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ORIGINAL ARTICLE

Investigation of coated whey protein/alginate beads as sustained release dosage form in simulated gastrointestinal environment

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Abstract

Aim: The biopharmaceutical behavior of new formulations based on both food-grade polymers, whey protein (WP) and alginate (ALG) was studied using different in vitro methods. The Biopharmaceutical Classification System (BCS) class I drug Theophylline was chosen as drug model. *Method*: Drug release was studied (i) at pH 1.2 (2 hours) followed by pH 7.5, and in simulated gastric fluid (SGF; 2 hours) followed by simulated intestinal fluid (SIF) using the paddle method and (ii) in an artificial digestive system. *Results*: Freeze-dried mixed WP/ALG (62/38) beads were coated with WP or ALG with encapsulation efficiency 34.9% and 18.3%, respectively. At pH 1.2, coated beads exhibited gastroresistant properties (< 10% of drug released after 2 hours) followed at pH 7.5 by a sustained release behavior (< 60% of drug released at 24 hours) controlled by an erosion mechanism. In SGF, despite enzyme hydrolysis, drug release was still controlled due to ALG shrinkage. After transfer in SIF, formulations were completely degraded in less than 2 h with total drug release. In an artificial digestive system, coated beads appeared gastroresistant, intestinal part sustained drug release was controlled by erosion. *Conclusion*: Combination of in vitro methods allowed prediction of the in vivo potentialities of WP- and ALG- coated WP/ALG beads as oral sustained release systems.

Key words: *Alginate; beads; controlled release; in vitro gastrointestinal conditions; whey proteins*

Introduction

In recent years, development of natural polymer-based beads as oral controlled delivery systems for pharmaceuticals[1–3], nutrients[4], or others bioactive agents[5–7] has been extensively reported. The physiochemical properties of biopolymers ensure designing safe and highly functional delivery systems. One emergent natural polymer consists of whey proteins (WPs), well-known in food industry because of their high nutritional value and their ability to form foam, emulsion, and gel[8]. Numerous studies have explored and established the WP potentiality as encapsulating material[4,9–16]. However, in the case of encapsulation of active material such as drugs, WP beads rapidly release the active material in simulated gastric and intestinal media leading to nonoptimal biopharmaceutical behavior[2]. The association of polymers in the same matrix was recently considered to improve the biopharmaceutical properties of beads[17]. The most popular combination was protein/polysaccharide because interactions between both these polymers create systems with novel functionalities[18]. WP has been already associated with two polysaccharides, arabic gum[19] and alginate[20]. The

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association between WPs and alginate is interesting because both polymers exhibited antagonist behavior at acidic pH, WPs being instable and alginate stable[20]. Nevertheless, the high viscosity of protein solutions did not allow until now the production of WP-based beads with an industriable method. Thus, we have developed a formulation composed of WP and alginate (ALG) associated in the ratio 62/38 to be compatible with the viscosity requirement of a large-scale production method, the Encapsulator® (Inotech, Dottikon, Switzerland).

To influence the biopharmaceutical behavior of drug dosage, the addition of coating is also frequently considered to limit or control drug release and to achieve modified and delayed release systems. Coated WP beads have been already investigated using chitosan or alginate as coating material, but the coating protocols were not compatible with all drugs or biological agents because they required heat treatment (85°C, 20 minutes) or glutaraldehyde cross-linking[2].

Our strategy has been to develop, for the first time, multiparticulate sustained release drug dosage forms based on WP obtained from polymeric solutions compatible with the Encapsulator® requirement in terms of viscosity (viscosity has to be below 200 mPas) and further coated by organic solvent and toxic cross-linking agent free methods. Thus, mixed WP/ALG beads were prepared by extrusion/cold gelation method and coated with WP or ALG coating using a simple and nontoxic coating process to control drug release. Theophylline (TPH) was chosen as a drug model because as all highly soluble and highly permeable drug (Biopharmaceutical Classificaion System class I drug), the limiting factor for drug release is the dosage form.

The aim of this study was to evaluate the biopharmaceutical behavior of TPH-loaded mixed WP/ALG beads coated with WP or ALG to determine the contribution of digestive parameters such as pH or enzymes on drug release and to estimate the potentiality of these formulations to sustain and control drug release. This study was performed using different in vitro methods such as classical in vitro dissolution tests, described in Pharmacopoeia, and a multicompartmental, dynamic artificial digestive system already used to predict the in vivo behavior of immediate and sustained dosage forms[21,22].

Materials and methods

Materials

WP (Alacen® 845) was provided by NZMP (Wellington, New Zealand). WP content was 93% (dry matter basis) as determined by Kjeldahl method (N X 6.38). The sodium alginate (Manucol DH) was provided from ISP (Wayne, NJ, USA). Pepsin 1:10,000 (from porcine gastric mucosa), pancreatin (from porcine pancreas), bile salts (from porcine extract), and calcium chloride dihydrate were obtained from Sigma-Aldrich (Saint-Louis, MO, USA). Lipase was purchased from Amano Pharmaceuticals. TPH was supplied from Pierre Fabre Medicament Laboratory (Labège, France).

