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Enhancement of melatonin photostability by encapsulation in lipospheres

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Abstract

The effect of lipid microparticle carrier systems on the light-induced degradation of melatonin was investigated. Microspheres loaded with melatonin were prepared using tristearin or tripalmitin as the lipid material and hydrogenated phosphatidylcholine or polysorbate 60 as the emulsifier. The obtained lipid microspheres were characterized by scanning-electron microscopy and differential scanning calorimetry. Free or microencapsulated melatonin was incorporated in a model cream formulation (oil-in-water emulsion) and irradiated with a solar simulator. The extent of photodegradation was measured by high-performance liquid chromatography. The photolysis experiments demonstrated that the light-induced decomposition of melatonin was markedly decreased by encapsulation into lipid microspheres based on tristearin and phosphatidylcholine (the extent of degradation was 19.6% for unencapsulated melatonin compared to 5.6% for the melatonin-loaded microparticles). Therefore, incorporation in lipid microparticles can be considered an effective system to enhance the photostability of melatonin.
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Keywords: Melatonin; Photodegradation; Lipid microparticles; Differential scanning calorimetry; Scanning-electron microscopy; Reversed-phase chromatography

1. Introduction

The hormone melatonin (*N*-acetyl-5-methoxytryptamine) is produced by the pineal gland and secreted into the blood in a circadian rhythm. Melatonin plays an important role in the regulation of several physiological processes, such as daily sleep induction, seasonal biological rhythm and aging [1,2]. Therapeutic applications of melatonin include sleep disorders, jet-lag syndrome and cluster headaches [1,2]. Moreover, due to its immunomodulatory and free-radical scavenging properties, melatonin has been used topically for protection of human skin against oxidative damage induced by exposure to the solar radiation [3–5]. However, the photolability of this hormone [2] is a major drawback for topical treatment of sunlight harmful effects. In fact, the photoprotective activity of melatonin is enhanced when the hormone is applied before irradiation of the skin [3,4]. Work from Maharaj et al. [6] demonstrated that although melatonin is rapidly degraded by exposure to UV light, the degradants retain the antioxidant properties of the parent compound against potassium cyanide-induced superoxide radical generation. However, the photolytic products of melatonin might not exhibit the same free radical scavenging activity of the hormone towards the highly toxic hydroxyl radicals [7], singlet oxygen [8] and reactive nitrogen species [9]. For instance, the photodegradant, 6-hydroxymelatonin [6] has been reported to be less efficient as hydroxyl radical scavenger than melatonin and to act also as promoter of hydroxyl radicals [7]. Therefore, there is a need for new systems exhibiting increased melatonin photostability.

The present investigation focuses on the use of lipidic microparticles (lipospheres) as a biocompatible delivery carrier for the prevention of melatonin photodegradation. Lipospheres consist of a solid fat core stabilized by a layer of surfactant molecules on the surface [10,11]. Incorporation in the lipid matrix enables increased drug stability and/or modified release [10,11]. Moreover, lipoparticles exhibit proper size for cutaneous performance [12,13] and are based on physiological lipids having affinity with the skin and hence minimal toxicity risk [10,11].

The present study reports on the preparation and characterization of lipid microparticles loaded with melatonin. In addition, the influence of the liposphere matrices on the light-induced decomposition of the hormone is presented.

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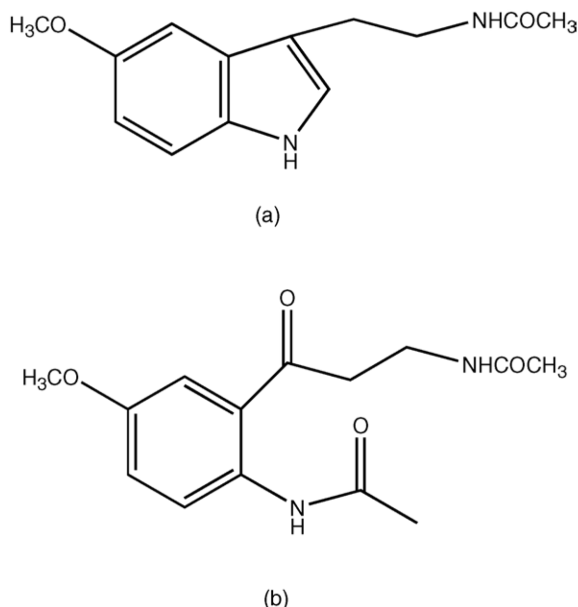

Fig. 1. Chemical structure of melatonin (a) and *N*-[3-(2-formylamino-5-methoxyl-phenyl)-3-oxo-propyl]acetamide (b).

