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ORIGINAL ARTICLE

Ionotropically cross-linked pH-sensitive IPN hydrogel matrices as potential carriers for intestine-specific oral delivery of protein drugs

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Abstract
Background: The oral delivery of proteins and peptide drugs is considered a major challenge. These types of therapeutics are readily degraded, if taken orally, due to the harsh high acidity of stomach and enzymatic attack in the upper small intestinal tract. *Methods*: Water-soluble copolymers of sodium acrylate (AAs) grafted onto carboxymethyl cellulose (CMC) were prepared and characterized using Fourier transform spectroscopy, differential scanning calorimetry, and X-ray diffraction. The obtained graft copolymers were then used in a combination with sodium alginate to develop a new series of pH-sensitive interpenetrating polymeric network (IPN) hydrogels through ionotropic gelation with divalent ions (Ca^{2+}). Morphology of the developed hydrogels was investigated using SEM. Swelling characteristics, at distinct compositions, were also studied at 37°C in two consecutive buffer solutions of pH 2.1 and 7.4 (similar to that of gastric and intestinal fluids, respectively). The release profiles of bovine serum albumin, as a model protein, from test IPN hydrogel films were studied in simulated gastric and intestinal fluids. In addition, the drug release process was confirmed by means of SEM. *Results*: Swelling studies of the developed IPN hydrogels at different pH values confirmed their pH-sensitive nature. The equilibrium swelling extents of the hydrogels were found to be dependent on the grafting yield of CMC/AAs graft copolymer. The IPN hydrogels attained equilibrium swelling percentages in the range 445–740%. In addition, the amount of bovine serum albumin released within 2 hours in pH 2.1 was relatively low (less than 18.1%). This amount increased up to 68% after 8 hours in pH 7.4. *Conclusions*: From the obtained preliminary data, it seems that the IPN hydrogels developed in this contribution can be tailored to act as good potential carriers for oral delivery of protein drugs. These hydrogels showed a promising protection of protein drugs from the harsh acidity of stomach and, at the same time, they conferred sustained drug release in the intestinal fluid.

Key words: Alginate, carboxymethyl cellulose, in vitro release, IPN, oral delivery, pH sensitivity, protein drugs

Introduction

The delivery of therapeutically active proteins and peptides by oral route represents a major challenge. These types of therapeutics are readily degraded, if taken orally, due to the harsh high acidity of stomach and enzymatic attack in the upper small intestinal tract. Also, the short half-life of protein drugs and their limited transit time in the gastrointestinal tract is even more problematic[1,2]. To date, several trials have been reported to overcome these drawbacks and to deliver peptide and protein drugs with maximum oral bioavailability. One of the main strategies is the formulation of these drugs in pH-responsive hydrogels[1,3–8].

Hydrogels are three-dimensional cross-linked hydrophilic polymers, which swell several times without dissolving when brought into contact with water or other biological fluids[9]. Over the last two decades, a great attention has been paid to the development and application of smart hydrogels which can respond to some

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physical, chemical, or biological stimuli, such as temperature, light, pH, and electric fields[1,10]. The pH-responsive hydrogels, a class of smart hydrogels, have witnessed an important potential use in oral site-specific delivery of drugs to the gastrointestinal tract[1]. The number of polymers suitable for the development of hydrogels for drug delivery purposes is very limited as compared to the total available polymers, due to inherent toxicity or lack of biodegradability.

Sodium alginate is a nontoxic natural 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. Alginate has a unique ability of gel formation in the presence of divalent cations such as calcium and barium ions[11]. A significant body of research has focused on investigating hydrogels based on calcium cross-linked alginate for protein drug delivery[3,12–14]. The swelling of these types of hydrogels was found to be minimal at pH 7.4[15], which limits drug release in the intestinal tract. Various studies have been reported to overcome this disadvantage by preparing hydrogels based on alginate with other polymers[3,12,14,15]. In our previous study[1], an attempt was made to overcome this shortcomings by developing hydrogel matrices based on calcium cross-linked mixture of alginate and carboxymethylated chitosan grafted with methacrylic acid. In another study[4], we also investigated the effect of further cross-linking/coating of hydrogel beads based on a combination of alginate with sodium acrylate/carboxymethyl chitosan copolymer onto the swelling behavior and drug release. This cross-linking/coating process was achieved using chitosan grafted with poly(ethylene glycol)[4]. The hydrogels developed in both studies showed enhanced swelling behavior in intestinal fluid as compared to that of hydrogels based on alginate alone. However, the swelling extent of most hydrogel formulations was relatively high in simulated gastric fluid (SGF), which led to a loss of significant amount (up to 50% in some formulations) of the loaded protein drug. This high swelling in gastric fluid was particularly attributed to the chitosan chains present in the hydrogel matrices. These chitosan chains induce more swelling in acidic medium due to protonation of their free NH_2 groups[16]. Therefore, in this study, a trial was carried out to develop and investigate a new series of interpenetrating polymeric network (IPN) hydrogels with limited loss of drug in gastric region toward more efficient oral delivery of protein drugs. In these IPN matrices, chitosan backbone was replaced by cellulose which has a very similar backbone but free of NH_2 groups.

Cellulose is one of the most abundant biopolymers in nature and estimated to be at levels approaching 10^{11} tons annually[17]. Carboxymethyl cellulose (CMC), also known as cellulose gum, is an anionic water-soluble cellulose derivative and it is available in a wide range of substitution[18]. In addition to the various superior characteristics of CMC including its low cost, the chemical modifications of it, mainly through grafting, can offer additional desired properties, enhance its overall characteristics, and increase its potential applications in many fields.

The new IPN hydrogels developed in this study are based on Ca^{2+} cross-linked mixture of alginate and modified CMC. The CMC was synthesized and further graft copolymerized with acrylic acid sodium salt (AAs) in a mild aqueous medium using ammonium persulfate (APS) as initiator. The rationale behind grafting was to increase the number of –COOH groups in CMC. Thus by developing Ca^{2+} cross-linked hydrogels based on alginate with CMC/AAs graft copolymer (CMC-g-AAs), these –COOH groups are expected to participate in minimizing the swelling at pH 2.1 (SGF) and maximizing the swelling at pH 7.4 [simulated intestinal fluid (SIF)]. The limited swelling in SGF will consequently decrease the loss of the loaded protein drugs in gastric region. Some studies also revealed that polymers containing –COOH groups have the ability to protect protein and peptide drugs from some protease enzymes such as trypsin and chymotrypsin[19]. This enzyme inhibitory effect was primarily attributed to the ability of these –COOH groups to bind with extracellular divalent cations (calcium and zinc)[19]. In addition, reduction of the concentration of extracellular divalent ions can help in opening the tight junctions and consequently improves the paracellular transport of protein drugs across the intestinal epithelium[20].

