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Tretinoin-loaded nanocapsules: Preparation, physicochemical characterization, and photostability study

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Abstract

The aim of this study was to prepare and characterize tretinoin-loaded nanocapsules as well as to evaluate the influence of this nanoencapsulation on tretinoin photostability. Tretinoin-loaded nanocapsules (0.5 mg ml^{-1}) were prepared by interfacial deposition of preformed polymer (poly-ε-caprolactone) using two different oily phases: capric/caprylic triglycerides and sunflower seed oil. Tretinoin-loaded nanocapsules presented drug content close to the theoretical value, encapsulation efficiencies higher than 99.9%, nanometric mean size with a polydispersity index below 0.25, and pH values between 5.0 and 7.0. Regarding photodegradation studies, tretinoin methanolic solution showed a half-life time around 40 min according to a first order equation, whereas tretinoin nanocapsule suspensions showed a half-life between 85 and 100 min (twofold higher than in methanolic solution) according to a zero order equation. Tretinoin-loaded nanocapsules improved tretinoin photostability, independently on the type of oily phase used in this study, and represent a potential system to be incorporated in topical or systemic dosage forms containing tretinoin.

Keywords: Nanocapsules; Nanoparticles; Photostability; Retinoic acid; Tretinoin

Tretinoin (all-*trans*-retinoic acid) is the active form of a metabolic product of Vitamin A, also called retinoic acid. It belongs to the first generation of retinoids along with isotretinoin, which is a *cis*-isomer of retinoic acid (Rigopoulos et al., 2004). This drug is effective in the topical treatment of different skin diseases such as acne vulgaris, ichtiosys, psoriasis, and neoplasias (Polano, 1974; Corbeil et al., 1994; Brisaert et al., 2001; Shin et al., 2005). The development and dermal administration of topical systems containing tretinoin present some drawbacks such as poor solubility, high chemical and photoinstability, which renders inactive metabolites, and irritation of the treated area (Elbaum, 1988; Brisaert et al., 1995; Brisaert and Plaizier-Vercammen, 2000; Lin et al., 2000).

Many efforts have been made over the last years to overcome some of these drawbacks. The association/inclusion of tretinoin with different kinds of delivery systems (liposomes, lipid nanoparticles, cyclodextrins, niosomes) has improved its solubility (Montassier et al., 1997; Brisaert and Plaizier-Vercammen, 2000), chemical stability (Brisaert and Plaizier-Vercammen, 2000; Lin et al., 2000; Brisaert et al., 2001; Manconi et al., 2003; Lim et al., 2004; Ioele et al., 2005), bioavailability and/or efficacy (Sacks et al., 1992; Ezpeleta et al., 1996; Lin et al., 2000; Shimizu et al., 2003; Shah, 2007).

Over the past 15 years, polymeric nanocapsules and nanospheres have been extensively studied as drug carriers in the pharmaceutical field (Schaffazick et al., 2003) as well as an efficient coating material to control the drug release from microparticles (Beck et al., 2007). Nanospheres are defined as a matricial polymeric structure, in which drugs can be entrapped or molecularly dispersed. On the other hand, nanocapsules are

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characterized by a lipophilic core surrounded by a polymeric layer, in which drugs can be dissolved in the oil, dispersed within the particle (Couvreur et al., 2002), or adsorbed at the interface particle/water (Pohlmann et al., 2002). When these systems were applied epicutaneously, they can alter the drug pharmacokinetic and biodistribution through skin. Besides, their small size facilitates their formulation in dermatological products and enables comfortable application to the skin (Perugini et al., 2002; Guterres et al., 2007).

Some works have been reported on the association of tretinoin in matrix type polymeric nanoparticles (Nam et al., 2003; Jeong et al., 2004; Seo et al., 2006). However, the feasibility to encapsulate tretinoin in vesicular polymeric nanoparticles (nanocapsules) and their potentiality to improve the photostability of this drug have not been yet evaluated. Hence, the aim of the present work was to prepare and characterize tretinoin-loaded nanocapsules prepared with two different oily phases (capric/caprylic triglyceride mixture – CCM and sunflower seed oil – SFO) and to evaluate their potential as topical delivery systems to improve the stability of tretinoin against photodegradation induced by UV light.

