Preparation and *In Vitro* Evaluation of New pH-Sensitive Hydrogel Beads for Oral Delivery of Protein Drugs

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ABSTRACT: New biodegradable pH-responsive hydrogel beads based on chemically modified chitosan and sodium alginate were prepared and characterized for the controlled release study of protein drugs in the small intestine. The ionotropic gelation reaction was carried out under mild aqueous conditions, which should be appropriate for the retention of the biological activity of an uploaded protein drug. The equilibrium swelling studies were carried out for the hydrogel beads at 37°C in simulated gastric (SGF) and simulated intestinal (SIF) fluids. Bovine serum albumin (BSA), a model for protein drugs was entrapped in the hydrogels and the *in vitro* drug release profiles were established at 37°C in SGF and SIF. The preliminary investigation of the hydrogel beads prepared in this study showed high entrapment efficiency (up to 97%) and promising release profiles of BSA. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 2828–2837, 2010

Key words: hydrogels; chitosan; grafting; alginate; protein drugs

INTRODUCTION

Controlled drug release enhances the safety, efficacy, and reliability of drug therapy. The oral route is one of the most convenient and comfortable ways for drug administration. However, in the case of peptide and protein drugs, the oral route exhibits many shortcomings. For instance, these types of drugs are readily degraded, if taken orally, due to the enzymatic attack in the upper small intestinal tract and the harsh high acidity of stomach. Also, the short half-life of the protein drugs and their limited transit time in the gastrointestinal tract represent major challenges.[1,2] Several attempts have been reported to overcome these shortcomings and to formulate protein drugs with maximum oral bioavailability.[3–7]

Hydrogels are crosslinked, three-dimensional hydrophilic polymers, which swell without dissolving when brought into contact with water or other biological fluids.[8,9] The pH-sensitive hydrogels, a class of the smart hydrogels, have potential use in the site-specific delivery of drugs to the gastrointestinal tract.[10] Polymers suitable for preparing hydrogels for the controlled release of drugs are quite limited, as compared to the total available synthetic polymers, because of the inherent toxicity or lack of certain properties such as biodegradability and swelling ability in a specific environment.

Alginate is a non-toxic biodegradable polyanionic copolymer. It consists of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues arranged either as consecutive blocks or in a random distribution.[11] Hydrogels based on calcium-crosslinked alginate has been widely investigated for protein drug delivery.[4,12,13] However, the swelling of the calcium-crosslinked alginate beads at pH 7.4 is minimal[14] due to relatively strong ionic interaction between the carboxylic groups on alginate and Ca^{2+}. This may limit the drug release at the intestinal tract. Various trials have been reported to overcome this disadvantage by preparing hydrogels based on alginate with other polymers such as chitosan (Cs) and its derivatives.[12–14]

Chitosan (Cs) is a cationic biopolymer obtained through alkaline N-deacetylation of natural chitin. Cs has been considered as a biodegradable, non-toxic, biocompatible, and environmental friendly material with many superior properties.[15–18] Carboxymethyl chitosan (CMCs) is a water soluble derivative of Cs. Because of the unique chemical, physical, and biological properties of CMCs, particularly its low toxicity and biocompatibility, it has been extensively used in many biomedical fields.[3,19–22]

Considerable interest has been focused on chemical modification by grafting synthetic polymers onto Cs[23–29] whereas very little reported work has discussed the graft copolymerization of vinyl

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monomers onto CMCs.[30–34] In this study, Cs was carboxymethylated and then graft copolymerized with methacrylic acid (MAA) in a mild aqueous medium using ammonium persulphate (APS) as initiator. An attempt was then made to produce and *in vitro* evaluate biodegradable pH-responsive hydrogel beads that would survive the harsh acidity of the stomach and preferably release the drug in the intestine. These hydrogels are based on CMCs-*g*-MAA with sodium alginate.

MATERIALS AND METHODS

Materials

Cs (molecular weight: 492 kD and N-deacetylation: 67.2%) and BSA were purchased from Acros Organics (NJ). Sodium alginate of high viscosity (~ 14,000 cps for a 2% solution at 25°C) and Bradford reagent were obtained from Sigma Chemical (St. Louis, MO). MAA and monochloroacetic acid were supplied by Riedel–De Haenag Seelze (Hanover, Germany). APS was obtained from AJAX Chemicals, Clyde Industries (Auburn, NSW, Australia). Isopropyl alcohol, acetone, methanol, acetic acid, calcium chloride, and all other reagents were of analytical grade and used as received.

Methods

Preparation of CMCs

Water-soluble CMCs was prepared by a modified method to that described by Xie et al.[34] In a typical procedure, 2 g of Cs were put in 500 mL reactor and suspended in 60 mL of isopropyl alcohol at room temperature for 5 h. To the swollen Cs suspension, 75 mL of aqueous NaOH solution (60% w/v) were added and then the whole mixture was refluxed at 85°C for 2 h. Then, 100 mL of aqueous monochloroacetic acid solution (60% w/v) were added over a period of 15 min. The mixture was heated with stirring, at 65°C for further 4 h. The reaction mixture was then neutralized using HCl solution (5*M*). After removal of the undissolved residue by filtration, CMCs was precipitated by adding methanol. The product was filtered, washed several times with a mixture of CH_3OH/H_2O (1 : 1), and freeze-dried.

