

Drug release behavior of a pH/temperature sensitive calcium alginate/poly(*N*-acryloylglycine) bead with core-shelled structure

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Abstract. In this study, a novel pH/temperature sensitive hydrogel bead with core-shelled structure, composed of calcium alginate (Ca-alginate) and poly(*N*-acryloylglycine) (PAG), was prepared using as a drug delivery system. The equilibrium swelling has indicated the distinct sensitivities of the beads to pH value and temperature. In pH = 7.4 phosphate buffer solution (PBS), the cumulative release amount of indomethacin loaded in the core of the beads was about 83.5% within 650 min, whereas this value only reached 16.6% in pH = 2.1 PBS. In addition, the release rate of indomethacin was much faster at 37°C than that at 24°C. The experimental results have showed that the Ca-alginate/PAG beads have a potential application for the pH/temperature-controlled drug release carrier in the biomedical field.

Keywords: smart polymers, drug release, core-shelled bead, pH/temperature sensitive

1. Introduction

A smart hydrogel is a three-dimensional polymeric network containing a large number of hydrophilic groups, which is capable of absorbing large quantities of water, but does not dissolve [1]. These hydrogels show the ability of abrupt change including the physical or chemical properties in response to the external stimuli such as pH [2–5], temperature [4–6], electrical field [7, 8], magnetic field [9], ion exchange [10]. The unique properties of a smart hydrogel imply that it can be used as a promising material in the biomedical and industrial applications. Some significant applications of hydrogels have been widely investigated including dialysis membranes [11], enzyme immobilization [12], tissue engineering [13], and drug controlled release systems [14–17] and so on. Among the smart materials, pH- and temperature-sensitive hydrogels have

been paid more attentions because the two physiological factors are very important for the human body [18–21].

To prepare a stimulus-sensitive polymeric hydrogel with biocompatibility and biodegradation, many efforts have been made to combine the thermo-sensitive polymers with some biopolymers such as β -cyclodextrin, starch, alginate and chitosan [22–24]. Alginate is a kind of water-soluble linear polysaccharide separated from brown sea weed and is composed of alternating blocks of 1–4 linked α -*L*-guluronic and β -*D*-mannuronic acid residues. Alginate can be cross-linked by the chelating effect of divalent cations such as calcium ion in the aqueous solution, leading to a facile preparation of hydrogel beads. As a pH-sensitive natural material, alginate is relatively low cost [25]. As a result, alginate is often selected as a matrix for the entrapment and

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delivery of proteins, drugs, and cells because of its biocompatibility and biodegradability under the normal physiological conditions [26–28].

Recently, it was reported that many amino acid moieties have been attractive candidates for synthesizing hydrogel materials to improve their biodegradability and biocompatibility [29–31]. For instance, a new enzymatically degradable temperature-sensitive biomaterial, poly(ethylene glycol)-*b*-poly(alanine-co-phenyl alanine) was synthesized by introducing amino-acid moiety as the main component [30]. Based on glycine, El-Sherbiny *et al.* [31] prepared a novel biodegradable pH/thermo-responsive poly(*N*-acryloylglycine-chitosan) hydrogel, for using as a controlled drug delivery carrier. In addition, our recent experiments have shown the injectable behaviors of glycine-based PAG with the temperature changing. PAG aqueous solution is in fluid state at higher temperature, while PAG solution reversibly turns into solid hydrogel at lower temperature. The H-bonding network among carboxyl (–COOH), amide (–CONH) groups in PAG chains and water molecules plays a crucial role in the transition of sol to gel.

In this investigation, we described the fabrication of a novel pH/temperature sensitive bead with core-shelled structure for the drug release carrier. The pH/temperature sensitive hydrogel beads were composed of Ca-alginate and PAG. The sensitive, core-shelled beads were prepared by three steps: the core formation, the swelling in sodium alginate solution and the cross-linking of shell. The pH-sensitivity of the beads originates from the shell composed of alginate and its temperature-sensitivity is mainly attributed to the core of PAG hydrogel. Theoretically, the combination of alginate and PAG could provide a new and efficient smart drug delivery system with a dual pH-temperature sensitivity. The release behavior of indomethacin from the bead with core-shelled structure was studied as function of the PAG concentration, temperature and pH in phosphate buffer solutions simulated to gastric (pH = 2.1) and intestinal fluids (pH = 7.4).

