New Route to the Preparation of Carboxymethylchitosan Hydrogels

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ABSTRACT: A new method to prepare CM–chitosan hydrogels was introduced with the use of steam. The procedure was simple and economical, with no toxic chemicals involved. The steam-induced crosslinking of CM–chitosan sodium salt involved the $-NH_2$ and –COONa groups, forming amide linkages (–CONH–), evidently supported by FTIR spectroscopy and other techniques. The hydrogels instantly imbibed a great deal of water. The degree of swelling (DS) of the hydrogels was found to be up to 36, depending on the harshness of steaming conditions used. Likewise, the coloration of the samples increased from light beige to brown with increasing temperature and duration of steam exposure. The overall efficiency of the steam method for the crosslinking of CM–chitosan sodium salt was quite high. The percentage weight loss was found to be less than 10 to obtain hydrogels with DS values around 20. No weight loss in the dry weight of the fractionated hydrogels was observed when the samples were steamed at 115°C or higher for 15 min or longer. J Appl Polym Sci 90: 4016–4020, 2003

Key words: carboxymethylchitosan; hydrogels; crosslinking; steam; swelling; polysaccharides

INTRODUCTION

Chitin is one of the most abundant polysaccharides found in nature. It is essentially extracted from crustaceous shells. Chitin materials (with a degree of acetylation > 0.5) are copolymers consisting of β-(1→4) D-glucosamine and *N*-acetyl-D-glucosamine units. Chitosan is derived from chitin by removal of *N*-acetyl groups on the copolymer by hydrolysis, so-called deacetylation. In general, chitosan dissolves in aqueous acidic medium below pH 6.5. Many areas have been explored to find possible uses for these polymers over the years. A biomedical use of chitin and chitosan in wound healing has long been investigated because of their physiological compatibility with living tissues.[1,2] Although they have been found to have an accelerating effect on the wound-healing process, they possess dissatisfactory water-sorption ability. Several attempts have been made to overcome such a problem.[3,4]

Carboxymethyl derivatives of both chitin and chitosan are water-soluble and of very low toxicity. These biodegradable polymers find various uses as metal ion chelating agents, drug carriers with controlled release, cosmetic ingredients, and medical aids.[5] In wound dressing application, carboxymethylchitosan (CM–chitosan) is usually used in a gel form that can greatly imbibe exudate. Such agents as those that are widely used for the crosslinking of chitosan are also employed to crosslink CM–chitosan (e.g., glutaraldehyde).[6] Another crosslinking agent used to produce covalently linked CM–chitosan is a carbodiimide.[7] Although effective, most of the stated reagents are known to be toxic or corrosive.

To avoid using noxious chemicals in the preparation of CM–chitosan hydrogels, a new environmentally friendly preparation route was discussed in this study. Steam generated by an autoclave was found to be a promising means to crosslink CM–chitosan sodium salt. To understand the effect of steam on the preparation of CM–chitosan hydrogels, different steaming temperatures and exposure times were used. The degree of swelling of the resultant hydrogels was simultaneously evaluated. The chemical structure of the steamed samples was confirmed by FTIR spectroscopy.

EXPERIMENTAL

Materials

Chitin from squid pens was prepared in our laboratory, and its degree of deacetylation was 0.25, determined by solid-state ^{13}C-NMR (Bruker DPX-300 spectrometer; Bruker Instruments, Billerica, MA). Other reagents were of analytical grade and used as received.

Preparation of CM–chitosan sodium salt

Water-soluble CM–chitosan sodium salt was prepared by a carboxymethylation reaction. Typically, 5 g

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of chitin powder was suspended in 150 mL of isopropyl alcohol at ambient temperature for at least 3 h. To the stirred slurry, 50% (w/v) aqueous NaOH was added and the solution was stirred at 80°C for 1 h. At this stage chitin was deacetylated to chitosan and 100 mL of 60% (w/v) aqueous monochloroacetic acid was slowly added over a period of 20 min. The stirring was continued for 3 h at 60°C. At the end, the whole solution was neutralized and then precipitated in CH_3OH. The mixture was filtered and the solid product was washed twice with 150 mL of 70% (v/v) CH_3OH/H_2O mixture. The washed product was eventually filtered and dried in a vacuum oven at ambient temperature. Yield of the resultant CM–chitosan salt product was 6 g. The molecular weight of the prepared sample was determined by gel permeation chromatography (GPC) with pullan as standard on a Waters 2410, 600E fraction collector II (detector: RI; columns: one ultrahydrogel linear column and one guard column; eluent: 0.1*M* NaOH; flow rate: 0.6 mL/min; operating temperature: 25°C; Waters Chromatography Division/Millipore, Milford, MA). The molecular weight of the product was found to be as follows: $\overline{M}_w$ = 1,307,000 g/mol and $\overline{M}_n$ = 65,000 g/mol.

