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Research paper

Influence of liposphere preparation on butyl-methoxydibenzoylmethane photostability

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Abstract

The incorporation of butyl-methoxydibenzoylmethane (BMDBM), one of the most efficient and frequently used UV-A blockers, into lipospheres was examined in order to decrease the light-induced sunscreen degradation. Lipospheres, obtained by the melt technique and using tristearin as the lipid material and hydrogenated phosphatidylcholine as the emulsifier, showed proper features in terms of size (10–40 μm), BMDBM loading level (21.63% ± 0.90%, w/w) and physical state. Photolysis studies, involving irradiation of lipospheres with simulated sunlight before and after their introduction in emulsion formulations, demonstrated a relevant enhancement of the encapsulated sunscreen photostability in comparison with unencapsulated BMDBM.

Keywords: Lipospheres; Sunscreens; Butyl-methoxydibenzoylmethane; Physical state; Photostability

1. Introduction

The increase in general knowledge and awareness of the harmful effects (i.e., erythema, cutaneous photoageing, immune suppression and skin cancer) induced by exposure to the sunlight UV radiation (290–400 nm) has led to the widespread use of topical sun protecting preparations [1]. The most common active ingredients in these products are organic sunscreen agents which attenuate the transmission of the solar energy to the skin by absorbing the UV radiation [2].

An essential requirement for the efficacy and safety of sunscreens is a high photostability, since the light-induced degradation of the UV filter not only decreases the expected photoprotective power but can also lead to the accumulation of harmful photolytic products on the skin [3]. However, published reports have demonstrated that several sunscreen agents undergo decomposition under sunlight exposure and consequently cannot maintain their initial absorptive capacity [3,4]. Different systems have been investigated to enhance the photostability of UV filters, including sunscreen combinations, inclusion complexes with cyclodextrins, polymeric microspheres and nanoparticles [2,4,5]. Solid lipid microparticles (lipospheres) represent an alternative carrier for preventing the irradiation-induced decomposition of sunscreens [6]. Lipospheres, based on naturally occurring lipids having chemical affinity with the skin, consist of a solid fat core stabilised by one layer of phospholipid molecule on the surface. They exhibit high entrapment capacity for lipophilic substances, synergistic protective effect [6] and proper size for both cutaneous performance and skin penetration prevention.

In particular, the present investigation focusses on butyl-methoxydibenzoylmethane (BMDBM), since it is the most efficient and widely used filter for protection against UV-A radiation (320–400 nm) [3] which contributes considerably to the sunlight-induced skin damage [1–3]. In fact, UV-A radiation is considered as one of the main causes of oxidative stress and tumour promotion [7]. However, BMDBM is not

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sufficiently photostable undergoing decomposition when illuminated with simulated sunlight leading to a decrease of its UV-filtering capacity [2,3]. In addition, published studies [8] have demonstrated that the photo-induced degradation of the sunscreen agent generates free radicals that inflict in vitro damage to DNA and bovine serum albumin. Consequently, there is a need for new carrier systems exhibiting enhanced BMDBM photostability.

The present study reports on the preparation and in vitro characterisation of lipospheres loaded with BMDBM. In addition, BMDBM photochemical stability, before and after liposphere incorporation in cutaneous formulations, was determined.

2. Materials and methods

2.1. Materials

For liposphere preparation, tristearin as the lipid phase and hydrogenated soybean phosphatidylcholine as the emulsifier were purchased from Fluka Chemie (Buchs, Switzerland) and Degussa Texturant Systems Italia (Padova, Italy), respectively. Butyl-methoxydibenzoylmethane (BMDBM) was supplied by Merck (Darmstadt, Germany). For the release study, caprylic/capric triglyceride (Miglyol 812) was kindly donated by Sasol Germany GmbH (Witten, Germany). For the cream formulation, sorbitan monostearate, polyoxyethylene sorbitan monostearate, butylated hydroxyanisole, isopropyl isostearate, cetearyl isononanoate and cetearyl alcohol were purchased from Henkel (Fino Mornasco, Italy). Methanol, acetonitrile and water were high-performance liquid chromatography (HPLC)-grade from Merck. All other chemicals were of analytical grade from Sigma (Milan, Italy).