Preparation of stock solutions

Protein solutions (10.0% and 11.0%, w/w) were prepared as follows: WP isolate powder was rehydrated in deionized water by gentle magnetic stirring for 1 hour at room temperature and then allowed to rest for 2 hours to ensure complete hydration of proteins. WP solutions were adjusted at pH 7.0 with NaOH, heated and maintained at 80°C for 40 minutes to denature proteins completely. The solutions were cooled overnight at room temperature. Sodium alginate solutions (1.5% and 3.0%, w/w) were prepared in deionized water and stirred gently overnight at room temperature.

Microparticle and coating preparation

Pure WP (11%, w/w) and pure ALG (1.5%, w/w) solutions were combined to prepare a solution with a 62/38 WP/ALG ratio to get the highest WP content but a viscosity below 200 mPas (e.g., Encapsulator requirement). TPH was added at 0.6% (w/w). TPH-loaded WP/ALG beads were obtained by extrusion/cold gelation technique using a needle (Terumo 25 G)/pump system as previously described[15]. Drops obtained by extrusion through the needle were collected in a gelling bath (0.1 M $CaCl_2$) saturated with TPH. The beads were maintained in the hardening solution for 5 minutes.

Loaded WP/ALG beads, collected by sieving, were further coated with WP or ALG by immersion. For WP coating, WP/ALG beads harvested from $CaCl_2$ solution were stirred for 10 minutes in denatured WP solution (10.0%, w/w) and then transferred to 0.1 M $CaCl_2$ solution for 5 minutes. For ALG coating, harvested WP/ALG beads were rinsed with deionized water immediately after production to eliminate excess Ca^{2+} ions, suspended in deionized water, and an equal volume of ALG solution (3.0%, w/w) was stirred in. Beads were stirred for 10 minutes and then transferred to 0.1 M $CaCl_2$ solution for 5 minutes. All coated beads were collected, rinsed with deionized water, and frozen at –80°C before freeze-drying in bottle. Freeze-drying was performed for 48 hours in a standard freeze-dryer as followed: beads were placed in pressure chamber (pressure less than 3 Pa) maintained at –34°C during 1 hour and progressively heated to 10°C during 24 hours. Then,

during the last 24 hours, temperature increased and was maintained at 25°C. Bottles were sealed directly in the pressure chamber.

Theophylline loading determination

Uncoated WP/ALG beads (300 mg) were accurately weighted and incubated in 20 mL of simulated gastric fluid (SIF, pH 7.5, pancreatin 1.0%, w/v, USP30). After complete microparticle degradation, samples were withdrawn, centrifuged (9000 rpm, 5 minutes), and TPH was measured by UV spectrophotometer at 273 nm (no interaction with other components of medium). For coated WP/ALG beads, the 300-mg sample refers to the uncoated mass. Each experiment was realized in triplicate. Encapsulation efficiency (EE) was calculated as follows:

$$\mathrm{EE} = \frac{\text{TPH amount in beads}}{\text{TPH amount in polymer solution extruded}} \times 100. \quad (1)$$

Microparticle size and morphology

Sizes of uncoated and coated WP/ALG beads were evaluated by taking photography using a digital camera (Olympus optical, Tokyo, Japan). Diameters of 60 beads were measured in triplicate by image analysis.

Classical in vitro release studies

Uncoated and coated WP/ALG beads (300 mg) were investigated using a USP2 apparatus (700 mL, 37°C, 50 rpm, $n = 3$). To simulate the gastrointestinal environment, beads were treated in a first experiment in pH 1.2 buffer (USP30) for 2 hours followed by pH 7.5 buffer (USP30) and in a second experiment in simulated gastric fluid (SGF, pH 1.2, pepsin 0.1%, v/w, USP30) for 2 hours followed by SIF. At predetermined time intervals, samples were withdrawn and the drug release was determined by UV spectrophotometer at 273 nm. Results of drug release were expressed in percentage of TPH encapsulated in each formulation.

