International Journal of Pharmaceutics 416 (2011) 507–514

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

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

M. Hamoudi[a], E. Fattal[a], C. Gueutin[a], V. Nicolas[b], A. Bochot[a,*]

[a] Univ Paris-Sud, CNRS UMR 8612, Physico-chimie, Pharmacotechnie, Biopharmacie, IFR 141, Faculté de Pharmacie, 5, Rue Jean-Baptiste Clement, Châtenay-Malabry 92296, France
[b] Univ Paris-Sud, Plateforme Imagerie Cellulaire, IFR 141, Faculté de Pharmacie, Châtenay-Malabry, France

ARTICLE INFO

Article history:
Received 4 November 2010
Received in revised form 28 January 2011
Accepted 31 January 2011
Available online 16 February 2011

Keywords:
Beads
Cyclodextrins
Oil
Oral route
Progesterone
Stability

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.

* Corresponding author. Tel.: +33 146835579; fax: +33 146835946.
E-mail address: amelie.bochot@u-psud.fr (A. Bochot).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.01.062

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-

taining: 10.3 g of sodium dihydrogen phosphate, 12.4 g of sodium chloride, 35 mL of sodium hydroxide solution 1 N, 7 g of sodium bicarbonate (to adjust pH to 6.5 when mixed with SGF in a ratio of 50/50; v/v) and completed with water to 1 L.

Pre-concentrated fasted state simulated intestinal fluid (pre-concentrated FaSSIF) simulating the fasted state containing: 10.3 g of sodium dihydrogen phosphate, 12.4 g of sodium chloride, 35 mL of sodium hydroxide solution 1 N, 3.2 g of Na-TC, 1.2 g of lecithin, 6 g of sodium bicarbonate (to obtain, a pH of 6.5 when mixed with SGF in a ratio of 50/50; v/v) and completed with water to 1 L.

Pre-concentrated fed state simulated intestinal fluid (Pre-concentrated FeSSIF) simulating the fed state containing: 17.3 g of acetic acid; 23.7 g of sodium chloride, 35 mL of sodium hydroxide solution 1 N, 16.1 g of Na-TC (five-fold higher than in FaSSIF), 1.3 g of lecithin, 14 g of sodium carbonate (to adjust pH to 5 when mixed with SGF in a ratio of 50/50; v/v) and completed with water to 1 L.

To facilitate the understanding of the results, we use the terms control SIF, FaSSIF and FeSSIF for the SGF/pre-concentrated control SIF, SGF/pre-concentrated FaSSIF and SGF/pre-concentrated FeSSIF mixtures (50/50; v/v), respectively.

Beads were collected at different time intervals: 5, 15, 30 and 55 min in SGF and 60, 70, 85, 120, 150, 180, 240, 360, 480, 960 and 1440 min in control SIF; at 60, 70, 85, 120, 150 and 180 min of incubation in FaSSIF and at 60, 65 and 70 min of incubation in FeSSIF. One hundred Nile red-loaded beads were used for each time and each medium and all the experiments were carried out in triplicate.

2.6.2. Determination of the proportion of intact beads

For each time and each medium, the proportion of intact (non disintegrated) beads was determined. Results are expressed as the mean percentage of bead remaining intact in the medium.

$$\text{residual amount of beads}\,(\%) = \frac{\text{intact beads}}{100\,\text{beads}} \times 100$$

2.6.3. Determination of the diameter of beads

For each time and medium, the mean diameter of the intact beads was determined using the optical microscope as described previously in Section 2.5.1 and micrographs were taken to describe their shape.

2.6.4. Determination of the percentage of remaining volume of beads

For each time and medium the mean volume of the intact beads was determined. Results are expressed as the mean percentage of the remaining volume of intact beads in the medium.

$$\text{remaining volume of beads}\,(\%) = \left[1 - \frac{(\text{initial bead diameter} - \text{bead diameter at time } t)}{\text{initial bead diameter}}\right]^3 \times 100$$

2.6.5. Observation of the beads with a confocal laser scanning microscope (CLSM)

Nile red-loaded beads incubated in SGIF were observed with a LSM-510 META (Zeiss, Germany) confocal laser scanning microscope equipped with a 1 mW helium neon laser, using a Plan-neofluar 10× (NA 0.3/dry) and a Plan-Apochromat 20× (NA 0.75/dry) objective lens equipped with differential interferential contrast (Nomarski). Red fluorescence was collected with a long-pass 560-nm emission filter under illumination from a 543-nm laser. The pinhole diameter was set at 1.0 Airy Unit giving a 12.8 μm and a 2 μm optical slice thickness for 10× and 20× objective lens respectively.

