

Development of hydroxyethylacryl chitosan/alginate hydrogel films for biomedical application

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Abstract Novel hydrogel films composed of hydroxyethylacryl chitosan (HC) and sodium alginate (SA) were prepared for biomedical application by using calcium chloride (CaCl_2) as a nontoxic ionic crosslinker to form a semi-interpenetrating polymer network (semi-IPN). HC was successfully prepared by following a Michael addition reaction of chitosan (CS) and hydroxyethylacrylate completely dissolved in distilled water at 70 °C. The distribution pattern of Ca^{2+} ions were well-dispersed within the hydrogel films examined by scanning electron microscope-energy dispersive spectrometry (SEM-EDS), implying uniformity of crosslinking. The swelling behavior of the hydrogel films in distilled water, simulated gastric fluid (SGF, pH=1.2) and phosphate buffer solution (PBS, pH=7.4) were investigated. The equilibrium swelling degree of the hydrogel films in distilled water increased with a decrease of either the SA content or the concentration of CaCl_2 . The hydrogel films showed pH-dependent behavior in that the shapes of the hydrogel films were stable in SGF while they degraded in PBS. The tensile strength and elongation of the hydrogel films reached 12.1 MPa and 162%, respectively, which presented reasonable mechanical properties during use and enough flexibility to follow skin movement. Cell viability of the hydrogels was measured using a methylthiazol tetrazolium (MTT) assay. The results indicated that the hydrogel films are not cytotoxic.

Keywords Hydrogel · Semi-interpenetrating polymer network · Biopolymer · Hydroxyethylacryl chitosan · Sodium alginate

Introduction

Biopolymers have been widely used for biomedical application in recent years [1–3]. Chitosan (CS) is a natural polysaccharide obtained from the deacetylation of chitin. It has been widely used for biomaterials owing to its biocompatibility, non-toxicity, biodegradability, its antibacterial properties and its ability to accelerate wound healing [4, 5]. However, acetic acid or other acids can facilitate dissolution of CS in water, resulting in cytotoxicity in the final product [6, 7]. Therefore, in order to enhance the solubility of CS in water, efforts to produce hydrophilic groups have been made by utilizing the covalent attachment of reactive amino groups at the $\text{C}_{2\text{nd}}$ position [8–10].

Several crosslinking methods for CS and its derivative have been employed to stabilize the shape and control release of the drug over a prolonged period of time, including chemical crosslinking. Various crosslinkers, such as glutaraldehyde [11], formaldehyde [12], ethyleneglycol diglycidyl ether [13] and N , N' -methylenebisacrylamide [14], have been used. Although treatment by those chemicals is effective in yielding a high degree of crosslinking, the residuals are cytotoxic, restricting their potential biomedical use.

To avoid the toxic crosslinking agent, many researches have contributed to photo-crosslink [15–17] and radiation crosslink [18–20] systems. These hydrogels are not pH sensitive and not effectively used in the controlled release of drugs. The formation of a semi-interpenetrating polymer network (semi-IPN) of uncrosslinked CS or its derivative and a well-gel forming polymer in the presence of a nontoxic ionic crosslinker is an alternative route for overcoming these

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limitations. Semi-IPNs are a way of blending two polymers where only one is crosslinked in the presence of another to produce an additional non-covalent interaction between the two polymers.

Sodium alginate (SA) is a natural non-toxic biodegradable anionic polysaccharide capable of forming hydrogels under mild conditions in the absence of toxic reagents [1, 21]. In the presence of multivalent metal cations, e.g., Ca^{2+} , Cu^{2+} , Fe^{3+} , etc., it easily forms three-dimensional networks due to formation of ionic bridges between the metal cations and the SA carboxylate groups of the same or different chains.

Hydrogels from the combination of CS and SA have been reported [22–24]. However, in regards to film-forming application, CS could not homogeneously blend with SA because they immediately form a gel via ionic crosslinking of the protonated amino moieties of CS and the carboxylate moieties of SA; this restricts their ability to form a hydrogel film. As such, water-soluble chitosan was usually replaced. Of the various types of water-soluble chitosan, carboxymethyl chitosan (CMC) has been used the most in the combination with SA [25–29]. Other types utilized include chitosan glutamate [30], N- α -glutaric acid chitosan [31], N-trimethyl chitosan [32] and galactosylated chitosan [33]. No attempt at using hydroxyethylacryl chitosan (HC) has been made. Moreover, most studies have been limited on the formation of bead hydrogel. A review of the literature shows very scarce research on film formation utilizing a semi-IPN of CMC and SA crosslinked by a relatively expensive genipin at the amino group residual in CMC [25].

In the present study, it is expected that the new type of hydrogel film can be developed via effective combination of HC with SA using the semi-IPN technique. Since HC does not easily dissolve in water or in simulated body fluids at temperatures approximating the human body it is reasonable to modify the uncrosslinked part in order to stabilize its final shape and efficiently control delivery of the drug; it can dissolve in water at a raised temperature, allowing it to mix well with other components without using an acidic solution. Michael addition reaction of CS and hydroxyethylacrylate in moderate conditions would be a useful technique for synthesizing HC soluble in water at temperatures higher than body temperature. G. Ma et al. [10] found that HC is an antimicrobial. Calcium chloride (CaCl_2) as the ionic crosslinking agent for the SA part was used due to its low cost, the hemostatic properties of the Ca^{2+} ion and the ability of the hydrogel to serve as a matrix for the aggregation of platelets and erythrocytes [1, 34]. Several characteristics of semi-IPN hydrogel films were evaluated to assess their potential use as a biomaterial; these characteristics included their swelling behavior in various fluids, their gel content, the state of water in the gel, the mechanical properties of the gel films in the wet state and their cytotoxicity.

Experimental procedure

Materials

CS with a degree of deacetylation and an average molecular weight of ~80% and 833 kDa, respectively, was purchased from Eland Co., Ltd. SA with an average molecular weight of 965 kDa was purchased from Carlo Erba Reagent. Hydroxyethylacrylate was obtained from the Thai Mitsui Specialty Chemicals Co., Ltd. Calcium chloride dihydrate was purchased from Merck Millipore, as were the phosphate buffered saline tablets. Simulated gastric fluid (SGF, pH 1.2) composed of 7 ml concentrated HCl, 2 g NaCl and 1,000 mL distilled water was prepared. All other reagents were analytical grade.

