Polymer Bulletin 47, 451-456 (2002)

Polymer Bulletin

Synthesis and characterization of pH/temperature-sensitive hydrogels based on chitosan derivative

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Received: 14 September 2001/Revised version: 18 October 2001/ Accepted: 22 October 2001

Summary

A series of pH/temperature-sensitive hydrogels composed of maleilated chitosan (MC) and N-isopropylacrylamide (NIPAAm) were prepared and characterized with respect to their swelling behaviors, change of lower critical solution temperature (LCST). These hydrogels showed broad pH-sensitivities below 32 °C, but above that temperature exhibited sharp pH-dependent phase transition behaviors which depended on the content of MC in the hydrogels. The change of ratio of NIPAAm to MC in weight did not affect LCST significantly, while it affected equilibrium water content (EWC) at high pH region. The hydrogels also exhibited a typical of reversibility depending on temperature and pH.

Introduction

Hydrogels are crosslinked hydrophilic polymer networks which can absorb large amounts of water within its structure without dissolution. Owing to its high water content and good biocompatibility, a wide range of biomedical and biotechnological applications of hydrogels have been studied including controlled drug delivery systems [1-3], contact lens [4], wound dressing [5], and a variety of other potential uses. Much attention has been focused especially upon the stimuli-sensitive hydrogels which can exhibit dramatic change in their swelling behavior, network structure, or mechanical strength in response to change in pH [6-7], temperature [8-9], electric field [10], and ionic strength [11]. Crosslinked poly (N-isopropylacrylamide) (PNIPAAm) has been known as one of the best temperature-sensitive hydrogels and shows a behavior of LCST at around 32 °C in aqueous solution [12]. The gel is swollen below the LCST, and becomes collapsed above the LCST. The LCST of PNIPAAm can also be modified by random copolymerization with hydrophilic [13-15] or hydrophobic [16] monomer in the gel composition. In particular, hydrogels prepared from random copolymerization of NIPAAm with ionic monomer such as acrylic acid (AAc) show a different LCST depending on the pH environment [13]. As the pH of solution increases the LCST of the hydrogel copolymerized with NIPAAm and AAc randomly shifts to a higher temperature due to increased hydrophilicity and charge repulsion. For higher than 10 mol % AAc, the LCST disappears at above the pK_a of AAc, because then the AAc exhibits hydrophilicity enough to offset the interaction of the

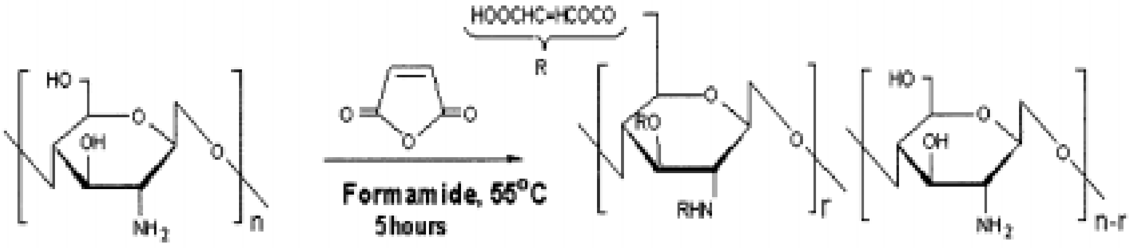


Figure 1. Reaction scheme for preparation of maleilated chitosan

hydrophobic temperature-sensitive components. However, copolymers composed of side-chains of PNIPAAm grafted onto Poly(AAc) show a constant LCST at either pH, independent of AAc content over a wide range of compositions [14]. It has also been found that the design of a new biocompatible and biodegradable stimuli-sensitive polymeric materials by use of polysaccharide such as dextran, or chitosan play a key role in the development of multi-stimuli responsive hydrogels.