Experimental method

Materials

Microgranular cellulose was purchased from W&R Ballston LTD, England. Acrylic acid 99%, Coomassie brilliant blue (G-250), bovine serum albumin (BSA), and APS were supplied by Sigma-Aldrich, St. Louis, MO, USA. Monochloroacetic acid 99% was obtained from Cambrian Chemicals (Oakville, ON, Canada). Alginic acid sodium salt was provided by Loba Chemie Pvt. Ltd., Colaba, Mumbai, India. Calcium chloride and all other reagents were of analytical grade and used as received.

Methods

Preparation of CMC-g-AAs

Carboxymethylation of cellulose. Water-soluble CMC was prepared by a modified method reported in our earlier study[21]. Briefly, 2 g of microgranular cellulose were put in a 500-mL round-bottomed flask and suspended in 60 mL of isopropyl alcohol at room temperature for 4 hours. To the swollen cellulose suspension, 75 mL of aqueous NaOH solution (60% w/v) was added and then the whole mixture was refluxed at 100°C for 3 hours. Then 100 mL of monochloroacetic acid solution (60% w/v) was added into the reaction mixture over a period of 15 minutes. The mixture was heated for 2 hours at 60°C with stirring. Then the resulting CMC was precipitated in methanol. The product was filtered, washed for several times with a mixture of $CH_3OH:H_2O$ (1:1), and dried.

Graft copolymerization. The graft copolymerization of acrylic acid onto CMC was carried out in a 500-mL two-necked flask using 0.2 g CMC. Before addition of the predetermined volume of monomer, AA, the monomer was converted into a sodium salt through neutralization with NaOH (2 M) and then made up to the volume of 20 mL with deionized water. The components were mixed and stirred for 20 minutes with bubbling of a slow stream of nitrogen gas. The flask was then placed in a thermostated bath at 70°C. Then, 7.5 mM of the initiator, APS (based on the total volume of reaction mixture), dissolved in 10 mL of deionized water was added dropwise with stirring. The used monomer concentrations based on the total volume of reaction mixture (30 mL) were 0.3, 1.2, and 2 mM. The copolymerization reaction was then carried out for 2 hours then the reaction was stopped by letting air into the flask and rapidly cooling down the reaction flask. Then the products were precipitated by pouring the reaction mixture into acetone. The precipitate was filtered off, washed with acetone, and the crude product was then dried and weighed. The formed homopolymer (polyacrylic acids, PAAs) was extensively extracted in a Soxhlet apparatus with methanol for 6 hours. The residual graft copolymer obtained was washed with methanol, dried, and weighed. The grafting percentage (G%) and the grafting efficiency (GE%) of the copolymers were calculated as follow[22]:

$$G\% = \left(\frac{W_g - W_0}{W_0} \right) \times 100 \tag{1}$$

$$GE\% = \left(\frac{W_g}{W_g + W_h} \right) \times 100 \tag{2}$$

where W_g, W_h, and W_0 are the weights of CMC-g-AAs copolymer, homopolymer (PAAs), and CMC, respectively.

Preparation of alginate/CMC-g-AAs hydrogel matrices

For preparation of the pH-responsive alginate/CMC-g-AAs hydrogel films, a predetermined amount of the CMC-g-AAs copolymer (with G%: 102%, 652%, or 1003%) was dissolved in deionized water and stirred for 5 minutes. Then the copolymer solution was mixed, with stirring, with a pre-calculated volume of 4% alginate solution. The obtained polymer mixture was left to degas and then casted in a Petri dish and dried at 40°C under vacuum. The pH-responsive IPN hydrogels were then obtained through the ionotropic gelation by immersing the resulting films in 20 mL of aqueous $CaCl_2$ solution (0.1, 0.2, or 0.3 M) for 20 minutes, washing with a certain volume of deionized water, and then drying again at 40°C under vacuum. The compositions of the IPN hydrogel formulations cross-linked with 0.2 M $CaCl_2$ (as examples for other formulations) are listed in Table 1.

Table 1. Composition of the developed IPN hydrogel matrices.

	CMC-g-AAs		Sodium alginate	
Sample code	G%	Weight (g)	weight (g)	$CaCl_2$ [M]
L1	102%	0.6	—	0.2
L2		0.4	0.2	0.2
L3		0.3	0.3	0.2
L4		0.2	0.4	0.2
M1	652%	0.6	—	0.2
M2		0.4	0.2	0.2
M3		0.3	0.3	0.2
M4		0.2	0.4	0.2
H1	1003%	0.6	—	0.2
H2		0.4	0.2	0.2
H3		0.3	0.3	0.2
H4		0.2	0.4	0.2
G2		—	0.6	0.2

Characterization

The prepared CMC and CMC-g-AAs graft copolymers were characterized by Fourier transform infrared spectroscopy (FTIR) (Mattson 5000 FTIR spectrometer) using KBr discs in the range of 600–4000 cm^{-1}. The differential scanning calorimetry (DSC) of CMC and CMC-g-AAs copolymers of different grafting yields were performed in a Du Pont model 2000 DSC analyzer. The samples (3–4 mg) were weighed into aluminum sample pans and sealed. An empty aluminum pan of approximately equal weight was used as a reference. The applied heating rate was 10°C/min up to a maximum temperature of 400°C under nitrogen atmosphere. All peaks were determined and the areas were converted into enthalpy values. The X-ray diffraction (XRD) patterns of both CMC and the prepared copolymers were also determined using a Philips apparatus PW 105 diffractometer (Philips Analytical, Cambridge, UK) using Ni-filtered Cu K_α radiation ($\lambda = 1.540$ A°) at an operating voltage of 40 KV. The surface morphology of the developed IPN hydrogel films before and after drug release was examined with the aid of a scanning electron microscope (SEM) (JEOL JXA-840A), electron probe microanalyzer. Samples were coated with gold under vacuum and then examined.

