Chitosan Coated Alginate Beads Containing Poly(*N*-isopropylacrylamide) for Dual-Stimuli-Responsive Drug Release

Jun Shi,[1,2] Natália M. Alves,[1,2] João F. Mano[1,2]

[1] 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

[2] Polymer Engineering Department, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal

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Abstract: Chitosan coated alginate beads containing poly(*N*-isopropylacrylamide) (PNIPAAM), were prepared to be used as a controlled pH/temperature sensitive drug delivery system with improved encapsulation efficiency and delayed release rate. The studied beads were characterized by differential scanning calorimetry, scanning electron microscopy, and Fourier transform infrared spectroscopy. Water uptake and release studies using indomethacin as a model drug were also performed. The drug loading efficiency of the beads with the polyelectrolyte complex coating is significantly higher (84%) than that of the uncoated ones (74%). The equilibrium swelling of the developed materials was found to be pH- and thermo-responsive. For all the conditions it was found that the release profile was slower for the coated beads, indicating that the polyelectrolyte complex coating could slow down the release rate effectively. These results suggest that the studied smart system has potential to be used as an effective pH/temperature sustainable delivery system for biomedical applications. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 84B: 595–603, 2008

Keywords: alginate; biomaterials; chitosan; drug delivery systems; pH/temperature responsive

INTRODUCTION

Recently, there has been a great deal of research activity in the development of stimulus-responsive polymeric hydrogels. These hydrogels respond to external or internal stimuli and the response can be observed through abrupt changes in the physical nature of the network.[1,2] Among these systems, pH or temperature responsive hydrogels have been extensively studied in the biomedical field because these two factors can be easily controlled and applicable both *in vitro* and *in vivo* conditions.[3–5]

Much research has been done to associate biopolymers (such as alginate and chitosan) with thermo-sensitive macromolecules in an attempt to prepare matrixes that present a dual and independent sensitivity to both pH and temperature. Poly(*N*-isopropylacrylamide), PNIPAAM, is one of the most widely studied temperature sensitive polymers, exhibiting a temperature-dependent volume phase transition at lower critical solution temperature (LCST) around 32°C.[6–8] Many studies concerning alginate/PNIPAAM dual responsive hydrogels have been reported.[5,9–11] However, relatively little work has been reported on the potential of both pH and temperature sensitivity in these systems, to control effectively the delivery of bioactive agents. In fact, such systems present both an independent and mutual influence of temperature and pH on the swelling and drug release of the system.[12–14] For instance, the presence of a thermo-responsive material in the developed system affects itself the pH response of the pH responsive material, even when the thermo-sensitive material is above the LCST. Moreover, the LCST can be adjusted to obtain a desired swelling/drug release profile.[12–14]

The temperature/pH responsive drug release behavior of the calcium (Ca)-alginate/PNIPAAM semi-interpenetrating (semi-IPN) beads were discussed in our former article.[15] However, the relatively low drug encapsulation efficiency and the high release rate of the Ca-alginate/PNIPAAM system were the main limitations of this dual-stimuli-responsive drug delivery system.

Chitosan, a polysaccharide derived from chitin by alkaline deacetylation, has been proposed as a useful excipient for either sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds. To achieve sufficient stability, chitosan gel beads are often chemically cross-linked with glutaraldehyde and

Correspondence to: J. F. Mano (e-mail: jmano@dep.uminho.pt)
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ethylene glycol diglycidyl ether.[16,17] However, residues of theses compounds in the chitosan beads may induce undesirable side effects. Recently, polyelectrolyte complexes have been proposed for the design of drug delivery systems.[18–25] The term "polyelectrolyte" is used for polymers carrying covalently bound anionic or cationic groups, and low molecular "counterions" securing for electroneutrality.[26] A polyelectrolyte complex can be prepared by the formation of complexes resulting from the interaction of oppositely charged polyelectrolytes. For instance, cationic chitosan can form gels with nontoxic multivalent anionic counterions, such as polyphosphate and sodium alginate, by ionic interactions.

In the present work, we prepared chitosan coated alginate beads containing PNIPAAM based on the electrostatic interaction between the carboxylate groups of alginate and the ammonium groups of chitosan. This procedure is an attempt to improve encapsulation efficiency and delay the release behavior and by this way overcome the main limitations of the previously reported temperature and pH-responsive Ca-alginate/PNIPAAM beads.[15] Such effects of electrostatic barriers have been reported by some authors for pH-responsive beads.[18,19] On the other hand, as the beads described in this study contain a chitosan coating it is necessary to analyze if their pH/temperature response is different than the response corresponding to the former system, which does not contain chitosan. So, the equilibrium swelling behavior of these modified beads (compared to unaltered alginate-PNIPAAM beads), as well as their performance as a drug delivery system, is investigated as a function of both pH and temperature. It should be mentioned that the LCST of the developed systems, which is around 31°C, could be adjusted to around 37°C or even higher temperatures in a future work. Such systems could be physiologically relevant, for instance, for targeted drug release of solid tumors in the intestinal track: (1) the drug administrated orally can bypass the acidity of gastric fluids without liberating substantial amounts of drug and go into the intestinal fluids (neutral conditions); (2) the tumor is subjected to local hyperthermia (typically between 37 and 42°C), which triggers drug release.