2.2. Methods

2.2.1. High-performance liquid chromatography

The HPLC apparatus comprised a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 10 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–Vis detector (Jasco, Tokyo, Japan) set at 320 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developments, Le Fontanil, France). Sample injections were effected with a Model 701 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5 μm Zorbax SB-CN column (150 × 3.0 mm i.d.; Agilent Technologies, Waldbronn, Germany) eluted isocratically, at a flow rate of 0.4 ml/min, with methanol–acetonitrile–water (55:25:20, v/v/v). Chromatography was performed at ambient temperature. The identity of the BMDBM peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardisation method.

2.2.2. Liposphere preparation

Unloaded lipospheres were prepared by adding hot (70 °C) phosphate buffer solution (0.2 M, pH 7.4) containing 2% (w/v) hydrogenated phosphatidylcholine (60 ml) to melted tristearin (4.8 g; m.p. 58–63 °C) at 70 °C under stirring (13,000 rpm, Ultra-Turrax, T25 Basic IKA-Werk, Labortechnik, Staufen, Germany). After 3 min, the obtained O/W emulsion was rapidly cooled under magnetic stirring to below 20 °C. The formed lipospheres, floating on the sample surface, were recovered by centrifugation (5000 rpm, 5 min), washed with water to remove the excess phosphatidylcholine, filtered and freeze-dried. Sunscreen-loaded lipospheres were obtained by dissolving BMDBM (1.8 g) in ethanol (1 ml, sample LE) or acetone (1 ml, sample LA) and adding the solution to the melted lipid phase. Alternatively, BMDBM was dissolved in the melted lipid phase at 80 °C (sample L).

2.2.3. Physical mixture preparation

A physical mixture containing 21% (w/w) BMDBM (sample PM) was prepared by mixing, suspending in water and freeze-drying the sunscreen together with unloaded lipospheres.

2.2.4. Liposphere morphology and size

Liposphere morphological structure was examined by both optical videomicroscope coupled with epifluorescence (N-400FL, Optika Microscopes, M.A.D. Apparecchiature Scientifiche, Bergamo, Italy) and Scanning Electron Microscope (SEM, XL-40, Philips, Eindhoven, The Netherlands). The particle size was determined by computerised image analysis (IMG-VIEW, CIGS, University of Modena and Reggio Emilia) of at least 200 lipospheres on SEM micrographs.

2.2.5. Thermal analysis

Thermograms were recorded on a differential scanning calorimeter (DSC-4, Perkin-Elmer, Norwalk, CT, USA) coupled with a computerised data station (Perkin-Elmer). Samples were heated in crimped aluminium pans at a scanning rate of 10 °C/min using dry nitrogen flow (30 ml/min). The reported data were averaged on three determinations.

2.2.6. Liposphere BMDBM content

Exactly weighed amounts of loaded lipospheres (30–50 mg) were dissolved in ethanol under sonication for 15 min. The obtained sample was diluted to volume (10 ml), filtered and assayed for BMDBM by UV spectrophotometry (Model lambda 3B, Perkin-Elmer) at a wavelength of 355 nm (unloaded lipospheres produced insignificant absorbance values at the same wavelength) and HPLC. Each sample was analysed in triplicate.

2.2.7. BMDBM dissolution and release

The sunscreen dissolution and release from the lipospheres were examined by adding BMDBM (5 mg) or loaded lipospheres containing an equivalent amount of

BMDBM to Miglyol 812 (100 ml) under mechanical stirring at 500 rpm and 37 °C. At fixed time intervals, 1 ml aliquots of the medium were withdrawn, filtered, diluted to 10 ml with ethanol and assayed for BMDBM concentration spectrophotometrically. All data were averaged on three determinations.