Chitosan is a polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine derived from N-deacetylation of chitin in aqueous alkaline medium. Due to its biocompatible, biodegradable, and nontoxic properties, chitosan and its derivatives have been examined extensively in biomedical [17] and pharmaceutical applications [18]. Chitosan has also been modified to extend its domain of application. It was the purpose of this study to prepare a new crosslinked hydrogels which were pH/temperature-sensitive from the polymerization of MC and NIPAAm in various contents, and to characterize its swelling properties depending on pH and temperature.

Experimental

Materials

Chitosan 100 was purchased from Wako Pure Chemical Co. Maleic anhydride and NIPAAm were purchased from Aldrich Chemical Co. Ammonium persulphate (APS), N,N,N,N-Tetramehylene diamine (TEMED), NIPAAm was purified by recrystallization from hexane-acetone mixture.

Preparation of maleilated chitosan(MC)

MC was synthesized according to the procedure reported by Berkovich, *et. Al* [19]. As shown in Figure 1, the reaction scheme, maleic anhydride (3.5 equivalent per chitosan unit) and chitosan were added to formamide. The quantity of chitosan in the reaction mixture amounted to 2 %. The heterogeneous reaction mixture was stirred at 55 °C. After stirring for 12 h, the reaction mixture was poured into methanol. The precipitate was filtered and washed with acetone. The product was resuspended in distilled water for purification, followed by adding an adequate amount of Na_2HPO_4 to give a clear solution of pH 8~10. After the solution was filtered the filtrate was dialyzed against deionized water for three days and lyophilized.

Synthesis of the hydrogels

Hydrogels composed of NIPAAm and MC were prepared by free radical polymerization in the presence of APS as an initiator and TEMED as an accelerator. The crosslinked hydrogels were prepared at various weight ratios of NIPAAm to MC given in Table 1. Polymerization mixture was bubbled through with nitrogen for 5 min and the mixture was immediately injected into the space between two glass plates. Polymerization was carried out at room temperature for 5 h. After the gelation was completed, the gel membrane was cut into disks, 8 mm in diameter, and then immersed in an excess amount of deionized water for 2 days to remove the residual NIPAAm unreacted. Swollen polymeric gels were dried at room temperature for 1 day, and these samples were further dried in a vacuum oven for 1 day at 40 °C.

Swelling measurement

For the pH dependent swelling studies, gels in triplicate were incubated in buffer solutions (ionic strength = 0.1M) ranging from pH 2 to 10 at a particular temperature. The incubation time was approximately 48 h. It was confirmed that 48 h equilibration was enough to reach the equilibrium swelling of the gel. For the temperature dependent swelling studies, gels were swollen in the particular buffer solution at various temperatures in the expected range of the LCST. Periodically, the gels were withdrawn from the buffer solution and weighted after removal of excessive surface water by blotting with a filter paper. After equilibration at one condition, gels were re-equilibrated at the other temperature. The EWC was calculated by the following equation,

$$\text{Equilibrium Water Content (EWC) (\%)} = [(W_s-W_d)/[W_s x100] \qquad (\mathbf{1})$$

where W_s and W_d were fully swollen gel and dried gel weights, respectively.

Results and discussion

Characterizations of MC and its hydrogels

The reaction of chitosan with maleic anhydride in formamide at 55 °C for 12 h led to the formation of maleilated chitosan, which was soluble in basic buffer solution but precipitated in acidic buffer solution. As shown in the FT-IR spectra (b) of Figure 2 the band at 1715 cm^{-1} can be assigned to α-unsaturated esters and the presence of vinyl groups in MC is confirmed by the bands at 1656 cm^{-1} and 810 cm^{-1}. The ^{1}H-NMR spectra shown in Figure 3 further confirms the chemical structures. The peaks at 5.8 – 6.7 ppm can be assigned to the protons of vinyl groups (–CH=CHCOONa) incorporated into the amine and hydroxyl groups of chitosan. The degree of substitution for maleic substituent carboxyl groups determined by the NMR spectra is

Table 1. Feed composition of a series of hydrogels

Sample code	MC (mg)	NIPAAm (g)	([COONa]/[NIPAAm+COONa]) × 100 (mol%)	APS (mg)
MC100-NIPAAm	100	1.2	5	10
MC350-NIPAAm	350	1.2	15	10
MC500-NIPAAm	500	1.2	20	10