2. Experimental part

2.1. Materials

Sodium alginate (viscosity of 2% solution at 25°C = 250 cps, Wuhan Sigma Chem. Co., Ltd., China) and indomethacin (Fluka Chem. Co., Ltd.) were used as received. Acryloyl chloride was supplied by Merck (Schuchardt OHG, Hohenbrunn, Germany). Glycine was purchased from Dongfang Health Materials Factory (Tianjin, China). All other chemicals were analytical grade and used without any further purification.

2.2. Measurements

¹H NMR spectrum of PAG was measured using a Bruker Avance400 (400 MHz) (Bruker, Switzerland) with tetramethylsilane (TMS) as internal reference. Fourier-transform infrared (FTIR) spectra were recorded on a Vector22 FTIR spectrophotometer (Bruker, Switzerland) in KBr pellet. Elemental analysis results were taken using a Elemental Analyzer CE-440 (Exeter Analytical, Inc., USA).

2.3. Preparation of the core-shelled beads

The composition of prepared beads was described in Table 1. Firstly, poly(*N*-acryloylglycine) (PAG) prepared from glycine and acryloyl chloride according to the described procedure [31] and sodium alginate were dissolved in deionized water, respectively. The sodium alginate concentrations were controlled at 1.5% (w/w) for all the samples in this study. Drug model, indomethacin was added into PAG solution at the ratio of 20% (w/w) (relative to the total weights of PAG). Secondly, using a syringe, the hot PAG-indomethacin solutions were extruded in the form of droplets into the cold acetate ethyl/chloroform under stirring at the rate of 60 rpm. The core of the titled bead was formed because of injectability of PAG. The indomethacin loaded beads (with the average diameter 3 mm) were transferred to sodium alginate solution at

Table 1. Composition and drug content of the core-shelled beads

Sample	Alginate concentration [% (w/w)]	PAG concentration [% (w/w)]	Drug feed to PAG [%]	Loading efficiency [%]	Drug content [%]
A	1.5	10.0	20.0	75.2	0.31
B	1.5	15.0	20.0	76.8	0.46
C	1.5	20.0	20.0	78.9	0.61

room temperature. After 1 hour, the bead swelled to some extent so as to get the shell structure of the bead. Lastly, the bead with core-shelled structure were immersed in the 1.5% CaCl_2 (w/v) solution for 30 minutes. The spherical, homogenous and core-shelled beads were obtained via the further crosslinking in the shell. After crosslinking, the beads were washed with deionized water repeatedly to remove the un-crosslinked sodium alginate.

2.4. Swelling investigation

In this study, the swelling behavior of the beads was studied in different pH PBS and temperature. The pH values were simulated to gastric pH = 2.1 and intestinal fluids pH = 7.4 in human body, respectively. The beads were immersed in the required medium. After reaching the swelling equilibrium, the beads were taken out and wiped with soft paper tissue to remove the water on the bead surface, and then weighted. The swelling ratio (SR) for each sample was calculated by using Equation (1):

$$SR [\%] = \frac{W_s - W_d}{W_d} \cdot 100 \quad (1)$$

where W_s and W_d represent the weights of hydrogels after and before swelling, respectively.

2.5. Loading content

The beads (about 25 mg) were crushed with a glass rod in a beaker and charged with 20 ml of PBS (pH = 7.4, containing 5% (v/v) ethanol) under stirring for 12 h. The clear supernatant was collected in another flask. Again, another 20 ml of PBS was added into the beaker for the further dissolving drug. The experimental process was repeated until no indomethacin was determined in the new clear supernatant by UV measurement. The amount of indomethacin loaded in the beads was determined

by UV spectrophotometry at 329 nm using a calibration curve constructed from a series of standard indomethacin solutions. The loading content [%] is the weight percentage of drug indomethacin relative to the beads in this study.