MATERIALS AND METHODS

Materials

N-isopropylacrylamide (NIPAAM, Acros Chem.), ammonium persulfate (APS, Sigma Chem.), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Sigma Chem.), "low viscosity" sodium alginate (viscosity of 2% solution at 25°C = 250 cps, Sigma Chem.), chitosan (medium molecular weight, 85% degree of deacetylation, Sigma Chem.), and indomethacin (Fluka Chem.) were used as received.

Synthesis of PNIPAAM

Poly(*N*-isopropylacrylamide), PNIPAAM, was prepared by redox polymerization as reported elsewhere.[10] Briefly, an aqueous solution was prepared by dissolving 35.3 mmol of *N*-isopropylacrylamide and 1.35 mmol of ammonium persulfate in 45 mL deionized water. The solution was then purged with nitrogen during 30 min to induce oxygen expulsion. Afterwards, 0.267 mmol of TEMED was added. The polymerization was carried out at room temperature for 7 h. After the reaction, the product was purified by precipitation in hot water and dissolved in water repeatedly. The resultant product was dried in air overnight and then vacuum dried at 40°C for 24 h.

Preparation of Uncoated Beads

The aqueous solutions of 1.5% alginate (w/v) and PNIPAAM were mixed in the composition of PNIPAAM: alginate = 1:3 (w/w). About 20% (w/w) of indomethacin (relatively to the total weights of alginate and PNIPAAM) were added into the above solution. Thereafter, the solution was extruded in the form of droplets using a syringe into 3% $CaCl_2$ (w/v) solution under stirring at 200 rpm. The smooth, spherical, and homogenous beads obtained were kept for 30 min in $CaCl_2$ solution under stirring. After crosslinking, the beads were washed with deionized water repeatedly. The resultant beads were dried in air overnight and then vacuum dried at 40°C for 24 h.

Preparation of Chitosan Coated Alginate Beads

The chitosan coated alginate beads were prepared based on the procedure referred in other works.[18,19] A homogeneous mixture of 1.5% (w/v) sodium alginate, PNIPAAM [PNIPAAM: alginate = 1:3 (w/w)], and 20% (w/w) of indomethacin (relatively to the total weights of alginate and PNIPAAM) were dissolved in deionized water. The pH was adjusted to 5.5 ± 0.1 by adding HCl solution (1*M*) to the earlier mixture. Homogeneous aqueous solutions of chitosan in 1% (v/v) acetic acid (0.2% and 0.4%) containing 3% $CaCl_2$ were used as coagulation fluid. The solution was mixed for 1.5 h before use. The pH of the coagulation fluids was adjusted to 4.5 ± 0.1 by adding NaOH solution (1*M*) to the earlier coagulation fluids. Thereafter, the mixture of alginate, PNIPAAM, and indomethacin were extruded in the form of droplets using a syringe into coagulation fluid under mechanical stirring at 200 rpm. The smooth, spherical, and homogenous beads obtained were kept for 60 min in $CaCl_2$ solution under stirring. The resultant beads were washed once with deionized water and transferred into a 0.08% (w/v) chitosan solution (previously obtained by dissolving 0.08 g of chitosan in 100 mL of 1% (v/v) acetic acid) for 30 min, then incubated in a 0.08% (w/v) alginate solution (previously prepared by dissolving 0.08 g of alginate in 100 mL of deionized water) for 30 min and at last put into 0.5% $CaCl_2$ solution for 30 min. The beads were washed with deionized water repeatedly. The resultant beads were dried in air overnight and then vacuum dried at 40°C for 24 h.

Scanning Electron Microscopy

The morphology of the beads was examined by scanning electron microscopy (SEM) (Leica Cambridge S 360) at an accelerated voltage of 15 kV. Before being observed by SEM, the beads were gold coated using a Hitachi coating unit IB-2 coater at 6 mA.

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy (FTIR) spectra of the samples were recorded with a double-beam Perkin-Elmer 1600 FTIR spectrometer in the range of 4000–400 cm^{-1} using KBr pellets.

