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# A novel pH-sensitive hydrogel based on dual crosslinked alginate/N-α-glutaric acid chitosan for oral delivery of protein

Renmin Gong[a,b], Chengcheng Li[a], Shengxue Zhu[a], Yinye Zhang[a], Yan Du[a], Jihong Jiang[b,*]

[a] College of Life Science, Anhui Normal University, Wuhu 241000, PR China
[b] The Key Laboratory of Biotechnology for Medicinal Plants of Jiangsu Province, Xuzhou Normal University, Xuzhou 221116, PR China

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ABSTRACT

A novel pH-sensitive hydrogel based on dual crosslinked alginate/N-α-glutaric acid chitosan (GAC) was prepared. The homogeneous alginate/GAC solution was dropped into calcium chloride solution and crosslinked by $Ca^{2+}$ ions. Sequentially, the crosslinked beads were suspended in sodium sulfate solution for forming dual crosslinked beads. The swelling behaviors of dual crosslinked beads were investigated in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF). Bovine serum albumin (BSA) was loaded in beads with the high loading efficiency. The sustained release profiles of BSA loaded beads were studied at different pH environment for simulating gastrointestinal condition. The amount of BSA released from the beads at pH 1.2 was relatively low (below 18%), while almost 100% of BSA could be released at pH 7.4. The preliminary results clearly suggested that the dual crosslinked alginate/GAC hydrogel may be a potential polymeric carrier for oral delivery of protein drugs.


## 1. Introduction

Along with the recent advance of recombinant DNA techniques, a wide range of bioactive molecules like proteins, peptides, and enzymes are commercially available as drugs. Although oral administration is considered as the most convenient and comfortable way for drug delivery, protein and peptide drugs still have to be administered by injection which is a costly way and has a very low patient acceptability. Oral administration of protein and peptide drugs is mainly confined by the acidic denaturation and enzymatic degradation of these drugs in the upper part of the gastrointestinal tracts. Various approaches have been proposed to protect protein and peptide drugs against acidic denaturation and enzymatic degradation. In the design of oral delivery of protein and peptide drugs, pH-sensitive hydrogel has recently received considerable attention.

Chitosan, produced commercially by deacetylation of chitin, is a natural polysaccharide composed of randomly distributed β-1,4-linked D-glucosamine and N-acetyl-D-glucosamine. Because of its non-toxic, biocompatible, biodegradable and mucoadhesive properties, chitosan is widely used in the pharmaceutical field. Chitosan has been used as carrier systems for hormones (Berthold, Cremer, & Kreuter, 1996; Cheng et al., 2005), proteins (Grenha, Seijo, & Remuñán-López, 2005; Yuan et al., 2007), enzymes (Chen & Chen, 1998; Jiang, Long, Huang, Xiao, & Zhou, 2005), and genes (Csaba, Köping-Höggård, & Alonso, 2009; Guliyeva, Öner, Özsoy, & Haziroğlu, 2006). Because of its apparent $pK_a = 5.6$, the application of chitosan in oral administration of protein and peptide drugs is restricted by its easy dissolution at low pH environment. Chitosan also loses the mucoadhesive and permeation enhancing capacities due to its deprotonation under physiological environment. Fortunately, the reactive amino group in chitosan can provide easy derivatization of this polysaccharide with some functional reagents for wider application.

Alginic acid is a natural copolymer containing β-1,4-linked D-mannuronic acid and α-1,4-linked L-glucuronic acid residues arranged randomly along the chain. Sodium alginate, a water soluble salt of alginic acid, exhibits a unique property of gel formation in the presence of multivalent cations such as calcium ions in aqueous medium or in lower environmental pH value. Due to the intrinsic properties of alginate calcium gel (biocompatibility, mucoadhesion, porosity, and ease of manipulation), much attention was focused on its therapeutic and pharmaceutical applications (Coviello, Matricardi, Marianecci, & Alhaique, 2007). The various complexes of alginate and chitosan have been used as the carriers for specific delivery and controlled release of drugs (Anal, Bhopatkar, Tokura, Tamura, & Stevens, 2003; Chen et al., 2004; Coppi & Iannuccelli, 2009; Coppi, Iannuccelli, Leo, Bernabei, & Cameroni, 2001; Dai, Li, Zhang, Wang, & Wei, 2008; Goycoolea, Lollo, Remuñán-López, Quaglia, & Alonso, 2009; Hari, Chandy, & Sharma, 1996; Lin, Liang, Chung, Chen, & Sung, 2005; Mi, Sung, & Shyu, 2002; Mladenovska et al., 2007; Murata, Miyamoto, & Kawashima, 1996; Park et al., 2005; Pasparakis & Bouropoulos,