2.6. In vitro indomethacin release

The drug release experiments were carried out by immersing the beads (about 0.040 g) in the desired 40 ml medium in a separate 100 ml flask. The flask was controlled at a required temperature and equipped with a magnetic stirrer. At the predetermined time interval, 4 ml release medium was taken out and the fresh medium with same volume was added to the flask to maintain the unchanged volume. The amount of indomethacin released from the beads was determined at 329 nm with a UV spectrometer. In addition, the data used in this paper were obtained from the average value of three determined value.

3. Results and discussion

3.1. Preparation of the bead with core-shelled structure

The fabrication strategy for the bead with core-shelled structure is illustrated in Figure 1. First of all, it is necessary to state that our recent experiment has indicated the obvious injectability of PAG selected in this work. PAG in water is in the form of solution at higher temperature, while PAG solution reversibly turns into hydrogel state at lower temperature. This transition of sol to gel is ascribed to the H-bonding interaction constructed by carboxyl ($-\text{COOH}$), amide ($-\text{CONH}$) groups in PAG chains and water molecules. It is also accepted that the density of crosslinking of PAG gel was increased with enhancing the PAG concentration, decreasing the temperature and pH value in the medium. The detailed study results about PAG are to be published in the other journal.

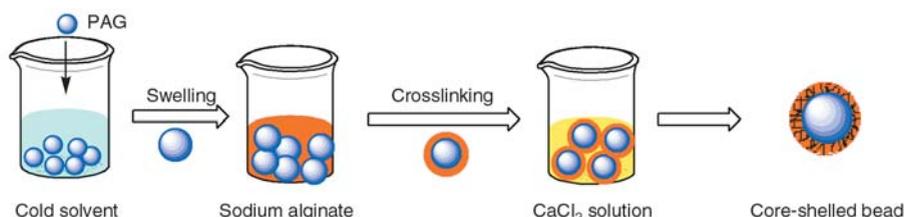


Figure 1. Schematic overview of synthesis of sensitive beads with core-shelled structure

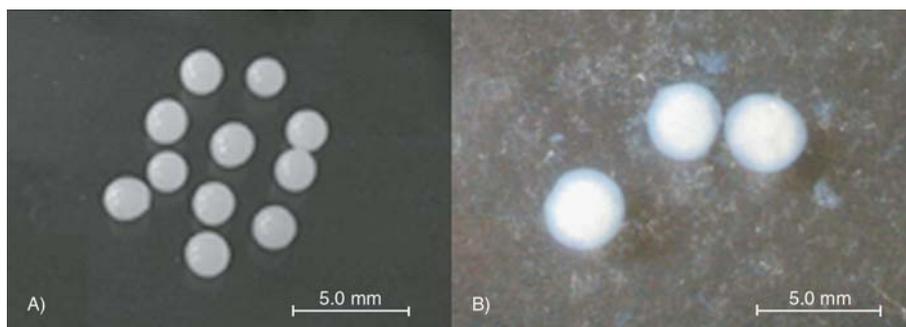


Figure 2. Photographs of the beads with the core-shelled structure (A: the core of PAG, B: the core-shelled bead)

In the process of preparing the PAG core, PAG solution was first heated up to 45°C, and then the hot PAG solution was extruded out by a syringe into a cold acetate ethyl/chloroform. To get the homogeneous spheres, some measures must be adopted in this study. The density of organic mixed solvent was controlled at about 1.0 with acetate ethyl and chloroform, avoiding the fast falling of PAG drops in the solution. In addition, the temperature of organic mixed solvent was regulated as low as possible. The PAG core hydrogel can swell to some extent in the sodium alginate solution, leading to the preliminary form of the shell layer. Obviously, the time interval of swelling for PAG core plays a key role in controlling the thickness of the shell. In the CaCl₂ solution, the cross-linked shell was formed by the chelating of Ca²⁺ with sodium alginate. Theoretically speaking, the beads contained a partial interpenetrating network layer composed of a little PAG chain and calcium alginate. Because of the unavoidable loss of indomethacin in the preparation, the loading efficiency was round 75–80% for all the samples (Table 1). To clearly illustrate the structure of the bead, we have obtained two photographs by a digital camera. The diameter of the prepared beads in this study was about 3 mm. Seen from Figure 2, the core-shelled structure of the beads can be observed distinctly (B). The core PAG is also homogeneous and glabrous spheres.

