European Journal of Pharmaceutics and Biopharmaceutics 73 (2009) 345–350

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

New amphiphilic and pH-sensitive hydrogel for controlled release of a model poorly water-soluble drug

I. Colinet [a], V. Dulong [a], G. Mocanu [b], L. Picton [a], D. Le Cerf [a,*]

[a] University of Rouen, Mont Saint Aignan, France
[b] "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania

ARTICLE INFO

Article history:
Received 12 May 2009
Accepted in revised form 17 July 2009
Available online 23 July 2009

Keywords:
Alginate-g-PCL
Hydrogels
Amphiphilic
pH-sensitive
Controlled release

ABSTRACT

This paper presents the development of new pH-sensitive, amphiphilic and biocompatible hydrogels based on alginate-g-PCL, cross-linked with calcium ions to form beads, prepared for controlled delivery of poorly water-soluble drug. We have focused our study on the effect of the length of PCL chains (530 and 1250 g mol^{-1}). Swelling profiles obtained clearly indicated that these hydrogels swell slightly (10–14%) in a simulated gastric fluid (pH 1.2), and strongly (700–1300% before disintegration) in a simulated intestinal fluid (pH 6.8). In both media, rates of swelling were lower for beads based on amphiphilic derivatives than for alginate/Ca^{2+} ones due to the hydrophobic PCL grafts, and decreased when hydrophobic character increased. A model drug, theophylline, was entrapped into these hydrogels and release studies were carried out. The drug was protected in acidic fluid (only 14–20% of release for alginate-g-PCL hydrogel against 35% of release for alginate hydrogel during 350 min). The drug is released completely in neutral fluid due to ion exchanges and disintegration of the hydrogel. PCL leads to decrease in the release kinetics in SIF (2 h for alginate-g-PCL/Ca^{2+} beads against 1 h for alginate/Ca^{2+} beads). It was demonstrated that the establishment of clusters inside beads by intramolecular interactions between PCL grafts of 530 g mol^{-1} in salt media allowed to retain the drug and to slow down its release considerably.

1. Introduction

One of the major challenges in the field of pharmaceutics is the elaboration of systems that protects, transports and finally releases a drug in a specific site in the human body with controlled kinetics release. There exist many systems to entrap and protect drugs for oral administration (nanoparticles [1,2], polymeric micelles [3,4], hydrogels [5,6], etc.). Among them, hydrogels have many advantages including simple drug formulations and administration procedures, no organic solvent, a sustained drug release behaviour, less systemic toxicity and some of them have a stimuli-responsive behaviour.

These systems, which swell or shrink in response to changes in the environmental conditions (temperature, pH, ionic strength, etc.) have been extensively studied and used as smart materials for various biomedical applications [7]. pH-sensitive systems can be used for oral drug administration when the oral delivery is pH-sensitive too [8–10]. The swelling of pH-sensitive hydrogels depends on variation of natural pH environment of gastrointestinal tract in human body, which varies from acidic in the stomach to neutral in the intestine. Thus, these systems allow releasing a bioactive compound at a specific site. They have the potential to reduce the side effects; thus, increasing pharmacological response. For this purpose, a variety of synthetic or natural polymers with acidic or basic pendant groups have been employed to elaborate pH-sensitive hydrogels [11,12]. Among them, alginate, a naturally occurring copolymer of guluronic (G) and manuronic (M) acid, is widely used as a pH-sensitive hydrogel in pharmaceutical applications [13]. The simple, mild, aqueous-based gel formation of sodium alginate, in the presence of divalent cations such as Ca^{2+}, occurs thanks to the stacking of the guluronate blocks in the alginate chains, with the formation of the "egg-box junctions" [14]. Such gels are widely used in a number of biotechnological applications involving the entrapment and the release of various biosystems [15,16]. Alginate beads have many advantages: non-toxic when taken orally, high biocompatibility and a good mucoadhesive agent that improves drug bioavailability and effectiveness [17]. Moreover, it was demonstrated that these beads can protect bioactive molecules from the acidic and harsh environment of the stomach [18]. However, the porosity of alginate beads results in a very low efficiency of incorporation with drugs having a low molecular

* Corresponding author. University of Rouen, Lab. Polymers Biopolymers Surfaces – CNRS FRE 3101 and FR 3038, 76821 Mont Saint Aignan, France. Tel./fax: +33 2 35 14 65 43.
E-mail address: didier.lecerf@univ-rouen.fr (D. Le Cerf).

0939-6411/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2009.07.008

weight and being water-soluble, resulting in a fast release. It was also demonstrated that alginate/Ca^{2+} beads disintegrate fast in intestinal media, which leads to a rapid release of drugs [19,20]. Consequently, the preparation of hydrogels based on hydrophobically modified alginate derivatives may be of great significance. For practical improvements, they might bring for existing or new application.