Graft copolymerization

Graft copolymerization reactions were carried out through a modified procedure to that reported by Sun et al.[30] The reactions were performed in a 250 mL two-necked flask using 0.1 g CMCs. Before addition of the predetermined amount of monomer MAA, the monomer was neutralized using NaOH (4*M*) and then made up to the desired volume with deionized water. The monomer concentrations used were in the range of (0.5–3*M*). The components were mixed and stirred for 30 min with bubbling of a slow stream of nitrogen gas. The flask was then placed in a thermostated oil bath at 70°C. Finally, the predetermined concentration of the initiator, APS (8 m*M* based on the total volume of reaction mixture) dissolved in 10 mL of deionized water was added dropwise with stirring. The graft copolymerization was carried out for 2 h at 70°C. The reaction was stopped by letting air into the flask and rapidly cooling down the reactor. The products were precipitated by pouring the reaction mixture into acetone. The precipitate was filtered off, washed with acetone, and the crude product was freeze-dried and weighed. The homopolymer formed (PMAA) was extensively extracted in a Soxhlet apparatus with methanol for 24 h. The residual graft copolymer obtained was washed with methanol, freeze-dried, and weighed. The percent grafting (*G*%) and the grafting efficiency (GE%) of the copolymers were calculated as follows:

$$G\% = 100[(W_g - W_o)/W_o]$$

$$\mathrm{GE}\% = 100[W_g/(W_g + W_h)]$$

where W_g, W_h, and W_o are the weights of graft copolymer, homopolymer, and CMCs, respectively.

Preparation of CMCs-*g*-MAA/sodium alginate hydrogel beads

The CMCs-*g*-MAA/sodium alginate hydrogel beads were prepared via the ionotropic gelation technique by using calcium chloride solution as the coagulation fluid. Homogenous aqueous CMCs-*g*-MAA/sodium alginate solutions (20 mL) with predetermined compositions (refer Table I) were prepared and left overnight to degas. Then, these prepared aqueous polymer solutions were used as dope and dropped into 80 mL of gently stirred (200 rpm) calcium chloride solution (0.1–0.3*M*) through a tip of micropipette (1000 μL). Ionotropic gelation reaction occurred instantaneously to form hydrogel beads. The beads that formed were allowed to crosslink with the Ca^{2+} in solution for 30 min. Then, the resulting calcium-crosslinked CMCs-*g*-MAA/alginate beads were collected and washed with distilled water to remove the unreacted calcium chloride. The beads were then freeze-dried and stored until further use.

Characterizations

The elemental analysis for Cs, CMCs, and the CMCs-*g*-MAA copolymers were performed with Carlo Erba EA 1108 elemental analyser (now CE

TABLE I
Different Compositions of the Prepared CMCs-*g*-MAA/Alginate Hydrogel Beads

Formulations	CMCs-*g*-MAA		Alginate (%)	$CaCl_2$ (*M*)	Entrapment efficiency (%)
	G (%)	(%)			
A1	570	1.0	1.0	0.1	29.25
A2		1.0	1.0	0.2	48.25
A3		1.0	1.0	0.3	37.50
A4		2.5	2.5	0.1	82.80
A5		2.5	2.5	0.2	95.10
A6		2.5	2.5	0.3	96.30
A7		2.5	1.0	0.1	69.43
A8		2.5	1.0	0.2	87.57
A9		2.5	1.0	0.3	87.00
A10		1.0	2.5	0.1	55.57
A11		1.0	2.5	0.2	74.57
A12		1.0	2.5	0.3	73.14
B1	1615	1.0	1.0	0.1	37.50
B2		1.0	1.0	0.2	44.00
B3		1.0	1.0	0.3	43.50
B4		2.5	2.5	0.1	87.10
B5		2.5	2.5	0.2	96.28
B6		2.5	2.5	0.3	97.40
B7		2.5	1.0	0.1	72.43
B8		2.5	1.0	0.2	83.29
B9		2.5	1.0	0.3	82.76
B10		1.0	2.5	0.1	70.00
B11		1.0	2.5	0.2	77.86
B12		1.0	2.5	0.3	79.86
C1	–	–	2.5	0.1	38.20
C2		–	2.5	0.2	43.00
C3		–	2.5	0.3	37.20

Instruments, Wigan, UK) with a flash combustion technique (Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand). Also, Cs, CMCs, and CMCs-*g*-MAA were characterized by FTIR (Perkin Elmer Paragon 1000 FTIR spectrometer). The crystallography patterns of Cs, CMCs, and CMCs-*g*-MAA copolymer were investigated using 2D-XRD equipment (Rigaku Micro Max 007 microfocus) imitating anode X-ray generator (Cu Kα) coupled with Osmic "Blue" confocal optics and a Rigaku RAxis (VI++) image-plate detector. Images were recorded and analysed with Crystal Clear (1.3.6-SPI, Pflugrath, JW, 1999, Acta Crystallogr. D50 1718-1725). The morphology of the prepared hydrogel beads was examined using an optical microscope (Zoom Stereo LEICA MZ12, Leica Microsystems GmbH). The size of three beads from each formulation was measured using a micrometer (MOORE & WRIGHT, Sheffield, England) and averaged.

Entrapment of a model protein drug

Hydrogel beads loaded with BSA as a model for protein drugs were prepared in the same manner as described earlier in section "Preparation of CMCs-*g*-MAA/sodium alginate hydrogel beads." Predetermined amounts of the BSA were added to the aqueous polymer mixture, stirred vigorously, and then the gelation reaction was carried out by dropping this drug-containing solution onto the $CaCl_2$ solution. The resulting hydrogel beads were collected, rinsed with 20 mL of distilled water, freeze-dried, and stored until further investigation.

Determination of the entrapment efficiency of BSA

The quantity of BSA entrapped in the CMCs-*g*-MAA/alginate beads was determined by the indirect method. After preparation of the beads, both the washings (20 mL) and the $CaCl_2$ solution (80 mL) were collected and filtered. Using the Bradford method,[35] the amount of BSA present was estimated from the absorption at λ_{max} 595 nm with the aid of a Varian Corg 50 scan (Palo Alto, CA) UV-Vis spectrophotometer. The difference between the amount of BSA initially added to the hydrogel beads and that estimated in the 100 mL (washings plus $CaCl_2$) was taken as a measure of the amount of BSA entrapped. The entrapment efficiency (EE%) of BSA was calculated as follows:

$$EE\% = 100(m_r/m_i)$$

where m_i and m_r are the amounts (mg) of the BSA initially uploaded and remain after beads washing, respectively.