3.2. Characterization of PAG

The structure of PAG was confirmed by ¹H NMR and IR measurements. As shown in Figure 3, the absorption peak at 3000–3600 cm⁻¹ was assigned to N–H stretching vibration of amide groups and O–H stretching vibration of carboxyl. The stretching vibrations of carbonyl (C=O) from carboxyl and amide groups were found at 1746 and 1640 cm⁻¹,

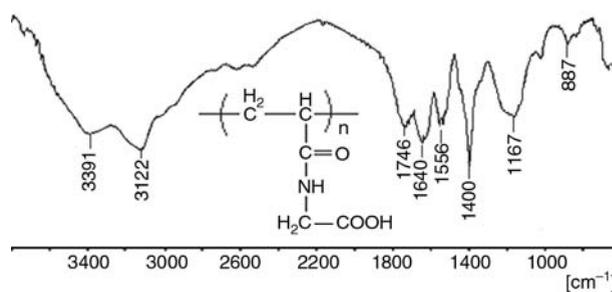


Figure 3. FTIR spectrum of PAG

respectively. Additionally, the characteristic peaks of ester (C–O–C) were also observed at 1167 cm⁻¹. In the ¹H NMR spectrum of PAG (Figure 4), the peaks at 3.6–4.2 ppm were ascribed to methylene group in [–NHCH₂COOH] repeating unit. The chemical shift of methylene and methine groups in [–CH₂–CH–] repeating unit was observed at 1.3–1.9 ppm and 2.0–2.5 ppm, respectively. Herein, the peak at 8.1–8.3 ppm indicated the existence of hydrogen atom in amide group of [–NHCH₂COOH] moiety. The absorption peaks ascribed to carboxyl (–COOH) was not found because of the complete deuteration of COOH with D₂O. Also, the ratio among hydrogen atoms except for amide and carboxyl was in good accordance with the molecular structure of PAG. In addition, the elemental analysis for PAG (C₅H₇N₁O₃)_n is listed as follow: Calcd: C 46.51, H 5.43, N 10.85; Found: C 46.39, H 5.52, N 10.67. Based on the above-mentioned data, it can

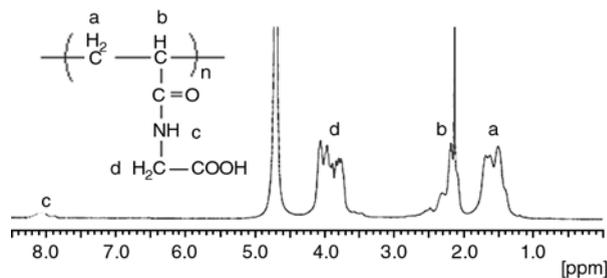


Figure 4. ¹H NMR spectrum of PAG (in D₂O)

be concluded that we have synthesized the titled polymer PAG.

3.3. Swelling study

In this study, the swelling ratio was obtained from the Equation (1). Table 2 has shown the dependence of swelling behavior of the beads on the different pH values and temperatures. A remarkably lower swelling ratio was found in the lower pH PBS. Because pK_a of alginate and PAG is about 3.2, most of carboxyl groups in the alginate and PAG exist in the form of COOH in the low pH medium (pH = 2.1). In the shell, the H-bonding constructed by –COOH of alginate led to the stronger interactions between polymer chains. Also, the H-bonding interactions among –COOH from PAG chains in the core predominated over the polymer-water interactions. Accordingly, the swelling ratio of the core-shelled beads in pH = 2.1 PBS is relatively low. In the higher pH PBS, the carboxylic acid groups were ionized and became –COO⁻ form. Thus, the weakened H-bonding interaction between polymer chains and electrostatic repulsion from –COO⁻ groups resulted in the higher swelling ratio [16].

