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Importance of using solid lipid microspheres as carriers for UV filters on the example octyl methoxy cinnamate

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Abstract

The aim of this study was to prepare solid lipid microspheres (SLM) of octyl methoxy cinnamate (2-ethylhexyl-*p*-methoxy cinnamate; OMC) to achieve controlled release, decrease penetration of this UV absorber from skin and improve its photostability. The influence of the carrier on the rate of release was studied in vitro with a cellulose acetate membrane and in vivo from excised rat skin with Franz diffusion cells. The release rate was decreased by up to 13–80% with the SLM formulation. In vivo, penetration of OMC into skin was investigated by HPLC method. It was found out that the rate of penetration is significantly dependent upon the formulation and could be decreased by up to 77% in SLM formulations. When different topical vehicles were compared, OMC was released and penetrated into rat skin more quickly and in greater amount from vehicles containing free OMC than in SLM form. Additionally, photostability was shown to be improved in SLM form.

Keywords: Octyl methoxy cinnamate; Solid lipid microspheres; Penetration from skin; Photostability

1. Introduction

Sun protecting substances are capable of protecting humans from harmful effects of solar radiation such as aging and skin cancers (Berardesca et al., 1991). Due to the depletion in the ozone layer, research regarding to sun protection has become a major concern (Horiot et al., 1995; Berset et al., 1996). Since these preparations are often applied on large skin areas even low penetration rates can cause significant amount of chemical UV absorber to enter the body (Watkinson et al., 1992). Sun protecting preparations need to achieve a controlled release (Fairhurst and Mitchnick, 1995). For this purpose, liposomes, microsponges, microspheres, nanocapsules and inclusion complexes have been used (Rogers, 1999; Scalia et al., 1999; Alvarez-Roman et al., 2001). An alternative system to former controlled release systems for topical administration are solid lipospheres as they have been used for cosmetics (Dingler et al., 1997). Additionally, sunscreen preparations need a high degree of resistance to photodecomposition on exposure to sunlight. A sunscreen must be effective in absorbing erythemogenic radiation in the 280–315 nm range without breakdown which would reduce its efficiency or give rise to toxic or irritant compounds (Wilkinson, 1973). In recent years, it was reported that spectral stability of some UV absorbers were investigated on stratum corneum sheets and photochemical stability was determined by examining the changes in

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absorption spectra of the substance (Kammeyer et al., 1987; Aberturas et al., 1987; Tarras-Wahlberg et al., 1999). In this study, it was attempted to prepare solid lipid microspheres (SLM) of octyl methoxy cinnamate (2-ethylhexyl-*p*-methoxy cinnamate; OMC). OMC is one of the most widely used lipophilic UVB absorber in sunscreen preparations. OMC as plain absorber and also in microsphere form was put into various vehicles and investigated and compared in respect of OMC release, penetration from skin and photostability.

2. Materials and methods

2.1. Materials

OMC was purchased from Merck, methanol (HiperSolv grade) and ethanol (AnalaR grade) were also from Merck. All other substances were of analytical grade having chemical purity. Cellulose acetate membrane was from Sartorius, Germany.

2.2. Preparation of formulations

2.2.1. SLM preparation

A modified method was used (Giannola et al., 1993). Lipid microspheres were prepared from warm oil in water microemulsions. Beeswax (6 g) was melted, Tween 80 (0.20 g) and OMC (3 g) were dispersed in melted beeswax and added to warm mixture (60 °C) of water. Microemulsion was mixed for 20 min at 500 rpm at room temperature. The beeswax was solidified enveloping OMC. Lipospheres produced were recovered by decantation, washed by water (40 ml) three times, filtered by sartocon filter and lyophilized until dryness. (Mean particle size was found as 250 μm.)

2.2.2. Microscopic analysis of SLM

Lipid microspheres were investigated under polarizing microscope with various magnifications.

2.2.3. Determination of OMC in SLM

Twenty milligrams lipid microsphere sample was dissolved in 10 ml ethanol and the UV filter content was determined by UV spectrophotometer at 308 nm against blank.

Table 1
Size distribution of lipid microspheres obtained expressed as %

Sieve range (μm)				
100	200	250	300	425
4.7	12.8	64.8	9.9	7.8

2.2.4. Topical formulations

Oleagenous cream (white soft paraffin 40:cetostearyl alcohol 25:liquid paraffin 33:Tween 20), carbopol gel (carbopol 940 2:propylene glycol 40:deionised water 58) and o/w emulsion (cetyl alcohol 25:white soft paraffin 25:propylene glycol 22:sodium lauryl sulphate 1:deionised water 37) were used as various topical vehicles containing 5% OMC.

2.3. Size distribution of microspheres

Drug incorporated microsphers (5 g) were placed on the top sieve of a series of six standard sieves ranging from 100 to 710 μm. The sieves were mechanically shaken for 15 min. The sieving analysis results obtained from the mean of five batches are shown in Table 1.