Determination of LCST

Differential scanning calorimetry (DSC) measurements (Setaram DSC 131) were conducted to determine the LCST of the uncoated semi-IPN and coated semi-IPN beads. First, all the beads were immersed in deionized water at room temperature and allowed to swell for 24 h. Then, the DSC analysis of the swollen beads was performed from 26 to 44°C at 3°C/min and under a nitrogen flow of 20 cm^3/min. Temperature and heat flow calibrations were carried out using a pure indium standard at the same heating rate of the experiments.

Swelling Studies

The swelling behavior of the beads was studied in phosphate buffer solutions (PBS) with two different pH, 2.1 and 7.4 (similar to that of gastric and intestinal fluids, respectively), and at two temperatures, 25 and 37°C. At predetermined time intervals, the swollen beads were weighed after wiped with soft paper tissue. The measurements were repeated at least three times, for each condition. The degree of swelling for each sample was calculated by using the following expression: Swelling ratio $= (W_s - W_d)/W_d$, where W_s and W_d are the weight of the swollen beads and that of the dried beads, respectively.

Determination of Indomethacin Encapsulation Efficiency of the Beads

The beads (10 mg) were dissolved in 100 mL of PBS (pH 7.4, containing 5% (v/v) ethanol) under stirring during 24 h. The amount of free indomethacin was determined in the clear supernatant by UV spectrophotometry at 320 nm using a calibration curve constructed from a series of indomethacin solutions with standard concentrations. Such experiments allow the calculation of both the loading efficiency and the loading content. The loading efficiency is defined as the weight percentage of loaded drug based on feed amount and the loading content is the weight percentage of drug relatively to the beads.

In Vitro Release Studies

The beads (10 mg) were suspended in 50 mL of PBS (pH 7.4 or 2.1). This dissolution medium was stirred at 50 rpm in a horizontal laboratory shaker and maintained at 37°C or 25°C. The sample (2 mL) was periodically removed and the withdrawn sample was replaced by the same volume of fresh medium. These experiments were performed at least three times. The amount of released indomethacin was analyzed with a spectrophotometer as described previously.

RESULTS

Characterization of the Beads

Three kinds of beads were prepared as listed in Table I. The wet beads just after preparation were found to be globular in shape; but upon drying in air at room temperature the sphericity was very much lost, as it is evident from SEM result of dried beads (Figure 1). It can be noted from Figure 1 that the beads are about 800–1000 μm in size and not spherical in shape. It appeared that the incorporation of chitosan in the Ca-alginate/PNIPAAM beads has greatly altered the surface texture of the beads [Figure 1(c–f)], when compared with the uncoated beads [Figure 1(a,b)]. For the uncoated beads [Figure 1(a,b)], the surface is found to be rougher than for the coated ones.

Figure 2 shows the FTIR spectra for alginate, chitosan, and the studied beads. By looking at Figure 2, it can be seen that the chemical structure of the beads is similar to alginate, which is the major fraction in the composition of the beads. For the studied beads, the characteristic peak of PNIPAAM at 1650 cm^{-1} (amide I) cannot be observed because of the strong peak of alginate at 1620 cm^{-1}, but a shoulder peak can be found at 1540 cm^{-1} associated to the amide II group of PNIPAAM. Additionally, the peak that appeared at 1365 cm^{-1} was assigned to the methyl group of PNI-

TABLE I. Composition, LCST, and Drug Loading Efficiency of the Studied Beads

Sample	Chitosan Concentration in Coagulation Fluid (%)	LCST[a] (°C)	Drug Percent (Feed, %)	Drug Content (mg/10 mg Beads)	Loading Content (%)	Loading Efficiency (%)
A	uncoated	30.7 ± 0.1	20	1.24 ± 0.03	14.8 ± 0.23	74.2 ± 1.15
B	0.2	31.1 ± 0.1	20	1.41 ± 0.03	16.9 ± 0.27	85.6 ± 1.20
C	0.4	31.1 ± 0.2	20	1.40 ± 0.02	16.8 ± 0.21	83.8 ± 1.05

[a]determined by DSC measurements.