In previous studies [21,22], we have reported the synthesis of new amphiphilic derivatives based on alginate grafted with poly(ε-caprolactone) chains of 530 and 1250 g mol^{-1}. In salt media, the aggregation behaviour of these samples is very different according to the length of poly(ε-caprolactone) (PCL) chains. As expected, for a PCL chain of 1250 g mol^{-1}, intermolecular hydrophobic associations are predominant and lead to the formation of aggregates that strongly increase viscosity of its solutions. At higher concentrations, these samples should have particular rheological properties. On the contrary, for a short PCL chain (M = 530 g mol^{-1}), an opposed and non-classical behaviour has been observed. Intramolecular associations are predominant, even in semi-dilute regime, and lead to a compact structure with the formation of hydrophobic clusters. In the present work, we studied the effect of the combination of the pH sensitivity of alginate and the hydrophobia of PCL on the release properties of a poorly water-soluble bioactive molecule in human body. We describe a detailed investigation of swelling and degradation behaviours of amphiphilic beads based on alginate-g-PCL cross-linked by calcium ions. The main objective was to get some idea about the possible behaviour of alginate-g-PCL/Ca^{2+} beads in gastrointestinal tract with respect to their stability. Then, we studied the drug release behaviour of these systems as a function of the incorporation rate and length of PCL, and their possible applications as a controlled release system in vivo for oral administration of drugs. The poorly water-soluble model drug used in this study was theophylline (TPH). This bioactive compound belongs to a class of medications called bronchodilators used in treating asthma and other airway diseases [23]. A differential scanning calorimetry study on TPH entrapped in hydrophobic dextran derivative microspheres has revealed that this drug partially interacted with the hydrophobic clusters of polymer [24]. The solubility of TPH is equal to 8 g L^{-1} in water. The TPH dose should not exceed 400 mg day^{-1} for an adult [25].

2. Materials and methods

2.1. Materials

The synthesis of alginate-g-PCLs was clearly described in a previous study [21]. Alginate with a ratio of M/G = 0.5 was purchased from Degussa Company (Baupte, France). The sample chosen is rich in guluronic acid because it was demonstrated that gelation occurs mainly with G residues [26]. Hydrochloric acid (HCl), potassium phosphate (KH_2PO_4) and sodium chloride (NaCl) were purchased from Sigma–Aldrich. Theophylline (TPH) and calcium chloride ($CaCl_2$) were purchased from Across Organics. All compounds and solvents were used without further purification. Water was purified with the Milli-Q reagent system (Millipore). Modified alginates are coded as following *X–Y*, where *X* is the Molar mass of the grafted PCL, and *Y* is the real incorporating rate of PCL. Hydrogels were based on 530-4, 530-8 and 1250-13.5 samples. Table 1 summarizes the characteristics of the modified polymers. The number average molecular masses indicate that no degradation occurs during the chemical modification of alginate. The weight average molecular masses and the Huggins' parameters show a strong aggregation, whereas intrinsic viscosities are in good agreement with intramolecular interactions in dilute regime.

Table 1
Physicochemical characteristics of alginate and alginate-g-PCL [21] (Mn: number average molecular mass, Mw: weight average molecular mass, [η]: intrinsic viscosity and K_H: Huggins coefficient).

	$\overline{Mn}$ (g mol^{-1})	$\overline{Mw}$ (g mol^{-1})	[η] (mL g^{-1}) (NaCl 0.1 M)	K_H (NaCl 0.1 M)
Alginate	194,000	342,000	1050	0.36
530–4	207,000	390,000	834	0.68
530–8	210,000	425,000	686	0.84
1250–13.5	387,000	993,000	370	1.15

These derivatives can easily undergo a sol–gel transition in presence of calcium ions due to the presence of more of 80% of carboxylate groups along the polysaccharidic backbone [27].

2.2. Preparation of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads

Polymer solutions of alginate and alginate-g-PCL at 20 g L^{-1} were prepared in water under vigorous stirring for 48 h at 40 °C. The pH was then adjusted to pH 7.0 with 0.1 M NaOH, and a solution of NaCl was added to obtain ionic strength of 0.1 M. The alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads were prepared by dropwise addition of 20 mL of alginate-g-PCL (or alginate) solution into 800 mL of ionic solution (NaCl 0.1 M and $CaCl_2$ 0.15 M) through a fine 21 gauge stainless steel needle. The distance between the edge of the needle and the surface of the calcium solution was 5 cm. Beads were prepared in salt media to ensure a homogeneous cross-linking density [28,29]. The droplets were slowly stirred for 1 h to allow a complete cross-linking. The alginate-g-PCL/Ca^{2+} (or alginate/Ca^{2+}) beads were then separated from the solution by filtration, washed with water and finally dried at 40 °C for 24 h. Drug-loaded beads were prepared by adding the same concentration of TPH to the alginate-g-PCL (or alginate) solution and to calcium chloride solution to avoid too much loss of TPH, thanks to osmotic equilibrium. We added 0.1 g of TPH for 0.1 g of alginate derivates.