Entrapment of a model for protein drugs

Drug-loaded alginate/CMC-g-AAs IPN-hydrogels were prepared by the same method used for preparing the drug-free hydrogels. As a preliminary in vitro drug release study, only one CMC-g-AAs copolymer sample with a moderate grafting yield (G%: 652%) was selected, as an example for other samples, for preparation of BSA-loaded IPN hydrogels. After mixing the predetermined volume of CMC-g-AAs solution (at G%: 652%) with alginate solution (4%), BSA was add, as a model for protein drugs, to the mixture at a ratio of 15% w/w (relative to the total weights of CMC-g-AAs and alginate) and then gently stirred for 15 minutes at room temperature. The obtained BSA/polymer mixture was then casted in a Petri dish and dried under vacuum at room temperature. The pH-responsive IPN hydrogels were then obtained, as described earlier, through ionotropic gelation using aqueous $CaCl_2$ solution (0.2 M) followed by washing with a certain volume of deionized water and drying under vacuum. Based on the chemical structure of the alginate and the CMC-g-AAs copolymer used in developing the IPN hydrogels, occurrence of copolymer–copolymer interaction is not expected. The hypothesized structure of the resulting IPN hydrogels is illustrated in Scheme 1.

Swelling studies

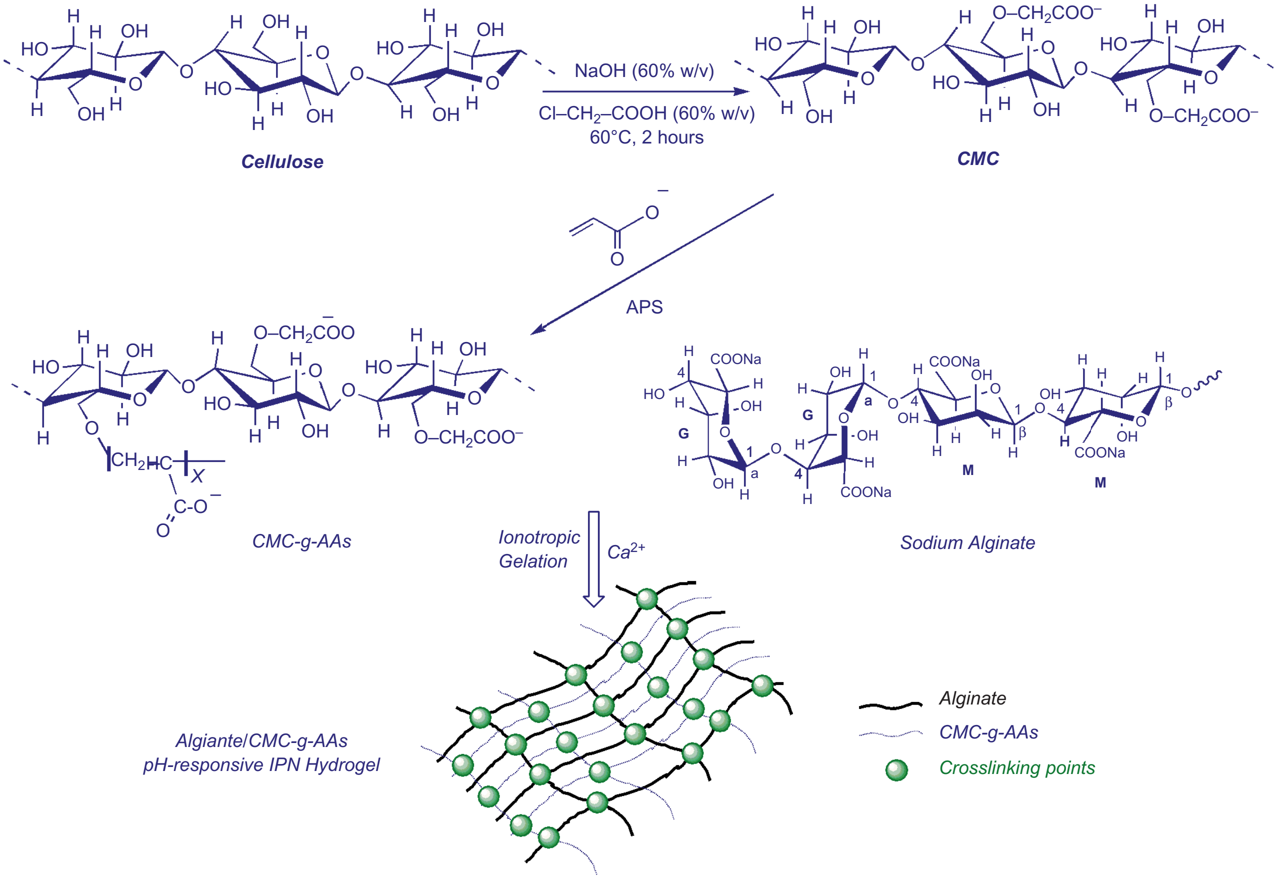
The swelling behavior of the developed alginate/CMC-g-AAs IPN hydrogel films was investigated in two consecutive buffer solutions (pH 2.1 and 7.4, similar to that of gastric and intestinal fluids, respectively). The buffer solutions were prepared from a mixture of phosphoric acid (0.054 mol), boric acid (0.040 mol), and acetic acid (0.042 mol) and then adjusted to the required pH value by the dropwise addition of 0.2 N NaOH solution. The pH values were precisely checked by a pH meter. A predetermined weight of the hydrogel film was immersed in 20 mL of buffer solution at pH 2.1 for 2 hours followed by transferring it to a buffer solution of pH 7.4 for 10 hours. At certain intervals, the swollen weights of the films were determined after removal of the surface liquid using tissue paper, until equilibrium swelling was attained. The percent swelling was calculated by the following equation:

$$\text{Swelling}\,\% = \left(\frac{W_t - W_0}{W_0}\right) \times 100 \qquad (3)$$

where W_0 is the initial weight and W_t the final weight of the film at time t.