2. Materials and methods

2.1. Materials

Melatonin (Fig. 1a) was supplied by Aldrich Chimica (Milan, Italy). Tristearin, tripalmitin and polysorbate 60 were purchased from Fluka Chemie (Buchs, Switzerland). Hydrogenated soybean phosphatidylcholine was a gift by Degussa Texturant Systems Italia (Padua, Italy). Methanol and water of HPLC grade were obtained by Merck (Darmstadt, Germany). All other chemicals were of analytical grade (Sigma, St. Louis, MO, USA).

2.2. 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 280 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 701 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Luna C_{18} column (150 mm × 4.6 mm i.d.; Phenomenex, Torrance, CA, USA) fitted with a guard column (5-μm particles, 4 mm × 2 mm i.d.) and eluted isocratically, at a flow-rate of 1.0 ml/min, with sodium acetate buffer (pH 4.0; 0.05 M)–methanol (50:50, v/v). The mobile phase was filtered through 0.2 μm nylon filters (Alltech Italia, Sedriano, Italy). Chromatography was performed at ambient temperature. The identity of the separated 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. Liposphere preparation

The unloaded lipospheres were prepared by adding hot (70 °C) phosphate buffer solution (pH 7.4; 60 ml) containing 2% (w/v) of surfactant to melted tristearin or tripalmitin (3.6 g) at 70 °C under stirring (10,000 rpm for 3 min; Ultra-Turrax, T25 Basic IKA-Werk, Staufen, Germany). The obtained oil-in-water emulsion was rapidly cooled under magnetic stirring to below 20 °C. The formed lipospheres were recovered by centrifugation (5000 rpm for 5 min), washed with water and freeze-dried. Melatonin-loaded lipospheres were obtained by dissolving melatonin (1.1 g) in 1 ml of ethanol and adding the solution to the melted lipid phase.

2.4. Liposphere characterization

Lipospheres were examined by scanning-electron microscope (SEM, XL-40, Philips, Eindhoven, The Netherlands). The particle size was determined by computerized image analysis of at least 100 lipospheres on SEM micrographs.

Thermal analysis was carried out on a differential scanning calorimeter (DSC-4, Perkin-Elmer, Norwalk, CT, USA) coupled with a computerized data station (Perkin-Elmer, Norwalk, CT, USA). Samples were heated in crimped aluminium pans from 30 to 150 °C at a scanning rate of 10 °C/min under dry nitrogen flow (30 ml/min).

The amount of melatonin entrapped in the lipospheres was determined by dispersing the lipospheres (30–40 mg) in ethanol under sonication (15 min). The obtained sample was diluted to volume (10 ml), filtered (0.45-μm membrane filters) and assayed for melatonin by HPLC, as outlined above.

2.5. NMR spectroscopy

^{1}H NMR spectra were recorded on a Varian Mercury Plus spectrometer (400 MHz). Samples were solubilized in $CDCl_3$. Chemical shifts are reported in ppm (δ) relative to TMS. Typical parameters for the ^{1}H NMR spectra were: 0.35 Hz/pt resolution, 1 s relaxation delay, 45° pulse.

2.6. In vitro release

Melatonin dissolution and release from the lipospheres were examined by adding previously sieved (100 μm) melatonin (7.0 mg) or lipospheres containing an equivalent amount of melatonin, to propylen glycol (100 ml) under mechanical stirring at 500 rpm and 37 °C. At appropriate time intervals, 1-ml aliquots were withdrawn, filtered and assayed for melatonin by HPLC.

