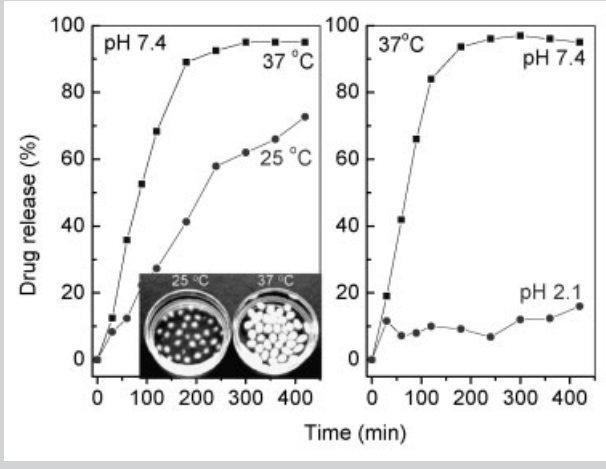
DOI: 10.1002/mabi.200600013

Summary: A series of semi-interpenetrating, polymer network (semi-IPN), hydrogel beads, composed of calcium alginate (Ca-alginate) and poly(*N*-isopropylacrylamide) (PNIPAAM), were prepared for a pH/temperature-sensitive drug delivery study. The equilibrium swelling showed the independent pH- and thermo- responsive nature of the developed materials. At pH = 2.1, the release amount of indomethacin incorporated into these beads was about 10% within 400 min, while this value approached to 95% at pH = 7.4. The release rate of the drug was higher at 37 °C than that at 25 °C and increased slightly with increasing PNIPAAM content. These results suggest that the Ca-alginate/PNIPAAM beads have the potential to be used as an effective pH/temperature sustainable delivery system of bioactive agents.



A summary of the temperature- and pH-dependence on the release of the drug over a period of 450 min. The effect of the temperature on the swelling of the beads is shown in the inset.

Drug Release of pH/Temperature-Responsive Calcium Alginate/Poly(*N*-isopropylacrylamide) Semi-IPN Beads

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Received: January 24, 2006; Revised: March 9, 2006; Accepted: March 10, 2006; DOI: 10.1002/mabi.200600013

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

Introduction

Stimuli-responsive hydrogels have attracted great interest recently due to their potential application in areas such as drug delivery, tissue engineering and biosensors.[1–4] 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 are applicable both *in vitro* and *in vivo* conditions.[5–7]

Alginate is a pH-sensitive and biocompatible hydrogel with a relatively low cost. Its dissolution and biodegradation under normal physiological conditions enables it to be used as a matrix for the entrapment and delivery of proteins, drugs and cells.[8–10] Recently, much research has been done to associate biopolymers 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 with the lower critical solution temperature (LCST) around 32 °C.[11–13] Several studies concerning alginate/PNIPAAM hydrogels have been reported.[7,14–19] For example, Ju et al.[7,18] studied the pH/temperature dependence of the swelling behavior of alginate/PNIPAAM hydrogels and Guilherme et al.[15] demonstrated the water

Table 1. Composition, LCST and drug loading efficiency of the prepared semi-IPN beads.

Sample	PNIPAAM/ alginate ratio	LCST[a)]	ΔH_c[a)]	Drug percent	Drug content	Loading content	Loading efficiency
	w/w	°C	$J \cdot g^{-1}$	feed, %	mg · (10 mg beads)$^{-1}$	%	%
A	Pure alginate	–	–	20	1.19	14.4	72
B	1:6	33.2	2.19	20	1.21	14.6	73
C	1:3	33.6	3.71	20	1.25	15	75
D	1:2	33.9	4.92	20	1.26	15.2	76
E	1:1.3	33.9	5.54	20	1.24	15	75
F	1:3	33.7	4.24	0	–	–	–

[a)] Values were determined by DSC measurements.

permeability of alginate/PNIPAAM membranes. However, neither of these above-mentioned studies investigated the potential of both pH- and temperature-sensitivity in these systems, in order to control more effectively the delivery of bioactive agents. Park and Choi[14] prepared interpenetrating, polymer network (IPN) hydrogel beads composed of alginate and PNIPAAM and investigated the temperature–modulated drug release with indomethacin. They only focused on the temperature-sensitive behavior due to the presence of PNIPAAM. In their case, the alginate was only used as a biocompatible and biodegradable polymer.