Swelling studies

The maximum swelling values of the CMCs-*g*-MAA/alginate beads were determined by immersing a predetermined weight of the sample in 20 mL buffer solution of pH 2.1 (SGF) or pH 7.4 (SIF) at 37°C until the equilibrium was attained. Then the weights of the swollen samples were determined after removal of the surface water using tissue paper. The swelling profiles of the hydrogels prepared using 0.2*M* $CaCl_2$ solution, as an example of other formulations, were also determined. In a typical procedure, a certain weight of the dried beads was immersed in 20 mL buffer of pH 2.1 (SGF) at 37°C for 3 h and subsequently transferred into another 20 mL buffer of pH 7.4 (SIF) at 37°C for 8 h. The swollen weights of the hydrogel beads were determined at intervals, after removal of the surface liquid using tissue paper. The percent swelling was calculated by the following equation:

$$\text{Percentage Swelling} = 100[(W_t - W_0)/W_0]$$

where W_0 is the initial weight and W_t is the final weight of the beads at time t. The data points represent Mean ± SD from three independent experiments.

In vitro cumulative release studies

The *in vitro* cumulative release patterns of the entrapped BSA were determined by placing the preweighed hydrogel beads loaded with BSA in 10 mL of buffer at pH 2.1 (SGF) at 37°C for 3 h and subsequently in 10 mL buffer of pH 7.4 (SIF) at 37°C for 8 h. At intervals, 100 μL aliquot was withdrawn and analyzed by the Bradford method at λ_{max} 595 nm using a UV-Vis. Spectrophotometer.[35] The withdrawn sample was replaced with an equal volume of fresh buffer, to keep the volume of the release medium constant. The data points represent Mean ± SD from three independent experiments.

Statistical analysis

The results were analyzed and expressed as Mean ± SD. Statistical analysis was performed by using factorial design for characterization of CMCs-*g*-MAA/alginate hydrogel beads. Effects of various parameters on the properties of the hydrogel beads were statistically analyzed by two-way analysis of variance (ANOVA) using the General Linear Models procedures of the SAS [SAS Institute (1997) SAS/STAT User's Guide: Statistics. Version 6.12. Cary, NC: SAS Institute]. Differences were considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Preparation of CMCs and CMCs-*g*-MAA copolymer

CMCs was prepared by a modified method to that adapted from Xie et al.[34] The intrinsic viscosity of the prepared CMCs in 0.1*M* aqueous NaCl at 30°C is ~ 5.1 dL/g. The structural changes of Cs and its derivatives (CMCs and CMCs-*g*-MAA) were confirmed by FTIR (Fig. 1). In the FTIR spectrum of Cs [Fig. 1(a)], the strong peak appeared at 3427 cm^{-1} was assigned to the N—H extension vibration, O—H

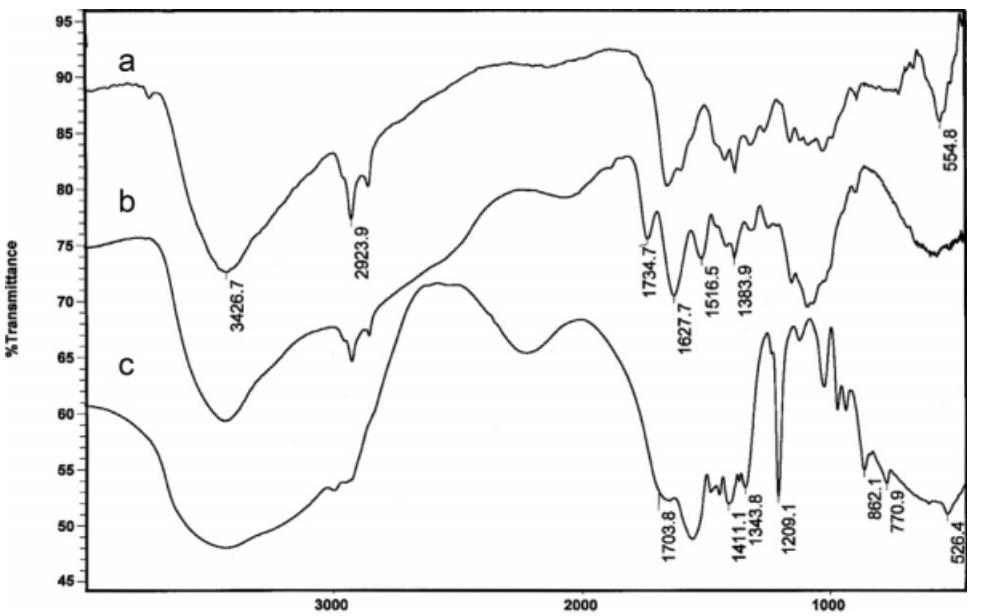
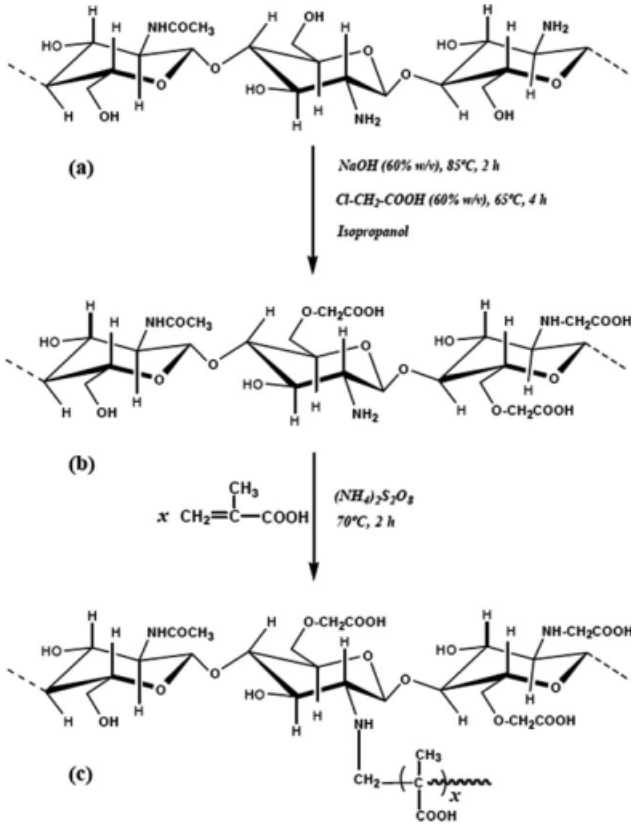


