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Beads made of cyclodextrin and oil for the oral delivery of lipophilic drugs: In vitro studies in simulated gastro-intestinal fluids

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ABSTRACT

The aim of this work was to investigate the stability in vitro, in simulated gastro-intestinal fluids, of beads, made of α-cyclodextrin and soybean oil, and to study the release of progesterone, a model of lipophilic drug. This was evaluated over time by the monitoring of the proportion of intact beads, their volume and the percentage of progesterone dissolved. Their incubation in the simulated gastric fluid provoked a moderate reduction of their number (20%) and a decrease of their volume (50%) after 55 min. Whatever the intestinal medium subsequently introduced, bead number and volume decreased more until bead disintegration that appeared faster in sodium taurocholate rich-medium. In such fluid, the amount of progesterone dissolved increased rapidly between 65 and 180 min, with both beads and emulsion to be equal after 85 min. With soft capsules, the increase was more gradual. In sodium taurocholate free-medium, more progesterone was dissolved from the emulsion than from beads or soft capsules. The release of progesterone from beads resulted from the erosion of their matrix and its partition equilibrium between oily micro-droplets and aqueous phase. The original structure of beads confers to this multiparticulate system interesting properties for the oral delivery of lipophilic drugs.

1. Introduction

Approximately 40% of new chemical entities exhibit poor aqueous solubility, which presents a major challenge for their use in therapeutics because it leads to low bioavailability (Abdalla et al., 2008). One possible strategy to improve the efficacy of such drugs is their incorporation into lipid-based systems (Tang et al., 2008).

The utility of lipid-based oral formulations in enhancing the bioavailability of hydrophobic and lipophilic drugs has been recognised for many years (Abdalla et al., 2008; Carrigan and Bates, 1973; Humberstone and Charman, 1997). Since the dissolution is the rate-limiting step in many cases, formulation design can be a useful approach to improve the oral bioavailability of this type of drug candidate (Pouton, 2006).

Two kinds of lipid system have been reported. One class is emulsified systems such as microemulsions (Constantinides, 1995), self emulsifying systems (SEDDS) (Gursoy and Benita, 2004; Kim et al., 2000) or nanoemulsions (Khandavilli and Panchagnula, 2007; Tiwari and Amiji, 2006), and the other class is lipid-based particulate delivery systems such as nanocapsules (Guterres et al., 1995; Nassar et al., 2009), lipid matrices (Savio et al., 1998), solid lipid nanoparticles (Luo et al., 2006; Muller et al., 2006) and nanostructured lipid carriers (Muchow et al., 2008; Yuan et al., 2007).

Among these systems, a new lipid carrier known as "beads", made of natural cyclodextrins (CD) and oil (Bochot et al., 2007) is openning up new prospects for oral delivery of lipophilic drugs. The bead composition is very rich in oil (Bochot et al., 2007), and as a result they are able to encapsulate lipophilic and fragile drugs such as retinoids (Trichard et al., 2007, 2008). Moreover, the bioavailability of isotretinoin was enhanced two-fold using this new delivery system compared with an oily solution (Trichard et al., 2007). However, complementary studies have to be carried out to understand better the behaviour of beads after their oral administration. The present work focuses on the preparation and characterization of Nile red and progesterone (PG)-loaded beads. PG was selected as a model of lipophilic drug. The behaviour of beads in simulated gastro-intestinal fluids (SGIF) was investigated in vitro to determine the mechanism involved in the release of PG. The influence of sodium taurocholate (Na-TC) on the stability of beads and PG release was studied.

Abbreviations: CD, cyclodextrin; FaSSIF, fasted state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; Na-TC, sodium taurocholate; PG, progesterone; SGF, simulated gastric fluid; SGIF, simulated gastro-intestinal fluids; SIF, simulated intestinal fluids.

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2. Materials and methods

2.1. Materials

Alpha-cyclodextrin (α-CD) (CAVAMAX® W6 Pharma) and soybean oil (Cropure®) were purchased from Wacker-Chimie (Lyon, France) and Croda (Trappes, France), respectively. Nile red, pepsin, progesterone (PG), sodium chloride (NaCl), sodium taurocholate (Na-TC) and 37% hydrochloric acid were supplied from Sigma Aldrich (Saint-Quentin-Fallavier, France). Lecithin EPC was obtained from Lipoid (Ludwigshafen, Germany). Sodium dihydrogen phosphate (NaH_2PO_4, $2H_2O$) was purchased from Fluka Chemika (Buchs, Switzerland). All the organic solvents used: methanol, tetrahydrofuran, acetonitrile and acetic acid, were purchased from Carlo Erba Reagents (Val de Reuil, France) and were of analytical grade.

Commercially available soft capsules containing 100 mg of PG were purchased from Ratiopharm® laboratory (Maisons-Alfort, France).