* Corresponding author. Tel.: +86 516 83403515.
*E-mail address:* jhjiang.xznu@163.com (J. Jiang).

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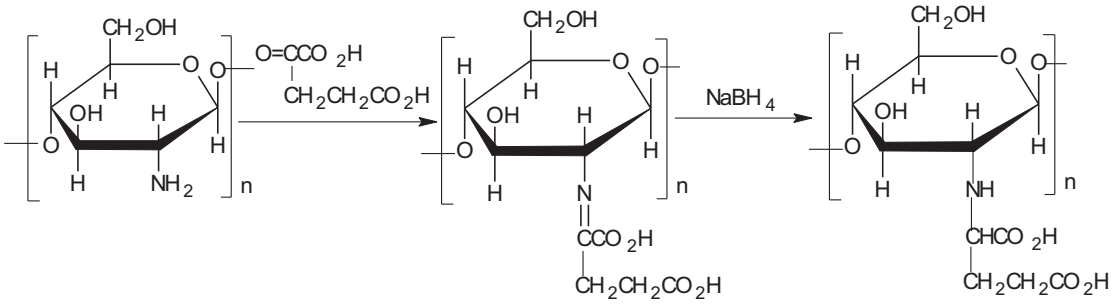


**Scheme 1.** The proposed synthetic route of GAC.

2006; Ribeiro, Silva, Ferreira, & Veiga, 2005; Sarmento et al., 2007; Tavakol, Vasheghani-Farahani, Dolatabadi-Farahani, & Hashemi-Najafabadi, 2009; Xu, Zhan, Fan, Wang, & Zheng, 2007; Yu et al., 2008).

In the study, a novel alginate/N-α-glutaric acid chitosan (GAC) hydrogel dual crosslinked by calcium chloride and sodium sulfate was reported. Swelling behaviors of the gel beads as a function of pH values were investigated. Additionally, release characteristics of bovine serum albumin (BSA), a model protein drug, from the tested gel beads were studied in simulated gastric and intestinal media. The purpose of this work was to investigate dual crosslinked alginate/GAC hydrogel as a potential carrier system for the oral delivery of protein drugs.

## 2. Experimental

### 2.1. Materials and reagents

Chitosan (MW: $8 \times 10^4$) with approximately 85% deacetylation was purchased from Koyo Chemical Co. Ltd., Japan. Sodium alginate (MW: $2.6 \times 10^5$) and BSA were acquired from Sigma Chemical Co., USA. α-Ketoglutaric acid and sodium borohydride were, respectively, supplied by Amresco Inc., USA and Sinopharm Chemical Reagent Co., Ltd., China. All other chemicals were of analytical grade and used without further purification.

### 2.2. Synthesis of GAC

The synthesis of GAC contained two steps, chitosan modified by α-ketoglutaric acid and reduced by sodium borohydride according to the method reported by Ding, Huang, Li, Liu, & Zeng (2006). Briefly, 7.2 g of α-ketoglutaric acid was added to 100 ml of 4.5% (w/v) chitosan solution prepared with 1% (v/v) acetic acid solution. Then the pH of the solution was adjusted to 4.5–5.0 using sodium hydroxide solution and stirred for 4 h at 37 °C. Afterwards, 2.0 g of sodium borohydride was added to the stirred mixture and the pH of the polymeric solution was adjusted to 6.5–7.0 using hydrochloric acid solution. The reaction system was further stirred for 24 h and then reaction was terminated by 95% alcohol. The precipitated polymer was filtered, washed three to four times with