In this work, the pH/temperature-sensitive release of indomethacin from semi-IPN hydrogel beads composed of Ca-alginate and PNIPAAM is investigated. The concept presented in this work includes the dispersion of indomethacin and PNIPAAM (previously synthesized) in an alginate aqueous solution. Then hydrogel beads were formed by precipitating the mixture into a Ca^{2+} solution. We hypothesize that the combination of the pH-responsive property of alginate and the temperature sensitivity of PNIPAAM could provide a new and efficient smart drug delivery system with dual stimuli response. The LCST of the developed system with different Ca-alginate/PNIPAAM weight fractions was determined by differential scanning calorimetry (DSC). The equilibrium swelling performance and dual sensitive release behavior from indomethacin-loaded beads were studied at different temperatures in phosphate buffer solutions (PBS) at pH = 2.1 and 7.4.

Experimental Part

Materials

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

Synthesis of PNIPAAM

PNIPAAM was synthesized by redox polymerization as reported elsewhere.[15] Briefly, an aqueous solution was prepared by dissolving 35.3 mmol of NIPAAM and 1.35 mmol of APS in 45 ml of deionized water. The solution was then purged with nitrogen over a period of 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 reactant 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 Semi-IPN Beads

At first, alginate and PNIPAAM were dissolved into deionized water separately. Then, their solutions were mixed in various compositions as described in Table 1. The alginate concentrations were maintained at 1.5% (w/v) for all the solutions. Indomethacin was mixed with the solution at the ratio of 20% (w/w) (relatively to the total weights of alginate and PNIPAAM) and then stirred gently for 6 h at room temperature. Thereafter, the solution was extruded in the form of droplets, using a syringe, into 1.5% $CaCl_2$ (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.

Determination of LCST

DSC measurements (Perkin-Elmer DSC 7) were conducted in order to determine the LCST of the alginate/PNIPAAM 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^{-1} and under a nitrogen flow of 20 $cm^3 \cdot min^{-1}$. Calibration for the temperature and heat flow was 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 PBS at two different values of the pH, pH = 2.1 and 7.4 (similar to that of gastric and intestinal fluids respectively), and at two temperatures, 25 and 37 °C. At pre-determined time intervals, the swollen beads were weighed after having been wiped with soft paper tissue. 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 for 24 h. The amount of free indomethacin was determined in the clear supernatant by UV spectrophotometry at 329 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 the feed amount and the loading content (%) is the weight percentage of drug, relative 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. The amount of released indomethacin was analyzed with a spectrophotometer as described previously.

Results and Discussion

Preparation and Characterization of the Semi-IPN Beads

Five kinds of beads were prepared as listed in Table 1. The wet beads, just after preparation, were found to be globular in shape; while the dried beads were not spherical, especially for those with a higher PNIPAAM content (D and E). Similar observations for Ca-alginate beads have been reported previously.[20] It was suggested that this phenomenon is a result of both the alginate/PNIPAAM/indomethacin interactions and the loss of entrapped indomethacin during the preparation process.[20] In the present work we also observed that the size of beads increased with increasing the PNIPAAM content. For all the samples, the loading efficiencies and the loading contents were around 75% and 25%, respectively (Table 1).

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.[8,13] 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 the PNIPAAM chains and the surrounding water molecules.[19] In the current work the LCST of the semi-IPN beads was determined by DSC measurements. The values of the onset temperature of the peaks (defined as LCST) and the endothermic enthalpies (ΔH_c) are shown in Table 1. For all the semi-IPN beads (with or without drug), a clear LCST can be observed between 33 and 34 °C, which is very close to the LCST of pure PNIPAAM. This indicates that there is no chemical bond or other strong interaction between alginate, PNIPAAM and the drug, which may change the balance between the hydrophobic and hydrophilic interactions in PNIPAAM. As expected, the value of ΔH_c increases with increasing the PNIPAAM content (Table 1).