Salt-free CM–chitosan was obtained after the aqueous solution of CM–chitosan sodium salt was acidified with 2*N* HCl to pH 2.

Preparation of CM–chitosan hydrogels

The prepared CM–chitosan sodium salt was subsequently employed in the preparation of CM–chitosan hydrogels. An aqueous solution of CM–chitosan salt was poured into molds. Afterward, the cast samples were freeze-dried to produce spongelike soft pads. The samples were kept in a desiccator for further analyses and uses. To crosslink the CM–chitosan sodium salt, the sponges were exposed to saturated steam at given temperatures for given periods.

To elucidate the mechanism of the crosslinking formation of CM–chitosan sodium salt by steam, the steam treatment of various powder materials, such as chitosan, chitin, and CM–cellulose sodium salt, was also performed under the same condition as that used for CM–chitosan sodium salt. The solubility of these steam-treated materials was evaluated.

Hydrolysis of amide-linked CM–chitosan hydrogels

An amide is typically hydrolyzed when heated with aqueous bases, yielding ammonia and a carboxylate salt. To verify that the crosslinking involved the formation of amide linkages, two steamed sponges (2 × 3 cm) were heated separately, one in 35% (w/v) aqueous NaOH and the other in H_2O, at 80°C for 2.5 h.

Structural analysis

The chemical structures of the prepared CM–chitosan hydrogels and their parent material (in both salt and acid forms) were characterized by FTIR. The IR spectra were recorded on a Perkin–Elmer System 2000 FTIR spectrophotometer (Perkin Elmer Cetus Instruments, Norwalk, CT) with KBr pellets.

Swelling study

The swelling ability of the CM–chitosan hydrogels was evaluated by a gravimetric method. Here, a steamed sponge of known weight was placed in water at ambient temperature. The weight of the swelled product was determined as a function of immersion time. The sample was quickly blotted with tissue paper to remove excess water on the surface and weighed on an electronic balance. The degree of swelling (DS) was calculated using the following equation:

$$\mathrm{DS} = \frac{W_s - W_d}{W_d}$$

where W_s and W_d are the weights of the swelled and dry samples, respectively.

To evaluate the efficiency of the steam treatment method for the crosslinking of CM–chitosan sodium salt, a steamed sponge of known weight was swelled in water at ambient temperature for 24 h. The water was repeatedly replaced with fresh one during the fractionation. The percentage weight loss (%WL) of a sample steamed at a particular condition directly represented the efficiency of that particular steaming condition for the crosslinking of CM–chitosan sodium salt. It was calculated using the following equation:

$$\%\mathrm{WL} = \frac{W_b - W_a}{W_b} \times 100$$

where W_b and W_a are the weights of samples before and after fractionation, respectively.

RESULTS AND DISCUSSION

Structural analysis

Figure 1 reveals the overlaid FTIR spectra of salt-free CM–chitosan, CM–chitosan sodium salt, and a steamed product. An absorption band at 1735 cm^{-1} corresponds to carbonyl stretching (>C═O), in Figure 1(a). The FTIR spectrum of CM–chitosan sodium salt, on the other hand, shows an absorption band of carboxylate ion ($–COO^-$) near 1650–1550 cm^{-1}. In both samples, notable functional groups were also detected: –OH around 3450 cm^{-1}, amide I band (–CONH–) around 1655 cm^{-1}, $–NH_2$ deformation at 1596 cm^{-1},