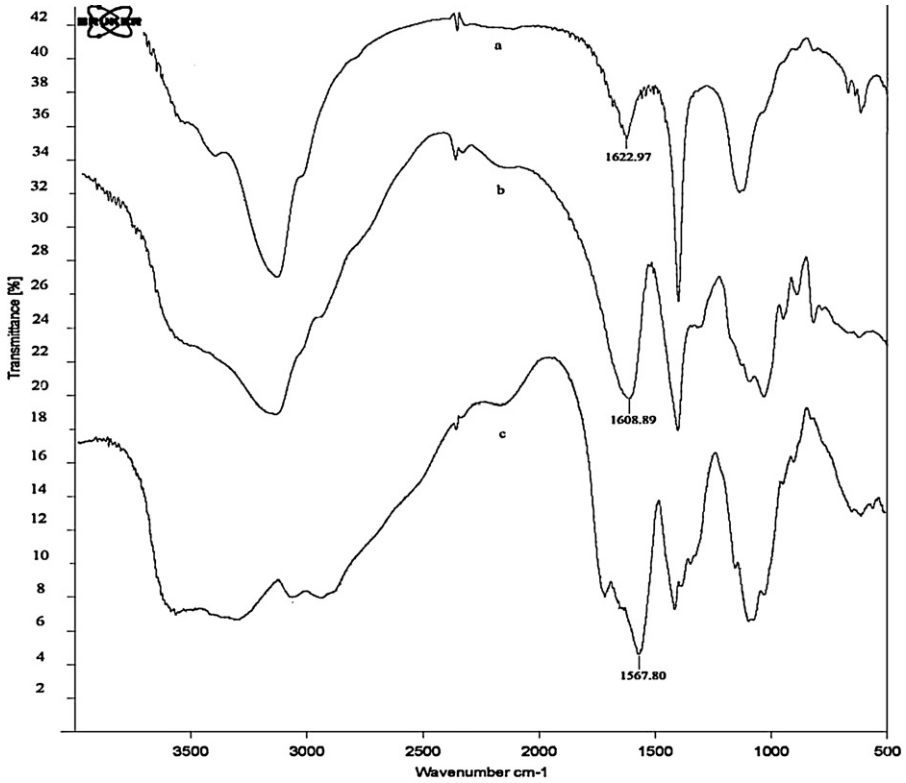


**Fig. 1.** The FTIR spectra of alginate/GAC (7:3), alginate and GAC.

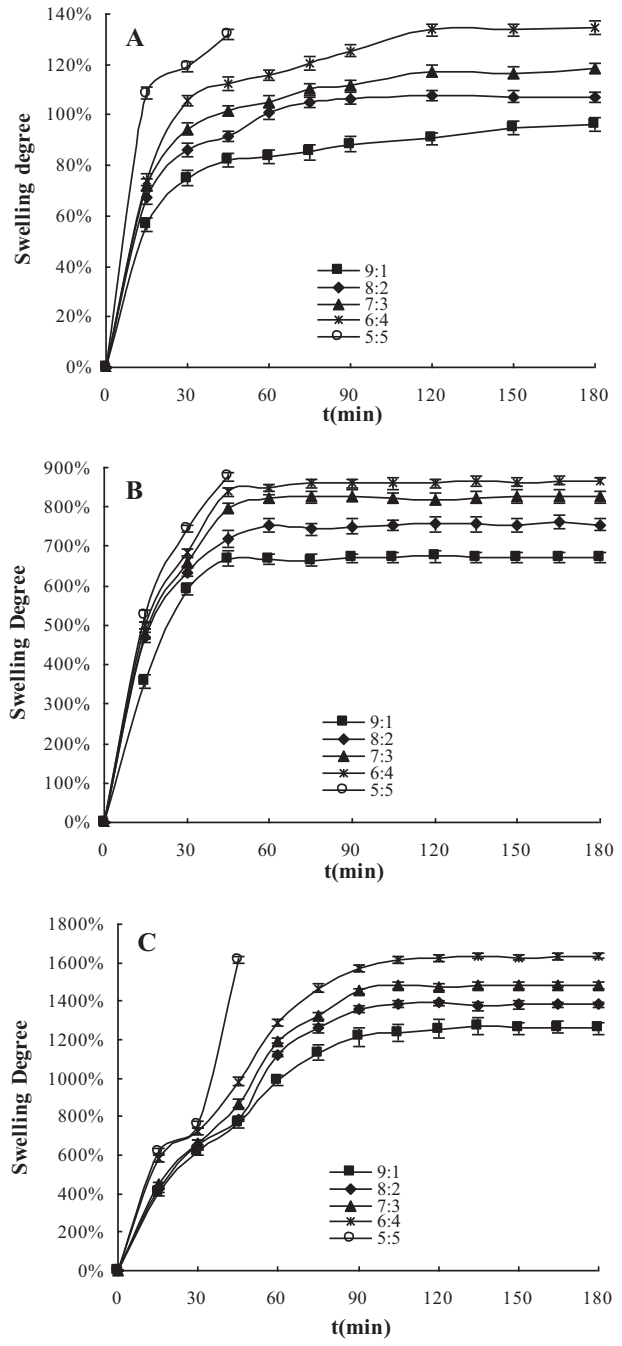


**Fig. 2.** The swelling curves of dual crosslinked blend beads in SGF, SIF and SCF.

ethanol and diethyl ether, respectively. GAC was dried in an infrared drier.

The chemical principle of GAC synthesis was illustrated in Scheme 1.

### 2.3. Preparation of dual crosslinked blend gel beads

The two percent alginate and GAC solution (w/v) was, respectively, prepared by dissolving 2.0 g sodium alginate or GAC in 100 ml deionized water or 1% (v/v) acetic acid solution. The pH value of GAC solution was adjusted to 5.0 by sodium hydroxide solution. Then alginate solution was poured into GAC solution according to definite alginate to GAC weight ratios (9:1, 8:2, 7:3, 6:4, 5:5) and mixed homogeneously. The uniform alginate/GAC solutions were dropped through a 0.45 mm syringe needle into 100 ml of

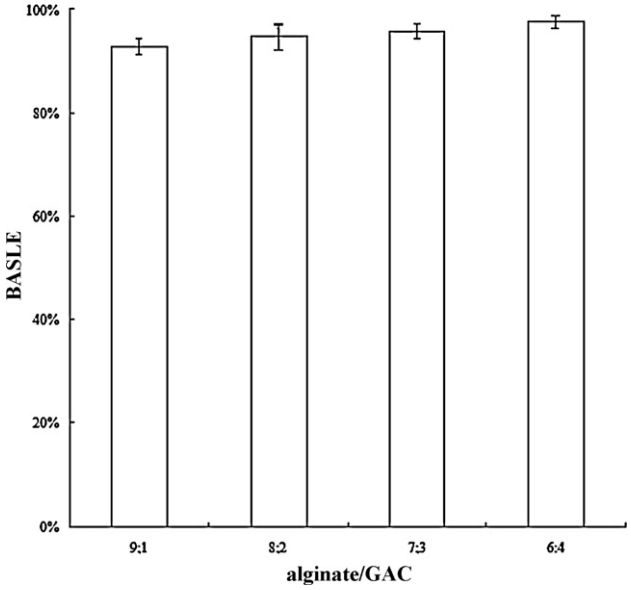


**Fig. 3.** The loading efficiency of BSA in dual crosslinked blend beads.

2% (w/v) calcium chloride solution with gentle stirring for forming smooth and spherical beads. The beads were allowed to crosslink with $Ca^{2+}$ in solution for 30 min under stirring. The $Ca^{2+}$ crosslinked beads were collected by filtration and washed with distilled water for several times to remove unreacted calcium chloride from the surface of beads. The prepared $Ca^{2+}$ crosslinked beads were subsequently suspended in 100 ml of 2% (w/v) sodium sulfate solution and stirred for 1 h. Then the $Ca^{2+}$ and $SO_4^{2-}$ dual crosslinked beads were recovered by filtration, washed with distilled water for several times, and air-dried at room temperature until the constant weight.

### 2.4. Preparation of BSA loaded dual crosslinked blend gel beads

In the preparation of the BSA loaded alginate/GAC gel beads, BSA with a final concentration of 0.4% (w/v) was added to the dissolved alginate/GAC solution with continuous stirring for forming the homogeneous alginate/GAC/BSA blend solution. The other processes were the same as the preparation of dual crosslinked alginate/GAC blank beads.