In vitro cumulative release

The in vitro cumulative release profiles of the entrapped BSA were determined by placing pre-weighed BSA-loaded hydrogel film in 20 mL of buffer at pH 2.1 (SGF) at 37°C for 2 hours and subsequently in 20 mL buffer of pH 7.4 (SIF) at 37°C for 8 hours. At intervals, 100 µL sample was withdrawn and assayed by Bradford method[23] at λ_{max} 595 nm using a photometer 5010 (Robert Riele



Scheme 1. The development of ionotropically cross-linked alginate/CMC-g-AAs IPN hydrogel matrices.

GmbH & Co. KG, Berlin, Germany). The withdrawn aliquot was replaced with an equal volume of fresh buffer, to keep the volume of the release medium constant. The data points represent the average of three independent experiments.

Statistical analysis

The obtained results were analyzed and expressed as an average from three independent experiments. Effects of various parameters on the swelling and drug release from the developed hydrogel matrices were statistically analyzed by one-way ANOVA using the Microsoft Office 2007. The differences were considered significant at the level of $P < 0.05$.

Results and discussions

The main target of this study was to develop a series of pH-sensitive IPN hydrogel matrices that can be used as smart potential carriers for intestine-specific sustained delivery of protein and peptide drugs. This smart hydrogel carrier system is composed of sodium alginate in a combination with a water-soluble cellulose derivative (CMC-g-AAs). The hydrogels were prepared through the ionotropic cross-linking using divalent ions (Ca^{2+}).

Preparation of CMC and CMC-g-AAs copolymers

Both CMC and CMC-g-AAs were prepared by modified methods described in our earlier study[21]. The structural changes of cellulose, CMC and the prepared graft copolymer, CMC-g-AAs were confirmed by FTIR (Figure 1-i). In the FTIR spectrum of cellulose (Figure 1-ia), the strong peak at 3439 cm^{-1} is due to the O–H stretching vibration and the intermolecular H-bonds of the polysaccharide moieties. The FTIR spectrum of CMC (Figure 1-ib) shows a strong new peak at 1728 cm^{-1} representing the carboxylate C=O asymmetric stretching. The signal at 1392 cm^{-1} could be assigned to the symmetric stretching vibration of carboxylate C=O. In case of IR spectrum of CMC-g-AAs (Figure 1-ic), there was a new absorption peak at 1264 cm^{-1}. No clear absorption due to vinyl unsaturation was observed at about 1640 cm^{-1}, and also no clear bands appeared in the range of 1400–1420 cm^{-1}, representing vinylic double bond in conjunction with carbonyl group. This tends to indicate the disappearance of the vinylic double bond of AAs due to grafting. From the obtained FTIR spectra, it also seems that the prepared CMC-g-AAs copolymer has characteristic peaks of both CMC and AAs. This tends to confirm the occurrence of grafting process. Also, as shown in Figure 1-ii, the intensity of C=O absorption in FTIR of CMC-g-AAs at 1728 cm^{-1} was increased with increasing the G% and this can be considered as another experimental evidence of grafting. Besides, the higher weight of the prepared graft copolymer than that of the starting CMC after the extensive removal of the homopolymer can be taken as evidence of grafting.

Figure 2 shows the DSC of CMC-g-AAs copolymer. From the figure, the thermogram of CMC-g-AAs shows a broad exothermic transition appeared at 78°C, which may be attributed to the loss of bound water. In addition, an endothermic peak was appeared at 217°C and a strong exotherm was found at 257°C reflecting the decomposition process of the synthesized copolymer.

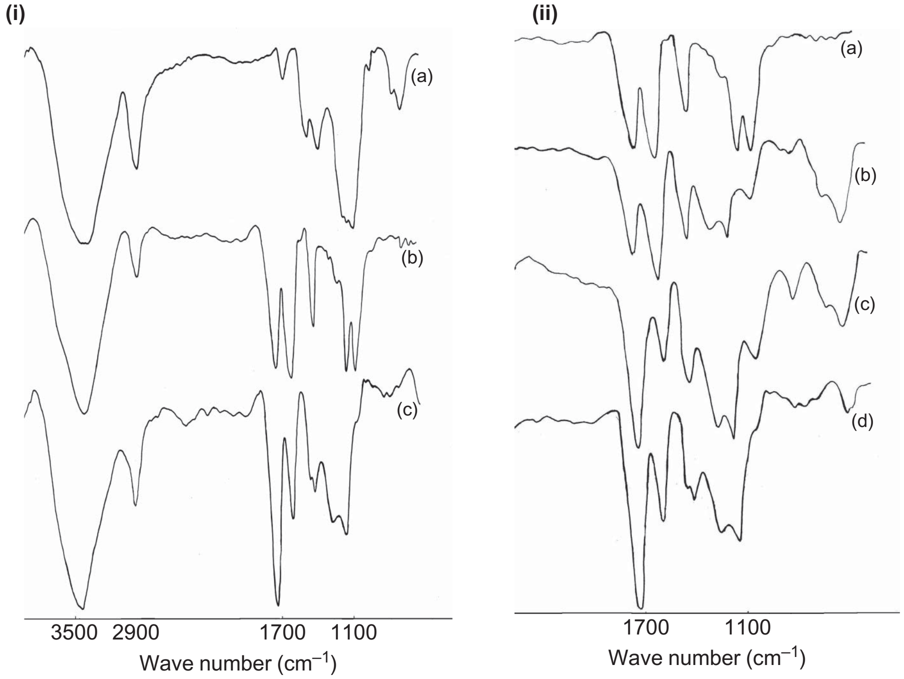


Figure 1. FTIR spectra of (i) cellulose (a), CMC (b), and CMC-g-AAs (G%: 1003%); (ii) CMC (a) in comparison with CMC-g-AAs of different G%; (b) 102%, (c) 652%, and (d) 1003%.