2.3. Determination of the amount of drug entrapped

The amount of TPH entrapped into the beads was calculated by measuring the absorbance of the gelling medium at 272 nm (λ_{max} of TPH) using a standard curve of known concentration in the range 0–0.08 M (Spectrometer UVIKON 860, Kontron Instruments). The amount of TPH entrapped was estimated by the difference between the initial and the final amount of drug in gelling media. Entrapment efficiency was expressed as the weight of drug entrapped in the beads divided by the initial weight of TPH in alginate-g-PCL solution. Moreover, it is important to notice that the drug exhibited the same λ_{max} for whatever the release medium used in this study, as the free drug in water and the presence of dissolved polymers did not interfere with the absorbance of the drug at this wavelength.

2.4. Study media preparation

Swelling and release studies of beads were carried out in three aqueous media: simulated gastric fluid (SGF, pH 1.2), simulated intestinal fluid (SIF, pH 6.8) and simulated gastrointestinal tract (GI) at 37 °C ± 0.5 °C. SGF (pH 1.2) was prepared by dissolving 2 g of sodium chloride and 7 mL of concentrated HCl in 1 L of water, and SIF was prepared by mixing 250 mL of 0.2 M KH_2PO_4 and 118 mL of 0.2 N NaOH according to the method described by Shantha et al. [23]. To mimic the GI tract, we took account data provided by Krishnaiah et al. [30], which was based on the scintigraphic studies in guar gum tablets using technecium-99 m-DTPA (99 m

Tc-DTPA) as tracer in human volunteers. We put a new batch of dried beads in the simulated gastric fluid (SGF, pH 1.2) for 2 h and then, after their filtration, we transferred them into simulated intestinal fluid (SIF pH 6.8) until complete degradation (e.g. complete loss of shape).

2.5. Swelling measurements

Accurately weighed amounts of beads (ranging from 0.1 to 0.2 g) were immersed in 40 mL of desired solution, and at fixed time intervals the beads were separated from the medium by filtration. Immediately, they were wiped gently with paper and weighed. The dynamic weight change of the beads with respect to time was calculated according to the formula:

$$\%\ \text{weight change} = \frac{W_t - W_i}{W_i} \times 100$$

where W_t is the weight of the beads in the swollen state and W_i is the initial weight of the beads.

Visually, in SIF, beads swell enormously and after that begin to shrink because of the disintegration of the beads. In the various curves plotted, the complete disintegration of beads was indicated by −100% weight changes. The data represent mean ± SD from three independent experiments with ±5% of incertitude.

2.6. Release studies

In vitro release studies were performed in SGF, SIF and GI at 37 °C. Accurately weighed amounts of dried drug-loaded beads (ranging from 0.1 to 0.2 g) were placed in beakers containing 1 L of the release medium at 37 °C. At periodic intervals 4 mL of aliquots were collected from the release medium, and the TPH concentrations were measured using a spectrophotometer at λ_{max} 272 nm. The percentage of cumulative amount of released TPH, obtained from three experiments, was calculated and plotted against time.

3. Results and discussion

3.1. Morphology of the beads

Pictures of wet pure alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads before drying are illustrated in Fig. 1. Alginate-g-PCL derivatives can lead to cohesive, stable and perfectly spherical beads, despite the complexity of hydrophobic interactions occurring in their solutions. Compared to the size of the wet beads (0.8 ± 0.1 mm), the average diameter of the dried beads was found to be around 0.2 ± 0.05 mm. For all these systems, air drying gave an acceptable spherical shape with a narrow size distribution.

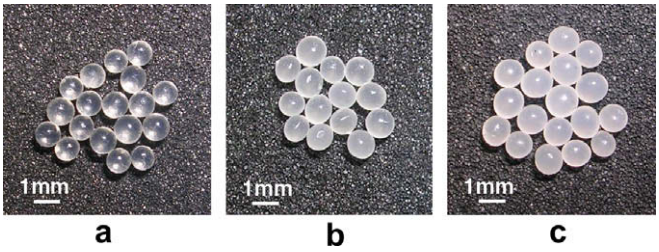


Fig. 1. Pictures of wet alginate/Ca^{2+} (a), 530-8/Ca^{2+} (b) and 1250-13.5/Ca^{2+} (c) beads.

3.2. Beads loading

The loading efficiency was higher for alginate-g-PCL/Ca^{2+} beads than for alginate/Ca^{2+} ones (Table 2). These results seemed to indicate hydrophobic interactions between TPH and PCL grafts in agreement with Miyazaki et al. [24]. The amount of TPH entrapped in alginate beads (near 0.1 g of TPH in 0.1 g of alginate derivate) lets to plan for industrial development.