### 2.5. Determination of loading efficiency (LE)

The BSA loaded gel beads (25 mg) were pulverized and incubated in 50 ml phosphate buffer (pH 7.4) for 24 h at room temperature with stirring. The sample was centrifuged and the amount of released BSA in supernatant was determined by UV–vis spectrophotometer (UV-2401PC, Shimadzu, Japan) at 280 nm. Supernatant from the blank beads was taken as correction. The loading efficiency is the percentage of BSA contained within the gel beads in relation to the initial amount of employed BSA. All samples were analyzed in triplicate. Assuming all loaded BSA being released during 24 h, the LE values were calculated according to the following equation:

$$\text{LE}\ (\%) = \frac{\text{total amount of BSA in beads}}{\text{total amount of used BSA}} \times 100 \quad (1)$$

### 2.6. Swelling characteristics of alginate/GAC gel beads

The swelling behaviors of gel beads were determined at 37 °C by incubating dried gel beads into a buffer solution at pH 1.2

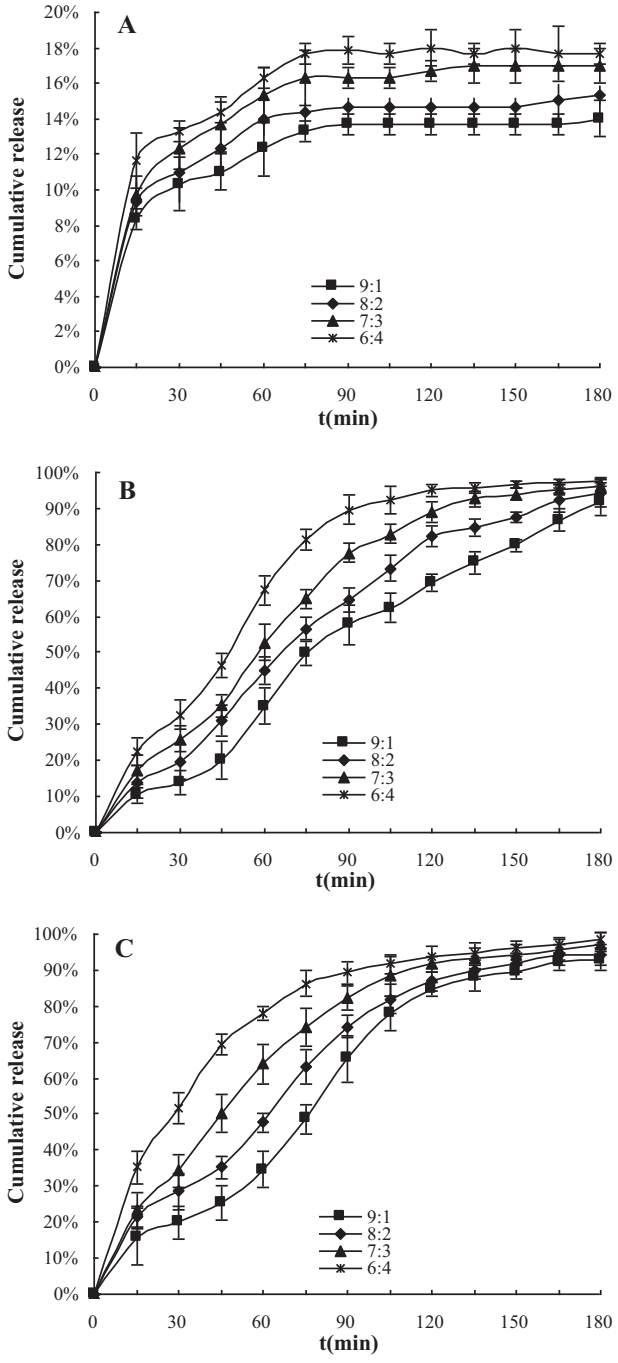


**Fig. 4.** The The cumulative release of BSA from dual crosslinked blend beads in SGF, SIF and SCF.

(HCl/KCl), 6.8 or 7.4 ($NaH_2PO_4/Na_2HPO_4$) with gentle agitation. At specific time intervals, the swollen beads were withdrawn from the swelling media and blotted with a piece of paper towel to remove excess water on the surfaces. The wet weight of swollen beads was weighed on an electronic balance. Each run of the experiments was replicated at least three times. The swelling ratios of beads at anytime were calculated using the following expression:

$$S(t) = \frac{W_t - W_0}{W_0} \quad (2)$$

where $W_t$and $W_0$ are the wet weight of beads at time $t$ and dried weight of beads, respectively.