Artificial digestive studies

The artificial digestive system (TIM1; TNO, Zeist, the Netherlands) consists of four serial compartments simulating the stomach and the three segments of the small intestine: duodenum, jejunum, and ileum[23]. Each compartment is composed of glass jacket with flexible wall between which heated water flows to control both the temperature inside compartment (37°C) and the pressure on flexible walls inducing the mixing of the chyme by alternate compression and relaxation (Figure 1). To control the transit of the chyme, a power formula ($f = 1 - 2^{-\beta(t/t_{1/2})}$, where f represents the fraction of chyme delivered, t the time of delivery, $t_{1/2}$ the half-time of delivery, and β a coefficient describing the shape of the curve) is used for gastric and ileal delivery. Chyme transit is then regulated by opening or closing the peristaltic valves that connect the compartments. The volume in each compartment is monitored by a pressure sensor connected to the computer. The pH is computer-monitored and continuously controlled by secreting either water or 0.3 M HCl (0.25 mL/min) into the stomach compartment and either electrolytes or 0.5 M $NaHCO_3$ (0.25 mL/min) into the three small intestine compartments. Simulated gastric (0.5 mL/min), biliary (0.5 mL/min), and pancreatic (0.25 mL/min) secretions (pepsin, lipase, bile salts, and pancreatin) are delivered into the corresponding compartment by computer-controlled pumps. All parameters of the system are adjusted to simulate the conditions found in the gastrointestinal tract of a healthy adult in the fasted state. The model is equipped with hollow fiber membranes (DICEA 90G, Baxter, Maurepas, France) connected to the jejunal or ileal compartments. Water and small molecules including the drug studied are removed from the lumen of the compartments by pumping dialysis fluid (10 mL/min) through the hollow fibers. About 100 mg of TPH encapsulated in freeze-dried WP- and ALG-coated beads were introduced into the gastric compartment simultaneously with 200 g of water to simulate the fasted state in an adult. During digestion, samples were collected directly in the stomach during the first 45 minutes and dialysis fluids were collected at 0.5 hours time intervals up to 4 hours. Dialyzed volumes were measured and TPH was detected by UV HPLC at 273 nm using the following protocol: 50 µL of sample were injected and analyzed on a Lichrospher RP 18 (5 µm) column by elution with mobile phase composed of 9% acetonitrile in sodium acetate buffer 0.01 N (pH 4) set up to a flow rate of 2 mL/min. The experiments of digestion were performed in triplicate for each formulation.

Kinetic release model

Drug releases from WP- and ALG-coated beads, investigated using in vitro dissolution testing and artificial digestive system, were fitted to equation proposed by Harland et al.[24] allowing modelization of the release mechanisms of beads.

$$\frac{M_t}{M_\infty} = A\sqrt{t} + Bt. \quad (2)$$

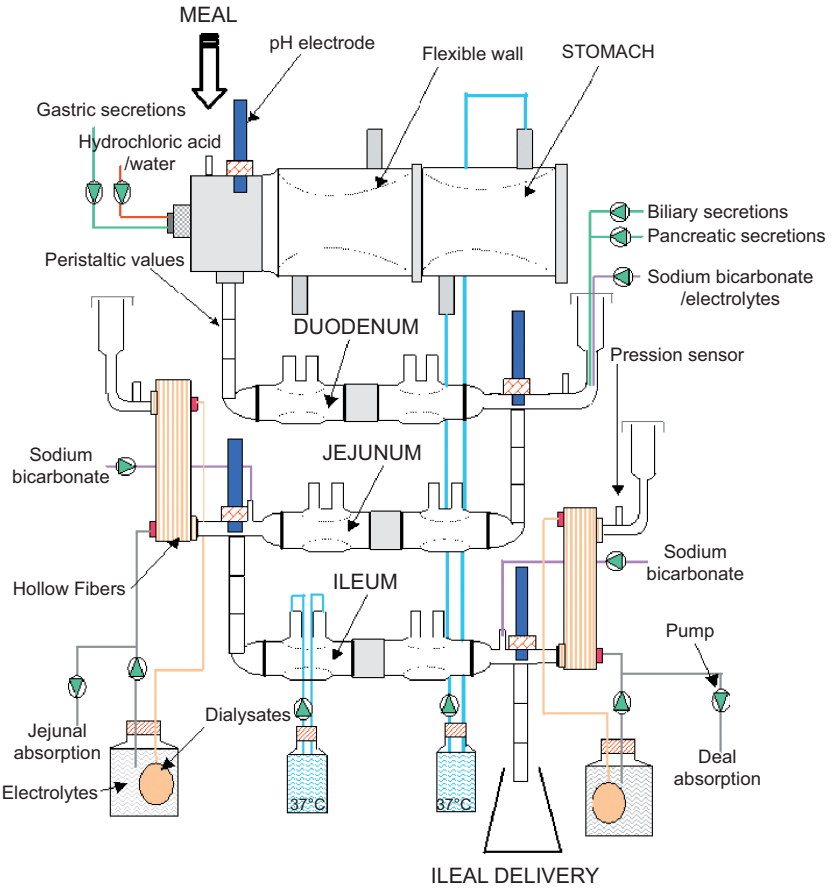

Figure 1. A multicompartmental, dynamic, computer-controlled gastrointestinal tract model consisting of a stomach compartment and the three segments of small intestine (duodenum, jejunum, and ileum) reproducing the in vivo digestive conditions of humans (adapted from Ref. 23).

In the above equations, M_t/M_∞ is the fraction of drug released at time t, A and B are diffusion and erosion terms. When $A > B$, the diffusion factor prevails in the release system, whereas when $A < B$, erosion predominates. If $A = B$, the release mechanism includes both diffusion and erosion equally.