2.7. Study of the PG release in SGIF

2.7.1. Determination of PG solubility in SGIF

PG solubility measurements were carried out in the SGIF described above. PG was added in excess to 10 mL of the SGF, control SIF, FaSSIF and FeSSIF in sealed tubes. Samples were then stirred at 37 °C for 24 h and centrifuged for 10 min at 10,000 rpm. The supernatant was filtered through a 0.45 μm Millex-HV filter (Millipore, France). A two-fold dilution of all the samples was made in methanol assay by HPLC (see Section 2.5.3). PG solubility was determined in triplicate in each SGIF.

2.7.2. Determination of PG partition coefficient between soybean oil and SGIF

The apparent soybean oil/SGIF partition coefficients of PG were determined by introducing 10 mL of the SGIF (SGF, control SIF, FaSSIF and FeSSIF) and 10 mL of soybean oil into a separating funnel. The funnel was shaken vigorously to allow the solutes to reach equilibrium. 9 mL of the saturated SGIF were collected into one vial while 9 mL of the saturated soybean oil were recovered into another. 100 mg of PG were introduced into the vial containing soybean oil and placed under magnetic stirring for 4 h. 8 mL of SGIF were introduced in the separating funnel and 8 mL of the oily solution of PG were gently deposited on the surface of SGIF. The funnel was shaken for 10 min and each phase was then collected separately (as described above). The PG distributed in SGIF was measured directly by HPLC without dilution. PG in soybean oil was extracted with THF/Methanol (v/v: 1/1) mixture, then diluted in the mobile phase (as described for PG extraction from beads prepared with the oily solution of PG) and finally assayed by HPLC. The determination of the partition coefficient of PG between soybean oil and SGIF was carried out in triplicate for each SGIF studied.

$$\text{partition coefficient of PG (soybean oil/SGIF), } P = \frac{\text{PG in oily phase (mg/mL)}}{\text{PG in SGIF (mg/mL)}}$$

2.7.3. Study of the PG release in SGIF

The study of the release of PG was performed in vitro in the SGIF, using the protocol reported in Section 2.5.1, on the following formulations: PG-loaded beads, PG-loaded emulsion and the lipid content of commercially available PG soft capsules composed of 100 mg of PG, 149 mg of peanut oil and 1 mg of soybean lecithin. A sample portion of each formulation corresponding to 10 mg of PG was introduced into 200 mL of SGF at 37 °C under stirring at a speed of 55 rpm. After 55 min, 200 mL of pre-concentrated SIF were added. For each formulation, two samples of 1.5 mL were withdrawn at different times of incubation over 24 h and were immediately filtered through Millex-HV 0.45 mm filters. An equal volume of fresh SGIF at 37 °C was then added to maintain a constant volume.All experiments were run in triplicate. The PG was assayed directly in the filtered samples, without dilution, by HPLC.

3. Results

3.1. Bead properties

Bead diameter (1.5 mm) and bead yield (83%) were similar for Nile red and PG-loaded beads (Table 1). However, the preparation of

Table 1
Properties of Nile red and PG-loaded beads ($n \geq 3$ batches; mean ± SD).

	Nile red-loaded beads	Beads prepared from an oily suspension of PG (140 mg/mL)
Bead preparation time (days)	3	9
Bead yield (%)	83 ± 1	82 ± 1
Bead diameter(mm)	1.50 ± 0.10	1.50 ± 0.10
PG encapsulation efficiency (%)	–	63 ± 2
PG loading (mg/g of beads)	–	90 ± 14

PG-loaded beads required a longer time than Nile red-loaded beads to achieve a monodisperse population of particles. Indeed, while only 3 days were necessary for the Nile red-loaded beads, 9 days were required for PG-loaded ones (Table 1). Quantification of PG in beads clearly showed that this drug was very efficiently encapsulated. The PG encapsulation efficiency and PG loading reached 63 ± 2% and 90 ± 14 mg/g of beads respectively (Table 1).

3.2. Stability of Nile red-loaded beads in SGIF

The incubation of Nile red-loaded beads in the SGF provoked a moderate reduction of their number (Fig. 1A) and a decrease of their volume (Fig. 1B) while preserving their shape (Fig. 2). Thus, 80% of the beads initially introduced in SGF were recovered after 55 min (Fig. 1A). At this time, the mean diameter of the beads was 1.19 ± 0.04 mm (Fig. 2) which corresponded to 50% of their initial volume (Fig. 1B). Whatever the medium (control SIF, FaSSIF or FeSSIF) introduced into the SGF after 55 min, the proportion of beads, their volume (Fig. 1A and B) and their diameter (Fig. 3) continued to decrease over time. However, the stability of the beads was affected differently by the different media: FeSSIF > FaSSIF > control SIF. The times of incubation in SGIF necessary to reduce the initial number of beads (100 beads) by half were 180, 120 and 60 min in control SIF, FaSSIF and FeSSIF (Fig. 1A) respectively. Moreover, the times required to get a loss of 85% of the initial volume of beads (which corresponded to a decrease of the initial diameter of beads by half) were 480, 150 and 60 min in control SIF, FaSSIF and FeSSIF respectively (Figs. 1B and 3). All beads were disintegrated after 24 h in control SIF (Fig. 1A) and only fragments in a range of 20–120 μm dispersed in oil were observed (Fig. 3M). In FaSSIF, the total disintegration of beads occurred in 180 min (Figs. 1A and 3J) and in 70 min in FeSSIF (Figs. 1A and 3F).