Preparation of hydroxyethylacryl chitosan (HC)

HC was prepared by partially referring to the procedure of G. Ma et al. [10]. Specifically, CS (3.0 g) was added into a solution of 1 wt. % acetic acid in distilled water (300 mL). Hydroxyethylacrylate (12 g) was then added to the solution. The reaction occurred at 60 °C for 48 h with continuous stirring. The solution was adjusted to a pH of 7 by using 10% w/v sodium hydroxide and then precipitated by pouring the solution into acetone. The precipitate was washed with plenty of acetone then dried under vacuum at ambient temperature overnight to obtain HC.

Preparation of HC/SA hydrogel films

The HC was dissolved in distilled water under continuous stirring at 70 °C. After cooling to room temperature, SA was added to the HC solution with the weight ratios of (HC:SA) 75:25, 50:50, 25:75 and 0:100 under continuous stirring to obtain an absolutely clear solution. The solutions were then poured into a Petri dish and dried at 40 °C to form HC/SA films with a thickness of 100 ± 5 μm . The films were then soaked in CaCl_2 solution (0.05, 0.1, 0.25 and 0.5 M) for 30 min and dried at 40 °C to obtain the HC/SA hydrogel films.

Characterization

The average molecular weight of HC was determined by gel permeation chromatography (GPC, Waters, Waters 600E, USA) using pullulans as a standard. The Fourier transform-infrared (FT-IR) spectra of HC and CS were received using a FT-IR spectrophotometer (FT-IR, Perkin Elmer, Spectrum GX, USA) using the potassium bromide (KBr) pellet method. FT-IR spectra were collected over a range of 400–4,000 cm^{-1} with a resolution of 4.0 cm^{-1} . The X-ray diffraction (XRD) pattern of HC and CS were studied at room temperature using an X-ray diffractometer from Bruker AG, D8 Advance, USA,

starting from 2θ of $5\text{--}40^\circ$ with a step size of 0.04° and a step time of 1 s. The distribution pattern of Ca^{2+} ions within the hydrogel films was examined by scanning electron microscope-energy dispersive spectrometry (SEM-EDS, LEO, LEO1455VP, USA). $^1\text{H-NMR}$ spectra of HC and CS were recorded by proton nuclear magnetic resonance ($^1\text{H-NMR}$, Bruker AG, NMR 300 ULTRA SHIELD, USA) using tetramethylsilane (TMS) as the standard. Degrees of substitution (DS) were calculated from the peak area at about 2.45 ppm of $-\text{CH}_2(\text{b})-$ proton against 2.8 ppm of $-\text{CH}(2$ and $2')$ -proton.

Swelling behavior

The hydrogel film's degree of swelling and equilibrium value were determined gravimetrically. Dried hydrogel films of an appropriate size were weighed, then immersed in distilled water at 37°C , phosphate buffer solution (PBS) with $\text{pH} = 7.4$ at 37°C and SGF with $\text{pH} = 1.2$ at 37°C ; to ensure complete equilibration, the samples were allowed to swell up to one day. After taking the swollen sample from the fluid, the samples were weighed after wiping surface water off with tissue paper. The degree of swelling and the equilibrium swelling degree were calculated as follows:

$$\text{Degree of swelling (DS)} = \frac{M_s - M_i}{M_i} \times 100\% \quad (1)$$

$$\text{Equilibrium swelling degree (ESD)} = \frac{M'_s - M_i}{M_i} \times 100\% \quad (2)$$

where M_s and M_i are the weights of the swollen wet hydrogel film and the initial dry hydrogel film, respectively. M'_s is the weight of the swollen wet hydrogel film at equilibrium swelling (one day).

Gel content

Dried hydrogel films of appropriate size were weighed, then immersed in distilled water at 37°C , and SGF with $\text{pH} = 1.2$ at 37°C , for 1 day. The sample was then dried and weighed. The gel content was calculated as follows:

$$\text{Gel content} = \frac{M_d}{M_i} \times 100\% \quad (3)$$

where M_i is the initial dry weight of the hydrogel film and M_d is the dry weight of the hydrogel film after immersion.

State of water

Differential scanning calorimetry (DSC, Mettler, DSC820, USA) was used to study the state of water in the hydrogels. The state of water in a hydrogel is normally classified into three types: free water; freezing bound water; and non-freezing bound water [35].

The swollen hydrogel samples at the equilibrium state were frozen inside the DSC instrument chamber at -80°C , and then heated to 50°C with a heating rate of $10^\circ\text{C}/\text{min}$. The equilibrium water content, freezing water content (including free and bound) and non-freezing bound water content were calculated as follows:

$$\text{Equilibrium water content (}W_e\text{)} = \frac{M'_s - M_i}{M'_s} \times 100\% \quad (4)$$

where M_i is the weight of initial dry hydrogel film and M'_s is the weight of swollen wet hydrogel film at equilibrium swelling (one day).

$$\text{Freezing water content (}W_f\text{)} = \frac{\Delta H_{\text{endo}}}{\Delta H_w} \times 100\% \quad (5)$$

where ΔH_{endo} is the area under the corresponding endothermic peak and ΔH_w is the heat of fusion of pure water (333.3 J/g [35]).

$$\text{Non-freezing bound water content (}W_{nf}\text{)} = W_e - W_f \quad (6)$$

MTT assay

Cytotoxicity of the hydrogel films was assessed by African green monkey kidney fibroblast (Vero) using an MTT assay. The films were immersed in PBS ($\text{pH} 7.4$) at 37°C . After 24 h, each liquor stock was obtained by filtering and then exposed in DMEM (Dulbecco's Modified Eagle Medium). Vero culture was cultured in a medium containing DMEM supplemented with 10% FBS (Fetal Bovine Serum) and seeded in a 96-well plate at $100 \mu\text{l}/\text{well}$ and subsequently incubated at 37°C for 24 h. Then, $100 \mu\text{l}$ of diluted liquor stock in DMEM was added to each plate and incubated at 37°C for 24 h. Then, $10 \mu\text{l}$ of MTT solution (5 mg/ml) was added in each well and further incubated at 37°C . After 4 h of incubation, the 9:1 mixture of DMSO:10% SDS was added in each well plate at a rate of $150 \mu\text{l}/\text{well}$ to dissolve the formazan crystals. Absorbance was measured at 570 nm using a

microtiter plate reader. The % cytotoxicity was calculated as follows [36]:

$$\% \text{ cytotoxicity} = \frac{A - B}{A} \times 100 \quad (7)$$

Where A is the absorbance of the control and B is absorbance of the samples.