The color of the semi-IPN beads in PBS (pH = 7.4) changed from almost colorless to white, when the temperature increased from below the LCST to above the LCST, in the present work; this is in line with previous observations in similar systems.[14,21] 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.[14,21] By using optical microscopy, Park and Choi[14] demonstrated that the beads were composed of a PNIPAAM-rich core and a hydrophilic alginate–rich shell layer above the LCST. This suggests that the concentric double layers in the semi-IPN gel beads are caused by the strong collapsing force of the PNIPAAM chain network above the LCST, but not affected by the presence of nearby physically-entangled alginate chains.

Swelling Study

Figure 1 displays the swelling behavior of the beads prepared at different pH values and temperatures. An obviously higher swelling degree can be observed at a higher pH value. In the low pH region (pH = 2.1) most of the carboxylic acid groups in the alginate were in the form of –COOH, as the pK_a of alginate is about 3.2. The hydrogen bonds between the –COOH in alginate and the –CONH– in PNIPAAM lead to polymer-polymer interactions predominating over the polymer-water interactions. As a result, the swelling ratio of alginate/PNIPAAM semi-IPN beads is relatively low. When the pH of the medium was changed to 7.4 the carboxylic acid groups became ionized and a small quantity of H^+ in water acts as the bridge among alginate, resulting in the increase of the swelling ratio.[16]

By looking at the swelling behavior of the semi-IPN beads at pH = 7.4 (Figure 1), it can be observed that at 25 °C there are no significant differences among the swelling ratios of all the samples. However, the swelling ratio decreases significantly from 52 (sample A) to 35 (sample E) at 37 °C. This phenomenon is attributed to the collapse of

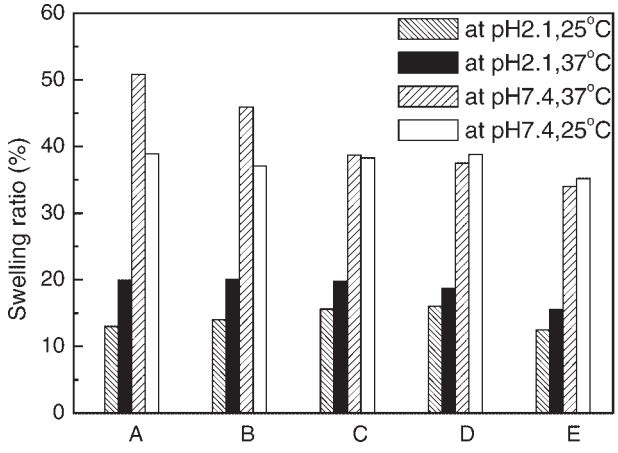

Figure 1. Temperature- and pH-dependent changes of the swelling ratio, for semi-IPN beads with different compositions.

the PNIPAAM chains at 37 °C, conducting the hydrogel to a more hydrophobic state.[22] A higher PNIPAAM content can tune the hydrogel more hydrophobic, leading to a decrease of the swelling ratio.

Drug Release Study

Figure 2 shows the indomethacin release profiles at 37 °C from sample C, at pH = 2.1 and 7.4. The amount of indomethacin released at pH = 2.1 is characterized by an initial burst (about 10%), after which almost no further release is observed. The initial burst release may be attributed to the release of indomethacin molecules loaded near the surfaces of the beads, and the low amount of the release is probably related to the low degree of swelling ratio of the beads in acidic conditions as shown in Figure 1. The amount of released drug at pH = 7.4 increases significantly (about 95% within 400 min), which may be related to the higher swelling ratio at neutral pH compared to pH = 2.1 as shown in Figure 1. A similar behavior has also been reported by other researchers.[23,24] Thus, on a more practical point of view, these biocompatible bead systems can bypass the acidity of gastric fluid without liberating substantial amounts of the loaded drug.