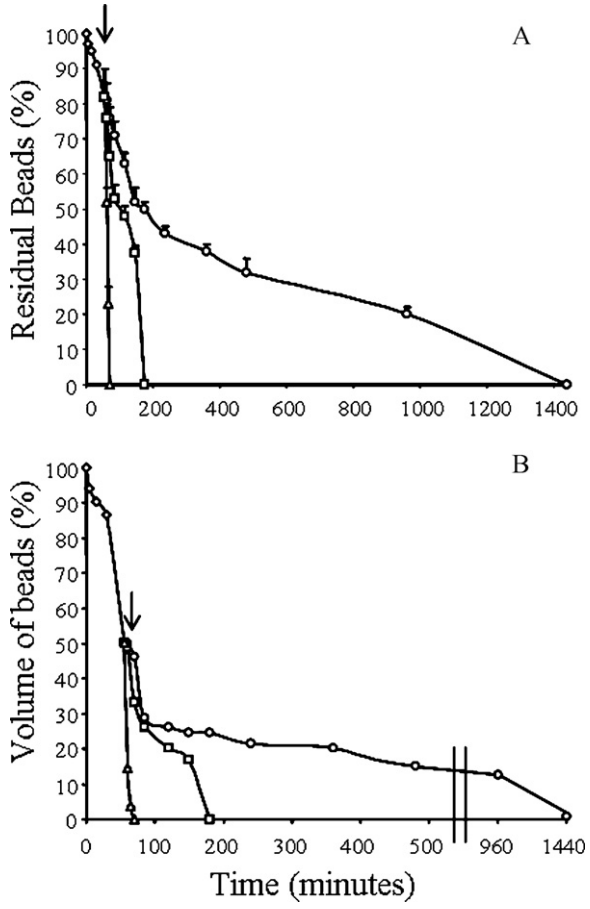

Fig. 1. Proportion (A) and volume (B) of intact Nile red-loaded beads remaining after incubation in different media: SGF (◊), control SIF (○), FaSSIF (□) and FeSSIF (△). Beads were incubated 55 min in SGF before addition of SIF.

Nile red-loaded beads observed by confocal microscopy 5 min after the addition of pre-concentrated FeSSIF to SGF (corresponding to a total incubation period of 60 min) showed that micro-droplets of oil were shed by the matrix (Fig. 4A). Moreover, numerous fragments of beads dispersed in oil were also seen (Fig. 4B). Similar observations were made in control SIF and FaSSIF. However, bead fragmentation and disintegration appeared more rapidly in FeSSIF than in FaSSIF and more slowly in control SIF (data not shown).

3.3. Study of PG release in SGIF

3.3.1. Evaluation of PG solubility in SGIF and PG partition coefficient (Log P) between soybean oil and SGIF

The solubility of PG (11 μg/mL) and the Log *P* value (3.7) were similar in SGF and control SIF (Fig. 5A). On the other hand, PG solubility was approximately 3 and 6 times higher in FaSSIF (Fig. 5B) and FeSSIF (Fig. 5C) respectively compared to that determined in SGF or in control SIF (Fig. 5A). Finally, the Log *P* value was lower in FeSSIF (Fig. 5C) than those measured in FaSSIF (Fig. 5B), SGF or control SIF (Fig. 5A). These results showed that Na-TC influenced the solubility of PG and its partition coefficient between soybean oil and SIF.

3.3.2. Study of PG release in SGIF

The study of the PG release in SGIF was performed with PG-loaded beads (90 mg/g), the PG-loaded emulsion (90 mg/g) and the lipid content (oily suspension) of commercially available PG soft capsules (soft capsules 400 mg/g). Only PG dissolved in SGIF was quantified.

In SGF, the emulsion allowed the dissolution of PG more rapidly and more importantly than from beads and soft capsules (Fig. 5A) to reach 16 ± 2%, 7 ± 1%, 5 ± 1%,respectively at time 55 min.