2.7. Photodegradation studies

Photolysis experiments were performed in cream preparations (oil-in-water emulsion) containing 0.5% (w/w) melatonin in conjunction with unloaded lipospheres or entrapped in the lipid microparticles. The formulation excipients were: sorbitan monostearate, polyoxyethylene sorbitan monostearate,

isopropyl isostearate, petrolatum, cetearyl alcohol, sodium benzoate, glycerin, propylen glycol, dehydroacetic acid, EDTA and water. The cream was prepared according to the common procedure used in compounding practice [14]. Empty or loaded lipospheres were dispersed in water and added in the cooling phase of the emulsion preparation at ca. 40 °C. The pH of the formulation was adjusted to 6.0 with citric acid. A portion (100 mg) of the test cream was transferred by means of a syringe 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 [15]. The solar simulator emission was maintained at 500 W/m^2. After the exposure interval, the beaker was removed and its content quantitatively transferred into a 10-ml calibrated flask with ethanol. The resulting sample was dispersed under sonication, diluted to volume and the remaining melatonin concentration was quantified by HPLC. All samples were protected from light both before and after irradiation. The degree of photodegradation was measured by comparing the peak areas of melatonin from the irradiated samples, with those obtained by analysis of an equivalent amount of the unirradiated preparations.

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. Lipoparticles preparation and characterization

Lipid microparticles loaded with melatonin were prepared through a melt technique [16] using tristearin or tripalmitin as lipidic material and hydrogenated phosphatidylcholine or polysorbate 60 as the emulsifier. The highest liposphere yield was obtained at a triglyceride/emulsifier ratio of 3:1. Investigations by scanning-electron microscope demonstrated that all the liposphere preparations showed spherical shape (Fig. 2), although some irregular fragments were present in the samples obtained using phosphatidylcholine as the emulsifier (Fig. 2a). The particle size was between 1–50 and 1–20 μm for the preparations based on phosphatidylcholine and polysorbate 60, respectively. The majority (>76%) of the population was in the 5–20 μm range, suitable for topical applications [12,13]. Additional characterization of the lipid microparticles was carried out by thermal analysis. The DSC profile of melatonin displayed an endothermic transition at 116 °C corresponding to its melting point (Fig. 3a). This peak was present in the physical mixture (melatonin with unloaded microspheres) curve (Fig. 3b) whereas a slightly outlined endotherm at about 101 °C was observed in the thermogram of the loaded lipospheres (a representative DSC profile is shown in Fig. 3c). These changes in the thermal behaviour suggest a molecular dispersion of the hormone into the lipoparticles. The amount of melatonin incorporated in the lipospheres was higher (15.71 ± 3.27%, w/w) for the systems involving hydrogenated soybean phosphatidylcholine as compared to microspheres made with polysorbate 60 (3.06 ± 0.59%, w/w). To evaluate the retention capacity of the lipid matrices, in vitro release studies were carried out using a medium (propylen glycol) in which melatonin was sufficiently soluble (to assure sink conditions) whereas lipospheres remain intact. Burst release was observed for the lipoparticle preparation based on polysorbate 60 (not shown), indicating adsorption of the drug on the microparticle surface. Conversely, the melatonin release rates from the lipospheres prepared with hydrogenated phosphatidylcholine were slower than the drug dissolution rate (Fig. 4), suggesting incorporation of melatonin in the lipid particle core. These results show that the type of stabilizing agent plays an important role in the encapsulation process. Between the liposphere samples containing phosphatidylcholine as emulsifier, the preparation based on tristearin produced the slower melatonin diffusion rate (Fig. 4).