Figure 1 FTIR spectra of (a) Cs, (b) CMCs, and (c) CMCs-*g*-MAA (*G*% = 1930%).

stretching vibration, and the intermolecular H-bonds of the polysaccharide moieties. The weak peak at 1655 cm^{-1} is due to the amide C=O stretching. The FTIR spectrum of CMCs [Fig. 1(b)] shows a strong new peak at 1735 cm^{-1} representing the carboxylate C=O asymmetric stretching. The signal at 1384 cm^{-1} could be assigned to the symmetric stretching vibration of carboxylate C=O. In case of FTIR spectrum of CMCs-*g*-MAA [Fig. 1(c)], the asymmetric stretching vibration of the carboxylate C=O of both CMCs backbone and poly(methacrylic acid), PMAA side chains (1550–1910 cm^{-1}) has overlapped with the amide C=O stretching (1655 cm^{-1}) to produce a strong peak at 1704 cm^{-1}. Also, the FTIR spectrum of CMCs-*g*-MAA shows some new absorption peaks at 1411, 1209, and 1160 cm^{-1}, which are characteristic for PMAA.[30]

In addition to the FTIR spectrum of CMCs-*g*-MAA, which had the characteristic peaks of both CMCs and PMAA, the higher weight of the graft products over that of the starting CMCs after the extensive removal of the homopolymer can be taken as an experimental evidence of grafting. Also, the occurrence of grafting can be deduced from the decreasing of *N*% upon comparing the elemental analysis data of CMCs (*C*%: 37.21; *N*%: 5.11; *H*%: 5.85) and the CMCs-*g*-MAA copolymers (*C*%: 33.62; *N*%: 0.65; *H*%: 4.77 for copolymer of *G*% = 1615). The preparation of CMCs and CMCs-*g*-MAA from Cs are shown in Scheme 1.



Scheme 1 The preparation of CMCs (b) and CMCs-*g*-MAA (c) from Cs (a).

2D-XRD

Figure 2 shows the 2D-XRD patterns of Cs and its derivatives, CMCs and CMCs-*g*-MAA. The diffractogram of Cs, Figure 2(a), shows three major crystalline peaks at 2θ values of 8.38, 11.49, and 18.25° in addition to a lot of weak and broad crystalline peaks. This diffraction pattern reflects a high degree of crystallinity for the Cs under investigation. Figure 2(b) shows the diffractogram of CMCs, from which it can be seen that CMCs has many crystalline peaks plus two broad and weak bands corresponding to 2θ values of about 21.43 and 26.33°. The diffractogram of CMCs seems to keep some of the characteristic peaks of Cs. For instance, CMCs still has the bands at 2θ values of 10.40, 11.49, 16.10, ~ 18.46 and ~ 21.43°. The grafting of MAA onto the CMCs backbone turned the resulting copolymer, CMCs-*g*-MAA into an amorphous material. The diffractogram of CMCs-*g*-MAA copolymer [Fig. 2(c)] shows two peaks, the first is corresponding to a 2θ value of 7.75° and the other one is patchy appeared at 2θ of ~ 21.65°. This resulting amorphous structure of CMCs-*g*-MAA may be attributed to the occurrence of the grafting in a random manner along the CMCs backbone and consequently destroying the regularity of the packing of the original CMCs chains leading to the formation of amorphous structure.

Preparation of CMCs-*g*-MAA/sodium alginate hydrogel beads

The resulting water-soluble CMCs-*g*-MAA was used in this study to prepare pH-sensitive hydrogel beads for the delivery of a model protein drug (BSA). Two CMCs-*g*-MAA copolymers of different grafting percents (570% and 1615%) were selected for the hydrogel preparation (refer Table I). The developed hydrogel is based on a combination of the CMCs-*g*-MAA copolymer blended with sodium alginate and ionotropically crosslinked by dropping this aqueous mixture into a Ca^{2+} solution.

Hydrogels based on calcium-crosslinked alginate have been widely investigated as drug delivery matrices.[4,12,13] However, it has been found[14] that the swelling of the calcium-crosslinked alginate beads at pH 7.4 is minimal due to the relatively strong ionic interaction between the alginate and Ca^{2+}. This may limit the drug release in the intestinal tract. In an attempt to overcome this shortcoming, Lin et al.[14] have developed a hydrogel complex composed of alginate blended with CMCs by dropping an aqueous mixture of the two polymers onto a Ca^{2+}

