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Research paper

Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection

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Abstract

Biodegradable polymer nanocapsules containing the lipophilic sunscreen, Parsol MCX (OMC), as the oil core were prepared by solvent displacement. We investigated the influence of polysorbate 85 (P-85) and poloxamer 188 (P-188) as stabilizing agents, the OMC loading capacity and the photoprotective potential of the formulations. The formation of nanocapsules is probably due to an interfacial instability arising from rapid diffusion of the solvent across the interface. The effectiveness of the stabilizing agents was attributed to their ability to inhibit coalescence during solvent diffusion. P-85 was a better stabilizer of poly(ε-Caprolactone)-nanocapsules than P-188. The OMC loading capacity was high (99 ± 1% of OMC initial concentration). The in vitro release of OMC-nanocapsules is governed by hydrophobicity and crystallinity of the polymer and by the high lipophilicity of the drug. The OMC-nanocapsules provided partial protection against UV-induced erythema, in a manner significantly better than a conventional gel. © 2001 Published by Elsevier Science B.V.

Keywords: Nanocapsules; Solvent displacement; Biodegradable polymers; Sunscreen; Photoprotection

1. Introduction

Colloidal drug carriers, including submicron emulsions, nanospheres, nanocapsules, liposomes and lipid complexes, have been attracting increasing interest in recent years, as vehicles for the intravenous administration of lipophilic drugs, as improved parenteral formulations, and as systems for site-specific drug delivery [1,2].

In general, two techniques have been used for the preparation of nanocapsules based on biodegradable polymers.

1. The emulsification-diffusion technique [3] consists of emulsifying an organic solution containing an oil, a polymer, and a drug in a aqueous solution of a stabilizing agent. The subsequent addition of water to the system induces solvent diffusion into the external phase, resulting in the formation of colloidal particles. Several difficulties have been encountered using this technique when working with certain stabilizing agents (e.g. poly-vinyl alcohol) and when using ultracentrifugation as the nanocapsule drug-loading technique.
2. The solvent displacement procedure [4]. In this process, the lipophilic drug, oil, polymer and optionally phospholipids, are dissolved in a water-miscible solvent (e.g. acetone). This solution is then poured with stirring into an aqueous solution containing a non-ionic surfactant (e.g. poloxamer 188). Addition of the acetonic-oily solution results in spontaneous emulsification and the formation of nanodroplets, probably due to an interfacial instability arising from rapid diffusion of the acetone across the interface [5].

Sunscreen preparations are usually applied to large skin areas to prevent sunlight-induced erythema. Therefore, effectiveness implies that sunscreen filters adhere to skin like a protective film. They should have a high affinity for the stratum corneum. The ideal medium in which an active ingredient is incorporated must provide not only the necessary solubility, but also maintain contact between the active ingredient and the skin. Topical administration of colloidal preparations, mainly liposomes and emulsions, has received little attention until relatively recently. The influence of the nature of the colloidal carrier, as well as the effects of size and surface charge, on drug penetration into the skin has

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been studied. The results obtained have shown clearly that the formulation can strongly impact the stratum corneum concentration and penetration of the UV filters [6–8].

The objective of this work was the preparation, optimization of an solvent displacement method for poly(ε-caprolactone) nanocapsules, using the lipophilic drug, octyl methoxycinnamate (OMC) as the oil core, and the determination the eventual release in vitro of the encapsulated sunscreen during the time of contact with the guinea pig skin. We then evaluated in vivo the ability of the OMC-nanocapsules to protect guinea pig skin against ultraviolet B (UVB) radiation.

2. Materials and methods

2.1. Chemicals

Poly(ε-Caprolactone) P(CL), with molecular mass of 10,000, was supplied by Fluka. Octyl methoxycinnamate (Parsol® MCX), OMC, was obtained from Givaudan-Roure (Vernier, Switzerland). Two non-ionic stabilizing agents were tested: Poloxamer 188, P-188, (Pluronic® F 68, BASF, Ludwigshafen, Germany) and Polysorbate 85, P-85, (Tween® 85, Atlas Chemie, Germany). Satiaxane CX 91 (SCX 91), acetone and ethanol (EtOH) were purchased from Fluka. Polysorbate 80, P-80 (Tween® 80, was supplied by Sigma–Aldrich Chimie).