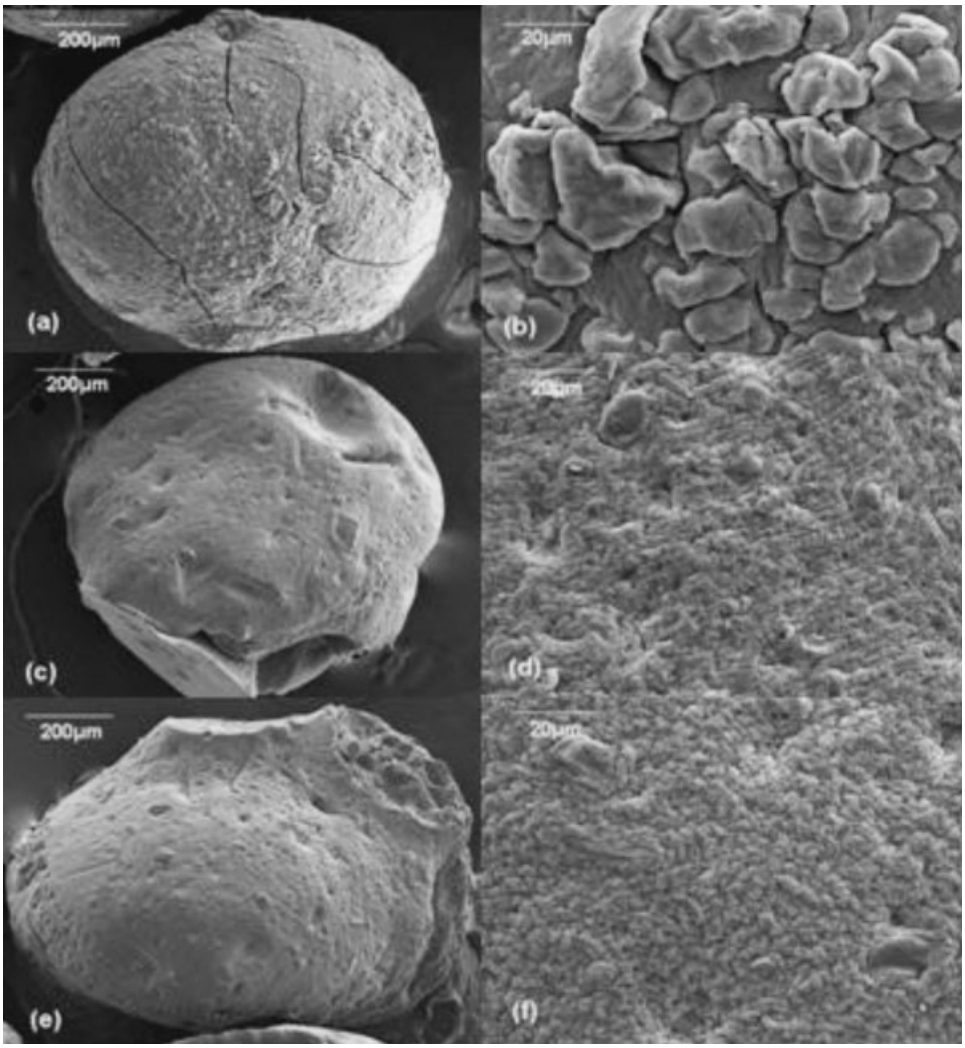

Figure 1. SEM micrographs of uncoated beads (a) and its surface morphology (b), chitosan (0.2%) coated alginate beads (c) and its surface morphology (d), chitosan (0.4%) coated alginate beads (e) and its surface morphology (f).

PAAM.[27] The peaks at 1688 cm^{-1} and 1070 cm^{-1}, which can be seen for the coated beads, are due to the presence of chitosan (see Discussion Section).

It can be observed from Table I that the loading efficiency of the uncoated beads is around 74%, while the values for the two coated beads are all around 84%. The drug within the coated beads seems to be more tightly entrapped as a result of the strong complex formed between the carboxylate groups of alginate and the protonated amine groups of chitosan.

LCST of the Beads

The LCST of the studied beads was determined by DSC measurements (Figure 3). The values of the onset temperature of the peaks (defined as LCST) are shown in Table I. The LCST was found to be around 31°C for all the beads, which is very close to the LCST of pure PNIPAAM. We also observed that the color of the studied beads in PBS (pH

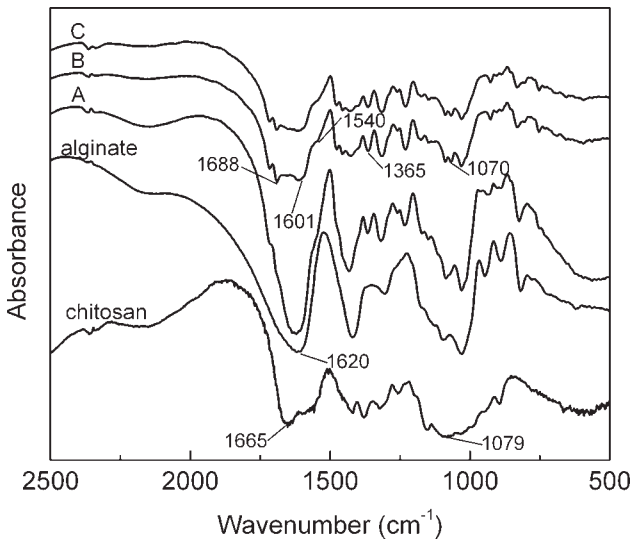

Figure 2. FTIR spectra of the studied semi-IPN beads (see Table I for the correspondence of the codes A, B, and C).