The nature of the medium added to SGF after 55 min strongly influenced the amount of PG dissolved (Fig. 5). Greater and faster effects were obtained in FeSSIF (Fig. 5C) and to a lesser extent in FaSSIF (Fig. 5B) compared to those seen in control SIF (Fig. 5A). In this medium, the percentages of PG dissolved increased slowly over time to reach values in a range of 12–32% at 24 h compared with 24–52% in FaSSIF and 40–79% in FeSSIF (Table 2).

More PG was dissolved from the emulsion up to 180 min in control SIF and up to 360 min in FaSSIF (Fig. 5A and B) compared to beads and soft capsules for which no real difference was observed (Fig. 5A and B). However, PG dissolved was around two-fold higher

Table 2
Percentages of PG dissolved from beads, emulsion and soft capsules, after 24 h of incubation in SGIF.

	Control SIF (%)	FaSSIF (%)	FeSSIF (%)
Beads	25 ± 5	40 ± 5	67 ± 7
Emulsion	32 ± 2	52 ± 2	79 ± 8
Soft capsules	12 ± 4	24 ± 1	40 ± 8

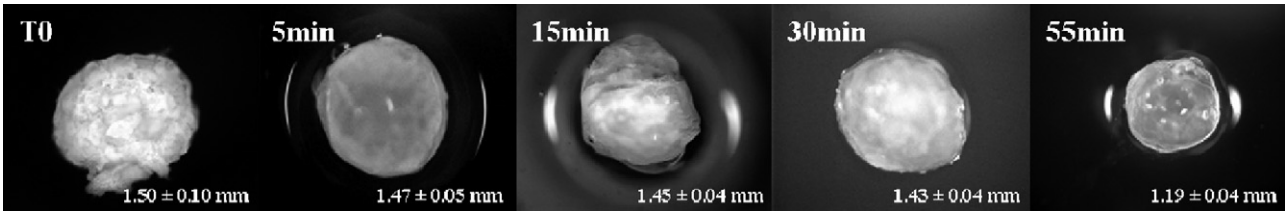

Fig. 2. Optical micrographs of Nile red-loaded beads incubated in SGF as a function of time of incubation. (⊢⊣ = 200 μm).