2.2.8. Photodegradation studies

Photolysis experiments were performed on the physical mixture (PM) and on lipospheres (samples LE, LA and L) before and after their introduction into a cream. The cream (O/W emulsion) was prepared according to the common procedure used in compounding practice. PM or lipospheres L were dispersed in water and added in the cooling phase of the emulsion preparation at about 40 °C. PM or lipospheres (about 30 mg, containing approximately the same amount of BMDBM) were spread onto the bottom of a beaker. A portion (100 mg) of the O/W emulsion containing 0.5%, 1.0% or 2.0% (w/w) BMDBM in PM or in lipospheres L was transferred by means of a syringe onto the bottom of a beaker. The samples were irradiated for 1 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 500 W/m^2. The applied UV energy was equivalent to 20 minimal erythemal dose (MED) which is considered representative of daily solar emission [3]. After the exposure interval, the beaker was removed, its content quantitatively transferred into a 10 ml calibrated flask with methanol, subjected to sonication for 15 min, and the remaining BMDBM concentration was quantified by HPLC as outlined above. All the samples were protected from light both before and after irradiation. The degree of photodegradation was evaluated by comparing the peak areas of BMDBM from the irradiated samples with those obtained by the analysis of an equivalent amount of unirradiated preparations.

Data were analysed for significance by using Student's unpaired *t*-test (Instat, Graphpad Software, San Diego, CA). *P*-values < 0.05 were considered significant.

3. Results and discussion

3.1. Liposphere preparation and characterisation

Lipospheres loaded with BMDBM were developed through a melt technique involving tristearin as the lipidic material and hydrogenated soybean phosphatidylcholine as the emulsifier. The highest liposphere yield was obtained at a triglyceride/phospholipid ratio of 4:1 in accordance with previous preformulative studies [9]. Since sunblockers act as absorbers in a molecular dispersion state, particular attention should be paid regarding their solubility in the vehicle. Moreover, since topical products do not support high amounts of powder components owing to applicability and good feeling performance problems, a relatively high entrapment capacity is required to achieve the effective dose. Therefore, different preparative conditions, including a melting temperature of 70 °C with co-solvents (samples LE and LA) or 80 °C without co-solvents (sample L), were adopted to provide high concentrations of BMDBM dissolved in the lipid. To evaluate BMDBM physical state, thermal analysis by DSC was carried out on lipospheres in comparison with the physical mixture. As thermograms show (Figs. 1 and 2), the crystallisation of tristearin in lipospheres led to a partial polymorphic modification from stable β-form (m.p. about 65 °C) to unstable α (m.p. about 48 °C) and β′ (m.p. about 62 °C) forms, as previously reported [9]. BMDBM melting peak ($T_{max} = 83.8 \pm 0.3$ °C) (Fig. 1a) shifted to lower temperature ($T_{max} = 77.7 \pm 0.3$ °C) in the physical mixture (Fig. 1c). Broad endotherms at the same temperature were observed in the DSC curves of loaded lipospheres (LE-$T_{max} = 77.3 \pm 0.3$ °C; LA-$T_{max} = 76.9 \pm 0.2$ °C; L-$T_{max} = 77.6 \pm 1.9$ °C) (Fig. 2). The endotherm at slightly higher temperature ($T_{max} = 83.2 \pm 0.1$ °C, typical of phosphatidylcholine) in the unloaded liposphere thermogram (Fig. 1b), which is not evident in the physical mixture and loaded liposphere profiles, suggests the presence of a small excess of the emulsifier. The BMDBM melting enthalpy in loaded lipospheres (LE $= 7.82 \pm 0.63$ J/g; LA $= 8.41 \pm 1.51$ J/g; L $= 7.94 \pm 1.38$ J/g) was significantly lower than that calculated in the physical mixture