Results

Size and drug loading

Spherical-mixed WP/ALG beads were obtained using standardized and reproducible conditions, and they were successfully coated with WP or ALG by a simple immersion process, providing spherical and individually coated beads. Freeze-drying preserved the spherical shape and the individual state of coated and uncoated beads (Figure 2). After freeze-drying, uncoated WP/ALG beads had a mean diameter of approximately 1559 µm and displayed a homogeneous size (variation coefficient 2.3%). WP- and ALG-coated WP/ALG beads were significantly higher with mean diameters of 1735 and 1757 µm, respectively, and a variation coefficient of less than 7%. The WP

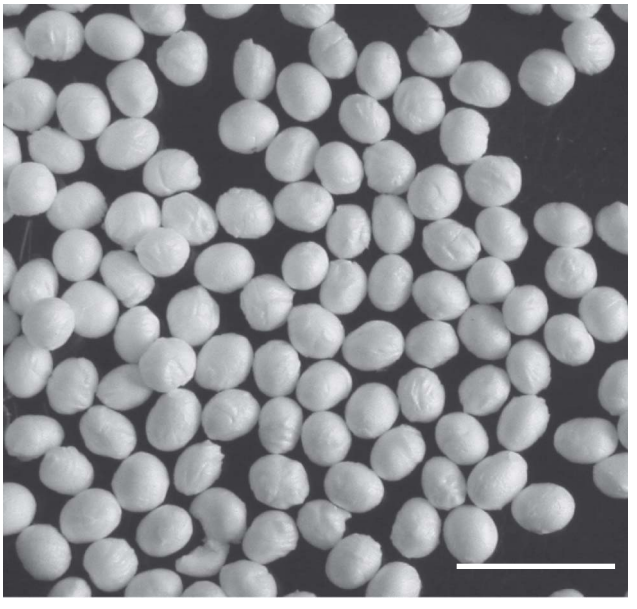

Figure 2. Photography of freeze-dried WP-coated WP/ALG microparticles. Scale bar represents 5 mm.

and ALG coating thickness were therefore estimated by calculation at 88 and 99 μm, respectively. No obvious difference of size was observed between both coatings.

TPH EE was about 88.8% for freeze-dried uncoated WP/ALG beads, whereas after coating, TPH EE fell to 34.9% and 18.3% for freeze-dried WP- and ALG-coated particles, respectively.

Classical in vitro release

As uncoated and coated beads were based on a protein matrix, they can be affected by changes in pH because of the presence of acidic (e.g., carboxylic acids) or basic (e.g., ammonium salts) groups in the polypeptide chains that either accept or release protons in response to environmental pH. To evaluate the influence of pH on the biopharmaceutical behavior, uncoated and coated WP/ALG beads were investigated using in vitro dissolution tests. The release of TPH from mixed WP/ALG beads uncoated and coated with WP or ALG was firstly studied in buffer at pH 1.2 for 2 hours followed by buffer at pH 7.5 for 22 hours (Figure 3). In buffer at pH 1.2, the release profile from uncoated WP/ALG beads was characterized by a burst release within the first 30 minutes followed by a plateau state (75% of TPH encapsulated). Because of rapid release, uncoated WP/ALG beads were not transferred in pH 7.5 buffer (Figure 3a). In contrast, no obvious release of TPH was observed from coated beads after 2 hours at acidic pH, regardless of polymer coating as less than 10% of the drug was released (Figure 3b). The subsequent transfer of coated beads in pH 7.5 buffer, simulating intestinal pH, induced the progressive release of TPH attaining 39% ± 10% and 52% ± 12% at the end of incubation from WP- and ALG-coated beads, respectively (Figure 3a). The pH-responsiveness of the vehicle was obvious as in pH 1.2, even after 20 hours the release was not significant (drug release below 10%, data not shown). As beads are protein-based hydrogels, proteases secreted in the stomach and intestine can attack protein matrix and thus influence the drug release rate. Therefore, the release of TPH from both coated WP and ALG beads was studied in SGF for 2 hours followed by SIF for a further 2 hours (Figure 4) simulating the gastrointestinal conditions according to USP specifications. In SGF, coated beads progressively released TPH at a faster rate for WP-coated microparticle reaching 38% *than for* ALG-coated beads at 2 hours (14%) (Figure 4). After transfer in SIF, the release of TPH was accelerated in both formulations with a total release in less than 2 hours with complete microparticle degradation.

Artificial digestive studies

The biopharmaceutical behavior of WP- and ALG-coated beads was evaluated using a multicompartmental and