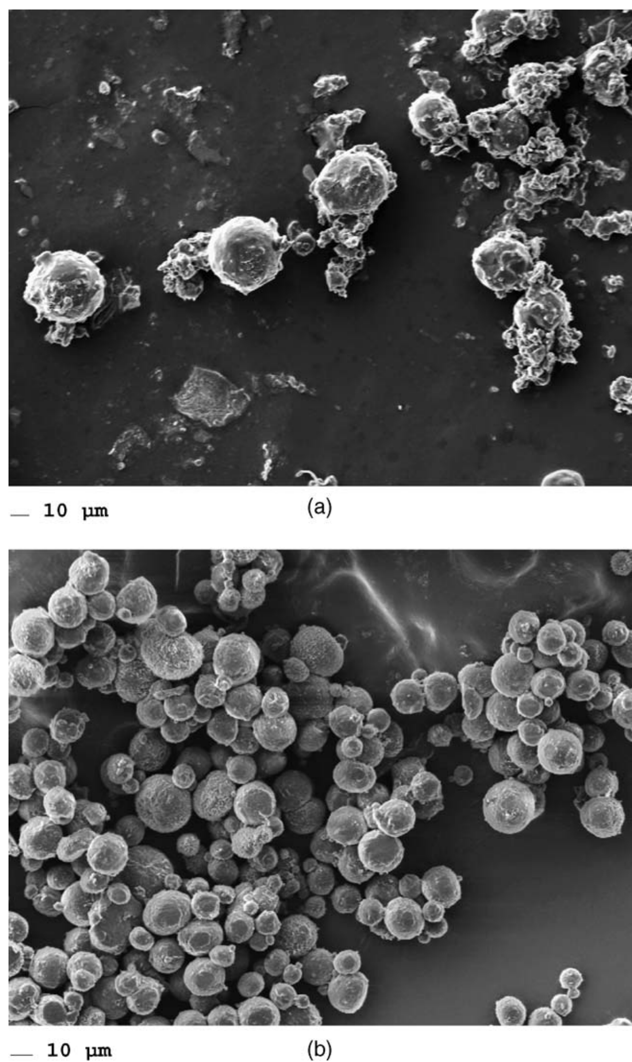

Fig. 2. SEM photomicrographs of tristearin lipospheres prepared with hydrogenated phosphatidylcholine (a) or polysorbate 60 (b).

3.2. Photodegradation studies

In order to study the effect of the lipidic particle matrix on the photochemical reactivity of melatonin, the photostability experiments were performed on a cream (oil-in-water emulsion) as a medium. This vehicle was selected as a model formulation

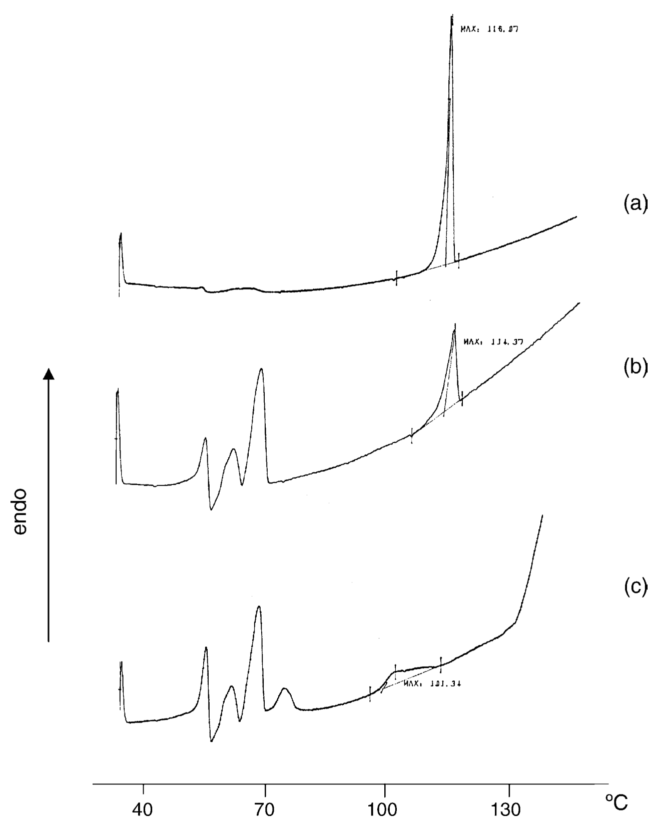

Fig. 3. DSC thermograms of melatonin (a), melatonin with unloaded lipospheres (b) and melatonin-loaded lipospheres prepared with tristearin and phosphatidylcholine (c).

since it represents the most common type of topical preparation [17]. Melatonin in combination with unloaded lipospheres or microencapsulated in the lipid particles was incorporated into the cream and exposed to the solar simulator. Microparticles made with polysorbate 60 were not examined because of the very low encapsulation efficiency, which limits their applicability to finished skin care products. During the light-

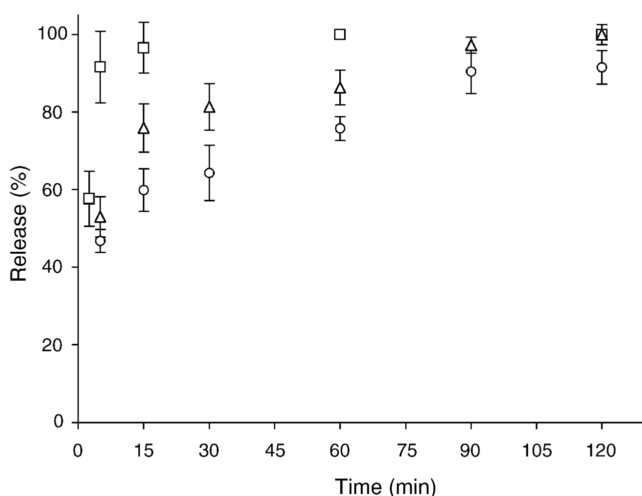

Fig. 4. Melatonin dissolution (□) and release from (△) tripalmitin- and (◯) tristearin-microspheres prepared with phosphatidylcholine, into propylen glycol ($n = 3$; mean ± S.D.).