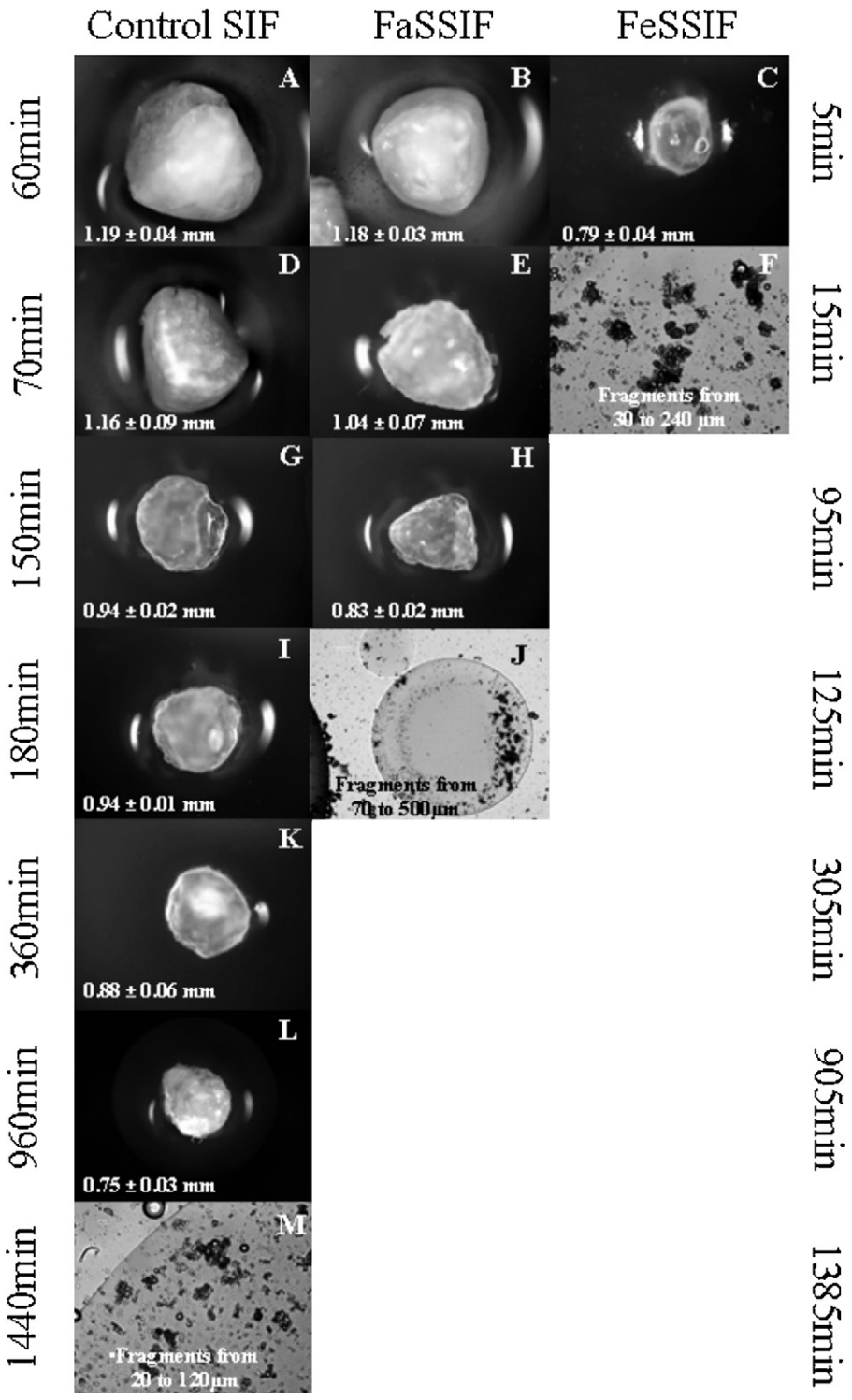

Fig. 3. Optical micrographs of Nile red-loaded beads incubated in control SIF (A, D, G, I, K, L and M), FaSSIF (B, E, H and J) and FeSSIF (C and F) as a function of total time of incubation. Beads were incubated 55 min in SGF before addition of SIF. (⊢⊣ = 200 μm).

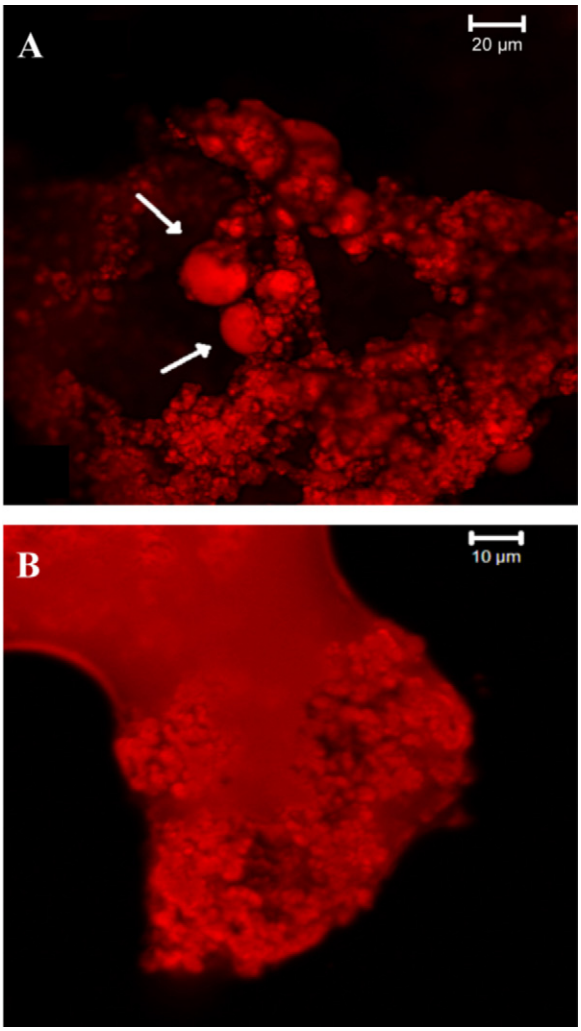

Fig. 4. Nile red-loaded beads observed by confocal microscopy after 60 min of incubation (55 min in SGF and 5 min in FeSSIF). Micro-droplets of oil released from the matrix (A) and fragments of beads dispersed in oil (B).

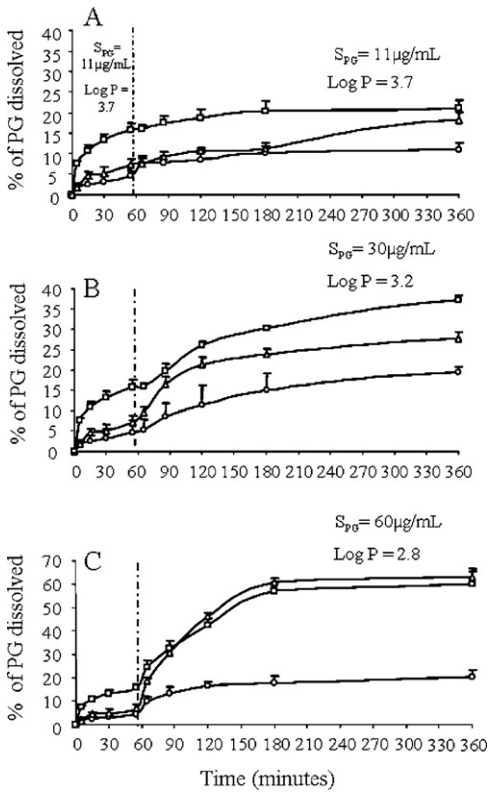

Fig. 5. Percentages of PG dissolved obtained from beads (△), emulsion (□) and from the lipid content of a soft capsule (○). Formulations were incubated 55 min in SGF before addition of control SIF (A), FaSSIF (B) and FeSSIF (C). PG solubility (S_{PG}) and the apparent partition coefficient for PG (Log $P_{(\text{soybean oil/SGIF})}$) in the different SGIF are given.

