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Fabrication and Evaluation of a novel Polymeric Hydrogel of Carboxymethyl chitosan-g-Polyacrylic acid (CMC-g-PAA) for Oral Insulin Delivery

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Abstract

A novel pH-sensitive hydrogel composing of carboxymethyl chitosan (CMC) and acrylic acid (AA) was fabricated *via* free radical grafting polymerization. The effective factors on the swelling ratios of hydrogels were studied. The successful fabrication of CMC-g-PAA hydrogel was confirmed with Fourier transform infrared spectroscopy (FTIR). The inner morphology of final hydrogel was observed with scanning electron microscope (SEM). Insulin (INS) was used as a model drug and encapsulated into the CMC-g-PAA hydrogel. The release profile of INS loaded-CMC-g-PAA (INS-CMC-g-PAA) hydrogel was obtained in phosphate buffer solution (PBS) with pH 1.2 and 7.4. The hypoglycemic effect of oral INS-CMC-g-PAA hydrogel was also studied. SEM photographs showed that CMC-g-PAA hydrogel had 3D network structures. The *in vitro* releasing tests showed that only 16.3 ± 2. 6 % of INS was released into in pH 1.2, while over 93.2 ± 3.8 % of INS was diffused into PBS (pH 7.4). This phenomenon illustrated that the INS-CMC-g-PAA hydrogel had a good pH responsibility, which could direct INS to release in basic environments. The animal experiments demonstrated that the oral INS-CMC-g-PAA hydrogel had a persistent and effective hypoglycemic effect. Therefore, the CMC-g-PAA hydrogel had a potential application on the oral delivery of protein drugs.

Key words: Carboxymethyl chitosan, pH-sensitive hydrogel, release, insulin

Abbreviations

CMC	Carboxymethyl chitosan
AA	Acrylic acid
PAA	Polyacrylic acid
CMC-g-PAA	Carboxymethyl chitosan-g-Polyacrylic acid
INS	Insulin
INS-CMC-g-PAA hydrogel	INS loaded-CMC-g-PAA hydrogel
FTIR	Fourier transform infrared spectroscopy
NMR	Nuclear magnetic resonance
SEM	Scanning electron microscope
PBS	Phosphate buffer solution
MBA	N, N-methylenebis acrylamide
C_{CMC}	CMC concentration
HPLC	High pressure liquid chromatography
C_{CMC}	CMC concentration
H-bonds	Hydrogen bonds
AR	Analytically pure

1. Introduction

Hydrogel is a kind of hydrophilic polymers that cross-linked *via* covalent bonds, hydrogen bonds, or Van der Waals' force, *et al* [1-3]. The cross-linking gives the polymeric system a 3D network structure, which makes the hydrogel to swell in water instead of dissolving [4-5]. Among the hydrogel families, pH-sensitive hydrogel can swell or shrink with the changing of surrounding pH levels. They usually contain many functional groups (*e.g.* carboxyl groups or amino groups), which can be hydrolyzed or protonated in water [6-7]. For example, in acidic conditions, hydrogen bonds (H-bonds) can form among the un-dissociated –COOH groups. The superposition of H-bonds prevents the hydrogels from swelling, which will result the hydrogel in a contraction state. In this case, only a little encapsulated drug can be released from the hydrogels. On the contrary, these –COOH will be ionized into $–COO^-$ moieties in basic conditions, and electrostatic repulsions among these ions will impulse the hydrogel swelling (as shown in Fig. 1). Then, most of loaded drug will be released quickly. Therefore, these properties can direct model drug to a target tissue site-specifically.

In the past few decades, the pH-sensitive hydrogels are employed as the release platforms to deliver model drugs into targeted sites [8-10]. The release behaviors can be controlled by drug-loading rates and the crosslinking degree of final hydrogels [11-12]. These pH-sensibility hydrogels containing –COOH groups can swell in alkaline or neutral conditions (*e.g.* intestinal juice) but shrink in acidic environment (*e.g.* gastric juice). In view of these, such hydrogels can be used as platform to direct

proteinic drugs (such as: INS, bovine serum albumin) or irritant drugs (such as: non-steroidal anti-inflammatory drugs) to intestinal environments [13-14]. Such process can prevent proteinic drugs from degradation by pepsase, or decrease the irritation of non-steroidal anti-inflammatory drugs on stomach. INS, an effective hypolycemic agent, can be destroyed by pepsase. Therefore, its oral bioavailability will be limited [15-16]. However, the pH-sensitive hydrogel provides an ideal solution for oral administration of INS. Recent years, different pH-sensitive hydrogels with various structures are developed to deliver INS to the intestinal environment. Shang *et al.* fabricates a novel pH-sensitive hydrogel *via* suspension polymerization [17]. The INS-loaded pH-sensitive hydrogel shows an excellent hypoglycemic effect administrated by oral method. S. S. Vaghani *et al*. uses CMC as a material to develop a novel hydrogel [18]. Their hydrogel shows excellent swelling ratios in the basic conditions. P.P. Kundu *et al*. uses pH sensitive N-succinyl chitosan grafted polyacrylamide hydrogel for oral INS delivery [20]. The INS-loaded hydrogel shows an excellent pH responsibility, retaining about 26 % of encapsulated INS in pH 1.2 but releasing of over 98 % of INS in pH 7.4. The oral administration of INS-loaded hydrogel is successful in lowering the blood glucose level of diabetic mice.

In this study, we fabricated a novel hydrogel with CMC and AA as the monomers, N, N-methylenebis acrylamide (MBA) as the crosslinking agent, and $K_2S_2O_8$ as an initiator. During the fabrication, CMC was grafted onto the main chains of PAA *via* a free radical polymerization. The influence factors on the swelling ratios of final hydrogel, such as: AA/CMA ratios, crosslinking, initiators, reaction times,

temperatures, and rheology of solutions were revealed, respectively. The pH-sensitivity and swelling-deswelling properties of final hydrogels were also carried out in different PBS with various pH levels. INS was employed as a model drug and encapsulated into the final CMC-g-PAA hydrogels to obtain a novel oral formulation (INS-CMC-g-PAA). The release behavior of INS from INS-CMC-g-PAA was performed in the simulated gastric juice (PBS, pH 1.2) and intestinal fluid (PBS, pH 7.4), respectively. The *in vivo* hypoglycemic effects of oral INS-CMC-g-PAA hydrogels were also revealed using diabetic rats models.

