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Research Paper

Performance of magnetic chitosan–alginate core–shell beads for increasing the bioavailability of a low permeable drug

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ABSTRACT

This work reports the synthesis and performance of magnetic chitosan–alginate core–shell beads for oral administration of small molecules in order to increase their bioavailability. For this purpose, we designed magnetic core–shell beads suitable for oral delivery that are resistant in acidic media (stomach pH), mucoadhesive, exhibit a superparamagnetic behavior and a very high entrapment efficiency. *Ex vivo* experiments were performed in Ussing chambers, to emphasize the effect of magnetic accumulation. The amount of drug permeated through the membrane exhibited a threefold increase with our novel drug delivery system. According to a correlation law, our *ex vivo* model showed that the adsorbed fraction (FA) in human is expected to reach 70% when using the magnetic retention system which is a great improvement when compared to the controls (FA = 20%).

1. Introduction

Oral administration still remains the route of choice for the majority of pharmaceutical dosage forms because of better patient comfort and reduced cost of treatment. The Food and Drug Administration (FDA) has introduced the Biopharmaceutical Classification System to categorize drugs in terms of their solubility and intestinal permeability [1]. Low permeable drug class exhibits poor absorption *in vivo*. This means that their concentration in the bloodstream is very low and, as a consequence, most of them have a poor bioavailability. Many common drugs such as antivirals (acyclovir), ant diabetics (metformin) or GABA analogs (gabapentin, baclofen) belong to this class [2]. Therefore there is a real need to develop new methodologies in order to address the problem of low permeable drugs for oral administration [3,4]. Most of these drugs have an absorption window located in the upper small intestine. However, passage through this region is rapid due to the gastrointestinal transit thus limiting the extent of absorption at this site [5]. As a result, efforts have been recently made to prolong the gastric residence-time of drugs by means of bioadhesives, size increasing and floating delivery systems [5–9]. These methodologies improving drug's bioavailability are based on retention of the formulations containing the drug in the stomach. This leads to an increased residence time on the stomach mucosa for mucoadhesive systems, over the stomach content for floating systems and at the pylorus junction with the small intestine for size increasing systems.

An alternative approach utilizes magnetic field to maintain the delivery system near their targeted zone [10–12]. Such a method has been used *in vivo* for the localization of a magnet containing pill in the rat's small intestine [13,14]. This system has also been used for monitoring the gastrointestinal transit of the pill [15,16]. Other experiments have employed magnetic polymeric microparticles to improve drug absorption. It was demonstrated that such improvements were the result of increased residence time and not increased absorption [17,18]. In fact, the drug is able to reach its intestinal absorption site over a longer period of time. The absorption of the drug through the membrane is not improved but occurs for a longer period of time and as a consequence the total amount of drug transported increases.

In this work we decided to use magnetic retention in order to increase the bioavailability of a low permeable drug across the intestinal membrane. For this purpose we designed magnetic core–shell beads suitable for oral delivery because they are both resistant in acidic media (stomach pH), mucoadhesive and exhibit a very high drug loading efficiency. We proved that an over-concentration of drug can be obtained near the intestinal membrane (in the upper part of the intestine called the jejunum) which is located near the absorption window of the drug by using a magnetic field. Using our methodology, the increase in bioavailability

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will be due to an increase in drug concentration (over concentration) and not to an increase in the residence time. The model drug we studied is an anti-diabetic molecule with no application up to date due to its low bioavailability. *Ex vivo* experiments showed that magnetic retention provided a threefold increase in drug apparent permeability when compared with free drugs and other controls. It was proven that this system could increase drug absorption without any alteration of the membrane. Furthermore according to a correlation law the predictive adsorbed fraction of the drug in human is expected to be strongly improved and to reach a very high value compared to the ones found in the literature using other retentive systems [19]. This opens new perspectives for a large number of drugs that had not yet been employed despite their high clinical potential because of their limited bloodstream access.

2. Materials and methods

2.1. Materials

All chemical reagents were purchased from Sigma–Aldrich, France. The model drug used in this study was synthesized and kindly provided by Technologie Servier. Physicochemical properties are as follows: Mw = 769 g/mol; p*Ka* = 7.4 and 3.5; partition coefficient: Log*P* = −1.11, Log*D* at pH 7.4 = −1.4; Solubility in phosphate buffer at pH 6 = 12 mg/mL at 25 °C. Parallelepiped neodymium magnet (NdFeB, 30 × 30 × 15 mm) was purchase from Supermagnete®, Germany.