with beads and the emulsion than with soft capsules after 24 h in these media (Table 2).

In FeSSIF, (Fig. 5C) the proportion of PG dissolved from beads and the emulsion increased rapidly between 65 and 180 min to be similar at time 85 min, while the increase was more gradual with soft capsules. The emulsion and beads remained more efficient at releasing PG than the soft capsules (79 ± 8%, 67 ± 7% and 40 ± 8%, respectively) (Table 2). Compared with beads and the emulsion, the amount of dissolved PG from soft capsules was not strongly influenced by FeSSIF up to 180 min since similar values were found in FeSSIF, FaSSIF and control SIF (between 5 and 18%).

4. Discussion

Our results demonstrate that the addition of Nile red or PG, previously dissolved or dispersed in soybean oil, affected neither the yield nor the diameter of the beads compared with unloaded ones (Trichard et al., 2007). This result is different from those obtained by Trichard et al. (2007, 2008) with other lipophilic drugs such as isotretinoin and adapalene that caused an increase of the initial diameter of beads. Although isotretinoin (Munoz Botella et al., 1996) and progesterone (Uekama et al., 1982) do not form inclusion complexes with α-CD, the physicochemical properties of drugs may influence the interactions between CD and the triglycerides at the oil/water interface necessary for bead formation (Bochot et al., 2007). As previously observed with retinoids (Trichard et al., 2007, 2008), the time required to achieve a monodisperse population of particles was longer in presence of PG. Lipophilic molecules in oil may delay the crystallization of α-CD molecules which is required for the formation of the matrix of beads. PG loading is much higher than that obtained with retinoids (Trichard et al., 2007, 2008). Thus, due to their high oil content (80%, w/w) (Bochot et al., 2007) the applications for these beads may not be limited to the encapsulation of low-dose highly potent drugs. Although PG loading of 200 mg/g could be reached from a concentration of PG of 300 mg/mL in oil (data not shown), a decrease of bead yield was observed. However, compared to other particulate lipid-based systems such as self-emulsifying pellets of 1.2 ± 0.2 mm of diameter (Abdalla et al., 2008), beads are at least 16 times more efficient at entrapping PG. This confirms their potential for the encapsulation of lipophilic compounds.

The main aim of this work was to evaluate in vitro the stability of beads in SGIF and to elucidate the mechanisms involved in the release of a lipophilic drug.

The beads do not behave as a fast disintegrating system since most of them are not destroyed after 55 min in SGF. Freeze-dried beads for which the inner structure consists of a partial crystalline matrix of α-CD molecules surrounding micro-droplets of oil (Bochot et al., 2007), undergo a hydration step in contact with SGF (Fig. 6A) followed by a progressive dissolution of α-CD molecules from the surface (Fig. 6B). This explains both the decrease of the volume of beads and the preservation of their shape. The erosion of beads induces the release of micro-droplets of oil (Fig. 6C), as observed by confocal microscopy. PG is then released from the oil according to its partition coefficient value in the SGIF (Fig. 6C). The