2. Experimental

2.1 Materials

CMC was purchased from Qingdao Haaga biotechnology Co., Ltd (Qingdao, China). AA (AR) was the product of Tianjin Yongda chemical reagent Co., Ltd (Tianjin, China). Before using, AA was refined by vacuum distillation to remove the polymerization inhibitors. MBA (AR) was obtained from Reading chemical technology (Shanghai) Co., Ltd. (Shanghai, China). Alloxan was bought from Shanghai Aladdin reagent Co., Ltd (Shanghai, China). INS was the product of Shanghai Li Rui biotechnology Co., Ltd (Beijing, China). Other inorganic reagents were bought from Tianjin Yongda chemical reagent Co., Ltd (Tianjin, China), and used as received without any further purification.

2.2 Fabrication of CMC-g-PAA hydrogels

The CMC-g-PAA hydrogels were fabricated *via* a free radical polymerization with CMC and AA as monomers, MBA as a cross-linking agent, and $K_2S_2O_8$ as an initiator (as shown in Scheme. 1). Under magnetic stirring, a mount of CMC was dissolved in alcohol to obtain solution (a). Next, a given mass of AA, MBA and $K_2S_2O_8$ were dissolved in water to give solution (b). Then, the mixture of solution (a) and (b) was oscillated in an ultrasonic cleaner to obtain a homogeneous solution (c). To remove bubbles, the mixture was centrifuged at the speed of 8, 000 rpm for 10.0 min. The final mixture was filled into several weighing bottles, separately. Then, these bottles were incubated in a constant temperature incubator at 37 ^{o}C. After 12 h of reaction,

the crude hydrogels were prepared. The crude hydrogels were cut into chips (8 mm^3) and loaded into dialysis bag (cutoff molecular weight of 8, 000). The bag was dialyzed in abundant of water to remove the un-reacted monomers and $K_2S_2O_8$. After lyophilization, the refined hydrogel chips were recovered and used for further studies.

2.3 Swelling ratios

The swelling ratios of CMC-g-PAA hydrogels were studied with a weighing method, and the swelling ratios were obtained from equation (1):

$$Swelling\ Ratios = \frac{weight\ of\ swollen\ hydrogels - weight\ of\ dried\ hydrogels}{weight\ of\ dried\ hydrogels} .....(1)$$

2.4 Characterization

FTIR analysis was carried out with a FTS-135 (BIO-RAD, USA) spectrometer, scanning from 4000 to 500 cm^{-1}. AA, MBA, CMC and CMC-g-PAA hydrogels were mixed separately with KBr at a 1:10 weight ratio, and KBr pellets were prepared under a hydraulic pressure of 400.0 kg. The ^1H NMR spectra of AA, MBA, CMC and CMC-g-PAA hydrogel were obtained to confirm the fabrication of CMC-g-PAA hydrogels. The ^1H NMR spectra of AA, MBA and CMC were obtained from an Avance 500 MHz NMR spectrometer (Bruker, Switzerland) with CH_3OH-d as solvents. The ^1H NMR study of CMC-g-PAA hydrogel was carried out with a solid-state NMR spectrometer (Bruker, Switzerland). The inner morphologies of CMC-g-PAA hydrogels were observed with an S-4800 SEM (Hitachi, Japan). Before observation, the specimens were gold coated to enhance their conductivities.

2.5 pH-sensitivity and reversible swelling-deswelling studies

PBS with pH values of 1.2, 3.0, 4.0, 6.8 and 7.4 were used to study the pH-sensitivity of CMC-g-PAA hydrogels. Hydrogel chips were immersed into above PBS for 24 h. At different intervals, these chips were taken out from PBS and cleaned the PBS on hydrogels' surfaces with filter paper. Then, the swelling ratios of hydrogel chips were calculated with equation (1).

2.6 *In vitro* drug release study

INS-CMC-g-PAA hydrogel was obtained from an adsorbing method. Therefore, the encapsulated efficiency of the INS-CMC-g-PAA hydrogel could be considered as 100.0 %. The *in vitro* release profile of INS was carried out in simulated gastric juice (PBS, pH 1.2) and simulated intestinal juice (PBS, pH 7.4), respectively. 0.2 g of INS-CMC-g-PAA hydrogel was placed into 50.0 mL conical flasks that pretreated with 30.0 mL PBS (pH 1.2). These flasks were vibrated at a speed of 100 rpm in a ZHWY-100H water-bathing constant temperature vibrator (Shanghai, China) at 37^{o}C. During the first 2 h, INS-CMC-g-PAA hydrogel was immersed in simulated gastric juice (PBS, pH 1.2). 2 h later, these hydrogels were transferred into simulated intestinal juice (PBS, pH 7.4) for another 8 h. At proper intervals, 5.0 mL PBS were withdrawn from the flasks and replaced with equivalent fresh PBS. The INS concentrations in these samples were measured with high pressure liquid chromatography (HPLC).

2.7 Modeling of diabetic rats

100 of male Wistar rats were used to construct the models of diabetic rats. After a 24 h of fasting, all the Wistar rats were administrated 200.0 mg/kg of alloxan *via* intraperitoneal injection. Then, all rats were fasting but free to drink water for another 48 h. Blood samples were drawn from caudal vein of Wistar rats. The blood glucose levels of Wistar rats were monitored *via* a glucometer and glucose test strip (HMD BioMedical Inc, Beijing). If the blood glucose level was over 16.67mmol/L, the diabetic Wistar rat was obtained.

2.8 Hypoglycemic effects of oral INS-CMC-g-PAA hydrogels

50 diabetic rats were divided into 5 groups at random. Rats in group A were used as control and treated with blank CMC-g-PAA hydrogel chips *via* gavage. Group B was administrated with INS solution (5.0 IU/Kg) by intraperitoneal injection. Group C - E were treated with INS-CMC-g-PAA hydrogel chips by gavage, and the dosages were 25IU/Kg, 50IU/Kg and 75IU/Kg, respectively. All rats were administrated once a day for 2 days. Then, the blood glucose levels of Wistar rats were monitored *via* a glucometer and glucose test strip (HMD BioMedical Inc, Beijing) following a 12 h of fasting.