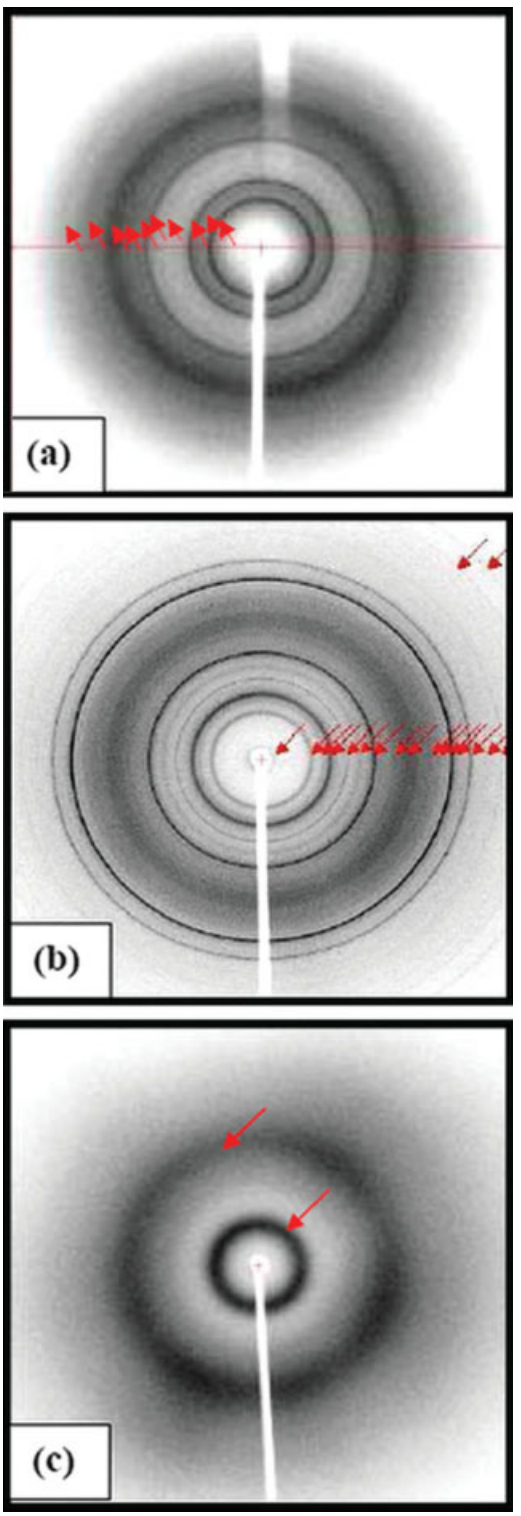
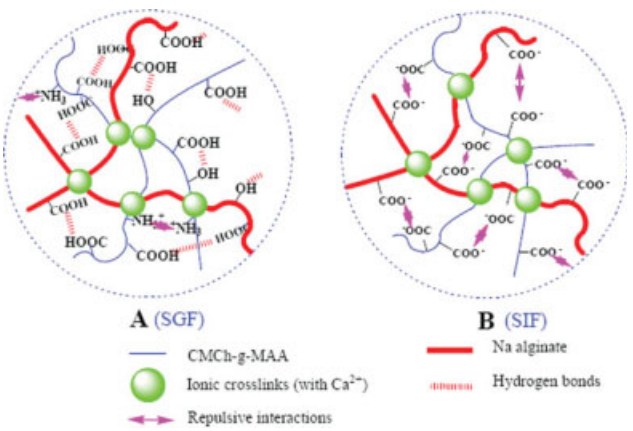


Figure 2 2D-XRD patterns of (a) Cs, (b) CMCs, and (c) CMCs-*g*-MAA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

solution. The resulting hydrogel beads showed enhanced swelling behavior in the intestine as compared to that of the hydrogels based on alginate alone. However, the swelling extent of these gel beads, prepared by Lin et al.[14] was relatively higher than alginate in the stomach. This may lead to the loss of some of the uploaded protein drug in the stomach. Therefore, in this study, CMCs was prepared and further modified via grafting of MAA onto its backbone. The main purpose of this modification is to increase the number of the carboxylic groups in the CMCs. Thus, by developing a hydrogel based on alginate with this modified CMCs, these carboxylic groups are expected to participate in minimizing the swelling at pH 2.1 (SGF) and maximizing the swelling at pH 7.4 (SIF). This expected role of the carboxylic groups in controlling the swelling extent of the prepared CMCs-*g*-MAA/alginate hydrogel is illustrated in Scheme 2.

During the early stage of this study, it was observed that the aqueous solution of CMCs-*g*-MAA can form hydrogels when dropped onto a Ca^{2+} solution. However, the resulting hydrogels had random shapes and were not symmetrical beads. Therefore, the hydrogel formulations based on CMCs-*g*-MAA alone were not investigated further in this study. In combination with alginate, as represented in Scheme 2, the hydrogel can form strong ionic crosslinks with Ca^{2+} at both pHs, 2.1 and 7.4. At pH 2.1 (Scheme 2A), the free carboxylic groups, which are not involved in the ionic crosslinking with Ca^{2+}, tend to form hydrogen bonds with each other and with the OH groups of the sugar moieties. Both of the ionic crosslinks and the hydrogen bonds act to minimize the swelling at pH 2.1. The repulsive interactions that may occur between the protonated NH_2 groups work to increase the hydrogel swelling. However, these repulsive forces are expected to be few, as the number of NH_2 groups remaining free, after the carboxymethylation of Cs and its further modification via grafting, should be quite limited. In contrast, at pH 7.4 (Scheme 2B), most of the free carboxylic



Scheme 2 A schematic representation of the different types of interactions in the CMCs-*g*-MAA/alginate hydrogel beads at both pH 2.1 (SGF) and pH 7.4 (SIF). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

groups would be ionized. Hence, strong repulsive forces are created by the electrostatic repulsion between these ionized carboxylate groups (COO^-). These repulsive forces are thus responsible for attaining the hydrogels higher values of swelling at pH 7.4.

Bead size measurements

The shape of all the prepared alginate and CMCs-*g*-MAA/alginate hydrogel beads is spherical. The average bead size of the different formulations is between 727 ± 75 μm and 1619 ± 25 μm. In most cases, for a certain bead composition, increasing the concentration of Ca^{2+} used in the hydrogel preparation led to a significant decrease ($P < 0.05$) in the size of the beads. For instance, according to the size, A10 > A11 > A12 [Fig. 3(a)]. For the majority of formulations, there is a slight difference ($P > 0.05$) in size between the beads prepared by 0.2*M* and 0.3*M* Ca^{2+}. Increasing the concentration of CMCs-*g*-MAA in the beads and/or the concentration of the whole polymer mixture forming the beads led to a significant increase ($P < 0.05$) in the size. From the results, it seems that, increasing the concentration of the CMCs-*g*-MAA in the hydrogel affects the bead size more than increasing the concentration of both the components of the mixture. For example, according to the size, A7 > A4 > A1 [Fig. 3(b)]. Increasing the *G*% of the CMCs-*g*-MAA from 570% (formulations A) to 1615% (formulations B) led to increasing the bead size. The size of the hydrogel beads based on alginate alone is less than that of the CMCs-*g*-MAA/alginate hydrogel beads [Fig. 3(c)].