2.2. Synthesis of magnetic core–shell beads

2.2.1. Synthesis of magnetic nanoparticles (MNPs)

The superparamagnetic particles were synthesized according to Massart's procedure [20]. Magnetite (Fe_3O_4) nanocrystals were prepared by alkaline coprecipitation of $FeCl_3$ (1.5 mol) and $FeCl_2 \cdot 4H_2O$ (0.9 mol) salts in alkaline solution (NH_4OH, 7 mol). In order to be completely oxidized from magnetite into maghemite (γ-Fe_2O_3), the solid phase was separated from the supernatant and immersed in a boiling solution of ferric nitrate (0.8 mol in 0.8 L, 30 min at 90–100 °C). After washing steps in acetone and diethyl-ether to remove the excess ions, the nanoparticles readily dispersed in water and formed a true "ionic ferrofluid" made of maghemite nanoparticles. The iron oxide surface bore positive charges due to adsorption of protons in acidic media, in that case a dilute HNO_3 solution at pH between 1.2 and 1.7. On the microscopic scale, this colloidal suspension of maghemite nanoparticles exhibits a Log–Normal distribution of diameters with parameters d_0 = 7.5 nm and σ = 0.35, as measured by vibrating sample magnetometry (VSM). The final iron content was checked by flame spectrometry (C_{Fe} = 1.1 mol/L). The colloidal suspension was ready to use and stable at room temperature for years.

2.2.2. Preparation of magnetic chitosan–alginate core–shell beads

Magnetic chitosan–alginate core–shell beads containing a model drug were prepared using a standard extrusion crosslinking method [21–26]. Chitosan solution (5% w/v) was prepared by dissolving 250 mg of chitosan in 5 mL deionized water containing 5% acetic acid (AA) (v/v) and 10% magnetic nanoparticles (MNPs) in water (v/v) under magnetic stirring. 250 mg of the drug (5% w/v) was then added and the mixture was allowed to stir for 30 min. The resulting solution was added dropwise in a gently stirred water bath (10 mL) containing 200 mg of sodium tripolyphosphate (TPP) (2% w/v) and 100 mg of alginate (AL) (1% w/v) by extrusion through a syringe needle (inner diameter of 0.45 mm). Size of the beads is controlled by the diameter of the syringe needle (0.45 G) and the flow rate (10 mL/min) of the syringe pump inside which is inserted the syringe containing the chitosan mixture. An optimal height of 30 cm was used to obtain spherical beads. The beads were then allowed to crosslink during 30 min under gentle magnetic stirring, collected by magnetic decantation and rinsed in deionized water. Beads were kept at 5 °C. The reticulation bath and washing water were kept for further analysis of the loading efficiency. Control beads without magnetic nanoparticles were synthesized using the same procedure except that the volume of MNPs was replaced by water. A mixture of chitosan 5% w/v and drug 5% w/v in water containing AA 5% v/v was added dropwise to a reticulation bath of TPP 2% and AL 1%. The other biopolymer magnetic beads were synthesized in the same way, using an extrusion crosslinking process and containing the same amount of magnetic nanoparticles and drug. For naked chitosan beads, the same mixture of chitosan 5% w/v, MNPs 10% v/v and drug 5% w/v in water containing AA 5% v/v was added dropwise to a reticulation bath of TPP 2% without alginate. For alginate naked beads, alginate mixture 2.5% w/v, MNPs 10% v/v and drug 5% w/v in water was added dropwise to a reticulation bath of calcium chloride ($CaCl_2$) 5% w/v. For alginate-chitosan core–shell beads, alginate mixture 2.5% w/v, MNPs 10% v/v and drug 5% w/v in water was added dropwise to a reticulation bath of calcium chloride ($CaCl_2$) 5% w/v and chitosan (1% w/v).

2.3. Characterization of the beads

2.3.1. Cell preparation for SEM analysis

Samples were dehydrated through graded concentration of ethanol (30–50–70–96–100%) before critical point drying (CPD 7501, Quorum Technologies). Samples were directly mounted on stubs, or manually freeze-fractured after a fast plunging in liquid nitrogen and then gold-sputtered (Scancoat Six, Edwards). Observation was carried out with a conventional SEM operating at 15 kV (Cambridge Stereoscan S260).

2.3.2. HPLC sample analysis

Concentrations of the drug were analyzed by HPLC (Waters, alliance) at a wavelength of 240 nm. The stationary phase was a Zorbax Eclipse XBD Phenyl, 150 × 4.6 mm, 5 μm column and the mobile phase was constituted by a mixture aqueous phase/acetonitrile 70/30 (v/v). The aqueous phase was prepared with ultra-pure water implemented with triethylamine (0.2% v/v) and equilibrated at pH 3.5 using phosphoric acid.

2.3.3. Drug content measurements

Drug entrapment efficiency in the beads has been measured by an indirect titration method. Magnetic beads were recovered by magnetic decantation. The supernatant was kept for analysis. Amount of free drug that has not been able to be encapsulated into the beads was measured in the crosslinking supernatant by HPLC using the standard HPLC titration method for the drug. Entrapment efficiency could then be calculated with the following equation [1],

$$\text{EE}\ (\%) = \frac{\text{Total drug amount} - \text{Amount of free drug in the supernatant}}{\text{Total drug amount}} \quad (1)$$

2.3.4. In vitro release study

To perform the *in vitro* release study, 10 beads were placed in 3 mL of Krebs Ringer media at pH 6.8 prepared by dissolution of the buffer powder in 1 L of distilled water at a controlled temperature of 37 °C in sink conditions (volume was 9× higher than the saturation volume). Concentration of the drug was measured for 2 h. At a predetermined 15 min time interval 750 μL of the media content was withdrawn and the concentration of the drug was determined using HPLC. 750 μL of fresh medium solution was