3.3. Swelling studies

Weight changes profiles of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads in SGF, SIF and GI are shown in Figs. 2a, b and 3, respectively. These hydrogels exhibit clearly a pH-sensitive swelling in agreement to the polyelectrolyte nature of alginate and alginate-g-PCL. In SGF (pH 1.2) (Fig. 2a), dried beads swell slightly in the first hour and gain weight due to the hydration of the hydrophilic carboxylate groups. Then, they reach the equilibrium state (10–20% of weight change), remain intact and do not show any signs of disintegration. These results can be explained by the conversion of the outermost layer of cross-linked hydrated alginate-g-PCL (or alginate) into a porous and insoluble alginic acid-g-PCL in SGF. This outer layer can slightly swell by hydration but forms a barrier that limits water uptake. The disappearance of electrostatic repulsions favours the shrinkage of beads that balances the slight swelling effect and, therefore, the diameter of beads only slightly changes. Moreover, the swelling rates of alginate-g-PCL/Ca^{2+} are lower than alginate/Ca^{2+} ones and decrease with increase in the hydrophobia (increase in the grafting rate and/or the length of PCL chains). The protonation of carboxylate groups also probably leads to a reinforcement of hydrophobic interactions between PCL grafts, and consequently to an increase in elasticity of the network and a decrease in swelling ability.

In SIF (pH 6.8) (Fig. 2b), beads based on alginate and its hydrophobically modified derivatives show a great water uptake in the first 80 min (700–1700% of weight change), and then start to disintegrate. This can be explained by an ion-exchange process of the Na^+ ions present in the external solution with Ca^{2+} ions, which are binding with COO^- groups mainly in the polyguluronate sequences. As a result, electrostatic repulsions among negatively charged COO^- groups increase, lead to a relaxation of chains and enhance the gel swelling in the initial phase. The chelating action of phosphate ions is supported by the highest affinity of phosphate for calcium than for alginate [31], and we observe some turbidity in the system due to the formation of calcium phosphate. This swelling occurs until the osmotic pressure equals the strength of the cross-linking bonds and physical interactions maintain the structure of the polymer network stable. Then, the beads start to disintegrate due to the loss of structure. Thus, the swelling curves in SIF begin to decline indicating the disruption of these systems. Bajpai and Sharma [19] and Dainty et al. [32] have previously reported this phenomenon.

However, the beads show a clear difference of maximum swelling degree in this media and weight changes of alginate-g-PCL/Ca^{2+} beads are clearly lower when the grafting rate and/or length of PCL chains increase. This can be explained by the establishment of a double network (Ca^{2+} ions and hydrophobic interactions between PCL grafts) evidenced by rheological measurements (data not shown). Moreover, the strength of hydrophobic interactions

Table 2
Loading efficiency of TPH in alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads.

	Alginate/Ca^{2+}	530-4/Ca^{2+}	530-8/Ca^{2+}	1250-13.5/Ca^{2+}
Loading efficiency (%)	72	89	87	91

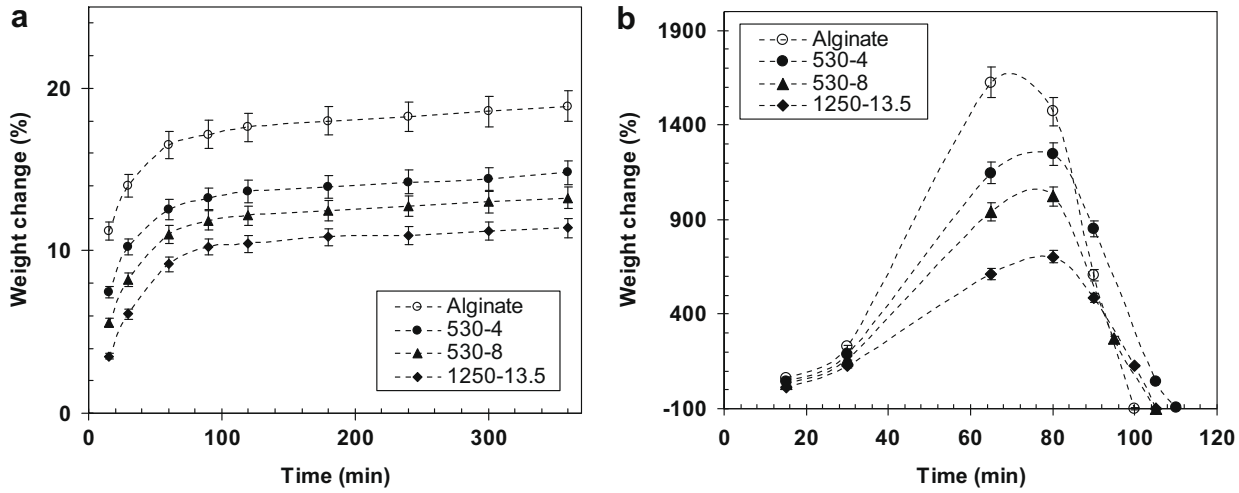


Fig. 2. Swelling profiles of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads as a function of time in (a) SGF, 37 °C and (b) SIF, 37 °C.

balances the osmotic pressure and decreased swelling ability of these systems. The loss of hydrophilicity of alginate-g-PCL/Ca^{2+} compared to simple alginate beads/Ca^{2+} can also explain the decrease in the network water uptake. However, despite a significant decrease in swelling of the amphiphilic systems compared to hydrophilic ones, the time of stability is approximately the same for whatever the system.