### 2.7. Release profiles of BSA from alginate/GAC gel beads

The *in vitro* BSA release profiles of gel beads were determined as follows: For simulating gastrointestinal tract conditions, 100 mg of BSA loaded gel beads in 20 ml of buffer solutions (pH = 1.2, 6.8 and 7.4) were incubated at 37 °C with continuous agitation. At appropriate time intervals, 5 ml of the supernatant was withdrawn and replaced by fresh buffer solution. The amount of BSA in supernatant released from the gel beads was determined at 280 nm using a UV–vis spectrophotometer. The percentage of cumulative amount of released BSA was evaluated from standard calibration curves. *In vitro* release experiments of BSA were repeated three times.

## 3. Results and discussion

### 3.1. FTIR spectrum characterization of alginate/GAC gel beads

The FTIR spectra of alginate/GAC gel beads, alginate, and GAC were shown in Fig. 1. Comparing the FTIR spectrum of alginate/GAC beads with alginate's, it was found that the asymmetrical stretching vibration absorption band of carboxyl groups at 1609 $cm^{-1}$ decreased obviously and drifted toward high wavenumber (blue shift). Comparing with the FTIR spectrum of GAC, it could been seen that in FTIR spectrum of alginate/GAC bead, the characteristic scissoring vibration absorption band of amino groups at 1568 $cm^{-1}$ decreased remarkably.

### 3.2. The swelling behaviors of alginate/GAC gel beads

The swelling behaviors of alginate/GAC gel beads (with different weight ratios of alginate/GAC) in buffer solution at pH = 1.2 (SGF), 6.8 (SIF) and 7.4 (SCF) were shown in Fig. 2. To three different pH values, the swelling ratios of test beads (alginate/GAC = 9:1, 8:2, 7:3, 6:4) increased along with increasing the amount of GAC in formulation of alginate/GAC gel beads except for the gel beads (alginate/GAC = 5:5) which disintegrated after 45 min. This interesting phenomenon might be attributed to that the GAC had more negative charge density than alginate and the side chains of α-glutaric acids introduced into chitosan reduced the weak interactions between polymer molecules. The high swelling ratio and disintegration of gel beads (alginate/GAC = 5:5) might fail to retain drug at the low pH environment in the upper part of the gastrointestinal tracts and cause rapid release of entrapped drug in the lower part of the gastrointestinal tracts.

From Fig. 2, it could also be seen that the swelling ratios of alginate/GAC gel beads in SIF and SCF were about 6 and 11 times higher than they in SGF, respectively. The carboxyl groups, dominant functional groups in alginate/GAC gel beads, existed with nonionic state at pH 1.2, so that electrostatic repulsion was weak and accordingly the swelling ratios were small. With increasing pH value up to 6.8 or 7.4, above their p$K_a$ 4.75, carboxyl groups (–COOH) were completely depronated into anionic form ($–COO^-$). The resulted strong electrostatic repulsive force between $–COO^-$ resulted in an increase of water uptake and produced high swelling ratios.

### 3.3. BSA LE of alginate/GAC gel beads

Ideally, a successful carrier system should have a high drug LE. Since the loaded bioactive molecules (including enzymes, peptides, and proteins) for such carrier systems were very expensive, low LE would cause a waste and limit the use of such carrier systems. In this study, BSA was loaded into alginate/GAC gel beads by physical incorporation. The LE of BSA on alginate/GAC gel beads was shown in Fig. 3. It could be seen that LE of BSA on alginate/GAC gel beads exceeded 92% in all test samples when the weight ratio of BSA to polymer was 20%, and the LE of BSA on alginate/GAC gel beads