Mechanical properties

The tensile strength, Young's modulus and elongation at the breaking point of the hydrogel films were measured by a universal testing machine (Lloyd Instrument, LR5K, UK) following ISO 2005. Hydrogel films in the fully hydrated state were cut into a dumbbell shape (type 2, gauge length 20 mm, width 4 mm). The samples were tested at a constant rate (5 mm/min) with a load cell of 100 N.

Results and discussion

Characterization of HC

The proposed route of HC synthesis is depicted in Fig. 1. The average molecular weight of HC obtained via GPC was reduced from 833 kDa of CS to 63 kDa. This suggests the CS chain was hydrolyzed by acetic acid. The solubility of HC in distilled water was evaluated. The results showed that HC completely dissolved in distilled water at 70 °C, while the original CS did not dissolve.

FT-IR spectra of CS and HC are shown in Fig. 2(a). Compared to CS, HC shows excluding absorption peaks at 1,724 cm^{-1} indicating the C = O stretching of part of the hydroxyethylacryl group, and absorption peaks at 1,570 and 1,409 cm^{-1} due to the asymmetrical and symmetrical stretching of the COO^- groups [10, 37].

Figure 2(b) shows the XRD patterns of CS and HC. CS shows two characteristic peaks around $2\theta = 10.4^\circ$ and $2\theta = 19.76^\circ$ assigned to crystal forms I and II, respectively. HC, on the other hand, had a broad halo pattern indicating lower crystallinity. It was ascribed to the presence of a bulky hydroxyethylacryl group that might hinder the formation of inter- and intra-molecular hydrogen bonds.

The ^1H NMR spectra of CS in $\text{CD}_3\text{COOD}/\text{D}_2\text{O}$ and HC in D_2O are shown in Fig. 3. After modification of CS, the peak corresponding to the $-\text{CH}_3$ (H_7) of *N*-acetylate was shifted from 1.79 to 1.89 ppm. The signals belonging to $-\text{CH}(2$ and $2')$ – protons were also shifted from 2.90 to 2.83 ppm. New signals at $\delta = 2.45$ and 3.15 ppm were assigned the to $-\text{CH}_2(\text{b})$ – proton and $-\text{CH}_2(\text{a})$ – proton, respectively. The

multiplet signals from 3.6 to 4.0 ppm were attributed to $-\text{CH}(3, 3', 4, 4', 5, \text{ and } 5')$ – and $-\text{CH}_2(6, 6', \text{ c, and d})$ – protons. The DS values based on the amine position were calculated by the relative intensities from the peak area at about 2.45 ppm of the $-\text{CH}_2(\text{b})$ – proton against 2.8 ppm of the $-\text{CH}(2$ and $2')$ – proton. The calculated DS is close to the value of 1.

Results confirm that HC was successfully prepared via a Michael addition reaction of CS and hydroxyethylacrylate.

Characterization of HC/SA hydrogel films

Cross-sections of the HC/SA hydrogel films was examined by SEM-EDS to investigate the distribution pattern of Ca^{2+} ions within the films. It can be seen in Fig. 4 that Ca^{2+} ions form a uniform distribution throughout the hydrogel films, implying uniformity of crosslinking. The results suggested that formation of calcium-crosslinked alginate at the surface of the films could not obstruct through diffusion of CaCl_2 (the Ca^{2+} ions) into the film matrix. However, the quantity of Ca^{2+} ions deposited in the hydrogel films depended not only on the concentration of the crosslinking reagent (CaCl_2) but also the ratios of HC to SA. The quantity of deposited Ca^{2+} ions increased with an increasing concentration of CaCl_2 , as seen in Figs. 4a and b. The higher amount of Ca^{2+} ions brought about a more ionic interaction between Ca^{2+} ions and the alginate chain carboxylate groups, resulting in more Ca^{2+} ions at the crosslinked points in the film. An increase of the HC to SA ratio in the films tended to decrease the quantity of Ca^{2+} ions, as seen in Fig. 4(b–d). It can be explained by the Ca^{2+} ions interacting with the carboxylate groups existing only in the alginate structure. Thus, Ca^{2+} ions can deposit on the films with higher SA content more so than they can on a film with a lower SA content. A schematic illustration of this network structure is proposed in Fig. 5.

Swelling behavior in distilled water at 37 °C

The influence of HC to SA ratios and of concentration of crosslinking reagent on the swelling behavior of the hydrogel films was studied. Dried films were exposed to distilled water at 37 °C; water penetrated into the films and the gel state was formed. Weight gain was calculated based on the initial dry weight of the film using Eq. (1). As shown in Figs. 6a and b, film samples could reach their equilibrium swelling in water within 30 min. Their equilibrium swelling degrees (ESD), which indicate the water retention ability of the films, are presented in Fig. 6c. The ESD of the films increased significantly when the HC to SA ratios were increased (Fig. 6a), but increased to a relatively lesser degree when the concentration of CaCl_2 was decreased (Fig. 6b). The maximum value was reached at approximately 600% by HC75SA25Ca0.05 (Fig. 6a). A lower ESD was obtained when a higher content of SA and/or a higher concentration of CaCl_2 were applied in