Finally, Fig. 3 depicts the dynamic uptake of water of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads in a medium of changing pH (GI). When the beads are transferred into simulated intestinal fluid after 2 h, the protonated, porous and insoluble alginic acid-g-PCL skin is converted to a soluble viscous layer. The surface of beads begins to dissolve causing the water uptake. After complete dissolution of the outermost layer, the ion-exchange process between the sodium ions of external buffer solution and Ca^{2+} ions present in the "egg-box" cavities of polyguluronate blocks of alginate or alginate-g-PCL occurs in the bulk of the beads. Then, the beads started to disintegrate and, after 2 h, they were completely destructed.

However, alginate-g-PCL/Ca^{2+} beads exhibit lower swelling rate in the intestinal pH than alginate/Ca^{2+} beads, and kinetics of disintegration are less rapid. This can be explained by the establishment of a dense network inside the alginate-g-PCL/Ca^{2+} beads in SGF due to hydrophobic interactions between PCL grafts.

As a result, the total stability of calcium ions cross-linked alginate-g-PCL beads in the media of varying pH is found to be nearly 200–250 min.

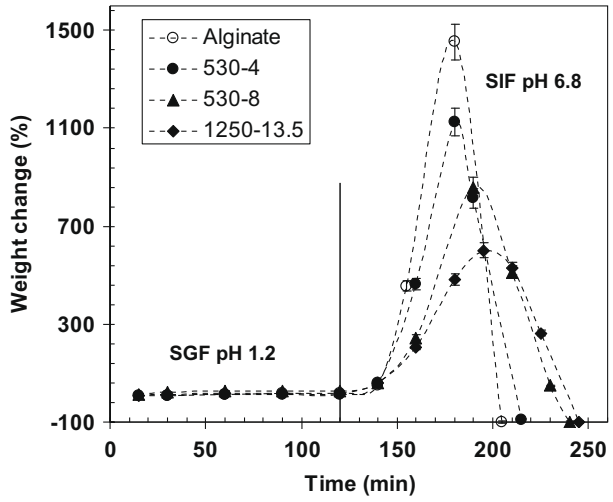


Fig. 3. Swelling profiles of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads as a function of time in GI, 37 °C.

3.4. Release studies

Sustained release profiles of cross-linked gel beads-loaded TPH as a function of time from alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads in SGF, SIF and GI are shown in Figs. 4a, b and 5, respectively. As shown in Fig. 4, the amount of theophylline released in SGF at pH 1.2 is very low. Only 10–18% of TPH is released from alginate-g-PCL/Ca^{2+} beads in the first 1 h against 30% from alginate/Ca^{2+} beads. At this pH, alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads shrink, scarcely swell and are not eroded. Consequently, drug is less released and the initial burst release may be attributed to the diffusion of the drug caused by the slight gel swelling and also by the release of drug adsorbed towards the surface of the gel matrices. Thereafter, the drug release is slowed down, due to reduced swelling in SGF and decreased pore size. Moreover, rates of drug released from alginate-g-PCL/Ca^{2+} beads are lower than for alginate/Ca^{2+} ones. This can easily be explained by the establishment of hydrophobic interactions between TPH and PCL grafts. As a result, the TPH entrapped in alginate-g-PCL/Ca^{2+} beads is protected almost completely from acid in gastric juice. This result can be compared to the works of Miyazaki et al. on the release of TPH in alginate solution and calcium alginate hydrogel at pH 1.2 [33]. Without calcium, the release of TPH is very fast (less than 120 min). Calcium alginate hydrogels with different polysaccharide concentrations (1–2% w/v) show a sustained release of TPH over a period of at least 360 min. For other matrices based on gelatin, agar and κ-carrageenan [34] and sclerogluran [35] the total release is also obtained after 360 min.

In our case, TPH is really entrapped in hydrophobic clusters in good agreement to the work of Miyazaki [24], and the diffusion of TPH is decreased by the alginic insoluble skin at the beads surface.

In SIF (Fig. 4b), the release of TPH from these hydrogels is much faster and 100% of drug is released within 80 min. This result can be correlated with the great swelling of the beads in this medium. However, release kinetics from amphiphilic systems are considerably slower than from alginate beads and start with a lag time followed by a rapid release due to swelling and disintegration of the