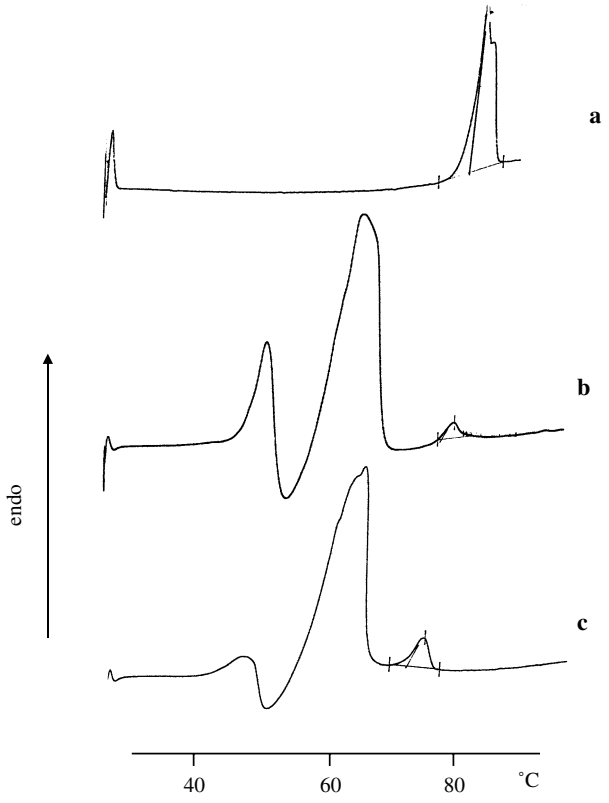

Fig. 1. DSC thermograms: (a) BMDBM; (b) unloaded lipospheres; (c) physical mixture.

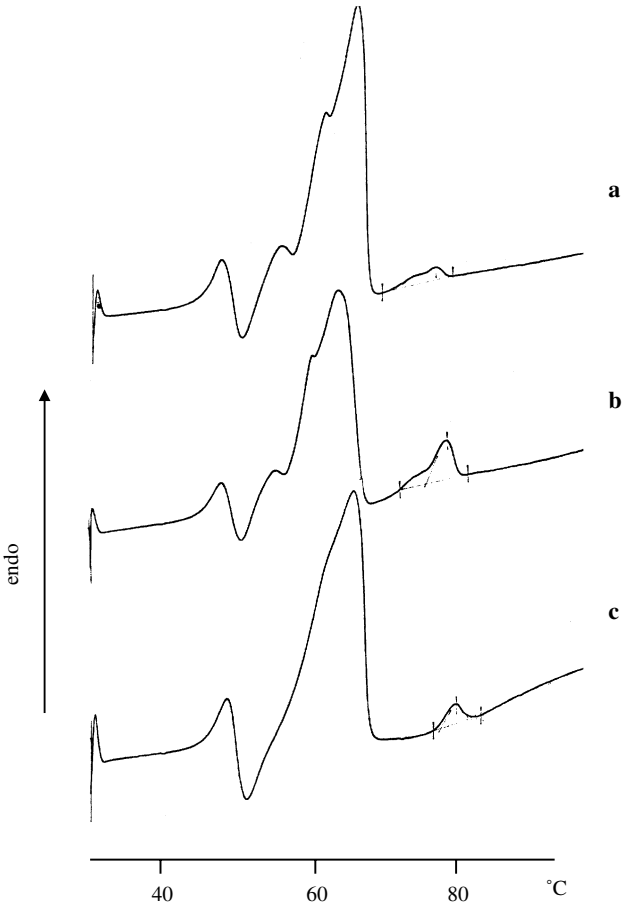

Fig. 2. DSC thermograms: (a) lipospheres LE; (b) lipospheres LA; (c) lipospheres L.

thermogram (24.41 ± 2.88 J/g), indicating a nearly molecular dispersed state of the sunscreen inside all liposphere samples.

All the liposphere samples were well formed showing spherical shape with a smooth surface (Fig. 3) and size between 1 and 100 μm, 80–90% of the population being in the 10–40 μm range, which is considered proper for topical applications. BMDBM loading was rather high for all the liposphere samples (21.63% ± 0.90%, w/w), corresponding to an encapsulation efficiency of about 77% (calculated as the percentage ratio between the quantity of BMDBM entrapped in the lipospheres and the amount of sunscreen added to the melted lipid phase). Such a loading level could be considered suitable for a suncare formulation with a good feeling performance. In fact, a 2% BMDBM concentration, considered sufficient to meet the Australian Standard requirements [10], could be achieved by introducing less than 10% lipospheres into the cosmetic formulation.

Optical microscopy and epifluorescent videomicroscopy photomicrographs were recorded on the same image of each liposphere sample. Regardless of the preparative parameters, BMDBM natural fluorescence appeared homogeneously distributed throughout the lipospheres (Fig. 4).