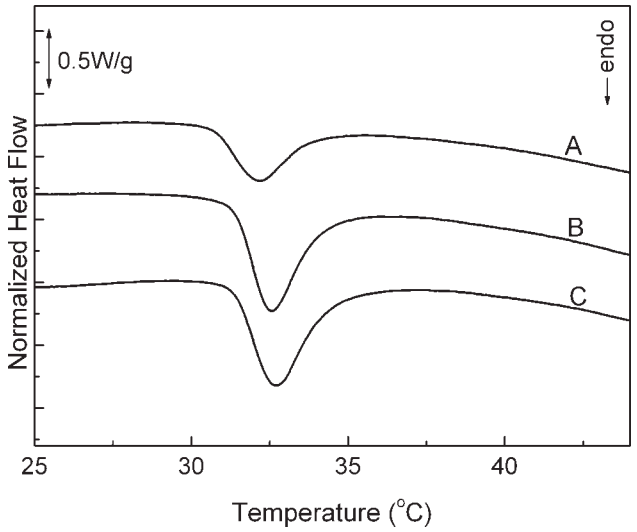

Figure 3. Temperature dependence of the normalized heat flow for the studied beads (see Table I for the correspondence of the codes A, B, and C) as studied by DSC from heating experiments at 3°C/min.

7.4) turned from almost colorless to white, when the temperature increased from below the LCST to above the LCST; this is in line with previous observations in similar systems.[9,15]

Swelling Study

Figure 4 shows the swelling behavior of the beads at different pH values and temperatures. The coated beads have a swelling property similar to alginate, since the fraction of alginate in the resultant beads is much higher than chitosan (as demonstrated by FTIR results). Figure 4 shows an obviously higher swelling degree at higher pH for all the beads. The mean values (and standard deviations) corresponding to the distinct samples can be found in Table II. Regarding the modifications in the swelling behavior after coating (Table II and Figure 4) it can be observed that the swelling ratio at pH 7.4 changes from ~30% to ~41% (25°C) and from ~32% to ~48% (37°C); at pH 2.1 the swelling ratio increases from ~11% to ~16% (either at 25°C or 37°C). Analysis by a Student's *t*-test showed that the differences between the swelling ratio of the coated and uncoated beads are statistically significant (greater than 95% confidence) both at pH 7.4 and 2.1. The *p* values were in the range 0.001–0.005 at pH 7.4 and varied between 0.02 and 0.04 at pH 2.1. So, we can say that the swelling ratio of the coated beads is higher than the one corresponding to the uncoated ones both at pH 7.4 and 2.1. Moreover, at pH 7.4 the swelling ratio of the coated beads is significantly higher at 37°C than at 25°C. Regarding the coated beads, the small differences in their swelling behavior are not statistically significant (*p* value > 0.1).

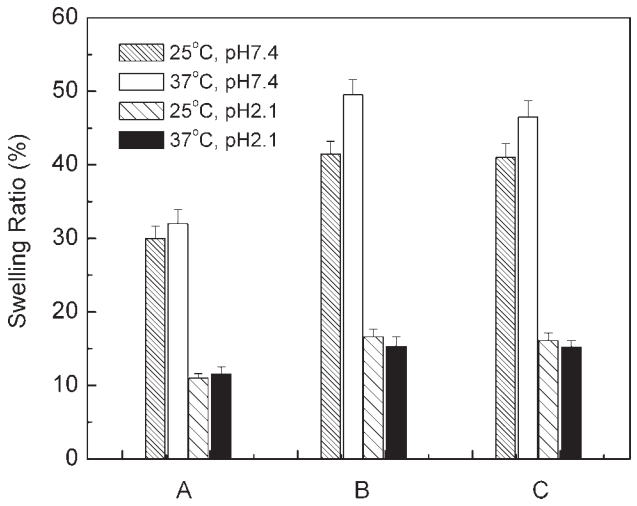

Figure 4. Temperature- and pH- dependent changes of equilibrium swelling ratio for the studied beads (see Table I for the correspondence of the codes A, B, and C).

An interesting property of the coated beads observed in our experiments is that they could keep intact during 240 min at pH 7.4 even though the swelling ratio is very high, while the uncoated beads can only maintain their integrity for about 180 min at the same conditions.