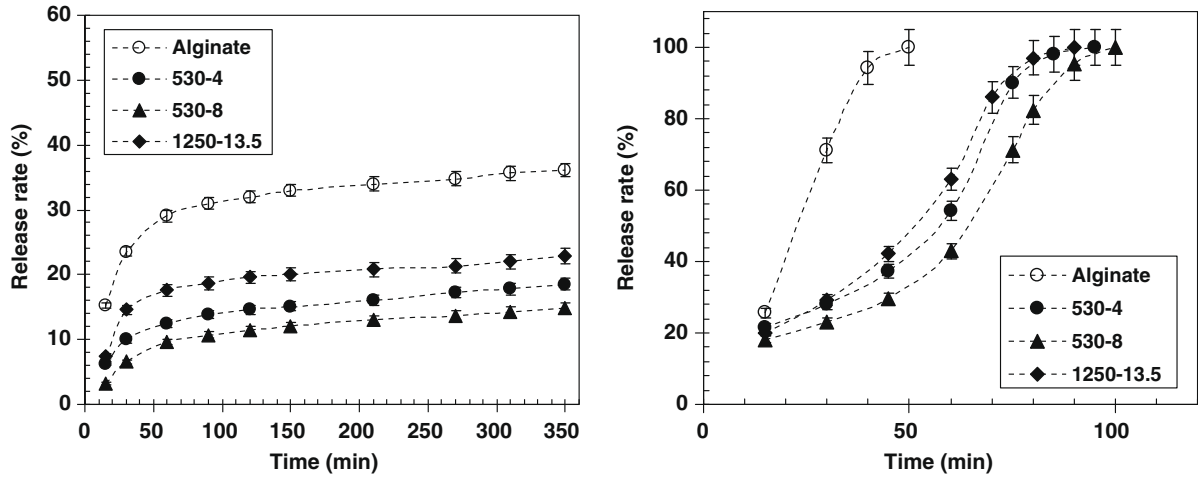
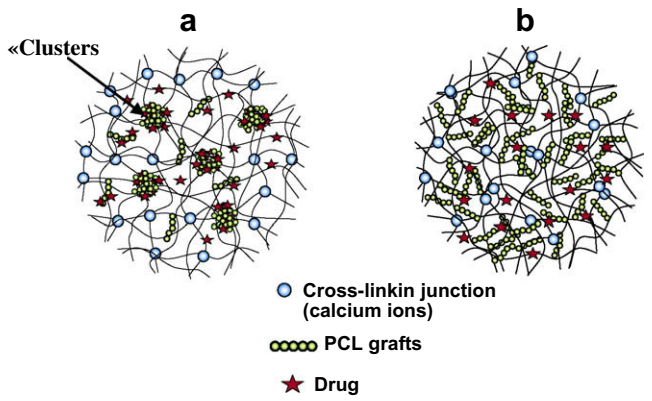


Fig. 4. Release profiles of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads as a function of time in (a) SGF, 37 °C and (b) SIF, 37 °C.

matrix. These systems allow releasing TPH for a time twice as long as systems based on alginate. These results can easily be explained by the lower swelling rates of alginate-g-PCL/Ca^{2+} beads and by hydrophobic interactions between PCL grafts and TPH.

However, it seems surprising to obtain an opposite behaviour for the two lengths of PCL. In both SGF and SIF, we obtained the lower release rates for 530-4/Ca^{2+} and 530-8/Ca^{2+} beads although the 1250-13.5/Ca^{2+} beads present the lowest swelling rates. Consequently, it seems that the drug release is not only controlled by a swelling/diffusion mechanism, but also depends on the microstructure inside the beads. In agreement with the physicochemical behaviour in salt media of alginate-g-PCL [21,22], we can assume the predominant establishment of intermolecular hydrophobic interactions in 1250-13.5/Ca^{2+} beads and predominant intramolecular interactions in 530-4/Ca^{2+} and 530-8/Ca^{2+} ones leading to hydrophobic microdomains (Scheme 1). These "clusters" probably entrap and retain TPH by hydrophobic interactions that could explain the slowing down of the release kinetics from the beads based on these derivatives.

The behaviour in GI is shown in Fig. 5. The samples exhibit a very slight drug release in the first 2 h in the artificial gastric fluid of pH 1.2. When these beads are transferred into the artificial intestinal fluid of pH 6.8, the beads release TPH with the same order of samples than Fig. 4. Consequently, although PCL grafts can diminish the swelling rates and allow slowing down the release, all the drug are finally released due to the disruption of the beads for 2 h in SIF.



Scheme 1. Possible schematic structures of 530-4/Ca^{2+} and 530-8/Ca^{2+} (a) and 1250-13.5/Ca^{2+} (b) loaded beads.

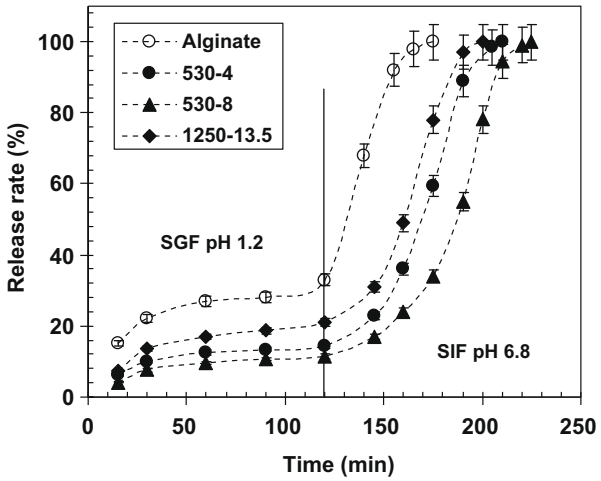


Fig. 5. Release profiles of alginate/Ca^{2+} and alginate-g-PCL/Ca^{2+} beads as a function of time in GI, 37 °C.