Additionally, no significant difference of the swelling ratios was observed among all the samples in pH = 7.4 PBS at 25°C. However, the swelling ratio increases significantly with increasing the temperature. For instance, the swelling ratios were 21.9 and 38.1% when the temperature was fixed at 25 and 37°C in pH = 2.1 PBS for the sample B. This observation is ascribed to the weakened H-bonding of polymer chains at higher temperature. As a result, the effective outspread of PAG and alginate chains at 37°C led to an increase of the swelling ratio.

Table 2. Dependence of swelling ratio of the beads on pH and temperature

Sample	pH = 2.1 25°C [%]	pH = 2.1 37°C [%]	pH = 7.4 25°C [%]	pH = 7.4 37°C [%]
A	21.5	39.8	87.1	108.0
B	21.9	38.1	86.4	103.2
C	23.1	35.6	83.2	98.9

3.4. Drug release study

As shown in Figure 5, the release behaviors of indomethacin from the beads have reflected a

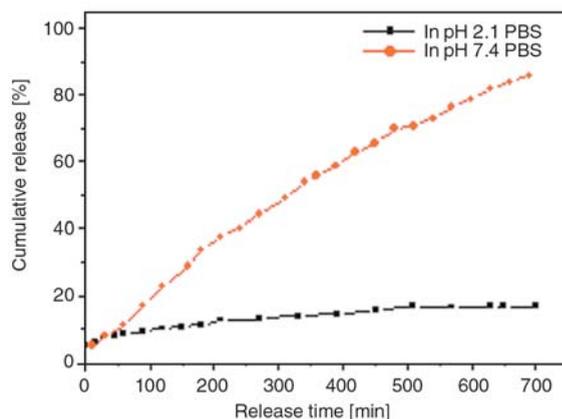


Figure 5. The effect of pH value on drug release behavior of the bead (B)

remarkable dependence on pH value at 37.0°C. In pH = 2.1 PBS, the amount of indomethacin released is described as an initial burst (about 10%), thereafter, almost no further indomethacin diffused out from the bead. The initial burst release behavior can be explained as the fast diffusion of indomethacin molecules in the surface layer of the beads. The low cumulative release of indomethacin is ascribed to the low swelling ratio of the beads in acidic conditions as shown in Table 2. The smaller channels for drug diffusion in the bead caused by lower swelling ratio hold back the effective drug release. In addition, the core PAG maintains a hydrogel state with higher density of crosslinking in the acidic medium. Thus, compared with the solution state of PAG, the core PAG with hydrogel state consequentially block the release of indomethacin to the outside. As an organic acid, indomethacin ($pK_a = 4.5$) also exists in the form of –COOH in pH = 2.1 PBS, The intermolecular H-bonding between indomethacin and PAG chains obviously block the drug release from the bead.

The cumulative amount of released indomethacin in pH = 7.4 PBS reached to as high as 83.5% within 650 min. This higher release rate may be related to the higher swelling ratio of the beads and the weak H-bonding interaction between drug and polymer network in the neutral PBS. A similar release behavior has also been reported in an inorganic-organic hybrid alginate bead and *N*-succinyl chitosan/alginate hydrogel systems [23, 24]. From the more practical point of view, the bead can overpass the acidity medium of gastric fluid without releasing substantial amounts of drug molecules, but release a mass of drug in the small intestine.

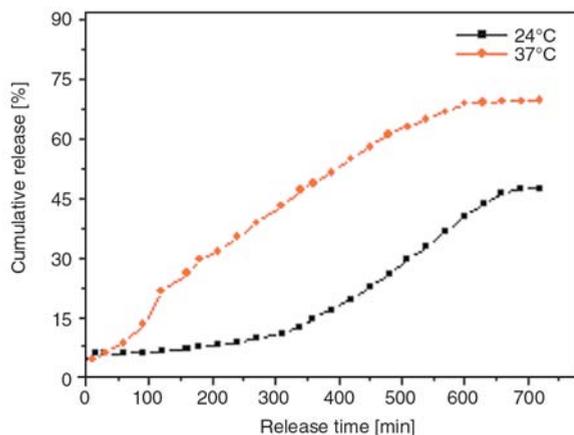


Figure 6. The effect of temperature on drug release behavior of the bead (B)