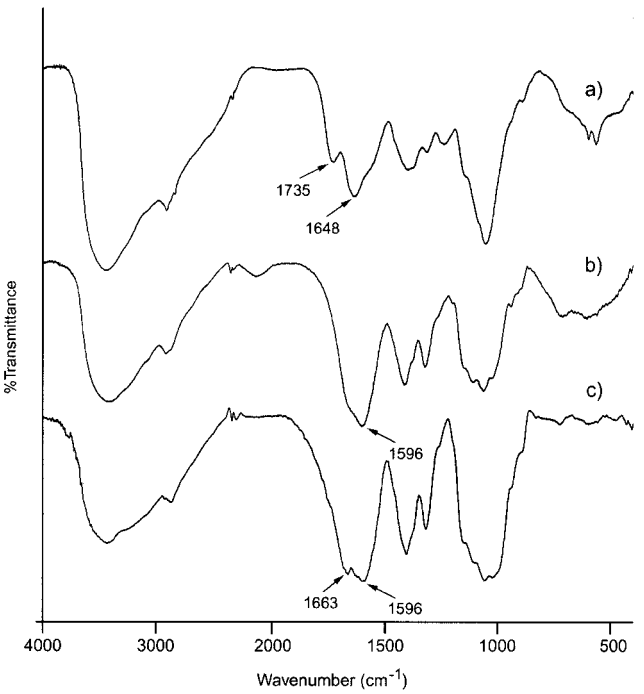

Figure 1 Overlaid FTIR spectra of (a) salt-free CM–chitosan, (b) CM–chitosan sodium salt, and (c) steamed CM–chitosan sodium salt (at 115°C, 15 min).

and bridge oxygen (C—O—C) at 1170–1114 cm^{-1}. Compared to that of CM–chitosan sodium salt, the amide I band in the steamed product seemed to intensify. This indicated that a steam-induced reaction in CM–chitosan sodium salt involved the $–NH_2$ and –COONa groups, forming amide linkages (–CONH–). It was noted, however, that there were no gross differences in the FTIR spectra of the steamed CM–chitosan salt samples after the different heat treatments (spectra not shown).

This was also proven by the hydrolysis of the steamed sponge by 35% aqueous NaOH at 80°C. The sponge collapsed and became completely soluble in 35% aqueous NaOH, whereas the other sponge in water at 80°C remained insoluble and perfectly retained its shape. The starting CM–chitosan sodium salt was initially soluble in water and 35% aqueous NaOH at room temperature. Chitosan (degree of deacetylation = 0.80 determined by solid-state ^{13}C-NMR) was also subjected to the hydrolysis in 35% NaOH under the identical condition; its $\overline{M}_w$ and $\overline{M}_n$ were found to reduce from 1,867,000 to 836,000 and 704,000 to 196,000, respectively. This suggested that the hydrolysis apparently induced the chain scission. This undesirable phenomenon could, however, scarcely make the redissolution of the hydrolyzed CM–chitosan hydrogel possible. Under the alkaline condition, amides were attacked by the strong nucleophilics, hydroxide ions, resulting in the breakup of the network and regeneration of the CM–chitosan sodium salt.

Another experimental evidence to support this crosslinking formation is illustrated in Table I. After heat treatment, chitosan, chitin, and CM–cellulose sodium salt remained soluble in their good solvents, whereas the steamed CM–chitosan salt became insoluble in water. CM–chitosan sodium salt differs from the other materials in that it contains both free amine and carboxylate groups along its polymeric chains. This strongly confirmed that the interchain crosslinking formation involved the –COONa and $–NH_2$ groups. It was previously reported that chitosan could be crosslinked by sulfosuccinic acid (SSA) that possessed three crosslinkable sites, two carboxylic acid groups and one sulfuric acid group, at room temperature after a 10-h reaction.[8] The crosslinking reaction proceeded evidently between the acidic groups of SSA and amine groups of chitosan. This amidization could turn a water-soluble chitosonium acetate film to a water-insoluble chitin upon heating.[9] It was noted that dry heat was not as effective as saturated steam, as seen in Table I, when used as the method of crosslinking. The sample heated at 150°C for 15 min in a

TABLE I
Solubility of Samples Heated at Given Temperatures

	Heating condition[a]		Solubility	
Heated sample	Temperature (°C)	Time (min)	Soluble	Insoluble
Chitin	110	15	X[b]	
Chitosan	110	15	X[c]	
CM–chitosan sodium salt	110	15		X[d]
CM–chitosan sodium salt	150[e]	15	X[d]	
CM–cellulose sodium salt	110	15	X[d]	

[a] Heating source was steam.
[b] Solubility testing in LiCl/NMP (*N*-methyl-2-pyrrolidone).
[c] Solubility testing in 0.1*M* acetic acid.
[d] Solubility testing in water.
[e] Heating source was dry heat from a convection oven.