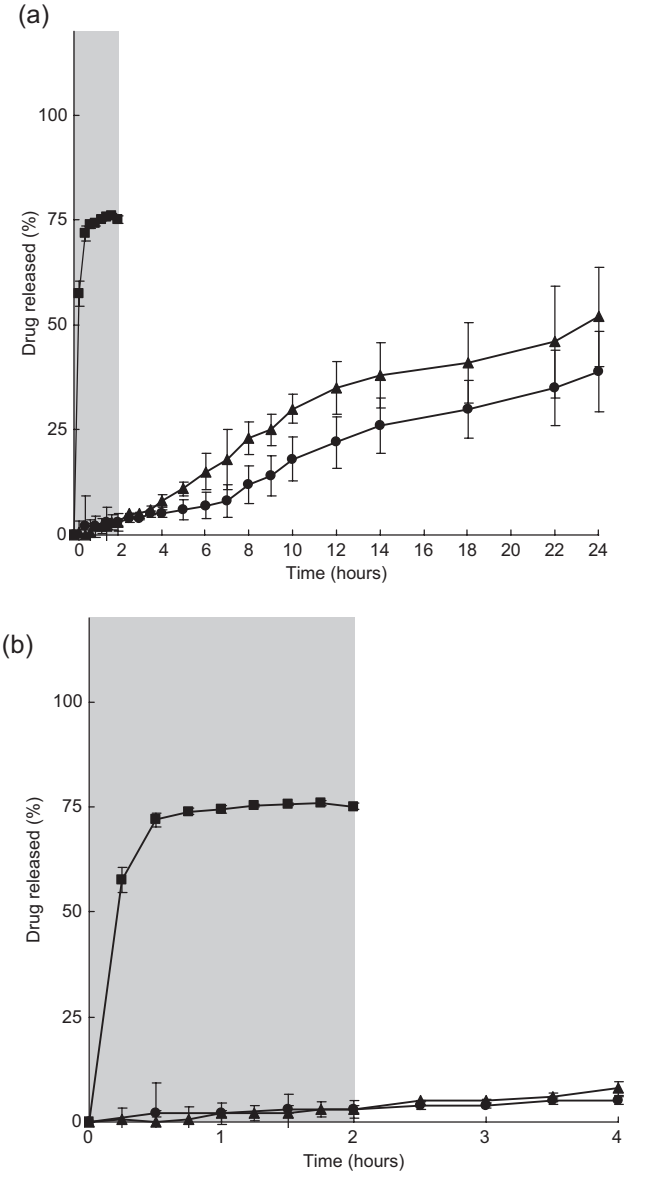

Figure 3. Drug released from (■) uncoated WP/ALG microparticles and (▲) ALG- and (●) WP-coated WP/ALG microparticles during incubation in (a) pH 1.2 buffer for 2 hours (grey zone) followed by pH 7.5 in USP2 and (b) was a zoom of drug release during the first 4 hours ($n = 3$).

dynamic system that closely mimics the in vivo conditions with regard to pH, enzyme secretions, fluid volume, and mixing intensity[23].

No significant drug release from WP- and ALG-coated beads was detected from samples collected in the stomach compartment during the first 45 minutes of digestion (data not shown). The cumulative percent of TPH absorbed in jejunum and ileal compartments are presented in Figure 5. The TPH concentration in jejunum and ileum dialysis fluids represents the drug released from the beads and dialyzed through hollow fibers considered thus as available for in vivo 'absorption'

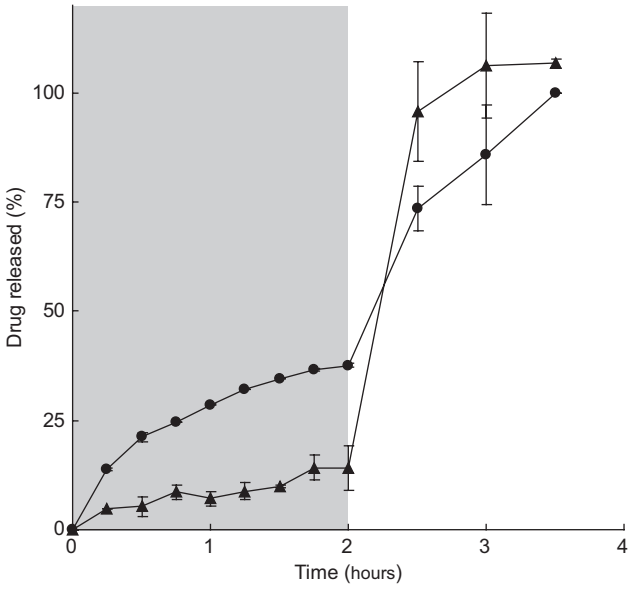

Figure 4. Drug released from (●) WP- and (▲) ALG-coated WP/ALG microparticles in SGF for 2 hours (grey zone) followed by SIF ($n = 3$) in USP2.

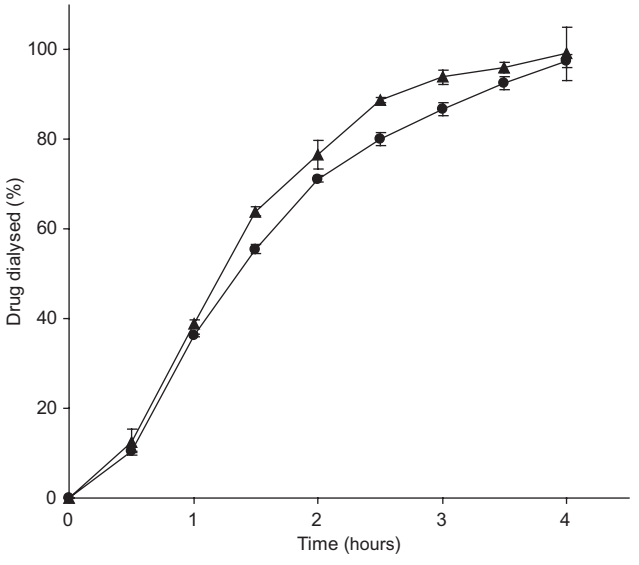

Figure 5. Cumulative drug 'absorption' during 4 hours digestion of (▲) ALG- and (●) WP-coated microparticles ($n = 3$) in TIM1.