Drug Release Study

Figure 5 shows the indomethacin release profiles of the studied beads at 37°C and pH 7.4. These results suggest that the amount of indomethacin released can be delayed in the coated beads. A Student's *t*-test analysis was conducted to support the previous statement. The *p* values obtained in the comparison between the data of sample A and sample B or C were in the range 0.001–0.048 for the time period between 60 and 180 min, i.e., the difference between the data of sample A and samples B/C is statistically significant (greater than 95% confidence) for the considered time period. The release amount reaches 90% within 180 min for the uncoated beads, while a drug release of 90% will take almost 270 min for the coated beads.

Figure 6 presents the drug release behavior at 37°C for all the samples at pH 2.1 and pH 7.4. A significant pH-depend-

TABLE II. Temperature- and pH- Dependent Changes of Equilibrium Swelling Ratio for the Studied Beads

	pH 7.4		pH 2.1	
	25°C	37°C	25°C	37°C
A				
Average value	30.0	32.0	11.0	11.5
Standard deviation	1.7	1.9	0.6	1.0
B				
Average value	41.5	49.5	16.6	15.3
Standard deviation	1.7	2.1	1.1	1.2
C				
Average value	41.0	46.5	16.1	15.2
Standard deviation	1.9	2.2	1.0	0.9

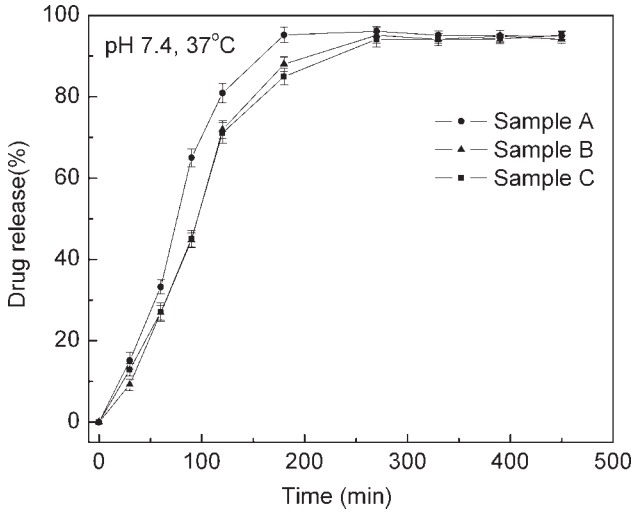

Figure 5. Release profiles of indomethacin from the studied beads (see Table I for the correspondence of the codes A, B, and C) measured at 37°C and pH 7.4.

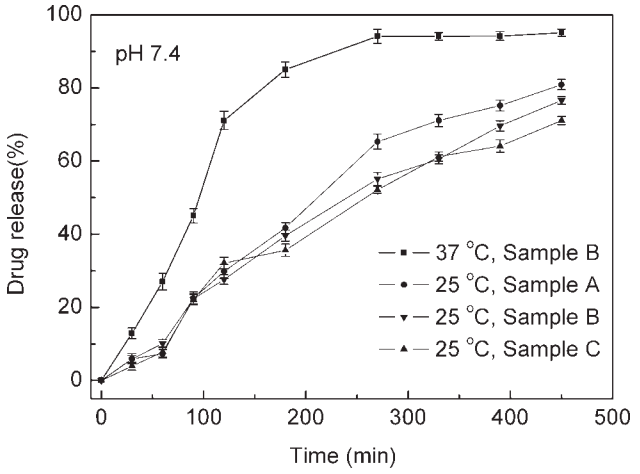

Figure 7. Temperature-dependent release profiles of indomethacin from the studied beads (see Table I for the correspondence of the codes A, B, and C) at pH 7.4 measured at 25°C and 37°C.

ent response can be observed for both coated beads, which is similar to the release behavior of the uncoated ones described in a previous article.[15] The release behavior at pH 2.1 is characterized by an initial burst after which a very slow release is observed. It can also be noted from Figure 6 that the release amount of the coated beads (about 3%) is lower than for the uncoated one (about 10%) at pH 2.1. Analysis by a Student's *t*-test showed that the differences between these results are statistically significant. The *p* values obtained in the comparison between the data of sample A and sample B/C were in the range 0.0003–0.04. It should be pointed that indomethacin solubility in acidic conditions is much lower than in neutral solutions. Therefore besides the response of alginate to pH, clearly detected in swelling (Figure 4), the release profile may also be dependent on pH due to the different solubility of indomethacin.[28]