Other systems as microspheres of chitosan and methylcellulose with TPH encapsulated give release in SIF, which is controlled by diffusion and not by degradation. Consequently, the total release is extended up to 12 h, but 40–60% of TPH is released in a burst effect in the first two hours at pH 1.2 [36]. A similar behaviour is obtained for non-pH-sensitive scleroglucan/borax hydrogels. The release is the same for whatever the pH [37].

4. Conclusions

A new pH-responsive drug delivery system based on hydrophobically modified polysaccharidic hydrogel was developed for oral drug delivery. We have also evidenced that the release of a poorly water-soluble drug from these systems was influenced not only by the swelling and erosion of alginate-g-PCL/Ca^{2+} gel matrices, but also by hydrophobic interactions between TPH and PCL grafts.

Release of TPH from alginate beads in low pH solutions was significantly reduced compared to alginate/Ca^{2+} beads, and these systems were able to protect effectively drug from acidic environment. In SIF, the release profiles obtained can directly be corroborated with the associative behaviour in salt media of alginate-g-PCL. It is the first amphiphilic system able to form hydrophobic domains by intramolecular associations in semi-dilute

regime. However, the rapid disruption of alginate-g-PCL/Ca^{2+} beads in intestinal media resulted in a final burst release of drugs. Therefore, it could be of great interest to improve the stability of these systems in SIF that could lead to their successful application for the localized oral drug delivery to the intestinal environment. This aspect will be discussed in a study of near future.

Acknowledgment

We are grateful to the French Ministry for Research and Technology for its financial support.