(named 'absorption'). The 'absorption' profile obtained depends on drug delivery system studied. The 'absorption' of TPH was not obviously different between WP- and ALG-coated beads ($T_{50} \approx 1.3$ hours) with a complete drug 'absorption' at the end of digestion.

Kinetic release model

As release of encapsulated material was clearly dependant on matrix behavior, TPH release was fitted with mathematical model (Harland equation, SAS software) to elucidate the mechanisms governing drug release during in vitro dissolution tests and in the artificial digestive system (Table 1). Drug release observed from all formulations was fitted with the Harland [Equation (1)] with correlation coefficient r^2 values of 0.9674–0.9962, with the worst correlation coefficient ($r^2 = 0.9292$) for ALG-coated beads investigated in SGF. As with buffer at pH 1.2, less than 2% of drug was released, no modelization was performed. In contrast, in SGF, drug release from WP- and ALG-coated beads was controlled by diffusion ($A > B$). At pH 7.5 as in SIF, TPH, release from both formulations appeared to be dependant on erosion mechanism ($A < B$). In the artificial digestive system, the drug was also released according to an erosion mechanism.

Table 1. Value of A, B, and r^2 calculated from Harland model for coated WP/ALG microparticles.

		Harland equation			
	Microparticles	A	B	r^2	Mechanisms
pH 1.2 buffer	WP coated	—	—	—	Release less than 2%
	ALG coated	—	—	—	
pH 7.5 buffer	WP coated	0.000	1.632	0.9757	Erosion
	ALG coated	0.899	2.123	0.9674	Erosion
SGF	WP coated	2.520	0.000	0.9962	Diffusion
	ALG coated	5.920	0.000	0.9292	Diffusion
SIF	WP coated	—	—	—	Complete degradation
	ALG coated	—	—	—	
Artificial digestive system	WP coated	0.000	0.594	0.9765	Erosion
	ALG coated	0.000	0.672	0.9793	Erosion

Discussion

The major goal of this work was to evaluate the biopharmaceutical behavior of a novel oral drug delivery system based on food-grade polymers using a class I drug model, TPH. This oral delivery system was obtained with organic solvent and toxic agent free methods using an emergent polymer, namely WP. Mixed beads were obtained with a ratio WP/ALG 62/38, which was the ratio with the highest WP content and a viscosity below 200 mPas which can allow, later, a mass production with the Encapsulator. Mixed beads were obtained by a classical extrusion/gelation process but were further coated with WP or ALG by innovative and simple immersion methods. The beads were then freeze-dried to obtain a dry and stable formulation adapted to oral route (i.e., hard gelatin capsule blend).

First, EE parameter was chosen as an indicator of suitability for the TPH formulation process. The high EE obtained for mixed WP/ALG beads indicated that

extrusion/cold gelation seemed to be a convenient method for encapsulating TPH. This high EE was only achievable because the external phase was saturated with TPH to decrease the concentration gradient and thus the diffusion of the encapsulated agent to the external phase during the microparticle formation. With this technique, the EE was in the same range than the EE obtained for another hydrophilic compound riboflavin with the emulsification/internal cold gelation method[20]. In contrast, the coating process based on immersion and washing steps impacted on drug content in mixed WP/ALG beads certainly because of drug efflux during washing steps. Because of its solubility, TPH might effectively be in solution in the polymer matrix making the diffusion out from beads during immersion or washing steps easier. Compared to WP-coated beads, ALG-coated particles exhibited a less important TPH EE indicating higher drug diffusion probably attributable to the additional washing step achieved during the ALG coating process. In the case of drug molecules with high molecular weight or lower solubility, drug loss might be less important as observed by Huguet and Dellacherie[25]. They have demonstrated that during a coating process based on immersion of ALG beads in chitosan solution, the loss of dextran (a linear polysaccharide of 40 kDa) was less than 5%. Thus, the coating steps presented in our work may be more adapted to other drugs than small molecules biopharmaceutical classification system (BCS) class I such as BCS class III less hydrophilic and/or larger molecules.