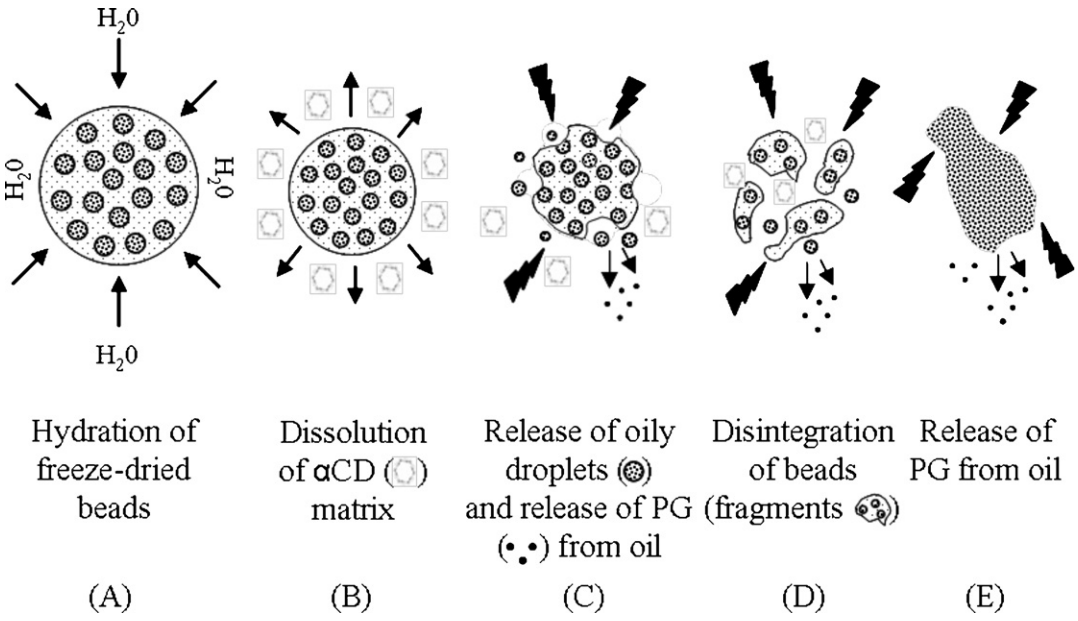

Fig. 6. Behaviour of beads incubated in SGIF. Na-TC ( ) acts intensely in steps C–E and reduces the stability of beads.

erosion also weakens the beads (Fig. 6C) as shown by the decrease of their number and the observation of fragments in the medium (Fig. 6.C). When the volume of beads reaches a critical threshold of around 12.5%, all the beads have disintegrated and only fragments are recovered (Fig. 6D). The fragments themselves act as a reservoir of oil containing PG as long as the α-CD matrix is not totally dissolved. Our results also show that Na-TC greatly reduces the stability of beads and increases the amount of PG dissolved. These effects are more pronounced at the high concentration of Na-TC in FeSSIF. The surfactant properties of Na-TC may facilitate the extraction of the oily droplets (Fig. 6C) and thereby accelerate the fragmentation of the beads (Fig. 6D) and then the release of PG from oil (Fig. 6E). Indeed, Na-TC in SIF decreases the partition coefficient of PG between soybean oil and SIF and improves its solubility, in particular in FeSSIF.

Both the emulsion and beads were prepared from the same components and with the same proportions. However, α-CD molecules in the emulsion formulation are not as well organized as in beads since X-ray diffraction peaks are more intense and narrower in beads (Bochot et al., 2007). It may explain why in SGF, release of PG from the emulsion was faster and two-fold higher than from beads. The release of PG from beads may be attributed first to the erosion of the matrix allowing the release of oily droplets and second to the affinity of PG for the SGIF. When beads have disintegrated they behave as the emulsion. In this study, the beads were also compared to the contents of the soft capsules which had a similar lipid composition (vegetable oil). Some differences between these two systems can explain why release of PG was faster and higher from beads than from soft capsules in FeSSIF and to a lesser extent in FaSSIF. Since beads are a multiparticulate system, the total surface exposed to SGIF is larger than that of the soft capsules. Moreover, beads release micro-droplets of oil which present a large exchange area with the SGIF as described for other dispersed formulations such as microemulsions (Gursoy and Benita, 2004; Tenjarla, 1999), self-emulsifying pellets (Abdalla et al., 2008), and solid self-emulsifying drug delivery systems (Tang et al., 2008). In SGIF, the lipid content of the soft capsule is released in an agglomerated form which floats on the surface of the medium, even in the presence of Na-TC. Thus, only small amounts of PG can be dissolved. The behaviour of beads in SGIF allows to understand better why isotretinoin-loaded beads were able to efficiently increase the oral bioavailability of this drug in rats compared with the lipid content of a commercial soft capsule (Trichard et al., 2007).

Since beads are a multiparticulate system with an original structure, they constitute an interesting lipid carrier potentially able to improve the oral bioavailability of lipophilic compounds. In such a system, the release of drugs results from the erosion of the α-CD matrix and its partition equilibrium between oil dispersed as micro-droplets and aqueous phase. In vivo studies are now in progress to compare the behaviour of drug-loaded beads in the fasted and fed states.

References

Abdalla, A., Klein, S., Mader, K., 2008. A new self-emulsifying drug delivery system (SEDDS) for poorly soluble drugs: characterization, dissolution, in vitro digestion and incorporation into solid pellets. Eur. J. Pharm. Sci. 35, 457–464.

Bochot, A., Trichard, L., Le Bas, G., Alphandary, H., Grossiord, J.L., Duchene, D., Fattal, E., 2007. Alpha-cyclodextrin/oil beads: an innovative self-assembling system. Int. J. Pharm. 339, 121–129.