References

[1] N. Anton, J.P. Benoit, P. Saulnier, Design and production of nanoparticles formulated from nano-emulsion, J. Control. Release 128 (2008) 185–199.
[2] G. Mocanu, D. Mihaï, D. Le Cerf, L. Picton, G. Muller, Synthesis of new associative gel microsphere from carboxymethyl pullulan and their interactions with lysozyme, Eur. Polym. J. 40 (2) (2003) 283–289.
[3] M.C. Jones, J.C. Leroux, Polymeric micelles – a new generation of colloidal drug carriers, Eur. J. Pharm. Biopharm. 48 (2) (1999) 101–111.
[4] W. Henni-Silhadi, M. Deyme, M.M. Boissonnade, M. Appel, D. Le Cerf, L. Picton, V. Rosilio, Enhancement of the solubility and efficacy of poorly water-soluble drugs by hydrophobically-modified polysaccharide derivatives, Pharm. Res. 24 (12) (2007) 2317–2326.
[5] N.A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, Hydrogels in pharmaceutical formulations, Eur. J. Pharm. Biopharm. 50 (1) (2000) 27–46.
[6] G. Mocanu, D. Mihaï, L. Picton, D. Le Cerf, G. Muller, Associative polysaccharides gels and their interactions with biological active substance, J. Control. Release 83 (2002) 41–51.
[7] N.A. Peppas, Hydrogels and drug delivery, Curr. Opin. Coll. Inter. Sci. 2 (1997) 531–537.
[8] P. Gupta, K. Vermani, S. Garg, Hydrogels: from controlled release to pH-responsive drug delivery, Research Focus 10 (2002) 569–579.
[9] I. Colinet, L. Picton, G. Muller, D. Le Cerf, pH-dependent stability of scleroglucan borate gels, Carbohydr. Polym. 69 (1) (2007) 65–71.
[10] V.P. Sant, D. Smith, J.C. Leroux, Novel pH-sensitive supramolecular assemblies for oral delivery of poorly water soluble drugs: preparation and characterization, J. Control. Release 97 (2004) 301–312.
[11] T. Inoue, G. Chen, K. Nakamae, A.S. Hoffman, A hydrophobically-modified bioadhesive polyelectrolyte hydrogel for drug delivery, J. Control. Release 49 (1997) 167–176.
[12] R.A. Siegel, M. Falamarzian, B.A. Firestone, B.C. Moxley, pH-controlled release from hydrophobic/polyelectrolyte copolymer hydrogels, J. Control. Release 8 (1988) 179–182.
[13] T. Coviello, P. Matricardi, C. Marianecci, F. Alhaique, Polysaccharide hydrogels for modified release formulation, J. Control. Release 119 (2007) 5–24.
[14] E.R. Morris, Molecular interactions in polysaccharide gelation, Brit. Polym. J. 18 (1986) 14–21.
[15] S. Shiraishi, T. Imai, M. Otagiri, Controlled-release preparation of indomethacin using calcium alginate gel, Biol. Pharm. Bull. 16 (1993) 1164–1168.
[16] I. Rousseau, D. Le Cerf, L. Picton, J.F. Argillier, G. Muller, Entrapment and release of sodium polystyrene sulfonate (SPS) from calcium alginate gel beads, Eur. Polym. J. 40 (2004) 2709–2715.
[17] S. Wittaya-Areekul, J. Kruenate, C. Prahsarn, Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone, Int. J. Pharm. 312 (1–2) (2006) 113–118.
[18] S.C. Chen, Y.C. Wu, F.L. Mi, Y.H. Lin, L.C. Yu, H.W. Sung, A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery, J. Control. Release 96 (2004) 285–300.
[19] S.K. Bajpai, S. Sharma, Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions, React. Funct. Polym. 59 (2004) 129–140.
[20] M. George, T.E. Abraham, Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan – a review, J. Control. Release 114 (2006) 1–14.
[21] I. Colinet, V. Dulong, T. Hamaide, D. Le Cerf, L. Picton, New amphiphilic modified polysaccharides with original solution behaviour in salt media, Carbohydr. Polym. 75 (2009) 454–462.
[22] I. Colinet, V. Dulong, T. Hamaide, D. Le Cerf, L. Picton, Unusual rheological properties of a new associative polysaccharide in salt media, Carbohydr. Polym. 77 (2009) 743–749.
[23] K.L. Shantha, D.R.K. Harding, Preparation and in vitro evaluation of poly(*N*-vinyl-2-pyrrolidone-polyethylene glycol diacrylate)-chitosan interpolymeric pH-responsive hydrogels for oral drug delivery, Int. J. Pharm. 207 (2000) 65–70.
[24] Y. Miyazaki, Y. Onuki, S. Yakou, K. Takayama, Effect of temperature-increase rate on drug release characteristics of dextran microspheres prepared by emulsion solvent evaporation process, Int. J. Pharm. 324 (2006) 144–151.
[25] <http://www.globalrph.com/pulmonary_theophylline.htm>.
[26] C.M. DeRamos, A.E. Irwin, J.L. Nauss, B.E. Stout, ^{13}C NMR and molecular modeling studies of alginic acid binding with alkaline earth and lanthanide metal ions, Inorg. Chim. Acta 256 (1997) 69–75.
[27] A. Sinquin, P. Hubert, E. Dellacherie, Amphiphilic derivatives of alginate: evidence for intra- and intermolecular hydrophobic associations in aqueous solution, Langmuir 9 (1993) 3334–3337.
[28] G. Skjåk-Bræk, H. Grasdalen, O. Smidsrød, Inhomogeneous polysaccharide ionic gels, Carbohydr. Polym. 10 (1989) 31–54.
[29] M. Rastello De Boisseson, M. Leonard, P. Hubert, P. Marchal, A. Stequert, C. Castel, E. Favre, E. Dellacherie, Physical alginate hydrogels based on hydrophobic or dual hydrophobic/ionic interactions: Bead formation, structure and stability, J. Coll. Inter. Sci. 273 (2004) 131–139.
[30] Y.S.R. Krishnaiah, S. Satyanarayana, Y.V. Rama Prasad, S. Narasimha Rao, Gamma scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers, J. Control. Release 55 (1998) 245–252.
[31] L.S. Liu, S.Q. Liu, Y.N. Steven, M. Froix, T. Ohno, J.J. Heller, Controlled release of interleukin-2 for tumour immunotherapy using alginate/chitosan porous microspheres, J. Control. Release 43 (1997) 65–74.
[32] A.L. Dainty, K.H. Goulding, P.K. Robinson, I. Simpkins, M.D. Trevan, Stability of alginate-immobilized algal cells, Biotech. Bioeng. 28 (1986) 209–216.
[33] S. Miyazaki, W. Kubo, D. Attwood, Oral sustained delivery of theophylline using in situ gelation of sodium alginate, J. Control. Release 27 (2000) 275–280.
[34] J. Liu, S. Lin, L. Li, E. Liu, Release of theophylline from polymer blend hydrogels, Int. J. Pharm. 298 (2005) 117–125.
[35] T. Coviello, F. Alhaique, C. Parisi, P. Matricardi, G. Bocchinfuso, M. Grassi, A new polysaccharide gel matrix for drug delivery: preparation and mechanical properties, J. Control. Release 102 (3) (2005) 643–656.
[36] A.P. Rokhade, N.B. Shelke, S.A. Patil, T.M. Aminabhavi, Novel interpenetrating polymer network microspheres of chitosan and methylcellulose for controlled release of theophylline, Carbohyd. Polym. 69 (2007) 678–687.
[37] T. Coviello, M. Grassi, R. Lapasin, A. Marino, F. Alhaique, Scleroglucan/borax: characterization of a novel hydrogel system suitable for drug delivery, Biomaterials 24 (2003) 2789–2798.