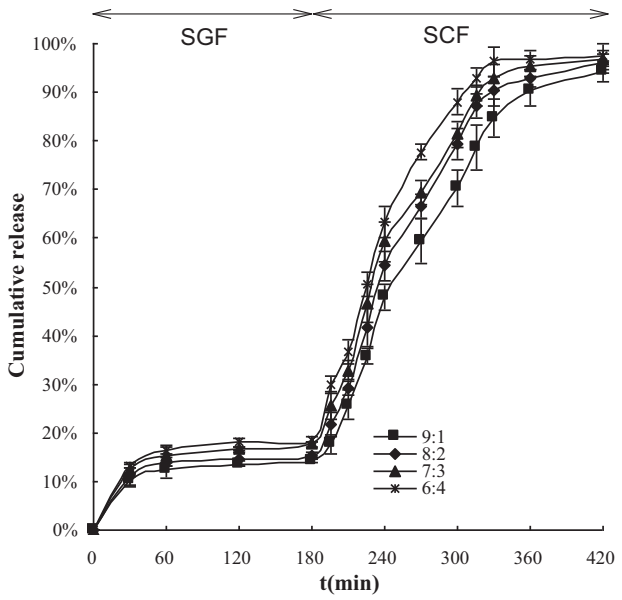


**Fig. 5.** The cumulative release of BSA from dual crosslinked blend beads in SGF followed in SCF.

seemed to increase with increasing the amount of GAC in formulation of gel beads. This higher LE of BSA on alginate/GAC gel beads was obviously attributed to ionic and other noncovalent interactions between BSA and carrier material. Because BSA was loaded in alginate/GAC gel beads under an acid condition (pH 5.0), which was above the isoelectric point of BSA (4.7) (Ang & Elimelech, 2007) but below the p$K_a$ value of amino groups of chitosan (6.3) (Wang, Turhan, & Gunasekaran, 2004), there was an electrostatic attraction between the negatively charged BSA and the positively charged amino groups of GAC chains, which resulted in an enhanced BSA LE.

### 3.4. *In vitro release profiles of BSA from alginate/GAC gel beads*

The cumulative release profiles of BSA from the dual crosslinked alginate/GAC gel beads as a function of time in SGF, SIF and SCF were shown in Fig. 4. The amount of BSA released in SGF was relatively low; only about 14–18% of BSA was released from different weight ratios of alginate/GAC gel beads within 75 min. Thereafter, the cumulative release of BSA kept almost no variety. The relatively low amount of protein released from the dual crosslinked alginate/GAC gel beads in acidic buffer solution was probably related to the comparatively low degree of swelling of the gel beads in SGF. In SIF and SCF, the amount of BSA released increased significantly because the swelling of the gel network increased considerably due to the ionization of carboxyl groups. Almost 100% of BSA was released from different weight ratios of alginate/GAC gel beads in SIF and SCF within 3 h.

For further simulating gastrointestinal tract conditions, the cumulative release behaviors of the dual crosslinked alginate/GAC gel beads were determined by immersing BSA loaded alginate/GAC gel beads in SGF for 3 h and then in SCF for additional 4 h. The BSA release profiles in simulating bioenvironmental conditions were shown in Fig. 5. The samples exhibited a very slight BSA release (<18%) in SGF within the first 3 h. When the beads were transferred into SCF, the release amount of BSA increased significantly and almost all of the BSA was released from beads within subsequent 4 h. This result indicated that the drug release might depend on the swelling of the gel beads where the mechanism of drug release might be due to the diffusion through the swollen gel network.

## 4. Conclusions

The novel pH-sensitive gel beads loaded with BSA based on dual crosslinked alginate/GAC were successfully prepared. The experimental results indicated that with the dual crosslinking of $Ca^{2+}$ and $SO_4^{2-}$, the alginate/GAC gel beads demonstrated excellent pH-sensitivity and drug release pattern dependent on the pH environment. The gel beads swelled slightly at pH 1.2 and the BSA released is less than 18%, however, they swelled more at pH 6.8 and 7.4 and almost 100% of BSA was released from different weight ratios of alginate/GAC gel beads within 3 h. The results clearly suggested that the dual crosslinked alginate/GAC gel could be an excellent candidate of polymeric carrier for oral delivery of bioactive protein drugs.

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