Biopharmaceutical behavior of uncoated and coated WP/ALG beads was evaluated by using classical in vitro dissolution testing to determine the contribution of digestive parameters such as pH and enzymes to the drug release. Because of the high TPH solubility, biopharmaceutical behavior of coated beads was not limited by the dissolution or diffusion of TPH. At pH 1.2, uncoated WP/ALG beads rapidly released TPH probably by drug diffusion as the beads were still intact after 2 hours. At pH 1.2, no breakdown of polymers occurred (no matrix weight loss and no WP release, data not shown), but the pure ALG matrix shrunk, whereas the WP one swelled slightly. At low pH, carboxyl groups of ALG were protonized and hence the electrostatic repulsions among these groups lessened, favoring matrix shrinkage[26]. In contrast, WP chains (isoelectric point of 5.2) were positively charged (pH < pHi: 5.2), which caused repulsive forces with cations binding polymer chains (Ca^{2+}) and subsequently matrix relaxation[13]. The release plateauing at 75% indicated that some TPH was still entrapped in polymeric matrix. This incomplete release was attributed to WP contribution and TPH–WP interactions as complete drug release was observed from pure ALG beads in pH 1.2 because of a rapid diffusion (data not shown). On the contrary, both WP and ALG coatings modified the particle biopharmaceutical behavior. WP- or ALG-coated beads released less than 10% of encapsulated drug within 2 hours, indicating that coating process or coating itself might be responsible for the sustained release of TPH. As less than 10% of TPH was released after 2 hours at pH 1.2, coated beads were considered as gastroresistant formulations according to USP specifications even if physiological gastric emptying time in human for multiparticulate forms in fasting state was estimated to be around 1 hour[27]. ALG is well known for its insolubility at acidic pH because of the conversion in gel structure. Thus, ALG coating can probably form a less porous structure because of shrinkage[28], which probably induced a more compact structure with a winding network. Concerning WP-coated beads, even if the drug release was similar to ALG-coated beads, this observation cannot be explained in the same way. WP cold set gels are widely influenced by pH[29] with an increase of protein–protein interactions at low pH[30], which probably impacted on WP coating structure. Structural changes might induce a more compact structure that limits drug release. TPH is a weak acid with a pK_a value close to 8.77[31], hence most of the drug is in the unionized form at the pH of the solution. No ionic interaction should occur between the drug and the polymers. As a consequence, drug release is only dependant on formulation behavior. By comparison, ALG-coated WP beads prepared using glutaraldehyde as cross-linking agent released the totality of TPH encapsulated within 2 hours at pH 1.2[32]. These authors attributed TPH release to the formation of cracks on the surface of coated beads caused by glutaraldehyde cross-linking. Thus, our coating seemed to be compact enough to retain TPH and was preserved during freeze-drying process. As expected, change of pH modified the biopharmaceutical behavior of these formulations; because at intestinal pH (pH = 7.5), TPH release occurred for both WP- and ALG-coated beads. However, the WP- and ALG-coated beads exhibited a prolonged release as only 12%–23% of TPH was respectively released at 8 hours and less than 60% at 24 hours. In these conditions, both coated particles exhibited a similar release profile shape with a more delayed release for WP-coated beads (T_{25} = 14 hours versus 9 hours for ALG-coated particles) probably because of a more stable structure for WP coating because on the one hand WP gel was stabilized by hydrophobic interactions and on the other hand above the isoelectric pH (pHi = 5.2), WPs were negatively charged which reinforced interaction with Ca^{2+} ions. Furthermore, ALG is well known to be sensitive to intestinal medium because of exchange between Ca^{2+} ions and cations of medium[33] leading to a swollen matrix. This ALG instability might explain the erosion mechanism governing TPH release shown by the Harland equation (Table 1).

This result is in accordance with visual observations of coated beads at the end of the 24-hour experiment, which still have a spherical shape but with a swollen gel aspect.

To challenge the system further, WP- and ALG-coated beads were studied in SGF for 2 hours then transferred to SIF. Because of the pepsin attack on polypeptidic chains, TPH was released from WP- and ALG-coated beads in SGF, but drug release was still controlled. As expected, pepsin was able to hydrolyze the WP matrix, but despite the large amount of pepsin used (10-fold higher than physiological concentration in human) and the long duration (2 hours), this enzyme was unable to completely and rapidly destroy the mixed structure. The relative slow break down of the WP coating could be because of the hydrophobic interactions between WP which mask hydrophobic amino acids corresponding to the pepsin action site. Fitting release data to Harland equation showed that the diffusion-controlled release mechanism governed the overall kinetics (Table 1) instead of the erosion mechanism as expected in the case of enzymatic hydrolysis. In fact, ALG inside mixed WP/ALG was responsible for drug diffusion release because of its shrinkage at acidic pH which limited enzyme penetration inside beads. Thus, ALG coating slowed down enzyme penetration inside beads more than WP coating (14% TPH released versus 38% TPH released from WP-coated beads). ALG has been widely used as encapsulation material. Coating was added to reduce the burst effect obtained from ALG microparticles. Different polymers were used to coat ALG particles by complexation with polycations such as chitosane, albumin, and poly-L-lysine[34]. In our work, encapsulation of a BCS class I drug in mixed and coated WP/ALG beads is for the first time described using WP as coating agent. These results obtained showed that it was possible to delay and control the drug release with natural polymers and a simple process. WP and ALG were more sensitive to SIF with complete drug release associated to complete microparticle degradation in less than 2 hours. Both pretreatment by pepsin, which started WP hydrolysis, and SIF conditions (ionic environment and pancreatin) caused coated microparticle disintegration by a synergic action on each polymeric matrix. On the one hand, ionic environment caused ALG matrix relaxation making pancreatin attack easier on WP and on the other hand, the high pancreatin concentration used in this study was 10-fold higher than physiological concentration and led to a very fast release of TPH in the 30 minutes after transfer into SIF.