2.2. Preparation of Nile red-loaded beads

Nile red-loaded beads were prepared by adding 5.8 mL of an oily saturated solution of Nile red to 20 mL of an aqueous solution (8.1%, w/v) of α-CD. The preparation was continuously shaken at 200 rpm in a gyratory shaker at 28 °C until a monodisperse population of beads was obtained. The resulting beads were washed by removing the dispersion medium with a pipette and by replacing it by water which was then withdrawn. The beads were freeze-dried for 48 h to eliminate water (Christ LDC-1 alpha1-4 freeze-dryer, Bioblock Scientific).

2.3. Preparation of PG-loaded beads

2.3.1. Determination of PG solubility in soybean oil

PG was added in excess to 10 mL of soybean oil in sealed tubes that were stirred for 48 h at room temperature. The samples were then centrifuged twice at 10,000 rpm for 10 min (centrifuge, Jouan, France). Portions of the saturated oil solution of PG were diluted in THF/Methanol mixtures (v/v: 1/1) for assay by HPLC (Section 2.5.3). PG solubility was determined in triplicate.

2.3.2. Preparation of PG-loaded beads from an oily suspension of PG

PG was dispersed (140 mg/mL) in soybean oil. PG-loaded beads were prepared, washed and freeze-dried as described in Section 2.2.

2.4. Preparation of PG-loaded emulsions

PG-loaded emulsion was prepared by adding 5.8 mL of an oily suspension of PG (118 mg/mL) to 20 mL of an aqueous solution (8.1%, w/v) of α-CD. The preparation was continuously shaken as described in Section 2.2 except that the shaking was stopped after 4 h. At this time, the preparation corresponds to an o/w emulsion (Bochot et al., 2007) which does not contain any beads (Bochot et al., 2007, Trichard et al., 2011). The emulsion was freeze-dried for 48 h.

2.5. Characterization of beads after freeze-drying

2.5.1. Bead size

The diameter of beads was determined on a sample of 50 beads using an optical microscope (Leitz Diaplan microscope, Leica Microsystèmes, France) equipped with a Coolsnap ES camera (Roper Scientific). For each bead, two diameters were measured and an average of the two was calculated for more accuracy.

2.5.2. Bead yield

Bead yield was calculated using the following equation:

$$\text{bead yield}(\%) = \frac{\text{weight of freeze} - \text{dried beads}}{\text{weight } (\alpha\text{CD} + \text{oily phase})} \times 100$$

2.5.3. PG encapsulation efficiency and PG loading

Separation and quantification of PG were carried out by HPLC. The analytical column was an Interchim (modulo-cart QS Uptisphere 10 ODB 300 mm × 4.0 mm, Montluçons, France). The system was equipped with a mobile phase delivery pump (binary HPLC pump, Waters 1525, Milford, USA), an auto sampler (Waters model 717 plus, Milford, USA), an on-line degasser, a column oven set at room temperature and a tunable absorbance UV detector (Waters model 2487, Milford, USA). The mobile phase was a mixture of acetonitrile and water (60/40: v/v). The injection volume was set at 20 μL, the flow rate was 1 mL/min and the absorbance measurement was performed at λ = 241 nm. The PG peak was identified on the chromatograms at a retention time of 7.5 min. Calibration curves were drawn up for PG from 1 to 150 μg/mL in methanol.

PG was extracted from the beads and the emulsion by the following method: freeze-dried beads and emulsion were weighed precisely (50 mg) and 5 mL of tetrahydrofuran was added to destroy the bead structure. An equal volume of methanol (5 mL) was then mixed in at room temperature. 500 μL of the resulting solution were diluted (1/20) with the mobile phase and the drug content quantified by HPLC. For each batch of beads, the extraction was performed on two replicate samples.

PG encapsulation efficiency and PG loading were calculated using the following equations:

$$\text{PG encapsulation efficiency}(\%) = \frac{\text{amount of PG within beads}}{\text{amount of PG in soybean oil}} \times 100$$

$$\text{PG loading}(\text{mg of PG/g of beads}) = \frac{\text{amount of PG within beads}}{\text{weight of beads}}$$

Bead diameter, bead yield, PG encapsulation efficiency and PG loading were expressed as mean and standard deviation values ($n \geq 3$).

2.6. Stability study of Nile red-loaded beads in simulated gastro-intestinal fluids (SGIF)

2.6.1. Protocol

The stability of Nile red-loaded beads in SGIF was determined using the apparatus 2 (rotating paddle apparatus conforming to European Pharmacopeia. The paddle rotational speed was set at 55 rpm and the temperature of the medium was maintained at 37 ± 0.5 °C.

One hundred Nile red-loaded beads were introduced into 200 mL of simulated gastric fluid (SGF). After 55 min, 200 mL of pre-concentrated simulated intestinal fluids (SIF) were added. The initially pre-concentrated SIF was thus diluted two-fold to yield the correct concentration. Four different media were used to simulate the composition of gastro-intestinal fluids:

Simulated gastric fluid (SGF) containing: 2 g of sodium chloride, 3.2 g of pepsin (385 units/mg), 7 mL of hydrochloric acid (37% HCl to adjust pH to 1.2) and completed with water to 1 L.

Pre-concentrated simulated intestinal fluid free of Na-TC and lecithin (pre-concentrated control SIF) used as control and con-