Figure 4 illustrates microscopic images of some CMCs-*g*-MAA/alginate hydrogel beads. From this figure, with increasing the Ca^{2+} concentration (from A4 to A6) the beads became smaller and more spherical with a smoother surface. This can be attributed to the increase in the extent of crosslinking. The same behavior can be noted also in A7–A9 and in D1–D3. This figure also confirmed that the difference in size between the beads prepared using 0.2 and 0.3*M* Ca^{2+} is non-significant ($P > 0.05$) than that between the beads prepared using 0.1*M* and 0.2*M*.

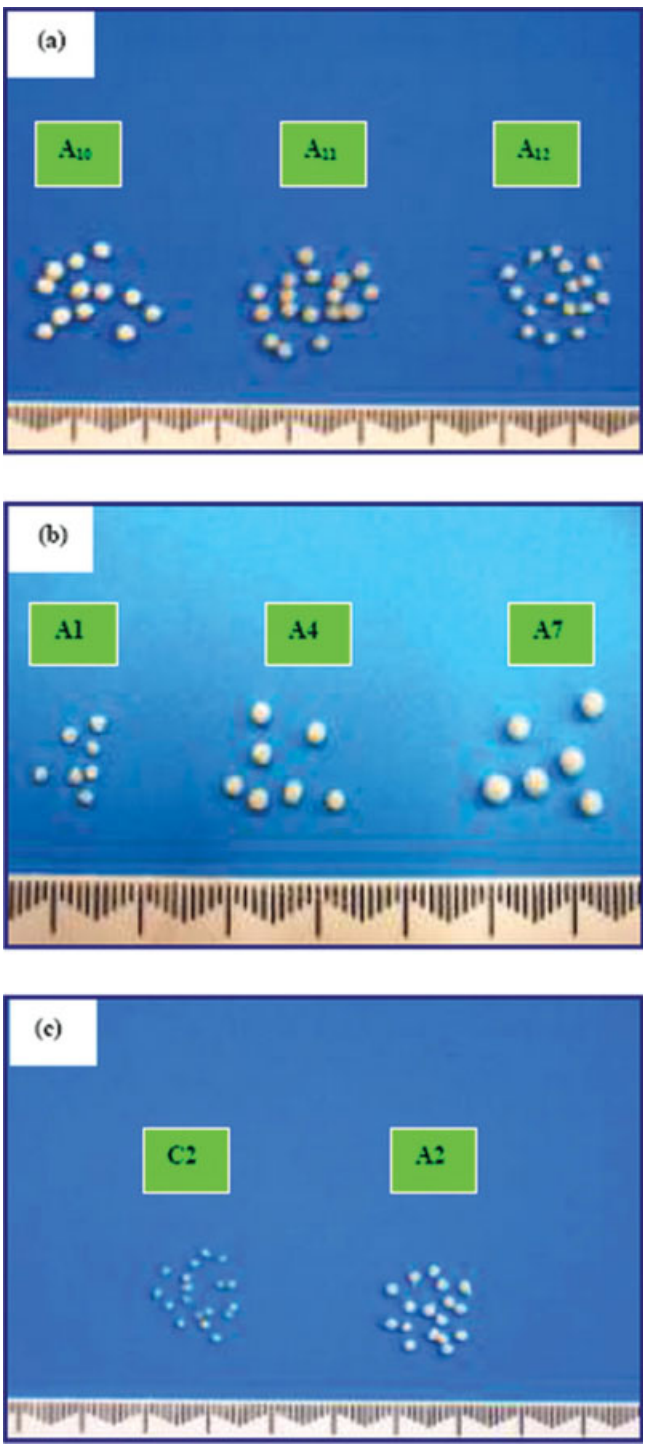


Figure 3 Photographs illustrating the differences in size of some dry drug-free CMCs-*g*-MAA/alginate beads. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Swelling characteristics

The swelling pattern of a hydrogel is one of the most significant characteristics that control the rate of drug release from this hydrogel. The swelling measurements of the prepared CMCs-*g*-MAA/alginate hydrogel beads were carried out for 3 h at pH 2.1 (SGF) followed by 8 h at pH 7.4 (SIF) at 37°C. From the swelling data obtained, at both pHs 2.1 and 7.4, increasing the concentration of $CaCl_2$ increased the crosslinking extent and consequently the equilibrium swelling percentage of the hydrogel beads decreased. It has been found that the difference in swelling at equilibrium between the beads prepared using 0.2*M* and 0.3*M* $CaCl_2$ is non-significant ($P > 0.05$) than the swelling difference between the beads prepared using 0.1*M* and 0.2*M* $CaCl_2$ ($P <$