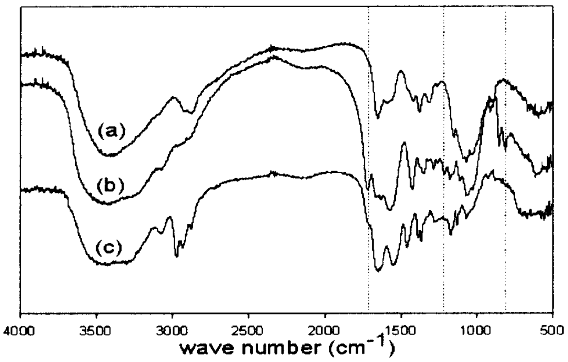


Figure 2. FT-IR spectrum of (**a**) chitosan, (**b**) MC, (**c**) MC500-NIPAAm

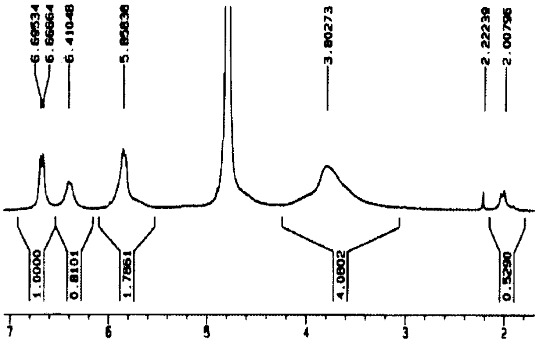


Figure 3. ^{1}H-NMR spectrum of maleilated chitosan

2.6 for three reactive sites in chitosan. As shown in Figure 2 (c), it is observed FT-IR peak intensity of the vinyl groups at 810 cm^{-1} decreases due to the formation of hydrogels. A large reduction of the peak at 810 cm^{-1} suggests that almost all vinyl groups were consumed by radical polymerization. Incorporation of NIPAAm into the hydrogels is confirmed by the increase of intensity of bands at around 1600 cm^{-1} and 1200 cm^{-1}, which are assigned to amide I band and isopropyl groups, respectively.