2.2. Methods

2.2.1. Nanocapsule preparation

125 mg of P(CL) and a specified quantity of OMC were dissolved in 25 ml of acetone and this organic phase was injected into 50 ml of an aqueous surfactant solution under moderate magnetic stirring. The aqueous phase immediately turned milky with bluish opalescence due to the formation of a nanocapsule suspension (NC-S). The acetone was then removed under reduced pressure at 35–40°C for approximately 30 min. The NC-S was concentrated to the desired final volume (10–20 ml) by removal of water under the same conditions. In order to optimize the process and to determine the effect of varying the OMC/P(CL) ratio, nanocapsules were prepared using different amounts of OMC.

2.2.2. Nanocapsule evaluation

- The mean size and polydispersity (index from 0 to 9) of the NC-S was measured with a Coulter Nano-sizer® (Coulter Electronics, Margency, France). Measurements were made in triplicate for all prepared batches.
- The physical stability of each NC-S was determined by assessment of particle size and polydispersity (index from 0 to 9) with a Coulter Nano-sizer® (Coulter Electronics, Margency, France) over a period of 1 month.
- The amount of the encapsulated drug was determined in the clear supernatant following separation of the NC from the aqueous medium by a combined filtration and centrifugation technique. OMC content in the NC was calculated by the difference between the total and free estimated drug concentrations. Measurements were made in triplicate for all prepared batches.
- The effect of the incorporation of different concentrations of OMC was also evaluated by measuring the particle size and polydispersity.

2.2.3. Sunscreens

Four sunscreen gels were used. (a) OMC-NC-gel, which contained NC-S with a total content of OMC of 3% and SCX 91 at 2% (w/w); (b) OMC-gel, with OMC at 3%, EtOH at 2% and SCX 91 at 2% (w/w); (c) the 'control', gel-free formulation of OMC; and (d) the 'control', gel-NC-free formulation of OMC. The OMC-NC-gel preparation was formed under constant and magnetically stirred of 50 rpm and thermostated at 25 ± 2°C for ensure the nanocapsules integrity.

2.2.4. In vitro experiments

The in vitro release profile of OMC from the OMC-NC-gel and OMC-gel was determined using a dialysis method. Approximately 0.5 ml of OMC-formulations was placed into a dialysis bag (cellulose membrane, cutoff = 15–20 Å, Merck, France) which was subsequently sealed and introduced into 400 ml of receptor medium (phosphate buffer/Tween 80 (5% w/w), pH 7.4). The system was thermostated at 37 ± 2°C and magnetically stirred at 200 rpm. Three milliliter samples of the receptor medium were taken at various times and assayed for OMC concentration by HPLC (Dionex, USA). A Hypersil BDS C8 (5 μm) 150 × 4.6 mm column (Supelco, PA, USA) was utilized and the mobile phase consisted of acetonitrile and water (85:15) at a flow rate of 1 ml/min. OMC was detected by its UV absorbance at 310 nm. In order to determine the NC integrity in the gel, an OMC-S release was studied at the same in vitro release conditions.

2.2.5. In vivo experiments

Guinea pigs were irradiated with a Biotronic UV 365/312 nm (Vilbert Lourmat, France) [9]. The UVB irradiance at the guinea pig skin was 0.43 J/cm^2. To study the photoprotective properties of the sunscreen preparations, they were applied to an area of 12 cm^2 at approximately 2 mg/cm^2. The formulations were allowed to dry for 15 min. Contralateral sites served as untreated controls. Erythemal responses at all irradiated sites were graded at $t = 24$ h using the following scale: 0 = No erythema; 1 = Minimally perceptible erythema without well-defined borders; 2 = Definite uniform erythema with well-defined borders; 3 = Intense erythema with edema.

Table 1
Properties of nanocapsules prepared using the solvent displacement procedure with different stabilizing agent/polymer systems (each formulation contained 0.51 g of OMC)

P-85 stabilizer[a] % w/v	Mean size (±SD)[b] nm	PI[c]	P-188 stabilizer[a] % w/v	Mean size (±SD)[b] nm	PI[c]
0.2	399 ± 3	2	0.2	427 ± 4	4
1.0	292 ± 3	2	1.0	360 ± 3	3
2.0	288 ± 2	1	2.0	344 ± 3	3
3.0	255 ± 3	1	3.0	330 ± 3	2
4.0	273 ± 4	3	4.0	342 ± 2	1
5.0	290 ± 3	2	5.0	416 ± 4	3

[a] In the aqueous phase.
[b] SD, standard deviation ($n = 3$).
[c] PI, polydispersity index expressed on a 0–9 scale.