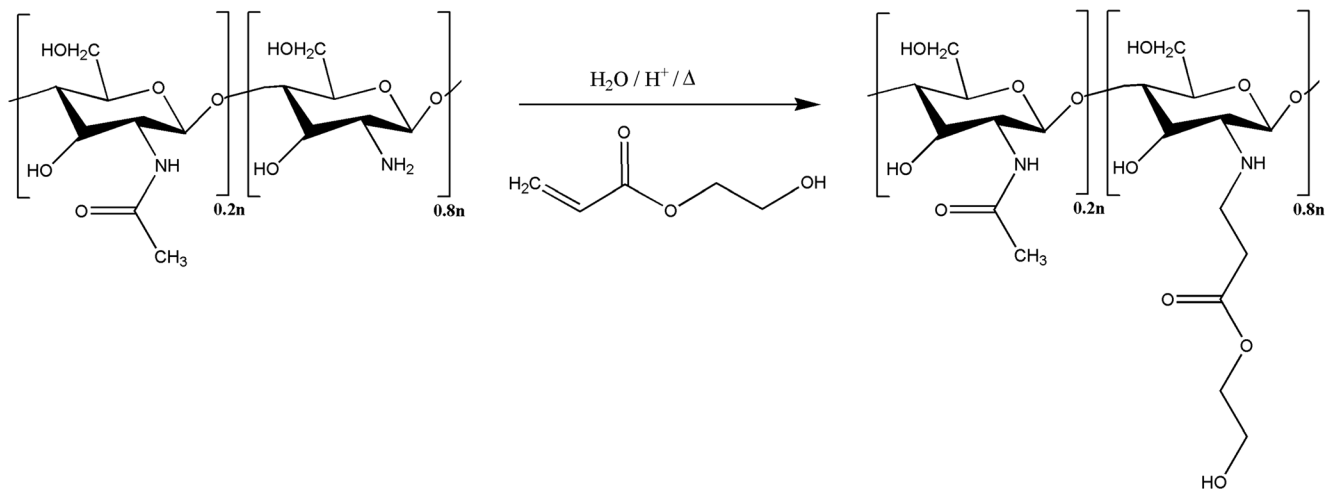


Fig. 1 Proposed route of HC synthesis

the films. The introduction of SA and CaCl_2 with a mole ratio of 1:2 induced crosslinking via ionic interaction between the Ca^{2+} ions and SA at the carboxylate position. The high SA content and high concentration of CaCl_2 brought about high crosslinking density in the film, thus, hindering chain expansion during swelling.

In vitro swelling behavior

Swelling behavior in PBS (pH 7.4 at 37 °C)

The swelling behavior of the hydrogel films in PBS was investigated using simulated wound exudate fluid and simulated intestinal fluid (SIF). The results (Fig. 7) indicate that HC/SA films rapidly absorb the liquids and then disintegrate. It was clear from the results that the films with a high HC content could reach the higher maximum degree of swelling and degrade faster than the low one. The maximum degree of swelling of HC75SA25 films in PBS was approximately 14 times compared with that in distilled water at around 6 times. It might be pointed that HC could remarkably swell and later

dissolve in PBS as inferred to CMC, one of the water-soluble chitosans with a structure similar to HC [7]. In addition, the degradation of calcium-crosslinked alginate could occur in PBS at a neutral pH since the affinity of PO_4^{3-} ions presented in the PBS to Ca^{2+} ions was higher than that of SA, resulting in breakage of the interaction between Ca^{2+} ions and the carboxylate groups of calcium-crosslinked alginate [38]. However, the results showed the effect of the presence of HC on the degradation of the films rather than the breakage of calcium-crosslinked alginate.

Swelling behavior in SGF (pH 1.2 at 37 °C)

Swelling behavior of hydrogel films in simulated gastric fluid (SGF) at 37 °C was also measured; results are shown in Fig. 8. The HC75SA25 film exhibited the highest degree of swelling at about 10 times within 20 min and later declined as the dissolution occurred. For lower HC content films, HC50SA50, HC25SA75 and SA films, the maximum degree of swelling decreased, respectively, and was maintained at the highest

Fig. 2 a FT-IR spectra and b XRD patterns of CS and HC

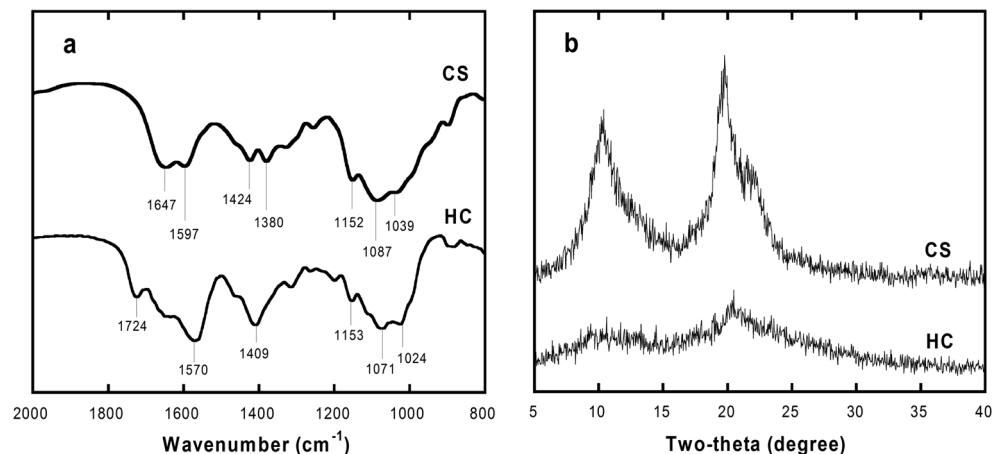
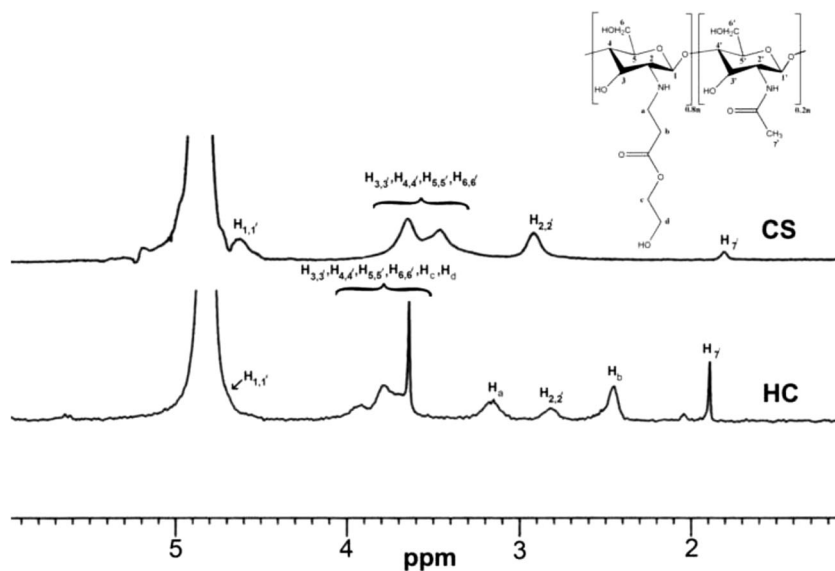