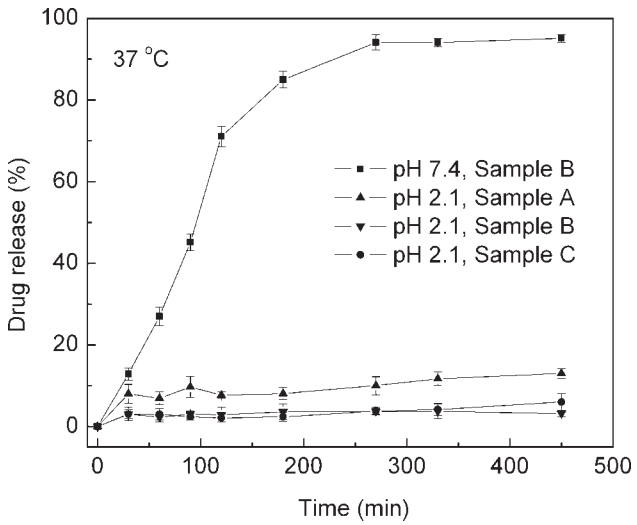

Figure 6. pH-dependent release profiles of indomethacin at 37°C from the studied beads (see Table I for the correspondence of the codes A, B, and C) measured at pH 2.1 and 7.4.

Figure 7 shows the drug release profiles from samples in a buffer solution of pH 7.4 at 37°C and 25°C. A temperature dependent response can be observed for both coated beads: higher release rates are obtained at 37°C, while lower release rates can be observed at 25°C. It can also be observed that for the coated beads the drug release rate seems to be lower than for the uncoated one at pH 7.4 and 25°C. Student's *t*-test analysis showed that the difference between the release of sample A and samples B and C is statistically different for times higher than 180 min (*p* values between 0.002 and 0.03). For the referred time period, the drug release was 7–25% higher for sample A than for samples B and C.

It should be pointed that no statistically significant differences were found in the drug release behavior at different pH and temperatures between the two coated beads (*p* value > 0.1).

DISCUSSION

Characterization of the Beads

The change in the texture of the outer surface for the coated beads [Figure 1(c–f)] when compared with the texture of the uncoated ones [Figure 1(a,b)] should be related to the formation of a polyelectrolyte complex coating between alginate and chitosan. We expect that this skin layer could reduce the loss of entrapped indomethacin during the preparation process, delaying the release of the drug in the following drug release experiments. The roughness observed for the uncoated beads probably results from the aggregation of indomethacin. Moreover, it seems that the surface texture of the coated beads is not affected by the different chitosan concentrations used in this study, because there are no obvious differences between Figures 1d and 1f.

Compared to the characteristic adsorption peaks of alginate and chitosan from the FTIR spectra of studied beads

(Figure 2), one can easily find that the chemical structure of the beads is similar to alginate. This result indicated that most of the bound chitosan was accumulated only at the surface due to the hindrance of macromolecular chitosan from further diffusing into the inner core.[20] For the uncoated beads (sample A), the characteristic peak of alginate is seen at 1620 cm^{-1} corresponding to the carbonyl (C=O) bond. After coating, the peak of 1620 cm^{-1} was replaced by a new broad band (1601 cm^{-1}), as a result of the interaction between the negatively charged $—COO^-$ groups of alginate (cross-linked with $CaCl_2$) and the positively charged $—NH_3^+$ groups of chitosan. Additionally, two new peaks at 1688 cm^{-1} and 1070 cm^{-1} can be seen for all the coated beads, which can be explained in terms of the unreacted chitosan.[22,29] These peaks are derived, respectively, from the peaks of 1665 cm^{-1} and 1079 cm^{-1} of chitosan. The slight shifts in the position of these peaks for the coated samples could be attributed to the electrostatic interaction between the carboxylate groups of alginate and the ammonium groups of chitosan after reaction. These results suggest the formation of a chitosan-alginate complex as a result of the ionic interaction.

As suggested from SEM results (Figure 1), the drug on the outer surface of the beads is covered by the polyelectrolyte complex membrane, which inhibits the loss of the entrapped drug during the gelling and washing processes. As a result, more indomethacin could remain in the beads. An increased load of other drugs in chitosan-coated alginate beads has also been reported.[18,19,23,30] It can also be noted from Table I that the chitosan concentration has little effect on the loading efficiency. So, the electrostatic interaction between chitosan and alginate tightens and stabilizes the surface of the beads. This means that polyelectrolyte complex coatings can improve the encapsulation yield effectively in such kind of systems.

LCST of the Beads

When a swollen PNIPAAM hydrogel is heated above the LCST, the PNIPAAM chains collapse and this is accompanied by a drastic contraction of the gel.[6,8] This behavior can be explained by the reversible formation (below LCST) and cleavage (above LCST) of the hydrogen bonds between —NH and C=O groups of PNIPAAM chains and the surrounding water molecules.[31] The LCST results listed in Table I indicates that there is no chemical bond or other strong interactions between the PNIPAAM fraction and the other components of the system, which could change the balance between hydrophobic and hydrophilic interactions in PNIPAAM.