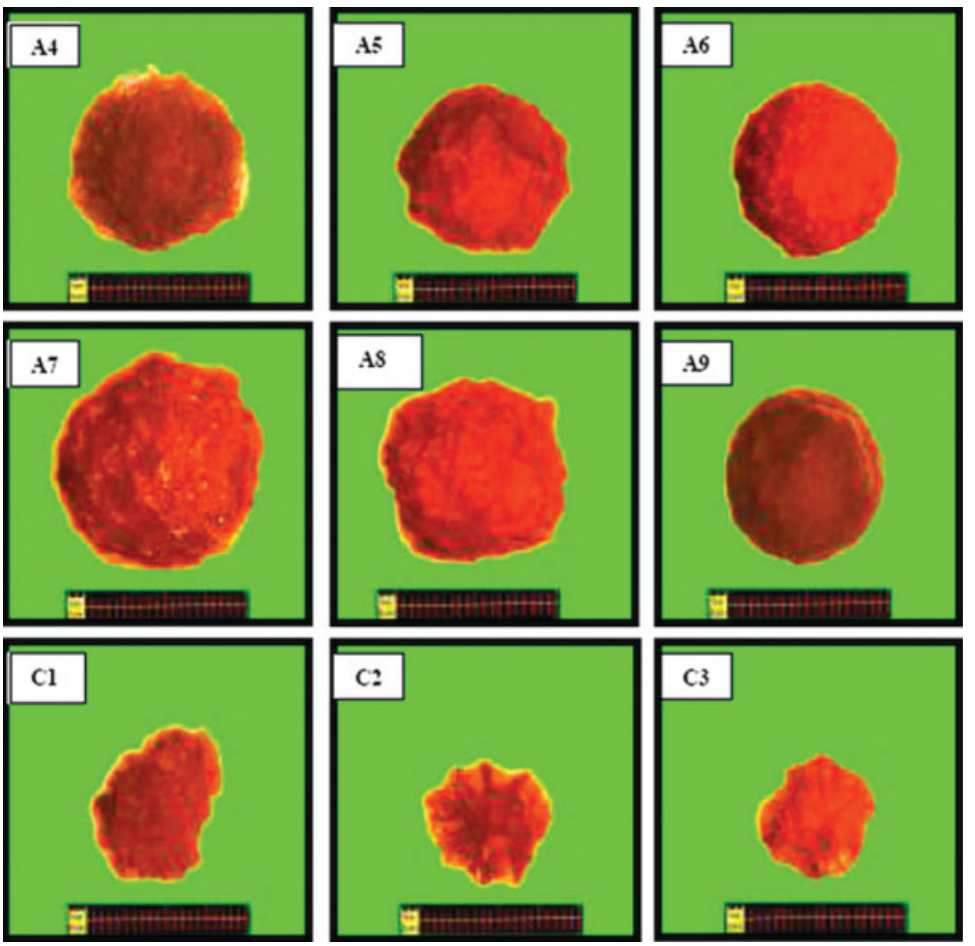


Figure 4 Microscopic photographs of CMCs-*g*-MAA/alginate hydrogel beads: 1 mm scale. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

0.05). The hydrogel formulations prepared using 0.2*M* $CaCl_2$, were selected, as an example for the other formulations, for the investigation of their swelling profiles. As shown in Figure 5, increasing the total percentage of all the polymer mixtures used in the preparation of the hydrogel beads made the beads more compact and consequently decreased their swelling values. For instance, in Figure 5(A), A5 (2.5% CMCs-*g*-MAA:2.5% alginate) attained swelling of 184% and 2978% at 37°C after 3 and 8 h in SGF and SIF, respectively, whereas A2 (1% CMCs-*g*-MAA:1% alginate) attained 201% and 3827% in SGF and SIF, respectively. The same behavior can be noted by comparing the swelling percentage of B5 and B2 [Fig. 5(B)]. Increasing the percent of the CMCs-*g*-MAA in the hydrogel beads relative to alginate led to increasing the swelling values in both SGF and SIF. For example, A8 (2.5% CMCs-*g*-MAA:1% alginate) attained swelling of 211% and 3500% at 37°C in SGF and SIF, respectively, whereas, A11 (1% CMCs-*g*-MAA:2.5% alginate) achieved swelling of 187% and 3432% in SGF and SIF, respectively. The same result can be also noted by comparing the swelling percentage values attained in both SGF and SIF for B8 and B11 [Fig. 5(B)].

From Figure 5(A), it can be seen that the gel beads prepared from alginate alone C2 (representative of

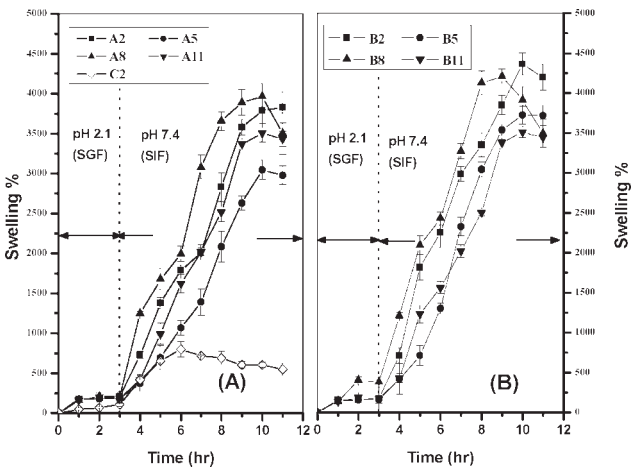


Figure 5 Swelling behavior of some formulation of CMCs-*g*-MAA/alginate beads in SGF for 3 h, followed by 8 h in SIF.

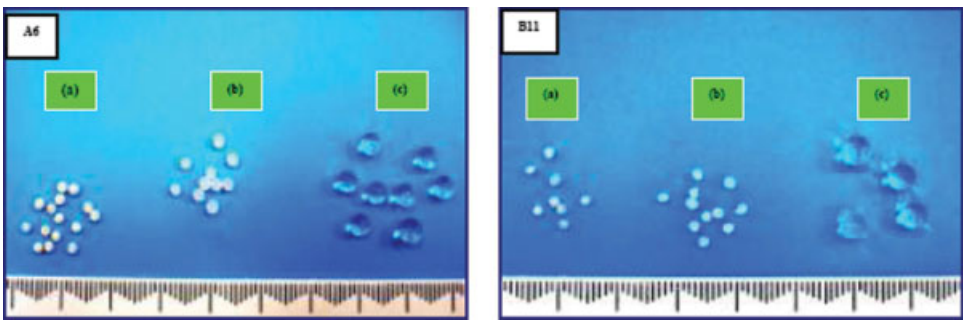


Figure 6 Illustrations of the difference in size between the dry and the swollen states of some drug-free CMCs-*g*-MAA/alginate beads; (a) dry, (b) after 3 h in SGF, and (c) after 8 h in SIF. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

C1–C3) attained relatively low swelling values in SGF as compared to the CMCs-*g*-MAA/alginate hydrogel beads. However, these alginate beads have limited swelling in the SIF which limit the drug release from them in the SIF. Therefore, it seems that, the CMCs-*g*-MAA/alginate beads, developed in this study, are more appropriate for the delivery of protein drugs to the intestine than the hydrogel beads prepared from alginate only. It seemed also, from the swelling results, that the CMCs-*g*-MAA/alginate gel beads prepared in this study achieved enhanced swellings (lower in SGF and higher in SIF) over those of the CMCs/alginate gel beads investigated recently.[14] For instance, in the majority of the prepared formulations in this study, the swelling percentage values attained at 37°C after 3 h in SGF by CMCs-*g*-MAA/alginate beads were less than 254% whereas in case of CMCs/alginate beads,[14] the swelling percentage at 37°C after 2 h in SGF were relatively higher (up to 800% in some formulations). Also, the CMCs-*g*-MAA/alginate beads, prepared in this study, achieved enhanced (higher) swelling (up to 4334 ± 75 as in B2) in SIF than that achieved by CMCs/alginate beads.[14]