3. Results and discussion

3.1. Nanocapsules

With the solvent displacement technique, it was possible to prepare reproducibly biodegradable OMC-nanocapsules with a mean size of 255–427 nm using P-85 or P-188 as stabilizing agents (Table 1). The loading capacity was 99 ± 1% of the initial OMC concentration.

The mean sizes after solvent evaporation were significantly different between the two stabilizers. With P-188, all NC suspensions were greater than 320 nm; when the NC were prepared with P-85, the mean size was smaller than 300 nm. This shows that the type and concentration of stabilizing agent play important roles in the formation of nanocapsules. It is possible that interfacial phenomena during diffusion contribute to the subdivision of the globule into nanodroplets before nanoparticle formation. The droplets are rapidly stabilized by the stabilizing agent, until diffusion of the solvent is complete and polymer aggregation has occurred. The smallest NC found in this work were obtained when the P-85 stabilizer was utilized at a concentration of 3% (w/v of the aqueous phase) (Fig. 1). The results in Fig. 2, show that the NC prepared with the two stabilizing agents were stable after evaporation, and that no aggregation was immediately detectable. However after 1 week, NC prepared with P-188 showed evidence of aggregation; this effect was independent of concentration. Quintanar-Guerrero [10] found similar results; the P-188 facilitates the formation of nanoparticles by exerting a stabilizing action during the diffusion of solvent globules (propylene carbonate) to the external phase, and thereby protecting the generated 'protonanoparticles'. The stability of the suspension depended only on the strength of the interactions at the particle surface. Therefore, if the stabilizer remains at the liquid-liquid interface, during the diffusion process, and if the interaction at the particle surface is strong, then stable nanoparticles will form. The stabilizer P-85 has a better P(CL)-surface affinity than P-188.

The solvent displacement technique has been proposed as an efficient means to encapsulate lipophilic drugs (e.g. indomethacin, dexamethasone, diclofenac [2,11,12] and, as shown in Table 2, this is also the case for OMC. The amount of OMC incorporated into the nanocapsules was gradually increased from 310 to 1342 mg. The absence of any aggregate or sediment at low OMC concentrations attested to good association of polymer with the oil. The polydispersity index of the nanocapsules increased when the quantity of OMC was increased beyond 516 mg, due to the presence of aggregates. The encapsulation efficiency also decreased at higher OMC concentration due to the formation of visible oil droplets. Based on these findings, 516 mg OMC was selected for the in vivo evaluation of the OMC-NC gel preparation.

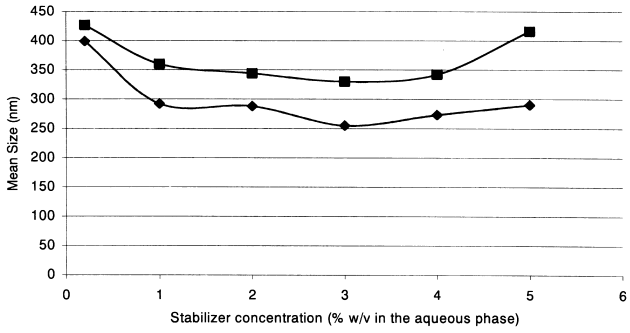

Fig. 1. Influence of the concentration of stabilizing agent on nanocapsule size. (◆) P-85 and (■) P-188.

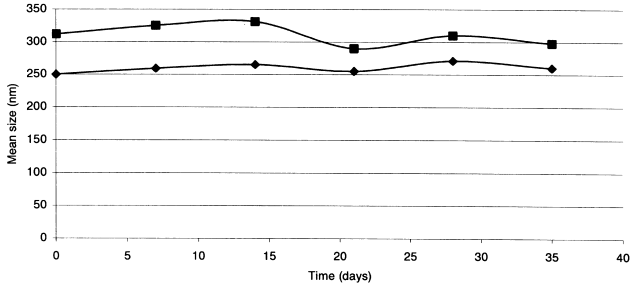

Fig. 2. Influence of time on nanocapsule size. (◆) P-85 and (■) P-188. Each formulation contained 0.51 g of OMC.