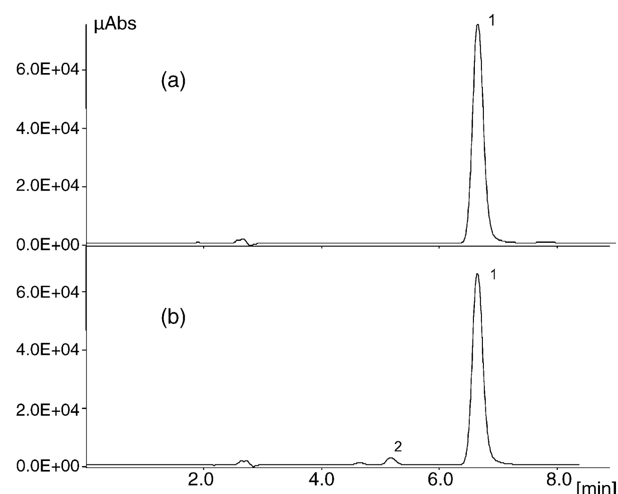

Fig. 5. HPLC traces of a cream product containing melatonin, before (a) and after (b) 2 h irradiation with the solar simulator. Other operating conditions as described in Section 2. Peaks: 1, melatonin; 2, *N*-[3-(2-formylamino-5-methoxyl-phenyl)-3-oxo-propyl]acetamide.

stability measurements, the applied energy was equivalent to 20 minimal erythemal dose (MED), which is considered representative of daily solar emission [18]. The degree of melatonin photodecomposition was measured by HPLC (representative chromatograms are shown in Fig. 5). NMR analysis of the major compound (retention time, 5.1 min) originated from the light-induced degradation of melatonin (Fig. 5b) revealed the presence of a resonance doublet at 8.41 δ(HC=O) which collapsed into a singlet after irradiation of the signal at 11.19 δ(NH). This finding provided evidence for the identification of the main photoproduct as the *N*-[3-(2-formylamino-5-methoxyl-phenyl)-3-oxo-propyl]acetamide (Fig. 1b), in accordance with previous studies [2,6]. In addition, in the chromatogram of the cream preparation exposed to the solar simulator, a minor peak was observed at 4.6 min (Fig. 5b), which can be probably traced to 6-hydroxymelatonin, according to Maharaj et al. [6]. In the formulation containing melatonin in conjunction with empty lipospheres, 19.6% of the drug content was lost following irradiation (Table 1). The photo-induced decomposition of melatonin was not significantly affected (Table 1) by its microencapsulation in the lipoparticles based on tripalmitin and phosphatidylcholine (18.9% degradation). Conversely, a remarkable reduction of the extent of photodegradation to 5.6% (Table 1) was obtained in the cream containing melatonin-loaded tristearin–phosphatidylcholine particles. Hence, the photostabi-

Table 1
Comparative photodegradation data for free and microparticle-encapsulated melatonin in cream preparations, after 2 h irradiation with the solar simulator

Sample	Melatonin loss[a] (%)	*P*[b]
Free melatonin	19.6 ± 4.3	
Melatonin incorporated in tripalmitin lipospheres	18.9 ± 5.9	>0.05
Melatonin incorporated in tristearin lipospheres	5.6 ± 4.1	<0.01

[a] Each value is the mean ± S.D. of six determinations.
[b] *P*-values (unpaired *t*-test) vs. free melatonin.

lization effects of the examined liposphere systems correlate with their diffusion modulation capacity (Fig. 4).

In conclusions, the results described in this study indicate that encapsulation of melatonin into lipid microparticles represents an effective strategy to enhance the photostability of the drug. Moreover, the biocompatibility and sustained release properties of the liposphere carrier system represent additional advantages for the formulation of skin care products.

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