All the animals used in experiments were fed in individual cages in controlled environments with free access to water and food. Animal experiments were carried out in accordance with the People's Republic of China National Standard (GB/T 16886.6-1997). All animal experiments were approved by the Administrative Committee on Animal Research (Hebei University of Science and Technology, Shijiazhuang, People's Republic of China). The informed consent was obtained for

any experimentation with human subjects.

3 Results and discussions

3.1 Single-factor experiments

The influences of monomer ratios, initiators, cross-linkers, and reaction temperatures on the swelling ratios and grafting ratios of final hydrogels were carried out (Fig. 2). Fixing the cross-linker at 1 wt% (weight ratios of monomers), initiator ($K_2S_2O_8$) at 0.5wt% (weight ratios of monomers), temperature at 60 ^oC, and reaction time of 12 h, the influences of CMC concentration (C_{CMC}) on the swelling and grafting of the CMA-g-PAA hydrogels were investigated (Fig. 2a). When C_{CMC} was 0.06 g/mL (viscosity = 1.15 mpa·s, Table. 1), the obtained CMC-g-PAA hydrogels showed the biggest swelling ratio (76.9-fold). However, the grafting ratio of CMC-g-PAA copolymers was only 67.2 ± 5.6 %. The low grafting of CMC-g-PAA copolymer usually had a low drug-loading. When C_{CMC} was increased to 0.07 g/mL, the obtained CMC-g-PAA hydrogels showed a swelling ratio of 61.4-fold, and their grafting ratio was up to 85.2 ± 6.0 %. Such swelling ratio and grafting ratio would give the CMC-g-PAA hydrogel an ideal drug-loading and release profile. Finally, the C_{CMC} of 0.07 g/mL was used in the fabrication of CMC-g-PAA hydrogel. Then, the optimum monomer ratio was also revealed (Fig. 2b). Fixing other influence factors at constant, the CMC/AA ratio of 1:6 obtained a good swelling ratio (65.5-fold of dry hydrogel) and grafting ratio of the final copolymers (62.2 ± 5.8 %). Therefore, the CMC/AA

ratio of 1:6 was used in the preparation of CMC-g-PAA hydrogel.

In this study, temperature was another influence factor on the swelling ratio and grafting ratio of final CMC-g-PAA hydrogel. The influence of reaction temperatures and time were carried out (Fig. 2c and d). It cloud be seen that the swelling ratios of final hydrogels decreased slightly with the temperature rising. This phenomenon might be due to the thermolysis of $K_2S_2O_8$. With temperature rising, more initiator was destroyed, and the reaction sites decreased. Therefore, the obtained hydrogel showed a poor swelling ratio and low grafting ratio. Therefore, the optimal temperature was fixed at 60 ^oC, and the obtained hydrogels showing a swelling ratio of 65.70-fold (weight of dry hydrogel) and a grafting of 65.2 ± 6.2 %. In addition, after 12.0 h of reaction, the swelling ratio and grafting ratio of CMC-g-PAA copolymer barely grown with time prolong. Finally, the optimal reaction time was confirmed as 12.0 h.

After cross-linking, the final CMC-g-PAA hydrogel would have a 3D network structure. Therefore, the cross-linking agent was a vital material in the preparation of CMC-g-PAA hydrogel. With cross-linking agents increasing, the swelling ratios of final hydrogels were in the trend of first increased then decreased (Fig. 2e). When less cross-linking agent was used, the final hydrogels could not form integrated 3D network structures [19]. The irregular inner structures of final hydrogels would exhibit poor swelling behavior. In reverse, overmuch cross-linking agents would form much cross-linking points. The high crosslinking density usually made the final hydrogel had a more dense structure, which would decrease the swelling ratios of

final hydrogels. Therefore, 1.0 wt% (percentage of monomers) was used in hydrogel fabrication. Then, the swelling ratio of hydrogel was 65.7-fold (weight of dry hydrogel), and the grafting of CMC-g-PAA copolymer was 65.2 ± 6.7%. Besides, it could be concluded from Fig. 2f that the optimal initiator was 0.5 wt% of monomers. Then, the crosslinking degree of the final hydrogel was 98.4 ± 6.3 % (as shown in Table. 1). In conclusion, the optimal formulation preparation of CMC-g-PAA hydrogel was shown below: a C_{CMC} of 0.07 g/mL, a CMC/AA ratio of 1:6, a cross-linking dosage of 1.0 wt% (monomers), an initiator ($K_2S_2O_8$) dosage of 0.5 wt% (monomers), a reaction temperature of 60 ^oC, and reaction time of 12.0 h.

3.2 FTIR and ^1H NMR spectra

To confirm the successful fabrication of CMC-g-PAA copolymers, the FTIR spectra of CMC, AA, MBA, and final CMC-g-PAA hydrogel were carried out (Fig. 3). In the FTIR spectrum of MBA (Fig. 3a), an adsorption peak due to the -C-N bond was found at the range of 3500 - 3180 cm^{-1}. The adsorption band of -C=C- distributed in 1690-1610 cm^{-1}. The wide band observed at 3447 cm^{-1} (Fig. 3b) was the combination peak of –OH and –N-H groups in CMC. The sharp band at 1680 - 1630 cm^{-1} was due to the stretching vibration absorption center of –C=O group. In the spectrum of AA (Fig. 3c), the absorption of –OH groups was observed at the range of 3600 - 2400 cm^{-1}. The absorption peak of double-bond was shown in the range of 1690 - 1610 cm^{-1}. By contrast, the absorption band of -C=C- disappeared but other typical characteristic peaks were all existed in the spectrum of CMC-g-PAA hydrogel (Fig. 3d). These data indicated that the CMC-g-PAA hydrogel was successfully prepared

without any un-reacted monomers left. The ^{1}H NMR studies were carried out (shown in Fig. 4). In the spectrum of AA, the peaks (δ = 6.0-7.0 ppm) were assigned to the protons of -CH=CH- groups. After polymerization, the -CH=CH- groups changed into -CH_2-CH_2- fragments, and these peaks would shift to about 3.7 ppm. Similarly, in the spectrum of MBA, the peaks at the δ of 5.7 ppm and 6.3 ppm were also shifted to about 5.0 ppm in the CMC-g-PAA hydrogel. Besides, all other peaks in the spectra of AA, CMC, and MBA were also found in the ^{1}H NMR spectrum of CMC-g-PAA hydrogel. These results also confirmed the successful fabrication of CMC-g-PAA hydrogel.