Carrigan, P.J., Bates, T.R., 1973. Biopharmaceutics of drugs administered in lipid-containing dosage forms I. GI absorption of griseofulvin from an oil-in-water emulsion in the rat. J. Pharm. Sci. 62, 1476–1479.

Constantinides, P.P., 1995. Lipid microemulsions for improving drug dissolution and oral absorption—physical and biopharmaceutical aspects. Pharm. Res. 12, 1561–1572.

Gursoy, R.N., Benita, S., 2004. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacother. 58, 173–182.

Guterres, S.S., Fessi, H., Barratt, G., Puisieux, F., Devissaguet, J.P., 1995. Poly (DL-lactide) nanocapsules containing nonsteroidal antiinflammatory drugs—gastrointestinal tolerance following intravenous and oral-administration. Pharm. Res. 12, 1545–1547.

Humberstone, A.J., Charman, W.N., 1997. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv. Drug Deliv. Rev. 25, 103–128.

Khandavilli, S., Panchagnula, R., 2007. Nanoemulsions as versatile formulations for paclitaxel delivery: peroral and dermal delivery studies in rats. J. Invest. Dermatol. 127, 154–162.

Kim, H.J., Yoon, K.A., Hahn, M., Park, E.S., Chi, S.C., 2000. Preparation and in vitro evaluation of self-microemulsifying drug delivery systems containing idebenone. Drug Dev. Ind. Pharm. 26, 523–529.

Luo, Y., Chen, D.W., Ren, L.X., Zhao, X.L., Qin, J., 2006. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J. Control. Release 114, 53–59.

Muchow, M., Maincent, P., Muller, R.H., 2008. Lipid nanoparticles with a solid matrix (SLN, NLC LDC) for oral drug delivery. Drug Dev. Ind. Pharm. 34, 1394–1405.

Muller, R.H., Runge, S., Ravelli, V., Mehnert, W., Thunemann, A.F., Souto, E.B., 2006. Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN) versus drug nanocrystals. Int. J. Pharm. 317, 82–89.

Munoz Botella, S., Martin, M.A., del Castillo, B., Menendez, J.C., Vazquez, L., Lerner, D.A., 1996. Analytical applications of retinoid-cyclodextrin inclusion complexes 1. Characterization of a retinal-beta-cyclodextrin complex. J. Pharm. Biomed. Anal. 14, 909–915.

Nassar, T., Rom, A., Nyska, A., Benita, S., 2009. Novel double coated nanocapsules for intestinal delivery and enhanced oral bioavailability of tacrolimus, a P-gp substrate drug. J. Control. Release 133, 77–84.

Pouton, C.W., 2006. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur. J. Pharm. Sci. 29, 278–287.

Savio, E., Dominguez, L., Malanga, A., Quevedo, D., Saldana, J., Camarote, C., Ochoa, A., Fagiolino, P., 1998. Lipidic matrix of albendazole: an alternative for systemic infections. Boll. Chim. Farm. 137, 345–349.

Tang, B., Cheng, G., Gu, J.C., Xu, C.H., 2008. Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. Drug Discov. Today 13, 606–612.

Tenjarla, S., 1999. Microemulsions: an overview and pharmaceutical applications. Crit. Rev. Ther. Drug Carrier Syst. 16, 461–521.

Tiwari, S.B., Amiji, M.M., 2006. Improved oral delivery of paclitaxel following administration in nanoemulsion formulations. J. Nanosci. Nanotechnol. 6, 3215–3221.

Trichard, L., Fattal, E., Besnard, M., Bochot, A., 2007. Alpha-cyclodextrin/oil beads as a new carrier for improving the oral bioavailability of lipophilic drugs. J. Control. Release 122, 47–53.

Trichard, L., Delgado-Charro, M.B., Guy, R.H., Fattal, E., Bochot, A., 2008. Novel beads made of alpha-cyclodextrin and oil for topical delivery of a lipophilic drug. Pharm. Res. 25, 435–440.

Trichard, L., Chaminade, P., Grossiord, J.L., Le Bas, G., Huang, N., Durand, D., Fattal, E., Bochot, A., Beads made of alpha-cyclodextrin and vegetable oils: oil composition and physicochemical properties influence bead feasibility and properties. JDDST (Journal of Drug Delivery Science and Technology). N°2, vol 21, 2011 In press.

Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M., 1982. Inclusion complexations of steroid hormones with cyclodextrins in water and in solid phase. Int. J. Pharm. 10, 1–15.

Yuan, H., Wang, L.L., Du, Y.Z., You, J., Hu, F.Q., Zeng, S., 2007. Preparation and characteristics of nanostructured lipid carriers for control-releasing progesterone by melt-emulsification. Colloids Surf B Biointerfaces 60, 174–179.