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Formulation of lipid core nanocapsules

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ABSTRACT

Polymeric nanoparticle aqueous suspensions have been proposed as drug carriers to improve the efficacy of medicines. Considering those nanocarriers, nanocapsules are vesicular structures containing an oil core surrounded by a polymeric wall. Recently, we proposed the supramolecular model for a new kind of nanocapsule prepared with triacylglycerol, sorbitan monostearate (SM), polyester and polysorbate 80. Varying the proportions of the raw materials in the organic phase, different kinds of colloids could be obtained. So, our objective was to formulate exclusively lipid-core nanocapsules (LNC) in aqueous suspensions. In this way, the analytical approach to verify the quality of the different formulations was based on light scattering measurements (dynamic light scattering, multiple light scattering and laser diffractometry) and density gradient. The increase in the SM concentration showed a slight tendency of both sedimentation and creaming, while the increase in the oil concentration resulted in creaming. For the latter, size distribution as function of time indicated the presence of nanoemulsion simultaneously with LNC. Finally, density gradient showed an exclusive band for formulations prepared using 1:4.1:2.6 (w/w) of SM, medium chain triacylglycerol and polyester, respectively.

1. Introduction

Recent interest has been focused on developing nanoscale biodegradable delivery vehicles capable of controlling the release of drugs. These nanoplatforms are supposed to obtain a higher effect with minimal toxicity due to the controlled delivery of the drug to the targeted site and to the decrease in its systemic distribution, as well as to protect the encapsulated drugs from early *in vivo* metabolization and elimination, improving their pharmacokinetic profile [1–7].

One of those extensively studied nanoplatforms is the polymeric nanoparticles that can significantly alter the drug pharmacokinetics and body distribution. While free drug distributes in all tissues and organs, the encapsulated drug distribution is imparted by the characteristics of the carrier [1,2,5]. Polymeric nanoparticles are colloidal systems that have received much attention owing to their potential use as drug carriers [8] and their ability in controlling the release of encapsulated drugs [9–11]. The term "polymeric nanoparticles" refers to vesicular or matricial colloids containing polymer as a domain in the system. Nanocapsules are vesicular carriers constituted of an oil core surrounded by a polymeric wall [8]. Recently, we developed a new kind of nanocapsules, named lipid-core nanocapsules, which are composed by a dispersion of sorbitan monostearate and medium chain triacylglycerol, in the core, enveloped by poly(ε-caprolactone), an aliphatic polyester as polymeric wall [12] (Fig. 1). Different from nanospheres composed by polymer or lipid-nanospheres, a dispersion of sorbitan monostearate and biodegradable polymer [13,14], those lipid-core nanocapsules are vesicular structures due to the presence of oil as raw material.

Polymer carriers represent one of the dominant classes of nanocarrier platforms capable of efficiently encapsulating and delivering a variety of drugs, peptides and proteins increasing stability and/or decreasing toxicity [15–17]. However, the qualitative composition of nanoparticles could influence either the drug *in vitro* release kinetic or the *in vivo* drug effect [18].

In previous reports our research group has studied the influence of the concentration of polymer in lipid-core nanocapsules on the release kinetic of indomethacin ethyl ester using the prodrug interfacial hydrolysis to simulate a sink condition [19,20].

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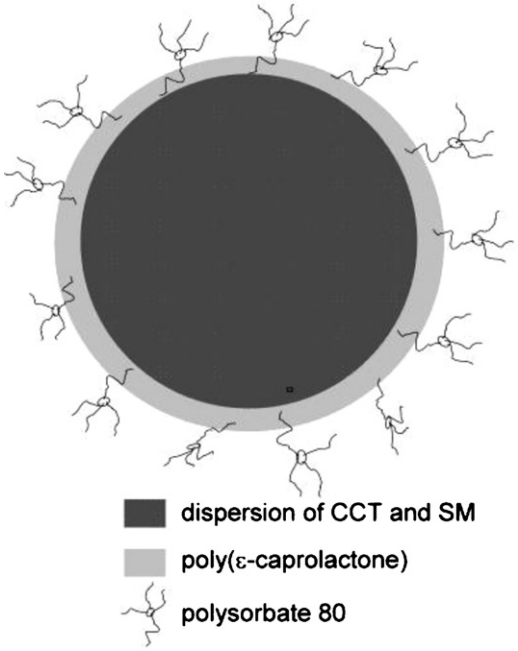

Fig. 1. Illustrative model for lipid-core nanocapsules.

The increase in the polymer concentration led to a slower drug release due to the reduction in the relative permeability of the polymeric wall of the nanocapsules [21]. DSC and SAXS analyses [22,23] showed that sorbitan monostearate is interacting with caprylic/capric triglyceride, in the core, and the interfacial hydrolysis of indomethacin ethyl ester as a function of the sorbitan monostearate concentration suggested that the core is, actually, a dispersion of the solid lipid in the oil [24]. Viscosity measurements carried out in sorbitan monostearate and caprylic/capric triglyceride mixtures in similar ranges used in the nanocapsule suspensions showed non-Newtonian behavior. So, the supramolecular model proposed for the lipid-core nanocapsules was confirmed [24].

Colloids and dispersions are complex and inherently unstable systems. The destabilization phenomena [25,26], affecting the dispersion homogeneity, are particle migration (creaming, sedimentation) and particle size variation due to aggregation, agglomerate or cluster formation (coalescence, flocculation or percolation). Furthermore, the characterization and stability evaluation of colloids are of prime importance, which are often studied by light scattering methods [27,28]. Multiple light scattering technique can be use without diluting the formulations to give information about their destabilization phenomena as a function of time [29,30].