3.3 TGA curves

Fig. 5 represented the TGA curves of the CMC and the final CMC-g-PAA hydrogel. It was clear that the CMC-g-PAA hydrogel showed good thermal stabilities up to 300 ℃, indicating the crosslinking structures of hydrogel increased its thermal stability. The final CMC-g-PAA hydrogel were cross-linked *via* MBA, which resulted in a more stability of final hydrogels. It was of interest to notice that the TGA curve of the final hydrogel did not show the thermolysis temperatures of the original CMC at around 280 ℃. It might be concluded that the thermal stability of the CMC units in the CMC-g-PAA hydrogel was improved through the grafting reaction.

3.4 SEM photographs

The hydrogel samples were immersed into PBS with pH of 1.2 and 7.4 for 24.0 h. After lyophilization, the inner morphology of hydrogel was observed with an S-4800 SEM. A contractive morphology could be seen from Fig. 6a, which indicated that the CMC-g-PAA hydrogel shank in the acidic PBS (pH1.2). All the 3D networks shut

down and only many wrinkles were observed on the hydrogel matrix. By contrast, obvious 3D network structures were shown in the SEM photograph of the hydrogel chips swollen in pH 7.4 (Fig. 6b). Such changing suggested that the hydrogel had a good pH responsibility. They could swell in the neutral or basic conditions but shrank in acidic environments.

3.5 pH responsibility

To study the pH-responsibility of CMC-g-PAA hydrogel, samples were immersed into different PBS with the pH levels of 1.2, 3.0, 4.0, 6.8, and 7.4, respectively. After 24 h swelling, the swelling-time curves were drawn (Fig. 7). All hydrogel chips swelled in different extent, and the swelling reached to equilibrium states after approximate 1.0 h. However, these chips showed different swelling curves in various PBS. In pH 1.2 and 2.0, the swelling ratios were less than 10.0-fold (weight of dry hydrogel). With the pH increasing, their swelling ratios rose sharply. When the pH of PBS increased to 7.4, the swelling ratio was up to 63.8 ± 0.9-fold. A *t*-test fitting indicated that a significant difference was in the swelling ratios between pH 1.2 and 7.4 ($P < 0.001$). These differences illustrated that the CMC-g-PAA hydrogel had an excellent pH sensibility.

3.6 Swelling/de-swelling curve

The fast swelling and de-swelling behavior is a unique property of the CMC-g-PAA hydrogel. The abundant of –COOH groups in CMC-g-PAA hydrogel made it to change quickly with the shifting of surrounding pH levels. When the hydrogel specimens were immersed in pH 7.4, rapid swelling behavior took placed (Fig. 8).

After 4 h swelling, the CMC-g-PAA hydrogel reached to the swelling equilibrium. When the swollen hydrogels were transferred into the PBS (pH 1.2), the hydrogels de-swelled quickly. The changing rates of swelling/de-selling ratios of the hydrogels were 0.04 h^{-1}. After two swelling and de-swelling circles, the hydrogels still had good swelling and de-swelling behaviors.

3.7 *In vitro* releasing profile

INS was used as a model drug and encapsulated into the CMC-g-PAA hydrogel *via* an adsorption method. After calculation, the drug-loading of the INS-CMC-g-PAA hydrogel was 216 mg/g, and its encapsulation efficiency was considered as 100 %. The *in vitro* releasing profile of INS-CMC-g-PAA hydrogel (Fig. 9) was carried out in simulated gastric fluid (PBS, pH 1.2) and intestinal juice (PBS, pH 7.4), respectively. In the first 2 h, the INS-CMC-g-PAA hydrogel was immersed in simulated gastric fluid (PBS, pH 1.2). In the acidic environment, the 3D networks in INS-CMC-g-PAA hydrogel would shut down, which prevented INS from releasing into the acidic medium. It could be seen from Fig. 9 that only 16.3 ± 2.6 % of encapsulated INS was released. In the subsequent releasing test, the INS-CMC-g-PAA hydrogel was transferred into simulated intestinal juice (PBS, pH 7.4) for another 8.0 h. In such condition, the INS-CMC-g-PAA hydrogel swelled and abundant INS diffused out off from the CMC-g-PAA hydrogel. The accumulated release amount of INS was up to 93.2 ± 3.8 %. Such release profile illustrated that the INS-CMC-g-PAA hydrogel could deliver INS to a target tissue (*i.e.* intestinal canal), site-specifically.

3.8 Oral hypoglycemic effects

The cytotoxicity of CMC-g-PAA hydrogel was carried out and the results were shown in Fig. 10. Comparing to control, the CMC-g-PAA hydrogel did not show obvious cytotoxicity, though the concentration of hydrogel was up to 10.0 mg/mL. After 12 hours of incubation at a hydrogel concentration of 0.5 mg/mL, the cell viability was 99.2 ± 5.6 %. When the hydrogel concentration was increased to 10 mg/ml, the cell viability was 95.4 ± 8.6 %. These phenomena illustrated that the hydrogel had a very low cytotoxicity, and the CMC-g-PAA hydrogel was suitable for oral administration.

The hypoglycemic effects of INS-CMC-g-PAA were measured with diabetic Wistar rats as the animal models, and their blood glucose levels were shown in Fig. 11. Animal experiments suggested that the blank hydrogel administrated by gavage did not show any obvious hypoglycemic effect. As to the positive control group (injection INS solution), an obvious hypoglycemic effect was observed, and the lowest blood glucose level was 27.2 ± 4.5 % of the original levels. The effect was lasted for about 5.0 h, and then the blood glucose reached to the original levels. By contrast, all the three groups of rats treated with INS-CMC-g-PAA hydrogel (50.0 IU/Kg, 75.0 IU/Kg, and 100.0 IU/Kg) by gavage showed obvious hypoglycemic effects. In group 1 (50.0 IU/Kg), the blood glucose decreased to 52.0 ± 6.1 % of the original levels, and the effect lasted for about 5.5 h. When Wistar rats were treated with 75.0 IU/Kg and 100.0 IU/Kg of INS-CMC-g-PAA hydrogels by oral route, the hypoglycemic effects were lasted over 9.0 h with the lowest glucose levels of 74.0 ± 5.8 % and 34.1 ± 4.7 % of the original levels. These data illustrated that oral administration of INS-CMC-g-PAA hydrogels could gain excellent hypoglycemic effects.