2.4. In vitro release and in vivo penetration studies from excised rat skin

Fifty mimcrometer cellulose acetate membrane and abdominal skin of rats were used on Franz diffusion cells (2 cm^2 surface area). 0.5 g vehicles containing microsphere samples were applied as donor compartment. Ethanolated (25%) phosphate buffer (pH 7.4) was used as receptor phase. Temperature was kept at 37 °C and stirred at 600 rpm. Three hundred microliters aliquots were collected and amount of OMC released and penetrated were determined by UV spectroscopy and HPLC at 308 nm, respectively for release and penetration experiments. The study was repeated by vehicles containing free OMC at 5% concentration.

2.4.1. HPLC analysis

The chromatographic conditions were as follows: the analytical column used was of stainless steel (250 mm × 4.6 mm i.d.) packed with LiChrosorb C-18

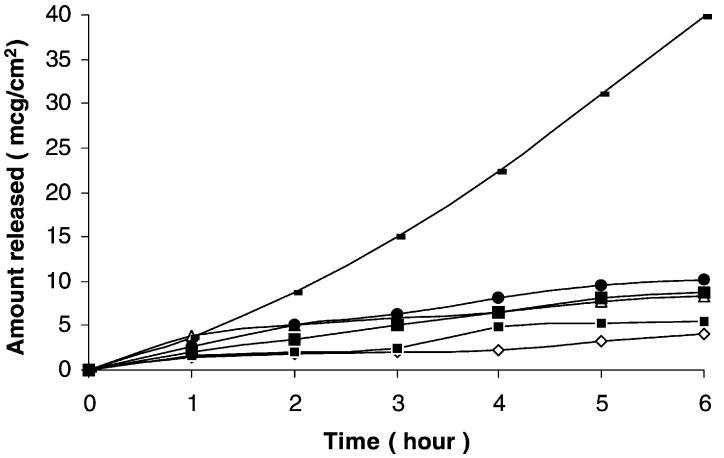

Fig. 1. Release profiles of OMC in oleagenous cream (■), gel (△), o/w emulsion (●), OMC in lipid microsphere from oleagenous cream (■), gel (◇), and o/w emulsion (■) ($n = 6$).

(Merck) of 5 μm. The sample injection volume was 5 μl. The detection wavelength was set at 306 nm and the eluent was methanol:water (83:17) containing glacial acetic acid (0.01% v/v).

2.5. Photostability studies

Five hundred milligrams portion of the test preparations containing both free OMC or in microsphere form was transferred into a quartz cuvette and exposed to the solar simulator (200 W Xenon-Mercury lamp fitted with focusing lens, to center the light on the sample with a filter ($\lambda > 290$ nm). Samples were placed in front of the filter and air-cooled during irradiation for 4 h. Photodecomposition products were determined by using thin layer chromatography (TLC).

2.6. TLC

One gram of the sample was extracted with 4 ml methanol and shaken for 1 h by using horizontal shaker. 0.5 g sodium sulphate was added and mixture left overnight. Five microliters was used for identification in TLC (Silicagel HF 254) solvent systems: (a) diisopropyl ether 20:*n*-hexane 80:acetic acid, and (b) ethyl acetate 65:methanol 25% 30:ammonia 5 were used as mobil phases. Anisaldehyde reagent (anisaldehyde 0.5:acetic acid 50:sulphuric acid 1) was used for identification.

2.7. Effectiveness of OMC in microspheres

Creams containig free OMC and in solid microspheres were applied on volunteers' inner arms and exposed to solar simulator for 15 min. In order to compare the effectiveness of OMC as free form and in solid microsphere. Erythema formed was measured by Mexameter (*n*:8) (Courage-Khazaka Inst., Germany).

3. Results and discussion

Investigations by means of polarized light microscope showed the spherical shape of lipospheres. Amount of OMC in lipid microsphere was found to be 87.52%.

In vitro release and penetration from skin of OMC from various vehicles are shown in Figs. 1 and 2. Release and penetration rates of OMC and the amount released and penetrated were decreased when OMC is in microsphere form. Differences in release and penetration rates were also observed due to vehicles. Release of free OMC was found to be highest from oleagenous cream and lowest from carbopol gel in lipid microsphere form (Table 2).