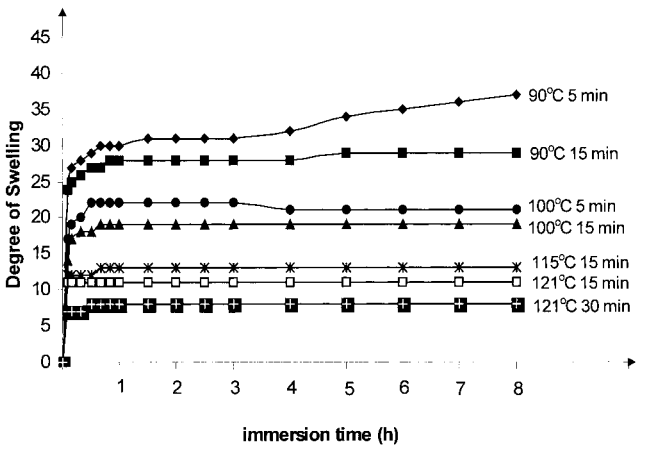

Figure 2 Relationship between degree of swelling and immersion time (h) of CM–chitosan hydrogels prepared under different steaming temperatures and durations of steam exposure.

convection oven was still soluble in water. This implied that water, in the form of steam, was essential for the crosslinking.

Swelling study

The effects of the steaming temperature and time on the degree of crosslinking of CM–chitosan sodium salt can be directly observed from the degree of swelling. The lower the degree of swelling detected, the higher the crosslinking density was. It was observed that the steamed samples started to develop color and had low affinity for water when they were exposed to high-temperature steam and a prolonged treatment. The coloration of the samples intensified from light beige to brown. This pigment formation was attributed to the Maillard reaction.[10] This thermally induced side reaction was also observed in chitosan when it was steamed at 115°C for 15 min.

All hydrogels swelled rapidly in water. The hydrogel could absorb water 36 times greater than its original weight. The degree of swelling of the CM–chitosan hydrogels is shown in Figure 2. The swelling of most hydrogels reached equilibrium within 2 h. The degree of swelling reduced from 36 to 8 as a function of steaming conditions from 90 to 121°C and 5 to 30 min. The increasing degree of crosslinking through the amide formation upon steaming accounted for the decrease in the swelling ability of the hydrogels.

In Table II, there was no net loss in the dry weight of the hydrogels when the samples were steamed at ≥115°C for ≥15 min. The fractionation process did not result in any dissolution of those CM–chitosan hydrogels. The overall efficiency of the steam method for the crosslinking of CM–chitosan sodium salt was quite high. The %WL was found to be less than 10 to obtain hydrogels with DS values around 20.

CONCLUSIONS

CM–chitosan sodium salt is a water-soluble derivative of chitin and chitosan. It is a very appealing material and has potential applications in industry, medicine, agriculture, and biotechnology. Like chitosan, CM–chitosan salt can be crosslinked by dialdehydes and carbodiimides. These reagents are either toxic or corrosive. In this work, a new route to the preparation of CM–chitosan hydrogels was established without the need for such noxious reagents. Steam generated by an autoclave was an alternative means to crosslink CM–chitosan sodium salt. The steam-induced crosslinking of CM–chitosan salt involved the $-NH_2$ and –COONa groups, forming amide linkages (–CONH–). The degree of swelling of the hydrogels was found to be up to 36, depending on the steaming conditions used. The overall efficiency of the steam method for the crosslinking of CM–chitosan salt was quite high. The %WL was found to be less than 10 to obtain hydrogels with DS values around 20. No weight losses in the dry weight of the hydrogels were observed when the samples were treated at 115°C or higher for 15 min or longer. With this environmentally friendly method, the prepared CM–chitosan hydrogel pads could be safely used as biomedical materials.

TABLE II
Percentage Weight Loss (%WL) of Hydrogels Prepared Under Various Steaming Conditions After Fractionation in Water for 24 h

	Steaming condition		Weight of sample (g)		
Sample	Temperature (°C)	Time (min)	Before fractionation	After fractionation	%WL
1	90	5	0.105	0.090	14
2	90	15	0.099	0.088	11
3	100	5	0.092	0.084	9
4	100	15	0.111	0.109	2
5	115	15	0.089	0.091	0
6	121	15	0.095	0.095	0
7	121	30	0.099	0.101	0

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