Fig. 3 $^1\text{H-NMR}$ spectra of CS and HC

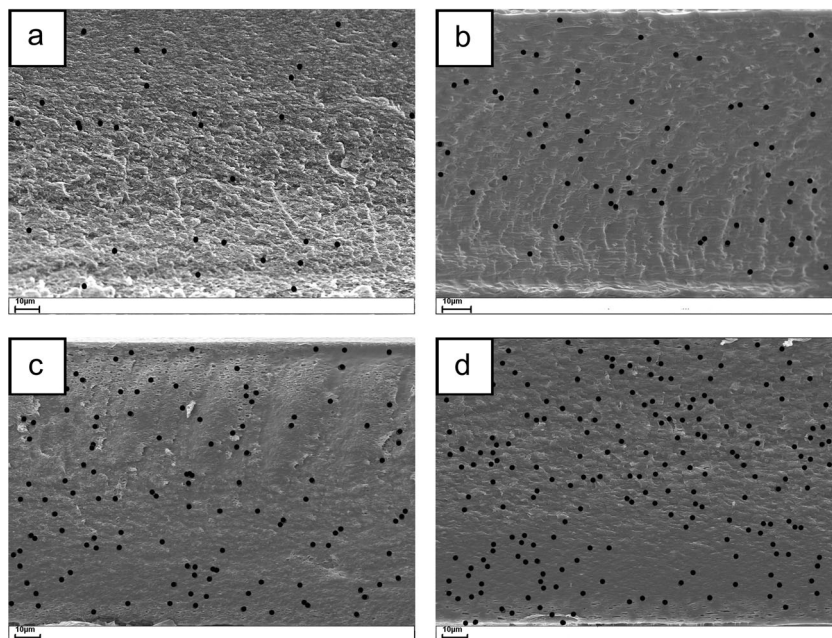


level. The concentration of crosslinking agent used showed less effect on the swelling degree. Compared to the swelling degree of a film with the same formulation in distilled water at 37 °C, films achieved a higher swelling degree in SGF than in distilled water, especially when high HC contents (HC75SA25 and HC50SA50) were used. Although the film with only SA crosslinked by CaCl_2 was applied, the swelling degree in SGF seemed slightly greater than that in distilled water. It might be that Na^+ and H^+ ions present in the SGF interfered and destroyed the interaction between Ca^{2+} ion and SA and brought about the collapse of partial crosslinking points.

Gel content

Gel content with various ratios of HC to SA and concentrations of crosslinking agent in distilled water at 37 °C are displayed in Fig. 9a. They were measured in order to imply the crosslinking degree of the polymer chains within the films. The values of all samples are higher than 60%, with the maximum values up to nearly 100%. Considering the various ratios of HC to SA, an increase in the content of SA caused an increase in the percentage of gel content because of the formation of crosslinks between Ca^{2+} ion and the carboxylate groups occurring only in SA. However, when the composition of HC to SA was fixed, the applied concentration of

Fig. 4 Two-dimensional distribution of Ca^{2+} ion on the cross-section of hydrogel films: **a** HC75SA25Ca0.05; **b** HC75SA25Ca0.25; **c** HC50SA50Ca0.25; **d** HC25SA75Ca0.25



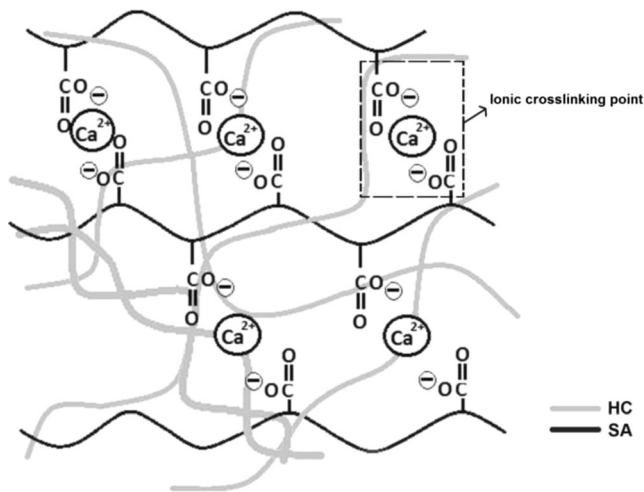


Fig. 5 Schematic illustration of the HC/SA network structure

crosslinking agent (0.05–0.5 M of CaCl₂) showed less effect on gel content. Results suggest their potential to be used in applications requiring permanent contact with water or biological fluids such as blood and wound exudate.

Figure 9b shows the gel content of the hydrogel films in SGF. It was appointed to investigate the stability of the crosslinking point within the hydrogel films. Compared to the gel content in distilled water, the gel content of the films in SGF is lower. It was due to the fact that HC could be protonated by cationic charges present in SGF, partially

destroying the crosslinked point in the film and, subsequently, causing dissolution of the uncrosslinked polymer from the films. Similar to the gel content of the films in distilled water, the gel content increased with an increase of SA, while the given concentration of crosslinking agent did not effect the gel content.

State of water in HC/SA hydrogel films

Although water content affects the quality and functionality of the hydrogels since most applications of hydrogels are based on their water absorption, the biocompatibility is definitely not simply a function of water content. As such, it is necessary to investigate the state of water in the hydrogels.