To evaluate the effect of the microencapsulation on the sunscreen in vitro release, a lipophilic medium (Miglyol 812) was selected in which BMDBM was sufficiently soluble (11%, w/w) to assure sink conditions, whereas lipospheres remain intact. Sunscreen release rate from the lipospheres became slower than BMDBM dissolution rate (Fig. 5) indicating the release modulation capacity of the lipid matrix. Moreover, the lack of burst-effect phenomena suggests that the sunscreen is

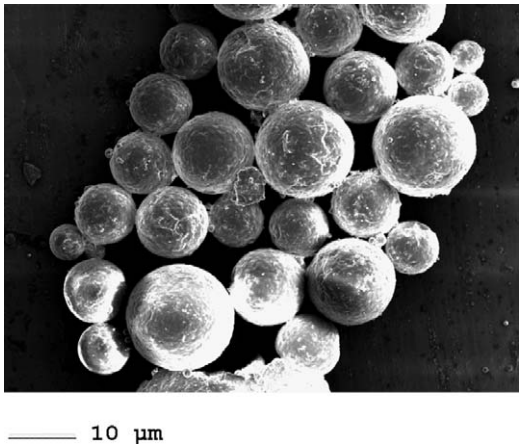

Fig. 3. SEM micrograph of lipospheres L.

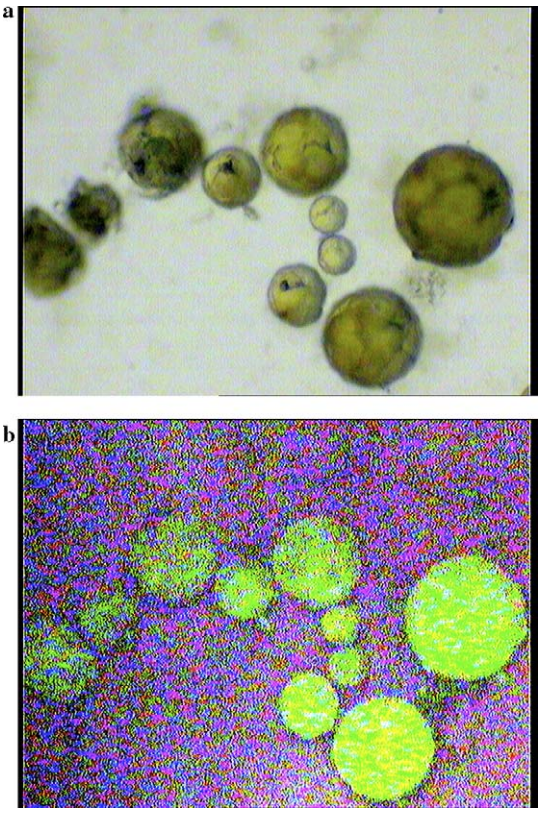

Fig. 4. Optical microscopy (a) and epifluorescent videomicroscopy (b) images of lipospheres L (magnification 400×).

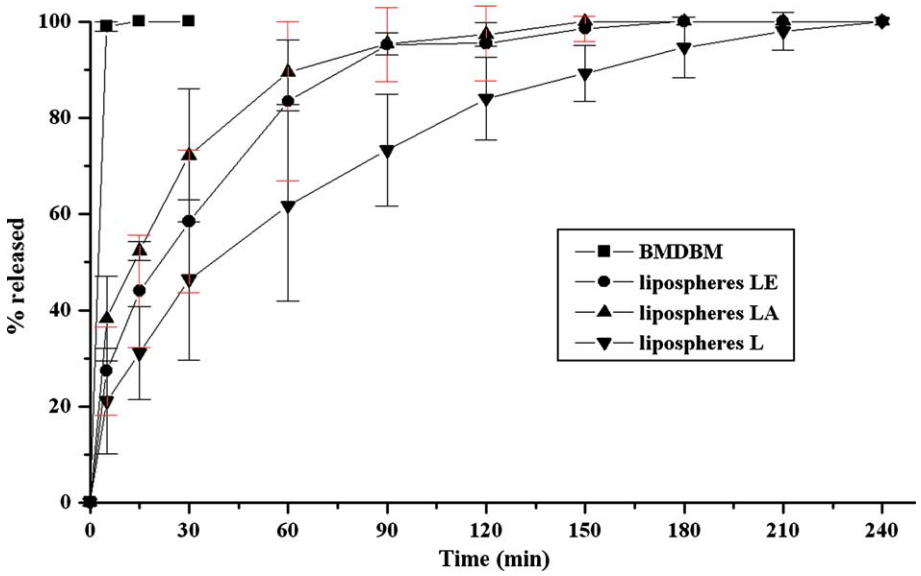

Fig. 5. BMDBM in vitro dissolution and release from lipospheres.

not present on the microparticle surface. No significant differences in sunscreen release behaviour were observed among the different liposphere samples.