3.1. In vivo penetration study from excised rat skin

Penetration of free OMC was found to be highest from oleagenous cream as in release studies whereas

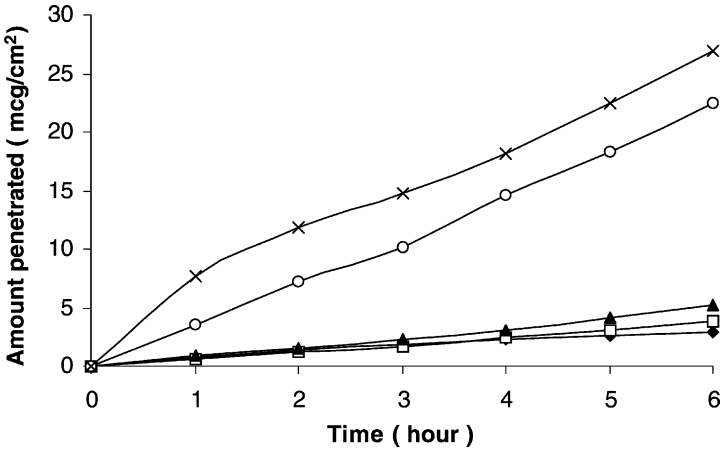

Fig. 2. Penetration profiles of free OMC in oleagenous cream (×), gel (□), o/w emulsion (◆) and OMC in lipid microsphere from oleagenous cream (▲), and gel (○) ($n = 6$).

Table 2
Released amount and release rate of free OMC and OMC incorporated in SLM from oleagenous cream, o/w emulsion and gel ($P < 0.05$)

Vehicles	Released amount (μg/cm^2)		Release rate (μg/cm^2/h)	
	Free OMC	LM	Free OMC	LM
Oleagenous cream	39.82 ± 1.13	5.35 ± 0.07	6.64 ± 0.02	0.89 ± 0.01
O/w emulsion	10.24 ± 0.09	8.87 ± 0.05	1.71 ± 0.01	1.48 ± 0.01
Carbopol gel	7.61 ± 0.08	2.46 ± 0.04	1.27 ± 0.01	0.41 ± 0.01

no penetrated amount was observed in the receptor phase in case of using OMC in lipid microsphere incorporated into o/w emulsion as shown in Table 3. Besides, the amount of free OMC penetrated from o/w emulsion was found to be 2.18 μg/cm^2 whereas the amount of free OMC penetrating was detected as 21.87 and 27.4 μg/cm^2 in carbopol gel and oleagenous cream, respectively. Penetration rate and the amount of sunscreens are dependent on the solubilizing effect of the vehicles in these agents. These differences also could be attributed to the interactions of the vehicles with skin structure.

3.2. Photostability studies

Taking into consideration the results of photostability studies, photodecomposition products of free OMC were observed whereas OMC was found stable in liposphere form exposed to a solar simulator as seen in Table 4.

Table 3
Penetrated amount and penetration rate of free OMC and OMC incorporated in SLM from oleagenous cream, o/w emulsion and gel ($P < 0.05$)

Vehicles	Penetrated amount (μg/cm^2)		Penetration rate (μg/cm^2/h)	
	Free OMC	LM	Free OMC	LM
Oleagenous cream	27.4 ± 0.06	6.38 ± 0.02	4.56 ± 0.02	1.06 ± 0.01
O/w emulsion	2.18 ± 0.01	–	0.36 ± 0.01	–
Carbopol gel	21.87 ± 0.03	4.82 ± 0.01	3.64 ± 0.01	0.80 ± 0.01

Table 4
Photodegradation of OMC in vehicles exposed to solar simulator for 30 min

Vehicles	Solvent system			
	a		b	
	Rf	Rfd	Rf	Rfd
Carbopol gel (OMC)	42	70	75	18
Oleagenous cream (OMC)	42	72	74	16
Carbopol gel (microsphere)	42	–	74	–
Oleagenous cream (microsphere)	40	–	75	–

Abbreviations: Rf, Rf of OMC; and Rfd, Rf of degradation product.

Table 5
Effectiveness of free OMC and in solid microsphere form ($P < 0.05$, differences between vehicles is significant)

Vehicles	Erythema value	
	Free OMC	OMC in lipid microsphere
Carbopol gel	577	581
Oleagenous cream	582	580
O/w emulsion	586	584
Control	631	631

3.3. Effectiveness of OMC in microspheres

Additionally, one of the most important aspects of sunscreen products is its effectiveness and it was concluded that incorporation of OMC into SLM has not changed its effectiveness against UV light in our experimental conditions. Table 5 reveals that the erythema values obtained by using the Mexameter was significantly different from control whereas no significant difference between erythema values of free OMC and OMC in lipid microsphere (*n*:8) was found.

4. Conclusions

Results of this study have shown that SLM can be suggested as carriers for OMC in order to decrease the release and penetration rate and amount of this UV absorber and choosing the suitable vehicle plays an important role in formulating a sunscreen product. Incorporation of OMC into SLM also enhanced the photostability of OMC compared to plain absorber in various vehicles. Another important aspect was the effectiveness of OMC in liposphere form which showed nearly the same protection as the free form after exposure to a solar simulator.

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