The state of water in water-swollen hydrogels has been demonstrated by many researchers [35, 39–41]. Three energetically distinct states of water have been identified: (i) free water that does not form hydrogen bonds with the polymer and can freeze or melt at the usual temperature of pure water; (ii) freezable bound water that interacts weakly with the polymer and freezes or melts as the temperature is shifted with respect to that of free water; and (iii) non-freezable bound water that is formed by the hydrogen bonds between water molecules and polar groups in the polymer and cannot freeze or melt within the normal temperature range of pure water. Among these three states, it has been verified that the

Fig. 6 Degree of swelling of HC/SA hydrogel films in distilled water at 37 °C. **a** varying ratios of HC to SA with 0.05 M CaCl₂; **b** HC50SA50 with varying concentrations of CaCl₂ as crosslinking agent; **c** at equilibrium

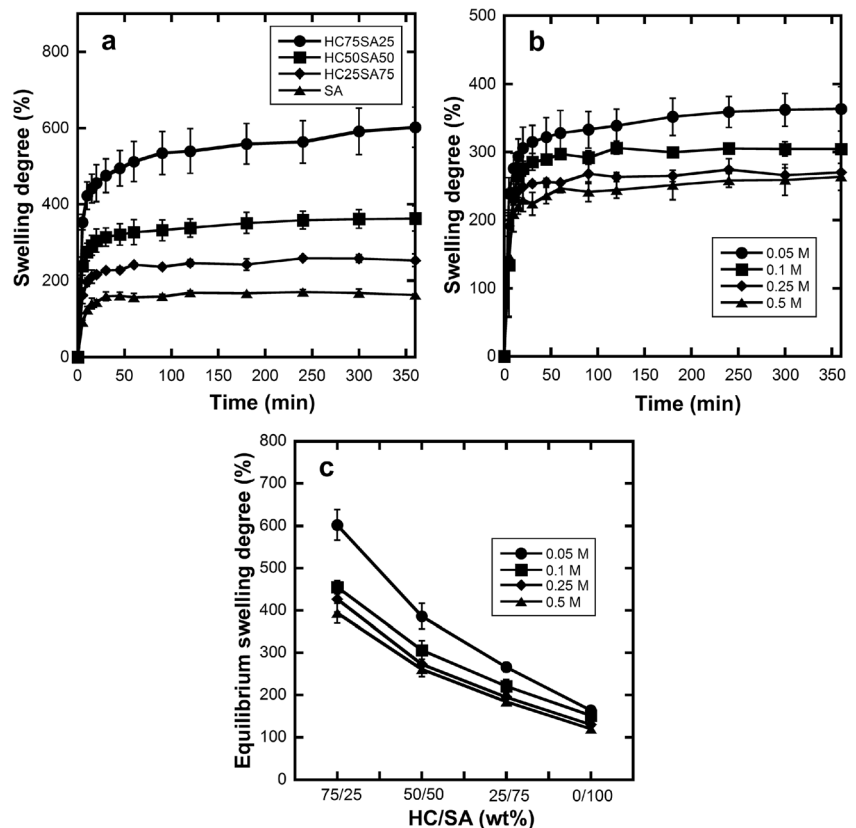
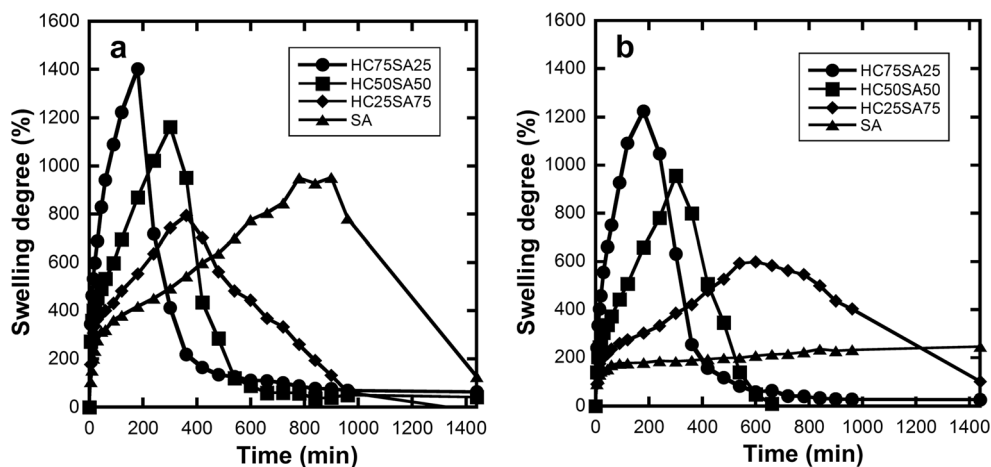


Fig. 7 Degree of swelling of HC/SA hydrogel films with varying concentration of CaCl_2 in PBS pH 7.4 at 37 °C. **a** 0.05 M CaCl_2 ; **b** 0.5 M CaCl_2



concentration of non-freezable bound water plays an important role in drug delivery [42].

We investigated the state of water in HC/SA hydrogel films by differential scanning calorimeter (DSC). Fig. 10 shows DSC thermograms of the melting behavior of freezable water in equilibrated, swollen HC/SA hydrogel films at various ratios of HC to SA and at different concentrations of CaCl_2 . It can be seen that the endothermic peak around 0–20 °C separated into two peaks. The sharp one is freezable free water that undergoes similar thermal transitions to that of bulk water. It can be seen from Fig. 10 that melting of water absorbed in all films starts at a temperature slightly lower than that of pure water (dash line). It may be the water in the films is not pure since there is some dissolution of low molecular weight polymers and CaCl_2 . This was confirmed by the slightly decrease in gel content remaining in the films after being swollen at equilibrium condition. Thus, the freezing point is depressed due to the colligative properties of the solution.

On the other hand, the broad one is designed for freezable bound water that undergoes a thermal phase transition at a temperature shifted with respect to that of bulk water. In case of various ratios of HC to SA in the films, the observed DSC

endotherms for the higher HC contents showed a broader and asymmetric peak than the lower HC one. This peak also moves to higher temperatures with higher HC content in the films. This tendency can be explained by the fact that higher water content at equilibrium was obtained when higher HC content was applied. Generally, for all studies on hydrogels of higher water content, the endothermic peaks are broad and structured [39]. It can be suggested that there are several states of water being bound with different binding sites of the HC and/or SA structures in different environments.

The state of water using different concentrations of CaCl_2 as a crosslinker during film forming was also considered. As discussed in the previous section, as a lower concentration of CaCl_2 was applied, a higher water content in the film was obtained. However, the influence of the concentration of CaCl_2 was not as strong as were the ratios of HC to SA. Thus, the broad endothermic peaks of the films crosslinked by the lower concentration of CaCl_2 were slightly broader than those of the films crosslinked by the higher concentration of CaCl_2 .