Figure 3 shows the drug release profiles from samples B and E in a buffer solution at pH = 7.4. For both samples higher release rates were obtained at 37 °C while lower release rates were observed at 25 °C. One plausible explanation is that the effective crosslinking density of the calcium-alginate network would be reduced by the precipitation of PNIPAAM, which would accelerate the drug release.[13] Therefore, the precipitation of PNIPAAM in the gel matrix plays a critical role in squeezing out the entrapped drug molecules from the gel beads at 37 °C. Another plausible explanation is that the disruption of the calcium-alginate gel matrix is apt to occur in PBS above

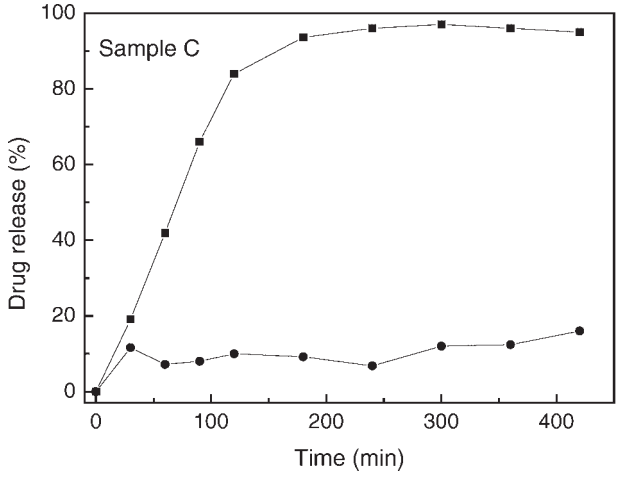

Figure 2. pH-dependent release profiles at 37 °C for sample C measured at pH = 2.1 (●) and 7.4 (■).

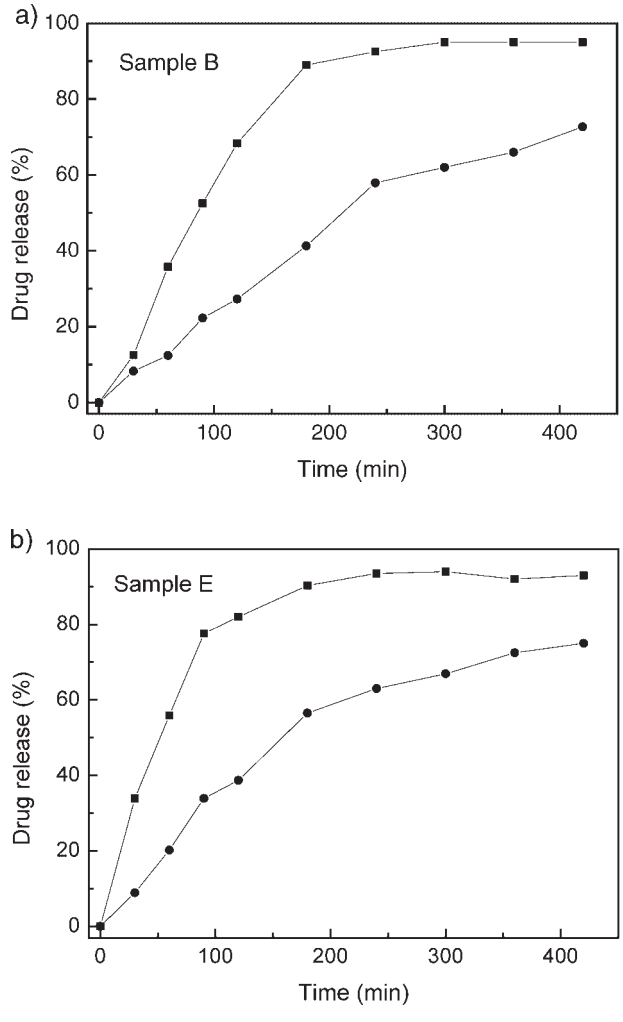

Figure 3. Temperature-dependent release profiles at pH = 7.4 for (a) sample B and (b) sample E, measured at 25 °C (●) and 37 °C (■).

pH = 5.5, due to the chelating action of the phosphate ions.[23] At these neutral pH values the affinity of phosphate for calcium is higher than that of alginate, and the solubility of the calcium-phosphate complex, once formed, increases. The squeezing property of PNIPAAM at 37 °C can break the balance of the semi-IPN network and, hence, accelerate the disruption of the beads. As a result, faster drug release rates can be observed from the matrix.