4. Conclusions

The CMC-g-PAA hydrogel was fabricated *via* a free radical grafting polymerization with MBA as a cross-linking agent. The optimal process conditions and formula were obtained: a C_{CMC} of 0.07 g/mL, a CMC/AA ratio of 1:6, a cross-linking dosage of 1.0 wt% (weight ratios of monomers), and an initiator ($K_2S_2O_8$) dosage of 0.5 wt% (monomers). The polymerization was carried out at 60 ^{o}C for 12.0 h. The FTIR and ^{1}H NMR spectra confirmed the successful synthesis of CMC-g-PAA hydrogels. The SEM photographs showed many 3D network structures in the hydrogels, which could give them excellent swelling abilities. Immersing the CMC-g-PAA hydrogels in different PBS, they exhibited various swelling ratios, which illustrated that such hydrogel had an excellent pH-responsibility. INS was encapsulated into the hydrogel to obtain a novel oral preparation. *In vitro* release studies showed that INS-CMC-g-PAA hydrogels could inhibit INS from releasing in acidic conditions but promote INS to diffuse in the basic environments. After administration by gavage, INS-CMC-g-PAA hydrogel showed a slow but long-acting hypoglycemic effect. After gavage with 100.0 IU/Kg of INS, the blood glucose levels of diabetic rats decreased to 34.1 ± 4.7 % of the original levels, and the effect was lasted for about 9.0 h. Therefore, the INS-CMC-g-PAA hydrogel had a potential application in the treatment of diabetes *via* oral administration.

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Tables

Table 1. Properties of CMA, AA and final hydrogels.

Samples	Concentration (g/mL)	Viscosity (mpa·s)	Morphology	Crosslinking(%)
CMC	0.07	1.15	yellow/transparent (L)	/
AA	/	1.21	transparent (L)	/
hydrogel	/	/	transparent (S)	98.4 ± 6.3

L: liqiud; S: solid.

Figure captions:

Figure. 1 Schematic presentation of swelling and insulin release pattern of the INS-CMC-g-PAA at pH 1.2 (stomach conditions) and pH 7.4 (intestine conditions).

Scheme. 1 Reaction scheme of CMC-g-PAA hydrogel. The final hydrogel was fabricated *via* radical polymerization, and the process included three stages: (1) chain initiation; (2) chain propagation; and (3) chain termination.

Figure. 2 Influence factors on the swelling ratios and grafting ratios of final CMC-g-PAA hydrogels. a) C_{CMC}, b) monomer ratios, c) reaction temperatures, d) reaction time, e) cross-linking agents, and f) initiators.

Figure. 3 FTIR spectra of CMC, AA, MBA and CMC-g-PAA hydrogels. The absorption bonds of hydroxyl groups in monomers were observed in the spectrum of final hydrogel. The absorption peaks of carbonyl groups were also found in the CMC-g-PAA hydrogel spectrum.

Figure. 4 ^{1}H NMR spectra of AA, CMC, MBA and CMC-g-PAA hydrogel. The characteristic peaks in the ^{1}H NMR spectra of AA, CMC and MBA were observed in the spectrum of CMC-g-PAA hydrogel.

Figure. 5 Inner morphology of the CMC-g-PAA hydrogel. a) Inner morphology of hydrogel immersed in pH 1.2 for 12.0 h; and b) inner morphology of hydrogel immersed in pH 7.4 for 12.0 h.

Figure. 6 TGA curves of CS and the final CMC-g-PAA hydrogel.

Figure. 7 Different swelling behaviors of CMC-g-PAA hydrogels in various pH levels. The swelling ratio of CMC-g-PAA hydrogel in pH 1.2 was less than 10-fold, while

these hydrogel swelled to 60-fold of the original ones.

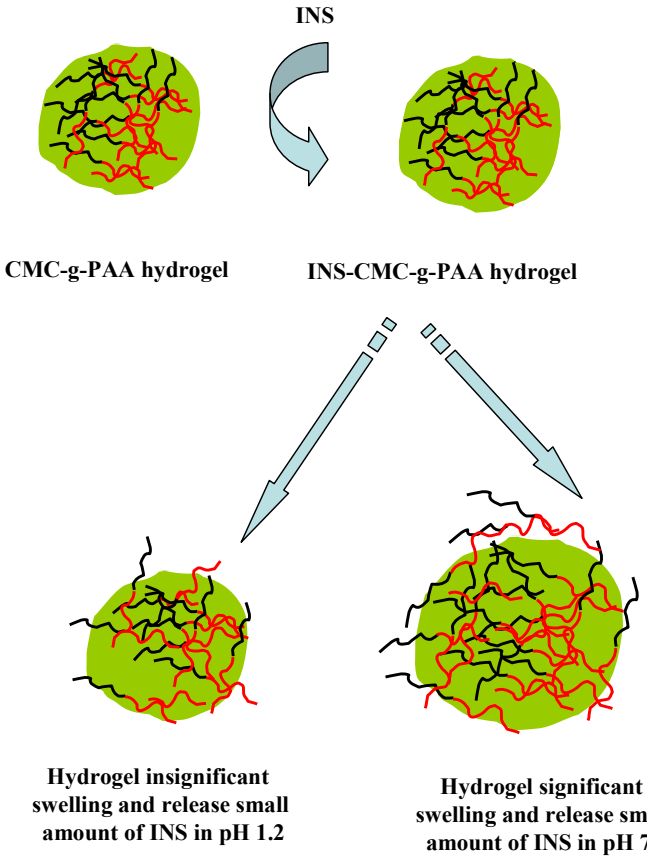
Figure. 8 Swelling-deswelling curve of the CMC-g-PAA hydrogels in pH 1.2 and 7.4. The CMC-g-PAA hydrogel swelled in the pH 7.4, but shrank in the pH 1.2. After two swelling-deswelling circles, the CMC-g-PAA hydrogel still had a good swelling behavior.