The freezable water content (free water and bound water) can be calculated from the area under the DSC peak using Eq. (5), representing the change in enthalpy. The non-freezing

Fig. 8 Degree of swelling of HC/SA hydrogel films with varying concentration of CaCl_2 in SGF, pH 1.2 at 37 °C. **a** 0.05 M CaCl_2 ; **b** 0.5 M CaCl_2

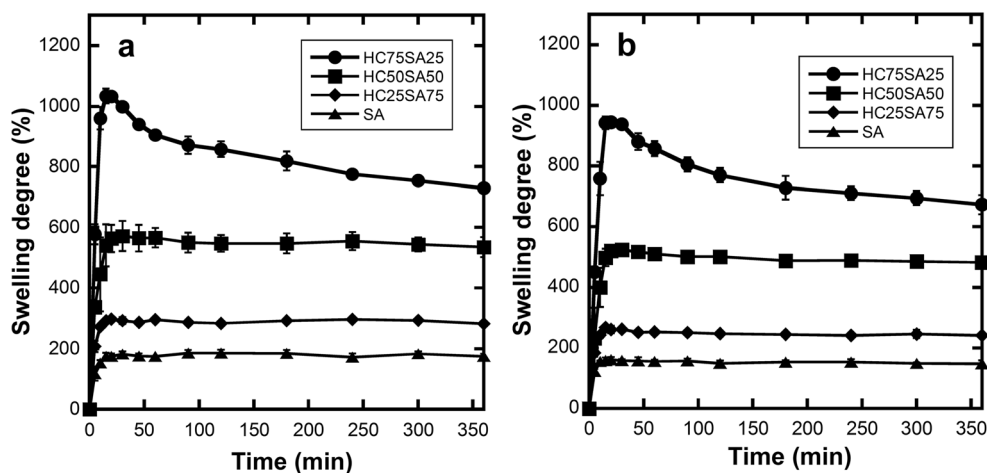
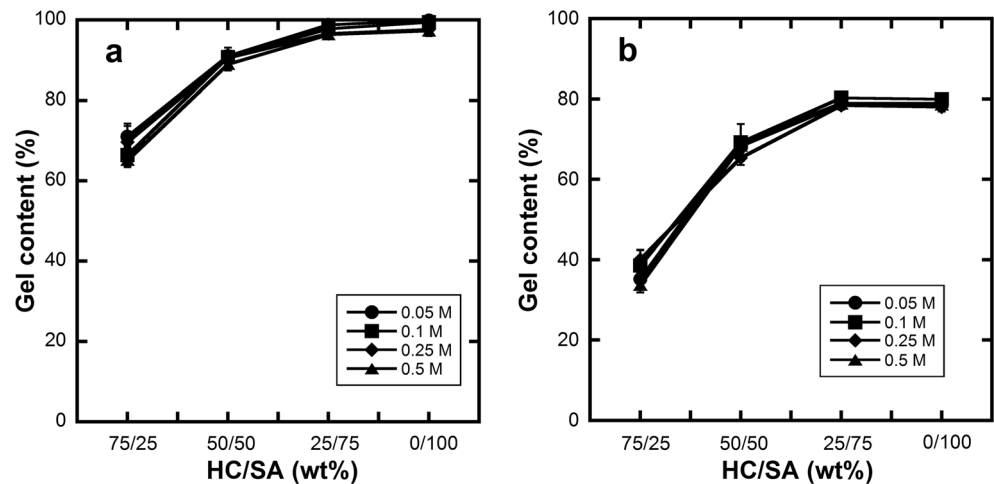


Fig. 9 Gel content of HC/SA hydrogel in: **a** distilled water at 37 °C; **b** SGF, pH 1.2 at 37 °C



bound water, however, cannot generally be observed by this technique. Alternatively, non-freezing bound water content can be obtained from Eq. (6). The freezable water and non-freezing bound water content are shown in Fig. 11. It can be seen that an increase in SA content brought about an increase in non-freezing bound water content. It is generally accepted that non-freezing bound water is formed by H-bonds between water molecules and polar groups in the polymer [40]. SA chains can be suggested to form H-bonds with water molecules better than the HC chains since it is known that interaction of water molecules with hydroxyl groups is stronger than with amine groups [41]. Moreover, the structure of HC consists of the bulky hydroxyethylacryl group that might impede formation of H-bonds between the hydroxyl groups and water. In addition, the concentration of CaCl₂ used as crosslinker is not or less influential since non-freezable bound water is not strongly dependent on the mesh size of the polymer network [35].

Mechanical properties

Tensile strength and elongation at the breaking point of the wet films are shown in Fig. 12. For biomedical applications,

tensile strength and elongation at the breaking point in the wet condition is very important. An idea for wound dressing is that the film should present reasonable mechanical properties during use and be flexible enough to follow skin movement. The tensile strength of the hydrogel films increased with an increasing SA content and/or concentration of CaCl₂ (Fig. 12a). This was due to the crosslinking reaction between Ca²⁺ ions and SA at its carboxylate groups. Thus, a high concentration of CaCl₂ and high SA content could possibility facilitate the crosslinking reaction, leading to improved film malleability and an increase in film strength.

By contrast, high SA content and high concentration of crosslinking agent bought about an increase in crosslink density, retarding molecule from movement and deformation, ultimately resulting in reduced elongation of the hydrogel films. As seen in Fig. 12b, a low SA content (HC75SA25 and HC50SA50) together with a high concentration of crosslinking agent (0.25 and 0.5 M) showed less impact on elongation of the hydrogel films. For a low SA content (HC75SA25), this was due to the lower crosslink density resulting in a lower tensile strength, at which point the films were easily broken when applied forces caused low elongation values at the breaking point. In the case of a high crosslinking

Fig. 10 DSC thermograms of HC/SA hydrogel films in a swollen state with varying concentrations of CaCl₂. **a** 0.05 M CaCl₂; **b** 0.5 M CaCl₂