From the results in Figure 5(A,B), it can be noted that, increasing the grafting percent (*G*%) of MAA onto CMCs and consequently increasing the number of the carboxylic groups led to improving (lower swelling percentage in SGF and higher swelling percentage in SIF, respectively) the swelling of CMCs-*g*-MAA/alginate beads. This behavior confirms the role played by the carboxylic groups in encouraging bead contraction in SGF by formation of H-bonds and the bead expansion in SIF due to the repulsion (Scheme 2). Some photographs that illustrate the difference in size between the dry and the swollen states of some drug-free CMCs-*g*-MAA/alginate beads are shown in Figure 6.

Entrapment efficiency

From the results of the drug loading efficiency (Table I), it can be seen, in general, that the amount of the BSA loaded in the beads seems to increase with increasing the concentration of Ca^{2+} in solution. However, in most cases, a plateau of BSA encapsulation was reached when the CMCs-*g*-MAA/alginate mixture was crosslinked using 0.2*M* Ca^{2+}. For this reason, the hydrogel beads prepared using 0.2*M* Ca^{2+} were selected for studying their release profiles.

In vitro cumulative release studies

Figure 7 shows the cumulative release profiles of BSA from the CMCs-*g*-MAA/alginate hydrogel beads at 37°C for 3 h in SGF followed by 8 h in SIF. The percent of drug released was much higher in SIF than in SGF because the release rate depends on the swelling of the hydrogel where the mechanism of drug release may be due to the diffusion through the swollen hydrogels. As discussed earlier, the swelling of the prepared hydrogel beads in SIF was greater than in SGF. From Figure 7(A), various amounts of BSA were released (lost) at 37°C within 3 h in the SGF depending on the hydrogel composition. For instance, A8 (2.5% CMCs-*g*-MAA:1%

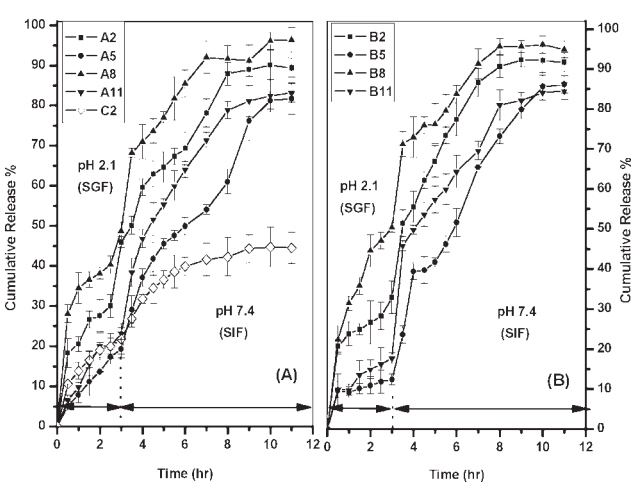


Figure 7 Cumulative release profiles of BSA from the CMCs-*g*-MAA/alginate hydrogel beads at 37°C for 3 h in SGF followed by 8 h in SIF.

alginate) released (lost from the gel) about 49% of the uploaded amount of BSA at 37°C after 3 h in SGF. Another high amount of BSA (46%) was also released from A2 (1% CMCs-*g*-MAA:1% alginate). In case of A11 (1% CMCs-*g*-MAA:2.5% alginate) and D2 (2.5% alginate) only 23.2 and 21.8% BSA were released in SGF, respectively. In the case of A5 (2.5% CMCs-*g*-MAA:2.5% alginate), only 19.3% of BSA was lost in SGF at 37°C. From these results, increasing the total percentage of the polymers used in the preparation of the hydrogel beads makes the beads more compact and consequently decreases their swelling in SGF and thus, it limits the drug loss in SGF. Whereas, increasing the percentage of CMCs-*g*-MAA in the hydrogel increased the swelling and consequently increased the loss of BSA in SGF.

In SIF, A2, A5, A8, and A11 released 89.4, 81.7, 96.3, and 83.1% of BSA at 37°C after 8 h in SIF, respectively. These values are much better than the amount of BSA released from alginate alone, D2, (44.5%). A similar release behavior was noted in case of the hydrogels of formulations B [Fig. 7(B)]. From Figure 7, some formulations such as A2, A8, B2, B5, B8, and B11 showed an initial burst release upon changing the medium and that may be attributed to the fast release of the model drug from the outer layer of the beads due to swelling.

Also, as appeared from both Figure 7 and the values of EE% shown in Table I, it can be noted that both the maximum percent of drug released after 8 h in SIF and the release profile are less dependent on the values of EE% while the composition of the different formulations plays the main role. For instance, the formulations A5 (EE%; 95.10%) and A11 (EE%; 74.57%) showed very similar maximum drug release after 8 h in SIF (about 82%). Figure 7 showed also that B5 is one of the formulations that achieved reasonably good release pattern (limited loss of BSA in SGF with a reasonable maximum release after 8 h in SIF).

CONCLUSIONS

The equilibrium swelling measurements of the prepared CMCs-*g*-MAA/alginate hydrogel beads at 37°C in SGF and SIF clearly showed the pH-responsive nature of them. The *in vitro* release profiles of BSA from the hydrogel beads were also estimated at 37°C in SGF and SIF. From this preliminary investigation, the CMCs-*g*-MAA/alginate hydrogel beads prepared in this study, showed promising release profiles of BSA, as a model for protein drugs. However, this hydrogel bead study requires more effort to limit the swelling and consequently the loss of drug in the SGF, to act as an excellent candidate for intestine-specific delivery of peptide and protein drugs.

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