Despite the detailed information collected for the biopharmaceutical behavior, classical in vitro release tests are not always suitable for predicting the drug release in vivo because of the complexity of the gastrointestinal tract. Among the various in vitro systems described in the literature to mimic the different part of the gastrointestinal tract, the artificial digestive system TIM1 has shown high in vivo behavior predictability. Thanks to this system, Souliman et al.[21,22] established a level A in vivo/in vitro correlation for immediate and sustained release formulations using BSC class I drugs with a high degree of predictability compared to USP in vitro methods. This artificial digestive system can reproduce certain parameters recovered in vivo as peristaltic movements which can impact erosion and diffusion mechanisms and thus drug release. Digestion of WP- and ALG-coated beads in artificial digestive system highlighted gastroresistance properties as observed with in vitro dissolution testing in pH 1.2 buffer. At physiological concentration of pepsin as used in the TIM1 compared to USP2, WP- and ALG-coated beads thus resisted enzymatic attack. Pure WP beads have already showed their ability to resist physiological pepsin concentration using an artificial stomach[15]. The results obtained with the coated beads, along the four segments of the artificial gastrointestinal tract, clearly demonstrated the sustained release properties of the beads. Fitting 'absorption' data with the Harland equation exhibited a release mechanism depending on erosion for both coated beads, and as observed with classical in vitro dissolution testing, both WP- and ALG-coated beads provided same release profiles depending on pH and enzyme hydrolysis. In the TIM1 apparatus, the drug release is under the influence of the variation of different parameters, this system being a dynamic system. Thus, the dosage form undergoes the impact of pH rising, enzymatic variations, peristaltic movements. Differences between performances of both coated formulations were erased by the TIM1. ALG-coated beads were supposed to be more sensitive to pH variations when WP-coated beads were probably more sensitive to enzymatic attack. As these parameters evolved at the same time, the resulting release from both formulations were similar but probably not for the same reason. Thus, the influence of digestive parameters simulated in the artificial digestive system was equivalent to drug 'absorption' from WP and ALG coating beads. However, TPH 'absorption' from coated beads was faster compared to TPH release from in vitro dissolution studies. This could be related to the intensity of peristaltic movements as well as motility patterns produced in the artificial digestive system compared to the paddle rotation from USP method, leading to more rapid matrix erosion.

Considering the in vitro/in vivo correlation already performed with the TIM1 with the fasted state protocol corresponding to in vivo healthy adult digestion parameters and further, assuming at least a rank order correlation, coated beads should exhibit in vivo a slow release profile regardless of coating material with a short T_{max} and a prolonged in vivo 'absorption' rate in the different

segments of the small intestine. Therefore, WP- and ALG-coated beads could be considered as gastroresistant formulations but also real sustained delivery systems with interesting potentiality for oral controlled delivery of different types of drugs.

Thanks to the in vitro experiments, behavior of coated multiparticles including interactions between polymers and drug in the formulation can be explained. The paddle method is suitable to explain the pH influence as well as the enzyme hydrolysis. Nevertheless, enzyme concentrations described in Pharmacopeia are too far from the physiological conditions to clearly simulate the real in vivo behavior of the dosage form. Artificial digestive systems that try to mimic the physiological conditions in the fasting state, with a standard unique protocol, allow us to predict in vivo behavior more accurately. The combination of the different in vitro studies and the in vivo potential of coated beads mean that this approach can be applied with confidence to the in vivo study.

Conclusion

Data from this work suggested that coated WP/ALG beads despite their sensitivity to pH and enzyme hydrolysis were gastroresistant and sustained release dosage forms. These properties were due to the coating addition with a natural polymer WP or ALG and the coating process itself. For the first time, the behavior of food grade particles was investigated in a complex gastrointestinal in vitro environment close to physiological conditions. In TIM1, drug 'absorption' was governed mostly by erosion mechanism, and no difference was observed between the two coating polymers used. This study demonstrated the potentiality of coated WP/ALG beads as oral sustained delivery system. Thus, even if the coating procedures developed here were not very adapted to TPH encapsulation in terms of EE, the in vitro performances highlighted the interest of coating and encouraged the development of a more suitable coating technique for BCS class I-loaded beads. In vitro studies are important tools to explain the biopharmaceutical behavior of drug dosage forms especially in case of new formulation with specific design and original excipients. Even if they are unable to replace the in vivo experiment, they could give powerful information concerning the influence of the main in vivo parameters encountered in the gastrointestinal tract.

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