Taking those considerations into account, our objective was to formulate aqueous suspensions exclusively composed by lipid-core nanocapsules (LNC). Indomethacin ethyl ester, an antinflammatory pro-drug [23,31], was used as lipophilic drug model due to its lipophilicity, leading to high encapsulation efficiencies. Furthermore, indomethacin ethyl ester-loaded lipid-core nanocapsules are mucoadhesive reservoir systems to delivery this pro-drug after oral administration [32]. In this way, we describe in this work an analytical approach to verify the quality of the formulations in order to select the optimized proportions of raw materials [sorbitan monostearate, caprylic/capric triglyceride and poly(ε-caprolactone)] used to produce exclusively LNC. Then, formulations were analyzed by light scattering techniques (dynamic light scattering, multiple light scattering and laser diffractometry) and density gradient measurements.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) (MW = 65,000) was supplied by Aldrich (Strasbourg, France). Caprylic/capric triglyceride (CCT) and polysorbate 80 were obtained from Delaware (Porto Alegre, Brazil). Span 60® (sorbitan monostearate, SM), dicyclohexylcarbodiimide (DCC), 4-(*N*,*N*-dimethyl)aminopyridine (DMAP) and indomethacin were obtained from Sigma (St. Louis, USA). All other chemicals and solvents used were of analytical or pharmaceutical grade. All reagents were used as received.

2.2. Synthesis of indomethacin ethyl ester

The synthesis of the indomethacin ethyl ester (IndOEt) was carried out as previously described [19,33]. A solution of indomethacin (5 mmol) in ethanol (20 mL) was added of DMAP (0.2 mmol) at 0 °C, under argon and magnetic stirring. After 10 min, DCC (5 mmol) was added in the medium. The reaction was carried out for 30 min at 0 °C and for 16 h 25 °C. The reaction was followed by thin layer chromatography (TLC). Then, the solvent was evaporated under reduced pressure and the residue added of dichloromethane (30 mL). The precipitate was filtered and the filtrate extracted by saturated $NaHCO_3$ aqueous solution (3× 10 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated. The product was purified by column chromatography (Silica gel 60, 70–230 mesh) using ethyl acetate and cyclohexane (1:1, v/v) as eluent. The isolated product was obtained as a solid (70% of yield) presenting a melting point (uncorrected) of 82–83 °C. The purity was determined as 99.2 ± 0.1% (HPLC).

^{1}H NMR analyses (Chloroform-d_1 99.8% atom D) confirmed the chemical identity of the product. NMR spectrum was obtained with a Varian YH-300 spectrometer, in a magnetic field of 7.0 T using a 5-mm multinuclear Varian probe at a temperature of 22 °C. Chemical shift values are expressed as δ (parts per million) relative to tetramethylsilane (TMS) used as internal standard.

^{1}H NMR 200 MHz (δ, ppm) $CDCl_3$: 7.66 and 7.46 (AB system, 2H and 2H, ArH *p*-chlorobenzoyl), 6.97 (d, 1H *J* = 2.5 Hz, H-4), 6.87 (d, 1H *J* = 9.0 Hz, H-7), 6.67 (dd, 1H *J* = 9.0 and 2.5 Hz, H-6), 4.16 (q, 2H *J* = 7.1 Hz, OCH_2), 3.84 (s, 3H, OCH_3), 3.65 (s, 2H, CH_2), 2.38 (s, 3H, CH_3), 1.27 (t, 3H *J* = 7.1 Hz, CH_3CH_2O).

^{13}C NMR 75 MHz (APT, δ, ppm) $CDCl_3$: 170.9 (CO-ester), 168.3 (CO-amide), 156.0, 139.2, 135.9, 134.0, 130.8, 130.7 and 112.7 (7 × Cq), 131.1 and 129.1 (4 × CH *p*-chlorobenzoyl), 114.9, 111.6 and 101.3 (3 × CH indol), 61.0 (OCH_2), 55.7 (OCH_3), 30.4 (CH_2), 14.2 and 13.3 (CH_3 and CH_3CH_2).

2.3. Preparation of the lipid-core nanocapsule formulations

Formulations were prepared by interfacial deposition of preformed polymer as previously reported [24]. At 40 °C, the polymer (PCL), the oil (CCT), sorbitan monostearate (SM) and indomethacin ethyl ester (IndOEt) (0.010 g) were dissolved in acetone (27 mL). This organic phase was injected into 53 mL of an aqueous phase containing polysorbate 80 under magnetic stirring at room temperature. After 10 min, acetone was eliminated and the suspension concentrated under reduced pressure. The final volume was adjusted to 10 mL. Nanocapsules were prepared using different CCT and SM concentrations (Table 1). For SM series (IC, IIC, IIIC, IVC, VC), the solid lipid (SM) concentrations varied from 3.85 to 11.50 mg/mL and for CCT series (IIIA, IIIB, IIIC, IIID, IIIE), the liquid lipid (CCT) concentrations were 16 to 47 mg/mL. Additional formulations were prepared as IIIE, but using 15.0 mg/mL of polymer (IIIEA) and 15.4 mg/mL of polysorbate 80 (IIIEB). Furthermore, optimized lipid-core nanocapsule suspensions were prepared using