Although all the liposphere preparations show satisfactory features (homogeneous distribution of the sunscreen in a molecularly dispersed state, suitable dimensional and loading properties and sustained release), sample L provides organic solvent avoidance as additional advantage.

3.2. Photodegradation studies

In order to investigate the effect of the microincapsulation process on the photochemical behaviour of BMDBM, the photolysis experiments were carried out initially on solid samples (physical mixture and lipospheres LE, LA and L). As reported in Table 1, in comparison with the physical mixture, the sunscreen entrapment in the liposphere matrices produced a marked decrease (86.5–98.7%) of the degree of BMDBM decomposition induced by illumination with simulated sunlight. This is probably due to physical scattering effect. It has been reported [6] that also solid lipid nanoparticles have the ability of scattering/reflecting incoming UV radiation. Although the differences observed among the examined liposphere preparations were small (Table 1), sample L provided the greatest photostabilisation effect and hence it was selected for further photolysis studies. In order to simulate the actual conditions in the finished suncare products, additional experiments were performed on a cream (O/W emulsion) which was chosen as a model formulation since it represents the most common type of sunscreen preparation [4]. BMDBM (1.0%, w/w) in combination with unloaded lipospheres or microencapsulated in lipospheres was incorporated into the cream and exposed to the solar simulator. In the formulation containing free BMDBM, $12.1\% \pm 2.6\%$ of the sunscreen content was lost following irradiation. A statistically significant reduction of the extent of photodecomposition to $4.3\% \pm 4.4\%$ was obtained in the cream containing the sunscreen-loaded lipoparticles. Additional photodegradation experiments performed at different BMDBM levels (0.5% and 2.0%, w/w) indicated that, in the examined range, the sunscreen concentration does not influence significantly the degree of photodecomposition (the percentage sunscreen losses in the formulations containing 0.5% and 2.0% BMDBM were, respectively, $14.1\% \pm 1.8\%$ and $12.2\% \pm 2.1\%$ for the unencapsulated UV filter, and $3.8\% \pm 2.8\%$ and $3.4\% \pm 3.0\%$ for the microparticle-entrapped BMDBM). These results demonstrate that the photostability enhancement effect achieved by the liposphere carrier was retained in the cream vehicle. However, the observed improvement in the sunscreen photochemical behaviour was not as marked as that measured for the lipospheres alone (Table 1). This is indicative of a reduction in the photostabilisation efficacy of the lipid microspheres in the emulsion matrix. This effect could be ascribed to the release of BMDBM from the lipospheres into the oil phase, which reduces the sunscreen fraction protected by the lipid microparticle matrix.

Table 1
Comparative photodegradation data for BMDBM in physical mixture (PM) and in lipospheres (samples LE, LA and L) after 1 h irradiation with the solar simulator

Sample	BMDBM loss[a] (%)	P[b]
Physical mixture	19.55 ± 7.22	
Lipospheres LE	2.64 ± 2.22	<0.001
Lipospheres LA	1.35 ± 2.54	<0.001
Lipospheres L	0.26 ± 0.23	<0.001

[a] Each value is the mean ± SD of eight determinations.
[b] P-values (unpaired t-test) vs. PM.

4. Conclusions

The results described in this study indicate that lipospheres as carrier for BMDBM represent a suitable approach to reduce the photodecomposition of the UV filter. Moreover, sample L, avoiding the use of organic solvent and exhibiting the highest photostability, could be considered as the optimum formulation.

The improved photostability combined with biocompatibility and sustained release properties of the carrier should minimise the cutaneous side-effects produced by such an UV-A filter.

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