Nanocapsule (NC) suspensions were prepared ($n = 3$) by interfacial deposition of preformed polymer method (Fessi et al., 1988). Briefly, an organic solution composed of tretinoin (0.0125 g), the oily phase, CCM or SFO (0.80 ml), sorbitan monooleate (0.194 g), the polymer (poly-ε-caprolactone) (0.25 g), and acetone (67.0 ml) was added to an aqueous solution (134.0 ml) containing polysorbate 80 (0.194 g) under moderate magnetic stirring (10 min). Then, the acetone was eliminated and the aqueous phase concentrated by evaporation under reduced pressure (40 °C) to a final volume of 25 ml (10 mg ml^{-1} of polymer and 0.50 mg ml^{-1} of drug). In order to verify the influence of the polymeric layer, nanoemulsions were prepared omitting the polymer. Blank formulations were prepared omitting the drug. All preparations were carried out protected from the light and kept in the dark during all the time.

Drug content (mg ml^{-1}) was determined ($n = 3$) after dissolution of nanocapsules or nanoemulsions in acetonitrile (1 ml of suspension to 25 ml of acetonitrile) and assayed by high performance liquid chromatography, HPLC. The chromatographic system consisted of a Gemini RP-18 column (150 mm × 4.60 mm, 5 μm, Phenomenex, Torrance, USA) and a Shimadzu instrument (LC-10AVP Pump, UV–vis SPD-10AVP Module, Class-VP Software, Shimadzu, Tokyo, Japan). The mobile phase at a flow rate of 1.0 ml min^{-1} consisted of acetonitrile/water (85:15%, v/v) containing 1% of glacial acetic acid. The volume injected was 20 μl and tretinoin was detected at 342 nm. The method was linear ($r^2 = 0.9999$) in the range of 1–20 μg ml^{-1}, accurate (recovery: 100 ± 3%) and precise (R.S.D.: <0.61% for repeatability and <1.72% for intermediate precision). The specificity was tested in presence of the colloidal suspension adjuvants and demonstrated that these factors did not alter the tretinoin assay. Free drug was determined in the ultrafiltrate (HPLC) after the separation of the nanoparticles by an ultrafiltration-centrifugation technique (Ultrafree-MC® 10,000 MW, Millipore, Bedford, USA), at 12000 rpm for 5 min. Encapsulation efficiency (%) was calculated by the difference between the total and free drug concentrations.

The pH values of the suspensions were determined and the particle sizes and polydispersity indices ($n = 3$) were measured by photon correlation spectroscopy (3 measures/batch; 2 runs of 30 s/measure at 25 °C) after adequate dilution of an aliquot of the suspension in water (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). The zeta potential values were determined (3 measures/batch; 10 runs/measure at 25 °C) after dilution (1:500) of the suspensions in 1 mM NaCl (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK).

All formulations appeared macroscopically homogeneous and their aspects were similar to a milky bluish opalescent fluid (Tyndall effect), regardless of the type of oily phase (CCM or SFO) or the vesicle structure (nanocapsule or nanoemulsion). The physicochemical characteristics of the formulations are presented in Table 1. The formulations presented drug content (mg ml^{-1}) close to their theoretical value (0.5 mg ml^{-1}) with high encapsulation efficiency (>99.9% for all drug-loaded formulations). Exact amounts of the tretinoin associated in nanocapsules could not be assayed because its concentration in ultrafiltrate was lower than the quantification limit (0.06 μg/ml) of the HPLC method.

Table 1
Physicochemical characteristics of drug-loaded and drug-unloaded colloidal systems (nanocapsules – NC and nanoemulsion – NE) prepared with capric/caprylic triglycerides mixture (CCM) or sunflower seed oil (SFO)

Formulation	Drug content (mg/ml)	Particle size (nm)	PDI[a]	Zeta potential (mV)	pH
CCM					
NC-tretinoin	0.500 ± 0.019	228 ± 08	0.16 ± 0.02	−7.27 ± 0.66	6.64 ± 0.31
NC	–	230 ± 20	0.21 ± 0.10	−4.45 ± 0.48	5.98 ± 0.28
NE-tretinoin	0.519 ± 0.013	210 ± 07	0.23 ± 0.06	−6.33 ± 0.83	6.48 ± 0.01
NE	–	259 ± 42	0.24 ± 0.09	−5.14 ± 1.08	6.82 ± 0.19
SFO					
NC-tretinoin	0.499 ± 0.001	222 ± 08	0.14 ± 0.02	−5.72 ± 1.36	6.66 ± 0.14
NC	–	222 ± 14	0.16 ± 0.03	−8.38 ± 3.00	5.68 ± 0.40
NE-tretinoin	0.509 ± 0.016	227 ± 08	0.21 ± 0.05	−6.26 ± 1.93	6.46 ± 0.16
NE	–	225 ± 08	0.22 ± 0.03	−6.13 ± 0.57	6.81 ± 0.06

Mean ± S.D.*: *represents the variation between the different batches ($n = 3$).
[a] PDI: polydispersity index.