It must be noted that the release rate obtained in the current work is lower than the rate reported by Park and Choi[14] at 25 °C and pH = 7.4. The distinct drug loading processes may be responsible for the different release rates. In the work of Park and Choi[14] indomethacin was loaded into the beads by the solvent sorption method, while in the present work the same drug was incorporated into the beads during the process of the bead formation. When the solvent sorption method is used, it is not easy for the drug to enter in the centre of the beads and most of the drug will probably be at the surface of the beads, leading to a fast release. By adopting the procedure of the present work, the drug could be easily incorporated into the centre of the beads, resulting in a relatively low release rate.

Figure 4 presents the effect of PNIPAAM content on the drug release behavior at 37 °C and pH = 7.4. A tendency for an increase of drug release with increasing PNIPAAM content can be observed within 100 min. Note that at this temperature PNIPAAM is above its LCST. There is no significant difference in the drug release behavior between samples A and B within 100 min, since the PNIPAAM content of sample B is very low and the squeezing property of PNIPAAM at 37 °C is not dominant in governing the drug release rate. It can also be found that there is almost no difference in the release behavior between samples D and E. It seems that the squeezing effect of PNIPAAM at 37 °C achieves the maximum level when the PNIPAAM:alginate ratio reaches 1:2.

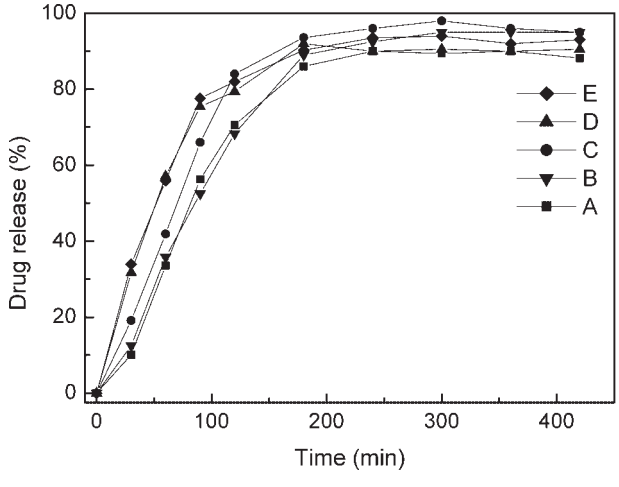

Figure 4. Effect of PNIPAAM content on the drug-release behavior for the samples at 37 °C and pH = 7.4.

Conclusion

A pH/temperature-sensitive drug delivery system based on semi-IPN hydrogel beads composed of Ca-alginate and PNIPAAM was proposed. The LCST of the beads measured by DSC was around 33 to 34 °C, being independent of the composition. The equilibrium swelling measurements of the beads clearly demonstrated the independent pH- and temperature-responsive nature of the materials. The drug release behavior of indomethacin from the beads was analyzed as a function of both pH and temperature. A drastic change in drug release was achieved by alternating the pH of the buffer solution and it was attributed to the change of states of ionic groups within semi-IPN beads. The release rate was much faster at 37 °C than at 25 °C due to the squeezing-out effect originating from the precipitation of PNIPAAM. Above the LCST, a tendency for an increase of drug release with increasing PNIPAAM content could also be observed. These results suggest that the Ca-alginate/PNIPAAM beads have the potential to be used as an effective pH/temperature-responsive drug delivery system in the biomedical field.

Acknowledgements: This work was partially supported by the *Portuguese Foundation for Science and Technology (FCT)*, through the POCTI/FIS/61621/2004 project and the POCTI and FEDER programs, and by the *European Union*-funded STREP Project HIPPOCRATES (NMP3-CT-2003-505758). J. Shi thanks the PostDoc Grant from *FCT* (SFRH/BPD/20872/2004/WN42).

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