-EHMC loaded nanoparticles made of PLGA were formulated in the same cosmetic preparations as above described for free trans-EHMC by dispersion into the aqueous phase of an equivalent amount of nanoparticles able to reach the same final concentration of sunscreen agent.

**Sunscreen photostability assessment**

Photodegradation studies were carried on formulations containing free or nanoparticle- loaded trans-EHMC. Furthermore, nanoparticle systems were investigated not formulated in order to investigate the influence of polymeric matrix in the photostability process. A portion of the test sample was transferred onto the bottom of a beaker and then irradiated for 2 h with a solar simulator (Suntest CPS+; Atlas, Linsengericht, Germany) equipped with a Xenon lamp, an optical filter to cut off wavelengths shorter than 290 nm and an IR-block filter to avoid thermal effects. The solar simulator emission was maintained at 250 W/m². The applied UV-B energy corresponded to 20 Minimal Erythemal Doses (MED) which is considered comparable to a daily solar emission (4). After the exposure interval (2 h), the beaker was removed and its content quantitatively transferred into a 10-ml calibrated flask with methanol and the remaining trans-EHMC concentration was quantified by HPLC, as outlined above. All samples were protected from light both before and after irradiation. The degree of photodegradation was evaluated by comparing the peak areas of trans-EHMC from the irradiated samples with those obtained by analysis of an equivalent amount of the unirradiated preparations. The percentage ratio of the photodegradation product peak area (cis isomer) to the trans-EHMC peak area was also used to evaluate the extent of photodecomposition (Figure 2). Statistical analysis of the results was carried out by Student's t-test. Significance was taken as P < 0.05.
Among the different formulations examined containing free trans-EHMC, the emulsion exhibited the highest degree of sunscreen photodegradation; on the other hand, the photo-instability of trans-EHMC was reduced in the emulsion-gel vehicle. The extent of photodecomposition observed in the gel preparation was intermediate between the values measured for the emulsion and the emulsion-gel (Fig. 3). On the basis of these results, the gel and emulsion-gel vehicles were selected to investigate the effect of nanoparticle encapsulation on the light-induced degradation of the sunscreen agent.

The salting out technique produced nanoparticles with narrow size distribution \( d_{90} = 2.05 \mu m, \text{S.D.}<0.3 \), and high encapsulation efficiencies (91.24\% ± 4.8\%).

Results of photodegradation studies performed on trans-EHMC loaded nanoparticles are summarized in the Figure 4. The lowest value for the cis-trans ratio, and hence the lowest degree of UV filter degradation, was achieved by the nanoparticles not included in the formulation. Both the emulsion-gel and gel matrices reduced the photostabilization effect achieved by the polymeric particle carrier, although the sunscreen agent decomposition measured in the preparations containing the nanoparticle systems was lower than that observed for the non-encapsulated trans-EHMC.
Fig. 3 Cis-trans ratio % recovered in photodegradation studies on formulations containing free trans-EHMC.

Fig. 4 Results expressed as cis-trans ratio % of photodegradation studies carried out on formulations containing trans-EHMC loaded PLGA nanoparticles compared to those containing free trans-EHMC.
DISCUSSION

In this work we evaluated the influence of formulation type on the photostability of trans-EHMC. It is known that the effectiveness of a sun filter depends on several factors like concentration, penetration, spreading and adherence and most of these characteristics are determined almost exclusively by the formulation (9). In fact, emollients used in cosmetic preparation can concentrate the sunscreen in the uppermost skin cutaneous layers due to their penetrating ability in the horny layer. An other factor strictly correlated to the emollient used is the formulation spreadability that could modify the layer thickness and thus the efficacy of sun-protection agent. Moreover, emollient contained in the lipid phase may also affect the stability of sunscreens under irradiation modifying their $\lambda_{\text{max}}$ and molar absorptivity (4,11).

Among the examined formulations, emulsion-gels made of a polyacrilamide polymer highlighted the best results, especially the formulation containing emulsifiers, indicating that excipient selection is very important in formulating cosmetic product containing trans-EHMC.

The results obtained from the photolysis experiments performed on preparations containing the sunscreen agent encapsulated in nanoparticles demonstrated that the preparation based on nanosystems loaded with trans-EHMC was effective in reducing the light-induced degradation of the sunscreen agent. Moreover, encapsulation in the polymeric particles should limit the interaction of the UV filter with the skin.

An other advantage correlated with the use of solid nanoparticles in sunscreen formulation is the physical scattering effect that could increase the sun protection factor of the formulation thereby reducing sunscreen concentration and their irritation potential.

In conclusion, although, at the present, an assessment of the photostability of sunscreen products is not a general requirements before marketing, results from our study indicated that the choice of formulation type and the excipients used play an important role in order to obtain a stable sunscreen agent into nanoparticle systems is effective in reducing its light induced degradation. Further studies are in progress in order to evaluate the effective sun protection factors of formulations containing trans-EHMC loaded nanoparticles.
Table 1. Composition of formulations tested

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Emulsion</th>
<th>Gel</th>
<th>Emulsion-gel A</th>
<th>Emulsion-gel B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropylmyristate</td>
<td>-</td>
<td>-</td>
<td>9.34</td>
<td>9.34</td>
</tr>
<tr>
<td>Lanolin</td>
<td>9.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>trans-EHMC</td>
<td>0.66</td>
<td>0.66</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Cetearyl octanoate</td>
<td>4.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitan stearate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polyacrylamide, C13-14</td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>isoparaffin, Laureth-7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.2</td>
</tr>
<tr>
<td>Polysorbate 60</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2.4</td>
</tr>
<tr>
<td>Phenoxyethanol, Methyl, Ethyl, Propyl and Butyl paraben</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Water</td>
<td>84.3</td>
<td>96.14</td>
<td>86.8</td>
<td>75.2</td>
</tr>
</tbody>
</table>

ACKNOWLEDGMENTS

This work was developed inside the project "Design and preparation of biocompatible systems for cutaneous application" granted from MIUR.
Technological strategies to improve photostability of a sunscreen agent

References


8) Schmidt T, Ring J. and Abeck D. (1998) Photoallergic contact-dermatitis due to combined UVB (4-methylbenzylidene camphor, octylmethoxycinnamate) and UVA (Benzophenone-3/butylmethoxydibenzoylmethane) absorber sensitization. Dermatology 196, 354-357


68
Author Address:

Dr. Paola Perugini
Department of Pharmaceutical Chemistry
University of Pavia
V.le Taramelli 12
27100 Pavia, Italy
fax +390382422975
e-mail: paola.perugini@unipv.it