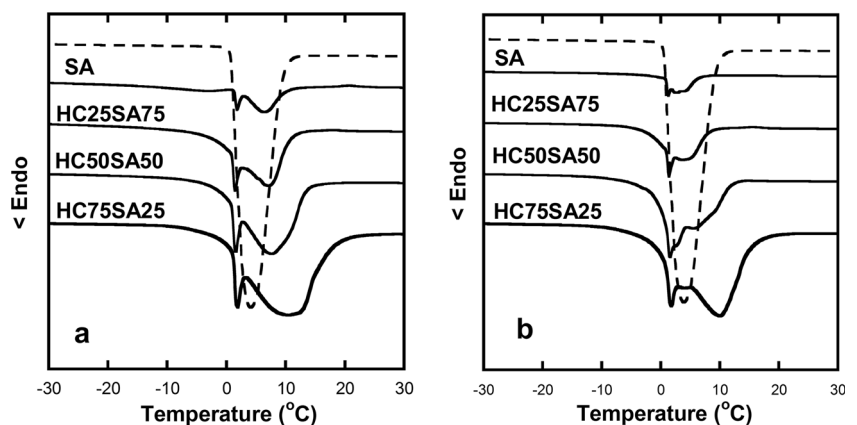
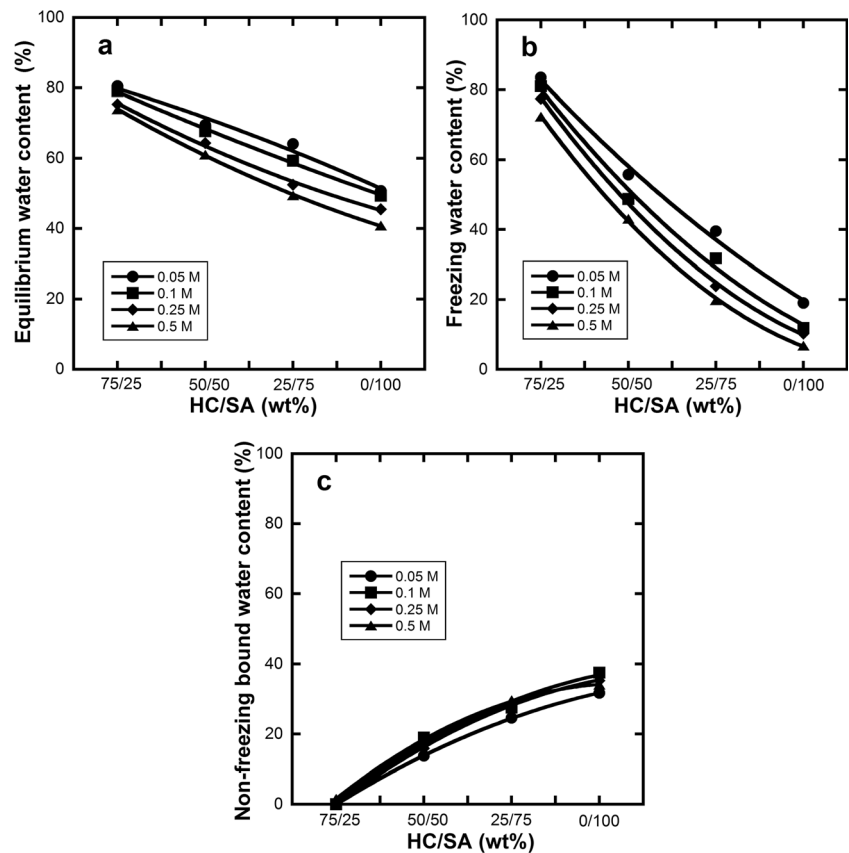


Fig. 11 Water content of HC/SA hydrogel films. **a** Equilibrium water content; **b** Freezing water content; **c** Non-freezing bound water content



agent concentration, the films should have high crosslink density with less deformation before breaking down.

Generally, the tensile strength of skin is usually in the range of 2.5–16 MPa [43], and elongation is approximately 70% in the most flexible zone [44]. It can be seen that most of the prepared hydrogel films meet or exceed these values, indicating potential biomedical use.

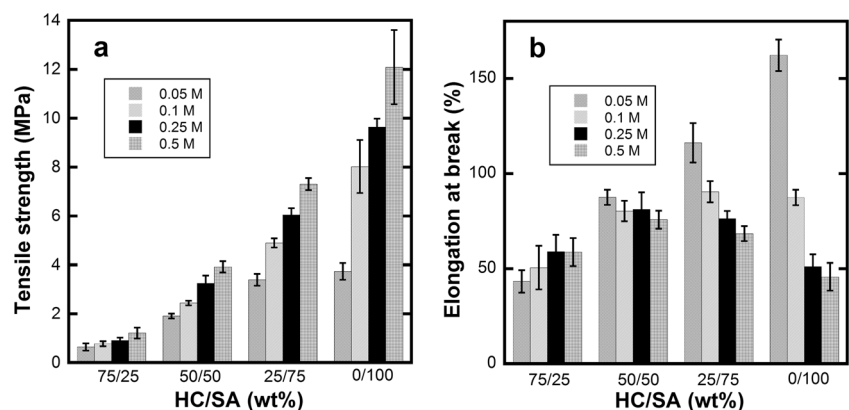
MTT assay

Cell viability of the synthesized hydrogels was determined following the MTT assay. The average percentages

of cytotoxicity of HC75SA25, HC50SA50, HC25SA75 and SA hydrogel films crosslinked by 0.5 M CaCl_2 are 5.56 ± 0.04 , 10.27 ± 8.17 , 7.43 ± 1.58 and 9.74 ± 1.98 , respectively. It can be concluded that the hydrogel films are not toxic to human cells. Hence, the results suggest potential application of these hydrogel films as biomaterials.

Consequently, the prepared hydrogel films possess excellent swelling capability, pH-sensitive properties, biodegradability, reasonable mechanical properties and nontoxicity, supporting their potential for use as biomedical materials.

Fig. 12 Mechanical properties of the hydrogel films: **a** tensile strength; **b** elongation at break



Conclusions

Semi-IPN HC/SA hydrogel films were successfully prepared by using CaCl_2 as a crosslinker. The systems investigated indicate a significant reduction in swelling as the SA and crosslinking reagent content is increased. Hydrogel films with higher HC content degrade faster in PBS than those with lower HC content; the hydrogel films were still stable in SGF. Moreover, all systems evaluated have been proven to be noncytotoxic. The comprehensive results of this study suggest their potential in healing of wounds and controlled drug release.

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