The studied beads in PBS (pH 7.4) turned from colorless to white when temperature changed from 25 to 37°C. It was suggested that the formation of the white core region generated above the LCST was due to the collapse and shrinkage of the PNIPAAM network out of the semi-IPN composite structure, which could lead to an increase of light diffusion.[9] The presence of nearby physically entangled alginate and chitosan polyelectrolyte complex chains do not affect the collapse of the PNIPAAM chain network above the LCST.

Swelling Study

In the low pH region (pH 2.1) most of the carboxylic acid groups in alginate are in the form of —COOH, as the pK_a of alginate is about 3.2. The hydrogen bonds between —COOH in alginate and —CONH— in PNIPAAM leads to polymer–polymer interactions predominating over the polymer–water interactions. As a result, the swelling ratio of the studied beads is relatively low. When the pH of the medium is changed to 7.4 the carboxylic acid groups become ionized and a small quantity of H^+ in water acts as the bridge among alginate, resulting in the increase of the swelling ratio.[32] The higher swelling ratio presented by the coated beads when compared with the uncoated ones, independently of pH or/and temperature values is attributed to the presence of chitosan at the surface. In fact chitosan by itself presents extremely high swelling and water uptake capabilities (~140% at neutral pH) as the work of Silva et al.[33] Also, a high temperature can help the water to penetrate into the semi-IPN beads, which contributes to the higher swelling ratio of the coated beads at 37°C when compared with their swelling at room temperature. Such observations have been already reported before.[15]

Drug Release Study

A slight, but significant, delay in the drug release can be observed for the coated beads at 37°C and pH 7.4 (Figure 5). This result is distinct from the results reported in other studies for chitosan-coated alginate beads without PNIPAAM,[18,19,22,23] which revealed that the drug release could be significantly delayed after coating. We expect that the difference in the release profile could be more evident in beads coated with more layers.[34] It was also observed no significantly different drug release behavior at 37°C and pH 7.4 for beads coated with different chitosan concentration.

The relatively low release amount of the studied beads at pH 2.1 (Figure 6) should be related to the low degree of swelling ratio of the beads in acidic conditions as shown in Figure 4. Although we did not expect the contribution of chitosan coating, which should dissolve at pH 2.1, we were able to observe that the coated beads exhibit a slightly slower release profile. The amount of released drug at pH 7.4 increases significantly (about 95% within 450 min), which can be related to the higher swelling ratio at neutral pH compared to pH 2.1 (Figure 4). The relatively low release amount of the coated beads (about 3%, compared to 10% for the uncoated ones) at pH 2.1 is attributed to the polyelectrolyte complex coating. So, although chitosan itself is expected to dissolve at pH 2.1, its dissolution may not be complete when it forms a physical network with alginate and/or PNIPAAM, and thus, can still provide the release barrier. Therefore, in an ingestible pharmaceutical application,

the coated beads are more efficient than the uncoated ones to bypass the acidity of gastric fluid without liberating substantial amounts of the loaded drug.

Regarding the results of Figure 7, the main reason to explain the higher release rate at 37°C and pH 7.4 is the precipitation of PNIPAAM above LCST, which leads to the squeezing out of the drug.[8,15,19] Additionally the swelling ratio of the coated beads is higher at 37°C than at 25°C as described in Figure 4, which can also result in a higher drug release rate. It was also found that for the coated beads the coating effectively decreases the drug release rate at pH 7.4 and 25°C.

It must be noted that the drug release results presented here were obtained with beads prepared with an alginate of low viscosity and low molecular weight (2 wt % = 250 cps). In fact, different results would be probably obtained if an alginate with a higher viscosity at 2 wt % (higher molecular weight) is used, because it is expected that the resulting variations in the molecular pore structure would affect the indomethacin diffusion. In terms of viscosity, it should be mentioned that to prepare such kind of beads (coated or uncoated) it is easier to use a low viscosity alginate (with values close to the one referred in the experimental section), otherwise it will be very difficult to extrude the mixture of alginate, PNIPAAM, and model drug with a syringe, in the form of droplets.

CONCLUSIONS

A pH/temperature sensitive drug delivery system, based on chitosan coated alginate beads containing PNIPAAM, was successfully prepared. The chitosan coating could (i) increase the loading efficiency and (ii) marginally decrease the release rate. Despite the chitosan coating, the alginate/PNIPAAM beads maintained the pH- and thermo- responsive behavior. The results obtained in this work could be useful in the development of smart hydrogels for controlled release of bioactive agents or for tissue engineering applications.

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