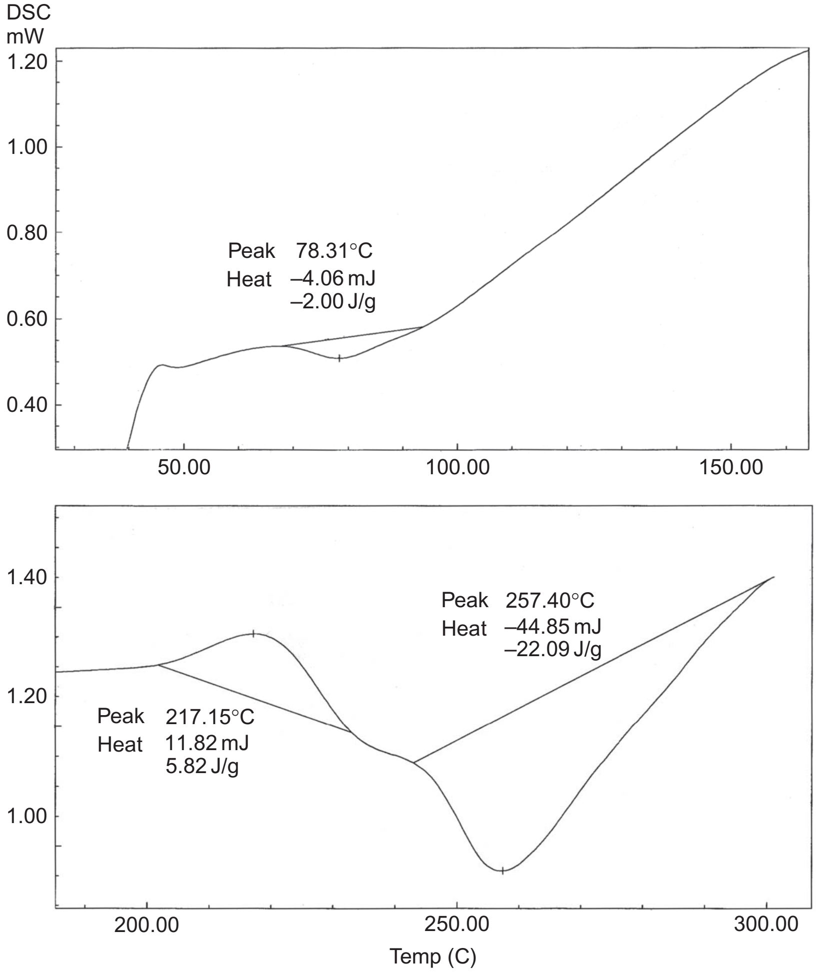


Figure 2. DSC of CMC-g-AAs copolymer (G%: 652%).

The XRD patterns of the prepared CMC-g-AAs copolymer in comparison to that of CMC are shown in Figure 3. In the diffractogram of both CMC and CMC-g-AAs, the halos centered on 2θ value of 12–13° represent the patterns of the amorphous CMC. In case of CMC-g-AAs, the new halo appeared at 2θ value of 20–22° can be attributed to the AAs side chains (inter-sodium distances) and its intensity reflects the level of grafting. The sharp reflections appeared at much higher values of 2θ in both diffractograms may be due to either the sample holder or some inorganic salt residues.

Preparation of alginate/CMC-g-AAs IPN hydrogels

A significant interest has been paid for investigation of different types of hydrogel matrices based on calcium cross-linked alginate for drug delivery[1,3,12–14]. However, many of these matrices showed inadequacies as carriers for oral delivery of protein drugs. These inadequacies include the minimal swelling in intestinal fluid (SIF, pH 7.4)[15], which may limit the drug release in the small intestine. Also, swelling of some of these reported hydrogel matrices was relatively high in the gastric fluid (SGF, pH 2.1), which leads to loss of relatively a large amount of the uploaded protein drug in the stomach. In an attempt to overcome these shortcomings, we have developed in this study, some new IPN hydrogel matrices and in vitro evaluated them as potential carriers for the oral delivery of protein drugs. These IPN hydrogels were prepared by the in situ ionotropic gelation reaction of two anionic polymers, sodium alginate and CMC-g-AAs, in the presence of Ca^{2+} solutions of different molar concentrations (0.1, 0.2, and 0.3 M). These three calcium concentrations were selected based on the results obtained from previous studies[1,15]. These studies reported that Ca^{2+} with a concentration of 0.1–0.3 M would be enough for cross-linking alginate solutions to produce alginate-based hydrogels. For developing of the IPN hydrogels, CMC was first prepared and then further modified through grafting of different ratios of AAs onto its backbone. The main reason of this modification was to increase the number of carboxylic groups in the CMC. Thus by developing a calcium cross-linked hydrogel matrices based on sodium alginate with this modified CMC, 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