Figure. 9 Cumulated release profile of INS from INS-loaded CMC-g-PAA hydrogel in pH 1.2 and 7.4. In pH 1.2, less than 20.0 % of loaded INS released from the hydrogel, but over 85.0 % of INS was released in pH 7.4.

Figure. 10 Cellular cytotoxicity assay of CMC-g-PAA hydrogel. Test data suggested that the CMC-g-PAA hydrogel had very low cytotoxicity.

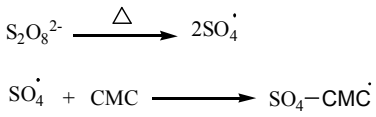
Figure. 11 Hypoglycemic effect of INS-loaded CMC-g-PAA hydrogel administrated *via* oral route. Results showed that orally administrated INS-loaded CMC-g-PAA hydrogel (100.0 IU of INS) gave an excellent hypoglycemic effect. The lowest blood sugar level decreased to 34.1 ± 4.7 % of the original levels and the effect was lasted for about 9.0 h.

Fig. 1

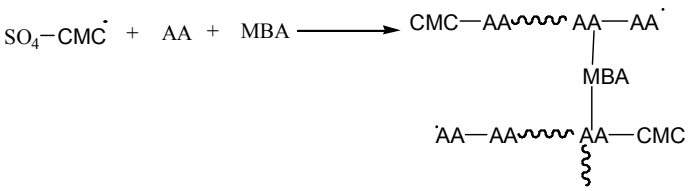


Sche. 1

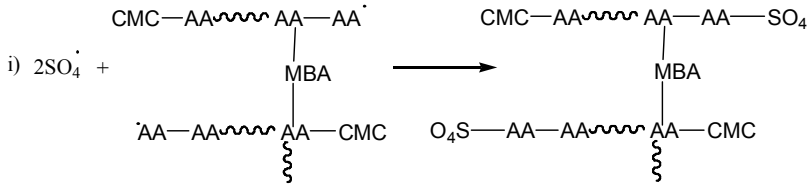
(1) Chain initiation



(2) Chain propagation



(3) Chain termination



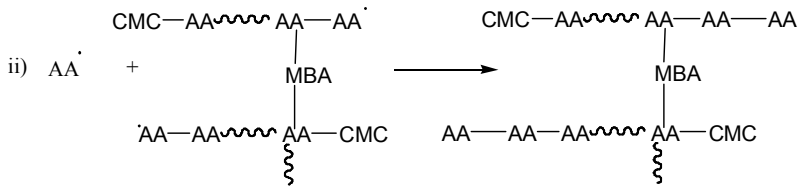