Table 2
Photodegradation rate constants (k) and half-lives ($t_{1/2}$) of free tretinoin (methanolic solution-MS) and tretinoin-loaded nanocapsules – NC or nanoemulsion – NE exposed to UV light for 1 h ($n = 3$)

Formulation	Kinetic order	k	$t_{1/2}$ (min)[a]	r^2
NC–CCM	Zero	2.8738 ± 0.1367	95.18 ± 4.34	0.9917
NC–SFO	Zero	3.0345 ± 0.4373	88.60 ± 13.22	0.9590
NE–CCM	Zero	3.2201 ± 0.3746	81.63 ± 9.57	0.9687
NE–SFO	Zero	3.7042 ± 0.8397	69.35 ± 14.36	0.9730
MS	First	0.0174 ± 0.0020	40.07 ± 4.23	0.9877

[a] $t_{1/2}$ calculated according to the equation related to kinetic order of reaction.

Tretinoin-loaded NC and NE presented similar mean diameters (210–230 nm), acidic pH values (5.60–6.90), negative zeta potentials (between −4.45 and −8.40 mV) as well as polydispersity indices below 0.25 indicating an adequate homogeneity of these systems (Alves et al., 2007).

The photodegradation of tretinoin was studied using an UV artificial lamp (Phillips TUV lamp–UVC long life, 30 W). The tretinoin methanolic solution (MS) or tretinoin-loaded nanoparticle formulations (2 ml in a 1 cm quartz cuvette perfectly stoppered) were exposed to UV radiation for 1 h at a fixed distance of 10 cm ($n = 3$). Two hundred microliters of the samples were withdrawn every 10 min and diluted with acetonitrile in order to quantify the tretinoin by HPLC according to the method previously described. In order to refute the hypothesis of thermal degradation, tretinoin MS and nanoparticle formulations covered by aluminum foil (protected from UV light) were also evaluated in the same way.

The photodegradation profile of tretinoin MS (36.7 ± 2.2% of the initial drug concentration remained after 1 h) was according to a first order kinetic (good linearity plotting logarithm of intact tretinoin as a function of time, r^2 0.9877), while the data obtained from the photodegradation experiments of the tretinoin-loaded NC or NE (56–67% of the initial drug concentration remained after 1 h) fitted better to the zero order kinetic (good linearity plotting intact tretinoin as a function of time, r^2 0.9590–0.9919). These results are in accordance with previous studies reported in the literature for tretinoin MS (Brisaert and Plaizier-Vercammen, 2000) and tretinoin-loaded niosomes (Manconi et al., 2003). When tretinoin in MS or loaded in formulations were protected from light, more than 93 % of tretinoin remained intact after 1 h of irradiation. The photodegradation rate constants (k) and half-lives ($t_{1/2}$) of free and tretinoin-loaded nanoparticles exposed to UV light for 1 h are shown in Table 2. Tretinoin MS showed a half-life time around 40 min, whereas the tretinoin-loaded NC suspensions showed half-life times between 85 and 100 min (twofold higher than in MS, ANOVA, $p \leq 0.05$). As can be observed in Table 2, the nanoemulsions also presented a potential to improve the photostability of tretinoin. However, this improvement was lower—$t_{1/2}$ between 69 and 82 min – compared to the nanocapsules – $t_{1/2}$ between 85 and 100 min (ANOVA, $p \leq 0.05$). These results show the importance given by the presence of the polymer to prevent the photodegradation of tretinoin. This better protection presented by the nanocapsules against UV-induced photodegradation of tretinoin could be attributed due to the crystallinity of the polymer, which has the ability of reflecting and scattering UV radiation (Perugini et al., 2002; Jimenez et al., 2004). Similar protection was earlier reported for other nanoparticulate systems like niosomes (Manconi et al., 2003), liposomes (Ioele et al., 2005), and complexes with cyclodextrins (Lin et al., 2000). However, up to now, this protection of tretinoin had not been previously reported for polymeric nanoparticles. Regarding the type of oily phase, the use of different oily phases did not show any statistical difference on the protection against photodegradation, both for nanocapsules or nanoemulsions (ANOVA, $p > 0.05$).

In conclusion, this work showed for the first time the feasibility to prepare tretinoin-loaded nanocapsules, at a concentration of 0.5 mg ml^{-1}, using two different oily phases (CCM or SFO). Tretinoin-loaded nanocapsules improved tretinoin photostability, independently on the type of oily phase used in this study, and represent a potential system to be incorporated in novel topical or systemic dosage forms containing tretinoin.

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