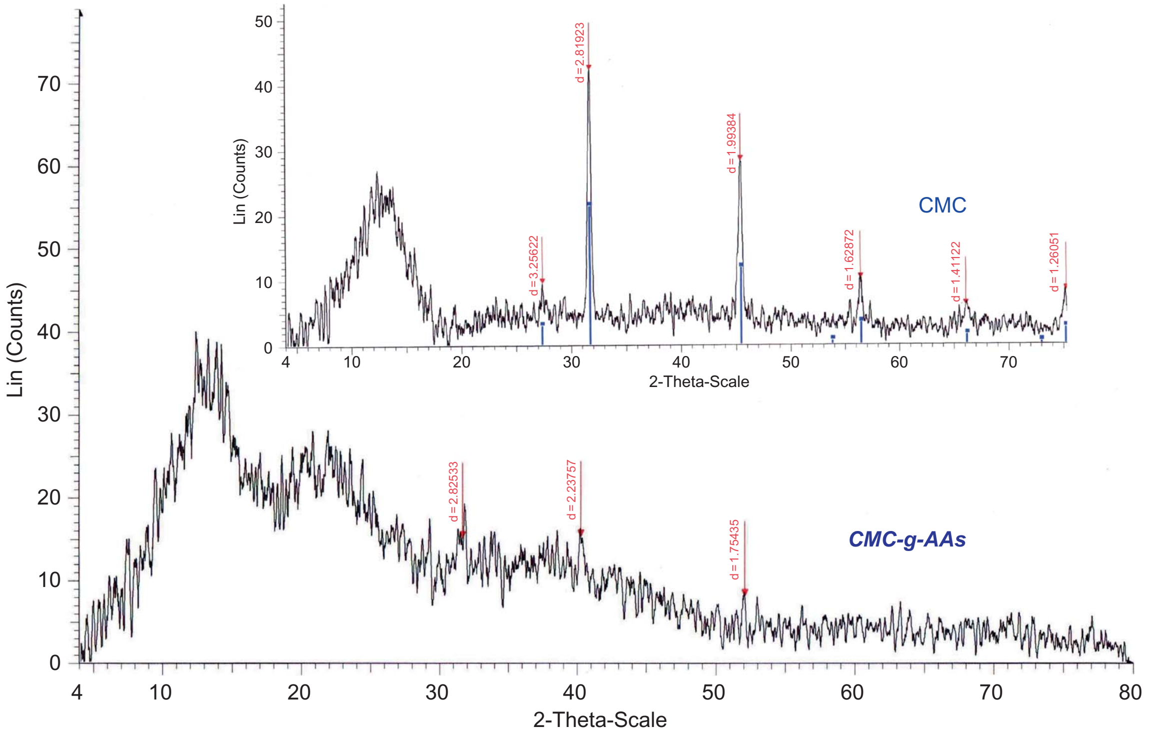


Figure 3. XRD patterns of CMC-g-AAs in comparison with CMC.

role of carboxylic groups in controlling the swelling extent of the developed hydrogel microparticles is illustrated in our earlier investigations[1,4].

An attempt was carried out to examine the ability of the synthesized CMC-g-AAs copolymer alone to form hydrogels through ionotropic interaction with Ca^{2+}. It was noticed that the aqueous solution of this graft copolymer, CMC-g-AAs could form hydrogel aggregations when dropped onto a Ca^{2+} solution. However, the resulting hydrogels were not stable enough and of very random shapes. For this reason, the hydrogels based on CMC-g-AAs alone were not further investigated in this study. This weakness of the CMC-g-AAs-based hydrogels may be attributed to both the linearity of the CMC backbones and the hindrance of cross-linking process that may arise from the grafted poly(AAs) side chains. Both alginate and CMC-g-AAs copolymer can form ionic cross-links with Ca^{2+} at both pHs 2.1 and 7.4. At pH 2.1, the free carboxylic groups, which are not involved in the ionic cross-linking with Ca^{2+}, tend to form hydrogen bonds with each other and with the –OH groups of the sugar moieties of both CMC and alginate[1]. Both of these ionic cross-links and the hydrogen bonds act to minimize the swelling at pH 2.1. In contrast, at pH 7.4, most of the free carboxylic groups would be ionized. Hence, strong repulsive forces are created by the electrostatic repulsion between these ionized carboxylate groups (COO^-)[1]. These repulsive forces are thus responsible for attaining the hydrogels higher values of swelling at pH 7.4. The development of the ionotropically cross-linked alginate/CMC-g-AAs IPN starting from the chemical modification of cellulose is illustrated in Scheme 1.

The morphology of the developed IPN hydrogel films (thickness about 25 µm) as investigated by SEM is shown in Figure 4. As apparent in the figure, the BSA-loaded IPN hydrogel films (Figure 4a) seem relatively integrated, dense, and smooth. This tends to show a reasonable homogeneity of the developed hydrogel matrices. This relatively smooth morphology has turned to show a highly rough surface with many holes and cavities after immersing the hydrogel film for 2 hours in pH 2.1 followed by 8 hours in pH 7.4 (Figure 4b). This morphology confirms the release process of the model drug, BSA.

Swelling study

The swelling behavior of a hydrogel is one of the most significant characteristics that control the drug release kinetics from it. The swelling behavior of the developed alginate/CMC-g-AAs IPN hydrogel matrices was investigated in pH 2.1 (SGF) for 2 hours followed by 10 hours in pH 7.4 (SIF) at 37°C. From the obtained swelling data, it was found that, at both pHs 2.1 and 7.4, increasing the concentration of $CaCl_2$ increases the cross-linking extent and consequently decreases the equilibrium swelling percentage of the hydrogels. However, it was noted that the swelling values attained at equilibrium for the hydrogels prepared using 0.2 and 0.3 M $CaCl_2$ are very similar (differences are statistically nonsignificant), but significantly different from that for the hydrogels prepared using 0.1 M $CaCl_2$. The IPN hydrogel formulations

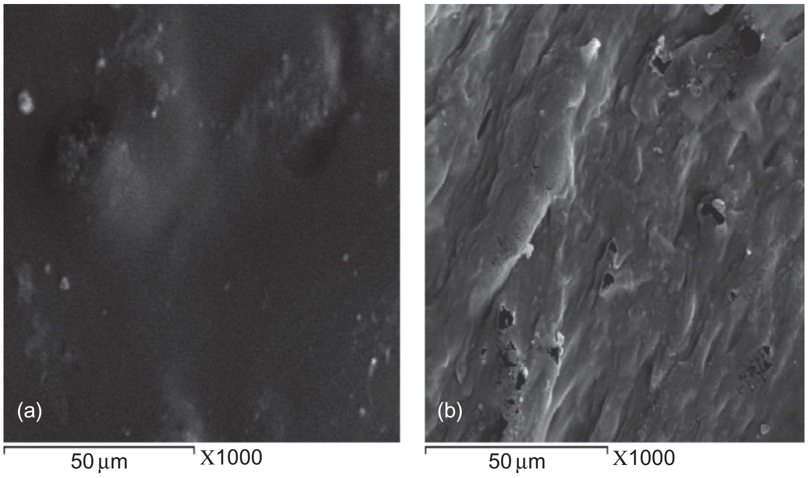


Figure 4. SEMs showing the surface of BSA-loaded IPN hydrogel film (M3) before (a) and after (b) BSA release from the film (the release solution was at pH 2.1 for 2 hours and subsequently in another solution at pH 7.4 for 8 hours).

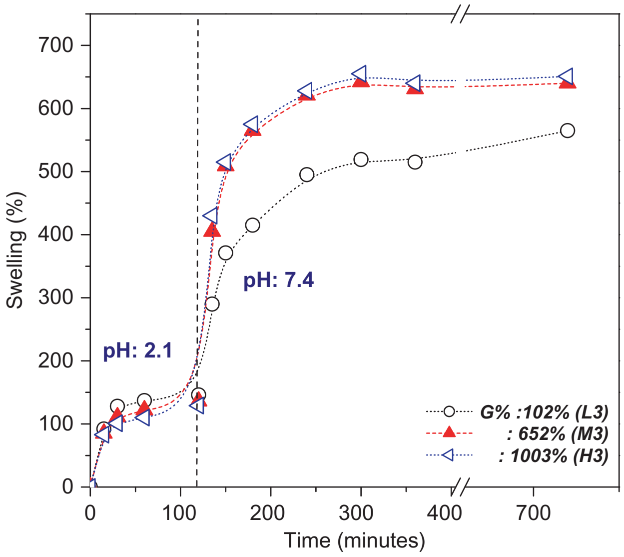


Figure 5. Swelling behavior of calcium cross-linked alginate/CMC-g-AAs IPN hydrogel films prepared at different G% for 2 hours in pH 2.1 followed by 10 hours in pH 7.4 at 37°C.