Fig. 2

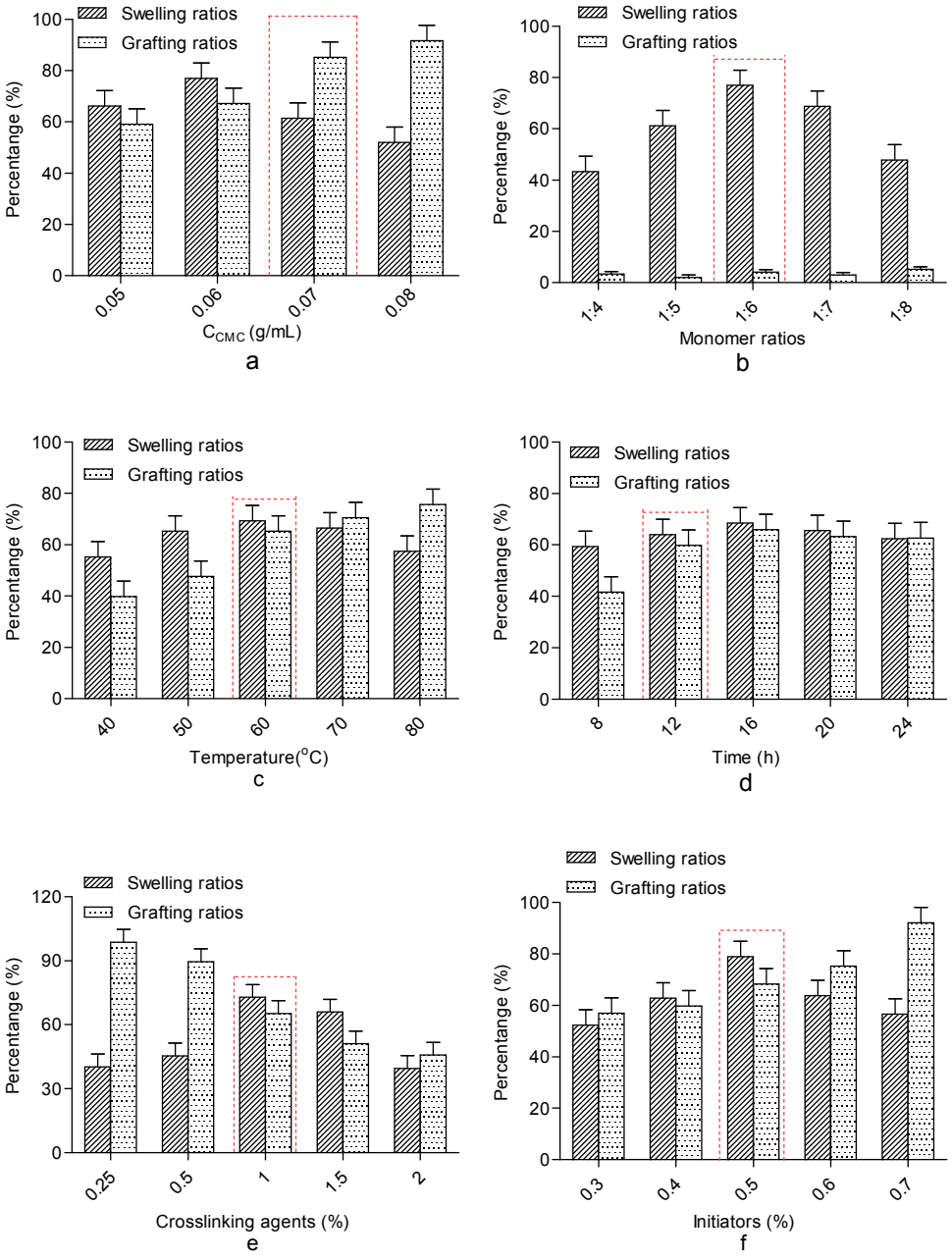


Fig. 3

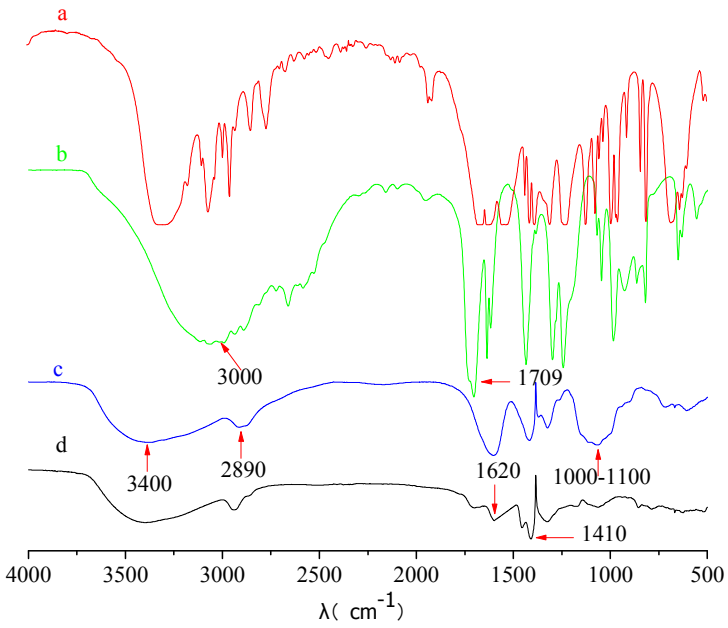


Fig. 4

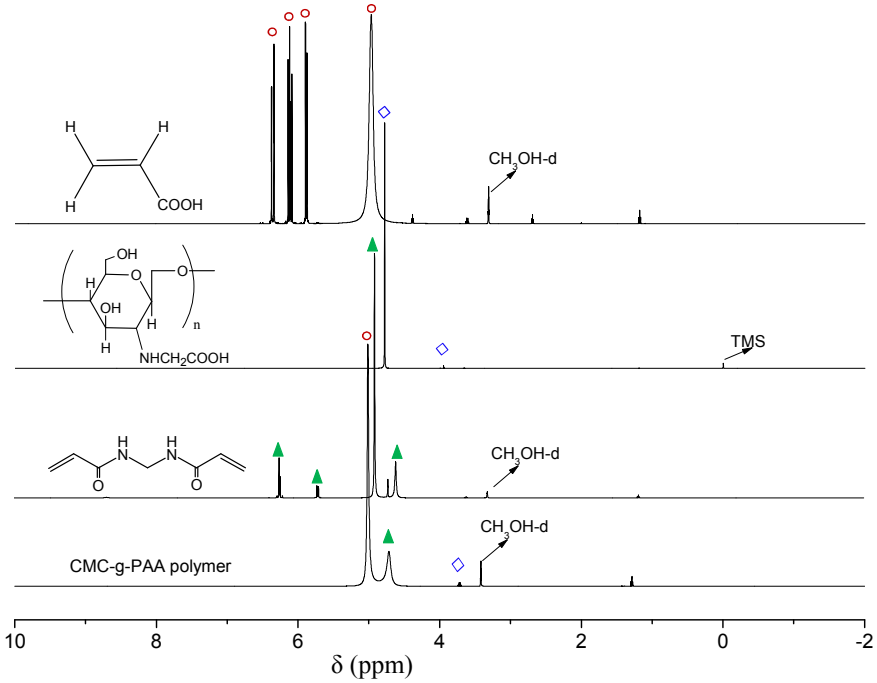


Fig. 5

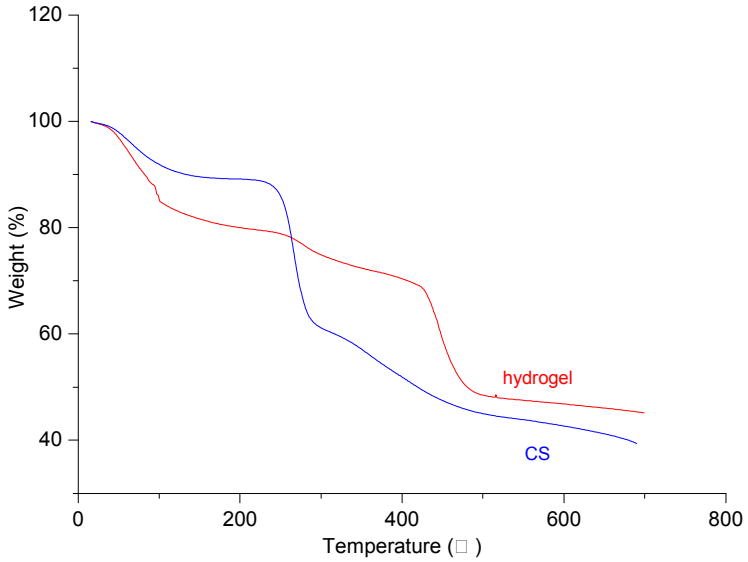


Fig. 6

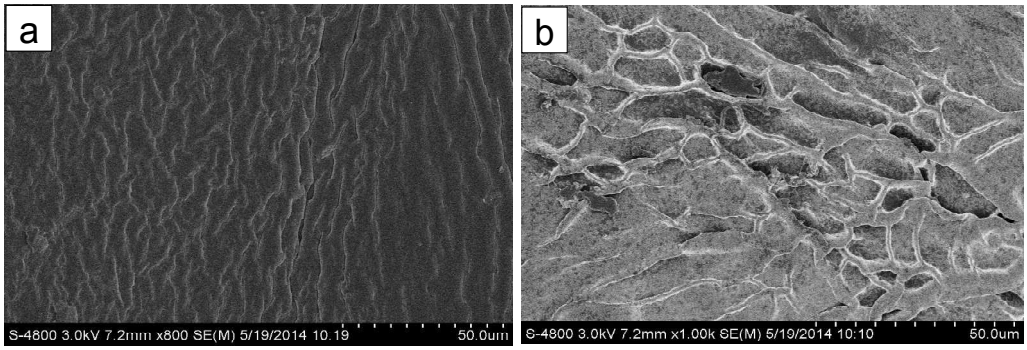


Fig. 7

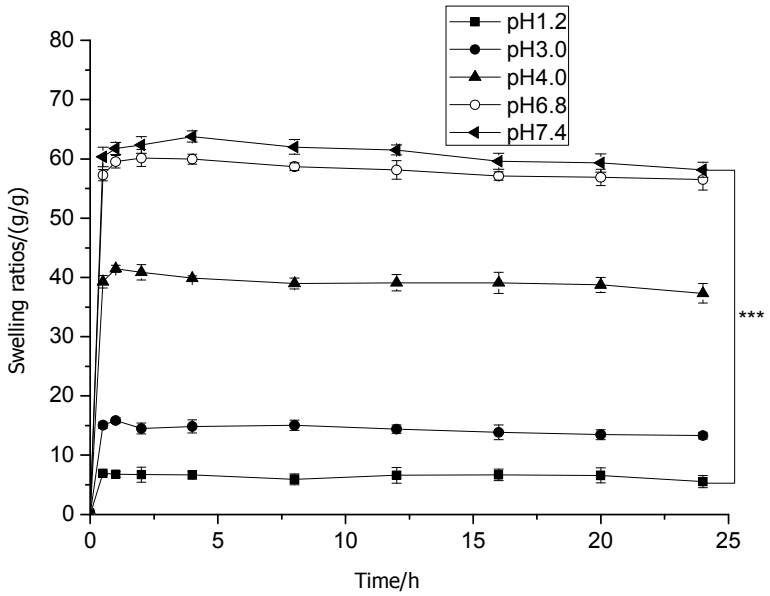


Fig. 8

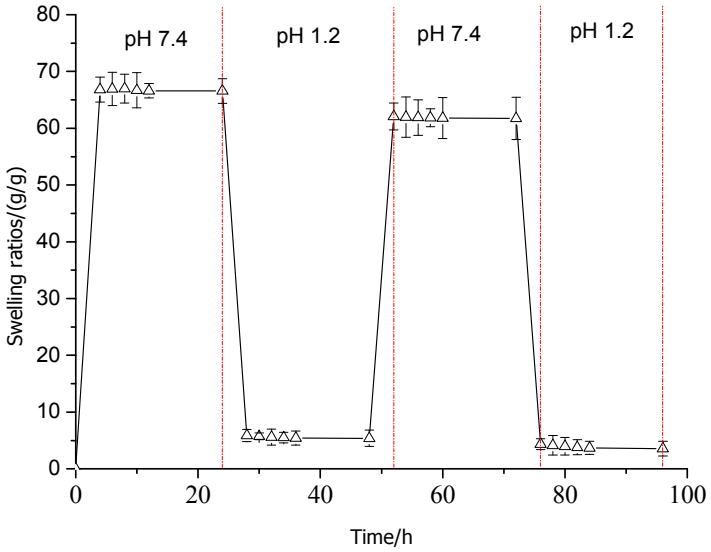


Fig. 9

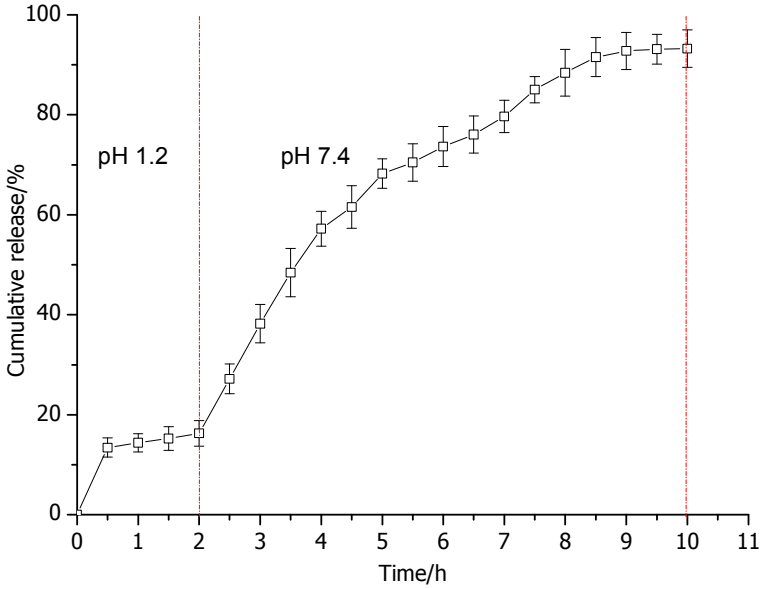


Fig. 10

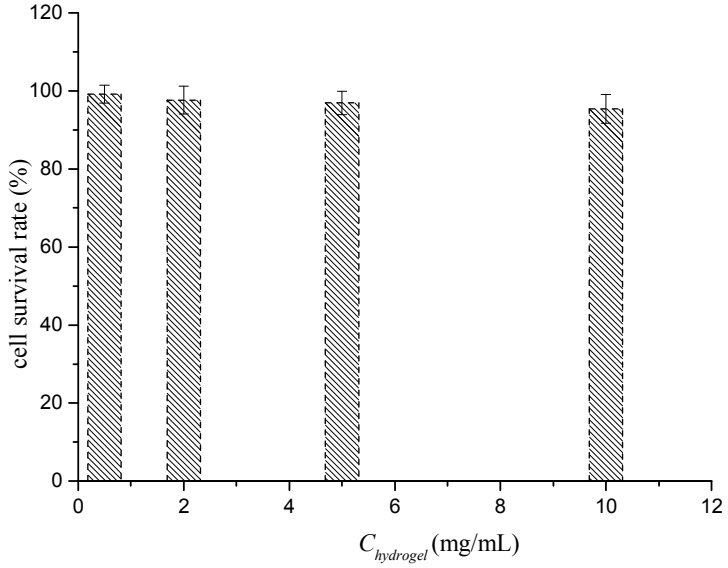


Fig. 11