The temperature-controlled release of indomethacin in pH = 7.4 PBS has been presented in Figure 6. The higher release rate was observed at 37°C while the release rate of indomethacin at 24°C was found to be much lower. For example, the amount of indomethacin released from the bead reached 46.4 and 12.1% within 330 minutes when the temperature was fixed at 37 and 24°C, respectively. For the core-shelled bead, the effective crosslinking density of PAG in the core constructed via H-bonding was decreased by increasing the temperature, which accelerated the drug release [13]. Accordingly, the transition of sol to gel of PAG or the cross-linking density formed by H-bonding in the core plays a key role in regulating the release of the entrapped drug molecules from the beads.

As for the shell composed of calcium-alginate, the disruption of the calcium-alginate in the bead is inclined to occur in PBS above pH = 5.5, via the chelating function of the phosphate ions from PBS [32]. The affinity of calcium to phosphate is higher than that to alginate in the neutral or alkaline PBS, and the solubility of calcium-phosphate complex increases at the higher temperature. Compared with 24°C, the stronger affinity of phosphate to calcium at 37°C accelerated the disruption of the shell. As a result, the faster drug release rate was observed from the beads with core-shell structure at higher temperature.

It is necessary to mention that the release rate of indomethacin in this work is lower than that reported by Park *et al.* [13] and Choi *et al.* [16] at 25°C. In the investigation of Park and Choi, drug was loaded into the beads by the sorption method, and most of the drug existed in the surface layer of

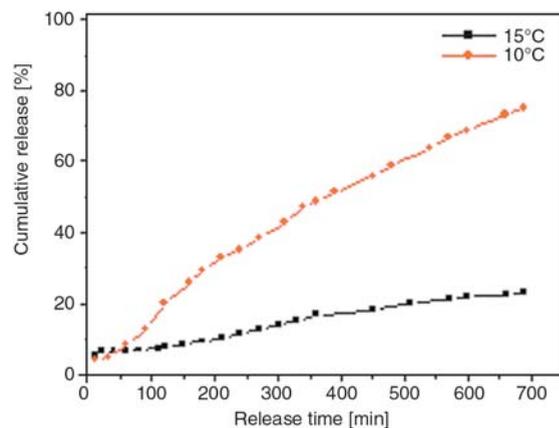


Figure 7. The effect of PAG concentration on drug release behavior of the bead (A and B)

the beads. In our study, the same drug was directly incorporated into the core of the beads during the preparation of bead. The release rate of drug loaded in the core was lower than that in the surface layer of the bead. Therefore, the different drug-loading methods may be responsible for the different release behaviors.

Figure 7 has indicated the effect of PAG concentration in the core on indomethacin release at 37°C and in pH = 7.4 PBS. It was found that the release of indomethacin was increased with a decreasing the PAG concentration in the core. For example, when the PAG concentration was fixed at 10 and 15%, the cumulative releases of drug were 69.4 and 21.8% within 600 minutes, respectively. It is necessary to state that the more the PAG concentration is, the higher density of crosslinking of PAG hydrogel in the core is. That is, the higher PAG concentration in the core leads to the stronger physically crosslinking constructed by H-bonding among amides and carboxyl groups, preventing the effective diffusion of drug molecule to the outside. Clearly, the PAG concentration in the core can be used as a measure to regulate the release rate of drug.

4. Conclusions

In this paper, a novel pH/temperature-sensitive hydrogel bead with core-shelled structure, composed of Ca-alginate and PAG, was prepared using as drug delivery carrier. The equilibrium swelling behaviors of the beads clearly indicated the remarkable sensitivity to the external pH and temperature. The drug release behavior of indomethacin from the beads was characterized as a function of pH,

temperature and the PAG concentration. 83.5% indomethacin in the bead was released within 650 min in pH = 7.4 PBS, while this value was only 16.6% in pH = 2.1 PBS. Additionally, indomethacin release rate was much faster at 37°C than that at 24°C because of the injectability of PAG in the core of the bead. It seems to expect that the Ca-alginate/PAG beads possess a potential application in the drug delivery systems controlled by pH or temperature.

Acknowledgements

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