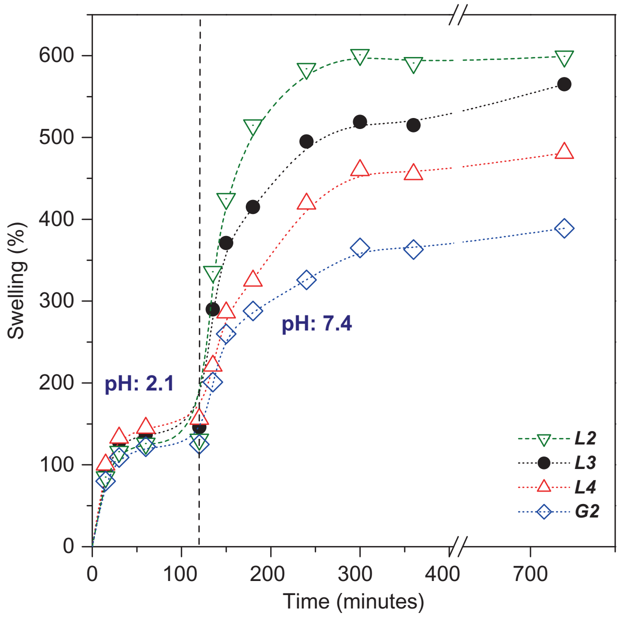


Figure 6. Swelling behavior of calcium cross-linked alginate/CMC-g-AAs (G%: 102%) IPN hydrogel films with different composition at 37°C.

prepared using 0.2 M $CaCl_{2,}$ were selected, as an example for the other formulations, as shown in Table 1 for the investigation of their swelling profiles. Figure 5 illustrates the swelling characteristics for some of the developed alginate/CMC-g-AAs hydrogel films of same compositions but differ only in the G% of the used CMC-g-AAs. As apparent from the figure, increasing the G% of AAs onto CMC backbone and consequently increasing the number of carboxylic groups tends, in general, to improve (reduce the swelling % in pH 2.1 and increase the swelling % in pH 7.4, respectively) the swelling of alginate/CMCs-g-AAs hydrogels. For instance, in pH 7.4, the cross-linked samples L3 (G%: 102%), M3 (G%: 652%), and H3 (G%: 1003%) attained swelling percents of 564%, 640%, and 651%, respectively. This performance confirms the role of the carboxylic groups in encouraging hydrogel contraction in pH 2.1 (SGF) by the formation of H-bonds and the hydrogel expansion in pH 7.4 (SIF) due to the repulsion[1]. In pH 7.4, it was found that the differences in swelling between M3 (G%: 652%) and H3 (G%: 1003%) are statistically nonsignificant.

Figure 6 shows the effect of the hydrogels composition on their swelling behavior. From the results in the figure, it can be noted that increasing the percentage of the CMC-g-AAs in the hydrogels relative to alginate led to increasing the swelling values in both SGF and SIF. This effect of the CMC-g-AAs content onto swelling was found to be more statistically significant in SIF than in

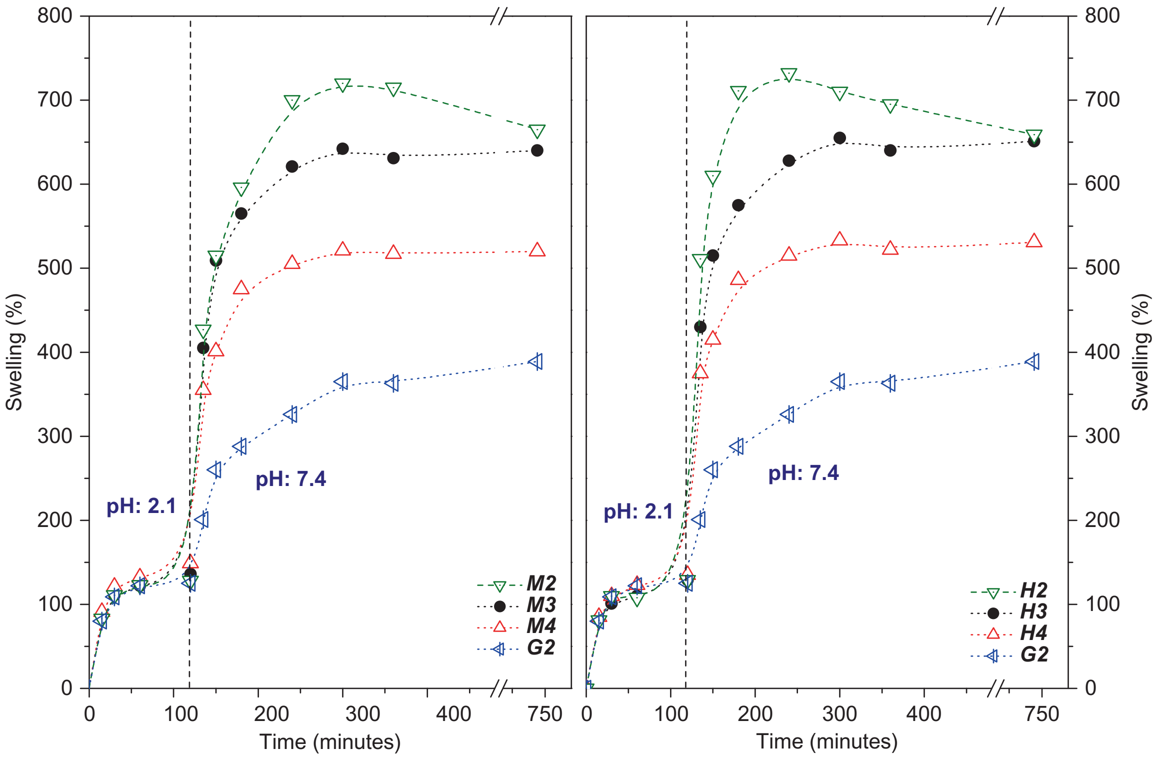


Figure 7. Swelling behavior of calcium cross-linked alginate/CMC-g-AAs: (G%: 652%—left) and (G%: 1003%—right) IPN hydrogel films with different composition at 37°C.