Table 2
Characteristics of OMC-nanocapsules prepared by the solvent displacement procedure with different concentrations of sunscreen (each formulation contained P-85 stabilizer at 3% (w/v in the aqueous phase))

OMC mg	Mean size (±SD)[a] nm	PI[b]	Aggregates[c]	Incorporation efficiency (% of initial conc.)[a] (±SD)
310	211 ± 2	1	–	99 ± 1
516	255 ± 3	2	–	97 ± 2
830	348 ± 5	5	+	73 ± 3
1032	475 ± 6	6	++	68 ± 2
1342	565 ± 5	8	+++	44 ± 3

[a] SD, standard deviation ($n = 3$).
[b] PI, polydispersity index expressed on a 0–9 scale.
[c] Aggregates, visible particles on the surface of the suspension.

3.2. In vitro experiments

The OMC release profiles from OMC-S and OMC-NC-gel were similar (Fig. 3), supporting the integrity of NC in the labile formulation. It is important to mention that the OMC-S release were faster than OMC-NC-gel release, this suggest that Satiaxane CX 91 in the OMC-NC-gel increased the viscosity into the gel-formulation, changing the diffusion velocity of OMC from the NC.

The two profiles that involved the OMC-NC-formulation showed a modest burst released of 2% (not shown). This can be explained by the high hydrophobicity and crystallinity of the caprolactone polymer [13,14] and by the high lipophilicity of the drug, preventing diffusion from the NC-S or OMC-NC-gel into the receptor medium supplying the OMC-NC-stability. In addition, the slow initial diffusion rate of OMC from the nanocapsules, can establish that the OMC was entirety encapsulated and not adsorbed at the external surface of the nanocapsules.

3.3. In vivo experiments

Sunscreens are used to prevent sunlight-induced erythema, and to reduce the risk of skin cancer [15–17]. Fig. 4 shows that the sunscreen preparations, OMC-NC-gel and the OMC-gel significantly reduced UV-induced erythema, compared to the corresponding OMC-free formulations. The results agree with those of Wolf [18], who have

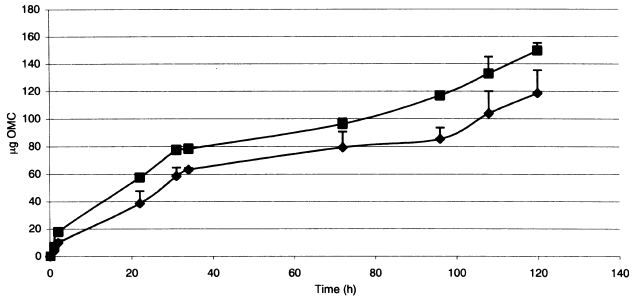

Fig. 3. Cumulative OMC release from (■) OMC-S and (◆) OMC-NC-gel into a phosphate-buffered solution ($n = 3$; mean ± SD).

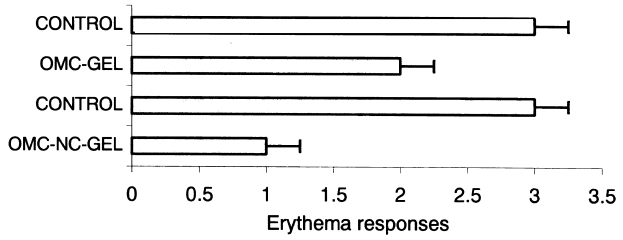

Fig. 4. Erythema response following exposure to UVB measured 24 h after irradiation and following pretreatment with either OMC-NC-gel or OMC-gel (and the corresponding sunscreen free controls) ($n = 6$; mean ± SD).

also evaluated the effects of OMC at a similar concentration. Furthermore, the OMC-NC-gel preparation resulted in a significantly better ($P < 0.05$) protection against UV-induced erythema than the simple OMC-gel. The effectiveness of sunscreen preparations against UV radiation has been extensively investigated. Effectiveness implies that the sunscreens adhere to skin as a protective film. The results presented here suggest that the NP, due to their high specific surface area, may be able to efficiently cover the skin surface and improve the sunscreen's ability to inhibit UV-induced erythema.

4. Conclusions

The present work has shown that biodegradable polymer nanocapsules containing the lipophilic sunscreen OMC as the oil core can be produced by the method of solvent displacement using P-85 as stabilizer. The nanoparticles formation process seems to be related to the interfacial turbulence generated during rapid diffusion of solvent. The advantage of this method is the instantaneous and reproducible formation of small nanocapsules exhibiting a high drug loading capacity.

We believe that topical application of a nanocapsule-gel preparation of OMC may be more effective in protecting against UV-induced erythema due probably at the NC-film formation on the skin surface. In conclusion, the results of this study emphasize the potential of sunscreen nanocapsules as new skin drug delivery systems.

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