SGF. For instance, in pH 7.4, the hydrogels L4 (0.2 g CMC-g-AAs), L3 (0.3 g CMC-g-AAs), and L2 (0.4 g CMC-g-AAs) attained swelling percents of 481%, 565%, and 595%, respectively, at equilibrium. The same result can be also noted from Figure 7a for the hydrogel matrices M2, M3, and M4 which were prepared using CMC-g-AAs of moderate grafting yield (G%: 652%). For instance, in the SIF (pH 7.4), the hydrogels M4 (0.2 g CMC-g-AAs), M3 (0.3 g CMC-g-AAs), and M2 (0.4 g CMC-g-AAs) attained swelling percents of 508%, 636%, and 652%, respectively, at equilibrium. In case of the hydrogels obtained using CMC-g-AAs of high grafting yield (G%: 1003%, Figure 7b), the formulations H2, H3, and H4 achieved equilibrium swelling values of 534%, 648%, and 661%, respectively. This effect of CMC-g-AAs content onto the swelling values can be attributed to the high hydrophilicity of CMC-g-AAs, which consequently increases the hydrophilic nature of the cross-linked hydrogel matrix leading to attaining higher swelling values at equilibrium. In addition, the Figures 6 and 7 revealed that the hydrogels prepared from alginate alone using same concentration of $CaCl_2$ (G2) attained limited swelling in the SIF as compared to the alginate/CMC-g-AAs hydrogels. This tends to limit the drug release from the alginate hydrogels in the intestine. Therefore, it seems that, the alginate/CMC-g-AAs hydrogels, developed in this study, are more appropriate for the delivery of protein drugs to the intestine than the hydrogels prepared from alginate only.

In vitro release profiles

The cumulative release profiles of BSA from the developed alginate/CMC-g-AAs hydrogels as compared to the hydrogels based on alginate alone are shown in Figure 8. The hydrogel matrices prepared using CMC-g-AAs copolymers of moderate grafting yield (G%: 652%) were selected as example for other formulations. The release study was carried out at 37°C for 2 hours in pH 2.1 (SGF) and then for 8 hours in pH 7.4 (SIF). As apparent from the figure, the percent of drug released was much higher in pH 7.4 solution than in pH 2.1. This can be attributed to the dependence of release rate from a hydrogel on its swelling pattern (see Figure 7), where the mechanism of drug release is primarily due to the diffusion through the swollen hydrogels. As can be noted also from Figure 8, different amounts of BSA were released at 37°C within 2 hours in pH 2.1 (SGF) depending on the hydrogel composition. Moreover, it is apparent from the figure that increasing the percentage of CMC-g-AAs in the hydrogel increased the swelling (see Figures 6 and 7) and consequently increased the amount of BSA released in SGF. For instance, M4 (0.2 CMC-g-AAs:0.4 alginate) released about 5.8% of the uploaded BSA at 37°C after 2 hours in SGF. In case of M3 (0.3 CMC-g-AAs:0.3 alginate) and M2 (0.4 CMC-g-AAs: 0.2 alginate), the released percents were 11.7% and 18.1%, respectively. In case of G2 (alginate only), the BSA released percentage was 0.63%. In SIF, M2, M3, and M4 released 64.8%, 28.8%, and 14.9% of BSA, respectively, at 37°C after 8 hours. Although these

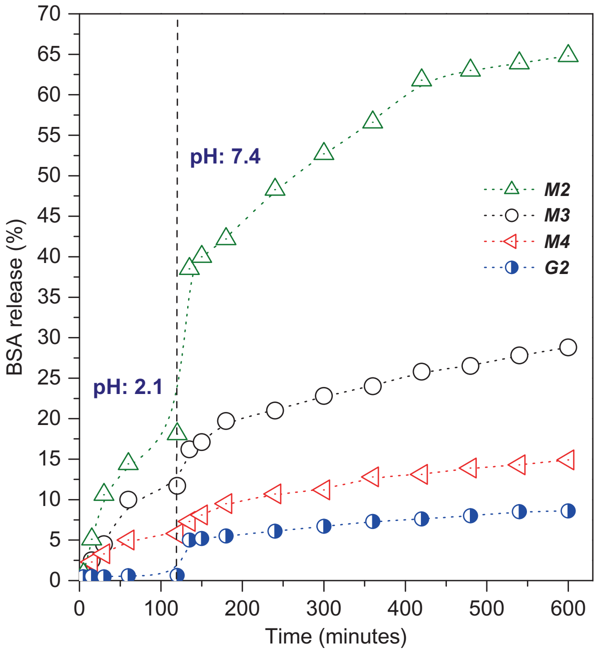


Figure 8. Cumulative release profiles of BSA from test films of calcium alginate/CMC-g-AA (G%: 652%) films prepared at distinct compositions determined by immersing dried test samples in a solution at pH 2.1 for 2 hours and subsequently in another solution at pH 7.4.

values of drug released in SIF are much better than the amount of BSA released from hydrogels based on alginate alone, G2 (only 8.6%), the alginate/CMC-g-AAs hydrogels developed in this study still need some modification to reduce the drug release (loss) in the gastric fluid (SGF).

Conclusion

Swelling measurements of the alginate/CMC-g-AAs hydrogels, developed in this study, at 37°C in pHs 2.1 and 7.4 clearly showed a pH-responsive nature of them. The in vitro release profiles of BSA from the hydrogels were also estimated at 37°C for 2 hours in pH 2.1 followed by 8 hours in pH 7.4. From this preliminary study, the developed hydrogel formulations showed promising swelling behavior and release profiles of the uploaded model protein drug, BSA in SIF, and it can be tailored to act as a good candidate for oral delivery of peptide and protein drugs.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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