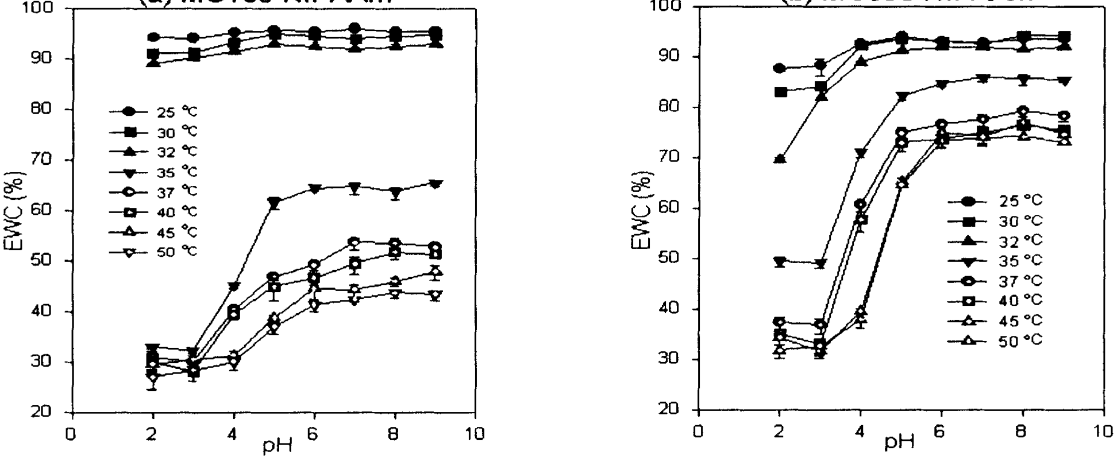
Swelling of the hydrogels

The EWC of this series of the hydrogels at different pH and temperatures is shown in Figure 4 (a), (b), (c). It can be seen that the EWC of the hydrogels was strongly dependent on both pH and temperature. Since negatively charged carboxyl groups of MC were incorporated into the polymer network, the gel swelled at high pH region due to ionic repulsion of the carboxyl groups, and collapsed at low pH values because of protonation of carboxyl groups. Figure 4 (a), (b), (c) also show that differences in EWC between low pH and high pH generally increased as the content of carboxyl groups in the hydrogels increased. Its transition pH shifted to a slightly higher pH in all hydrogels as the temperature increased since the gels hydrophobicity increased with temperature. This result agrees with that published by Hoffman [20].

Figure 4 (d) shows the EWC of MC500-NIPAAm hydrogel with different pH as a function of temperature. The LCST of the hydrogel with 20 mol % carboxyl groups appeared sharply at around 32 °C in low pH range. In high pH range, the LCST became noticeable though it was broad. In general, random copolymer gels composed of NIPAAm and AAc with more than 10 mol % AAc does not exhibit LCST behavior at the pH higher than pKa of AAc [13]. On the other hand, copolymers composed of PNIPAAm grafted onto a backbone polymer, PAAc, exhibit a constant LCST irrespective of the ratio of monomers [14-15]. In the present study, the LCST of PNIPAAm within the crosslinked network hydrogels was found to be relatively independent of MC because PNIPAAm segments and MC segments containing carboxyl groups consisted of two different polymer chains within the hydrogels like PNIPAAm and AAc in PAAc-g-PNIPAAm.

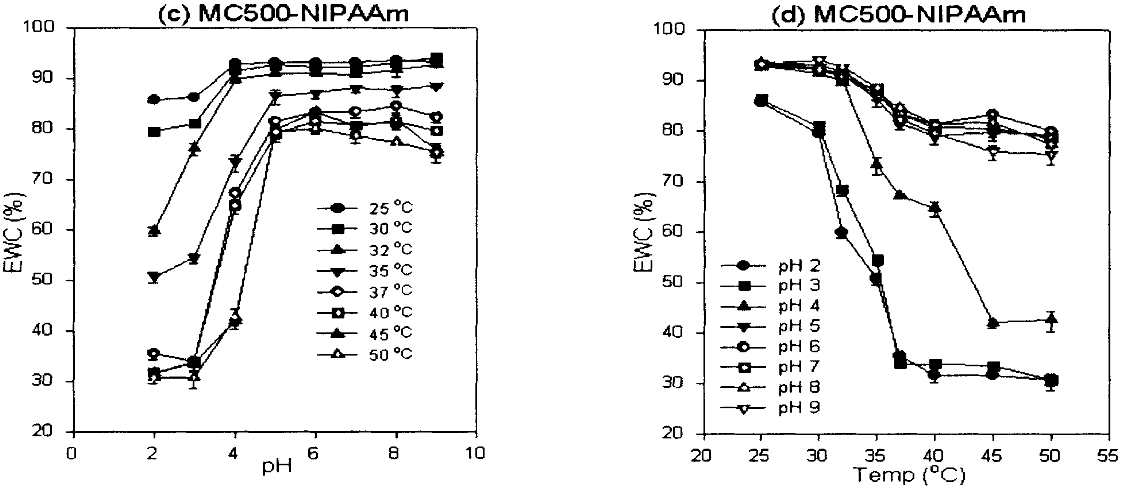
Effect of reversibilities on swelling ratio

Figure 5 (a) shows the change of EWC of this series of hydrogels when the hydrogels were immersed in pH 7.4 at 30 °C and 37 °C. It was found that the hydrogels could



(a) MC100-NIPAAm

(b) MC350-NIPAAm



(c) MC500-NIPAAm

(d) MC500-NIPAAm

Figure 4. (**a**), (**b**), (**c**) EWC of hydrogels as a function of pH; (**d**) EWC of MC500-NIPAAm as a function of temperature

swell and deswell over a period of time when temperature was varied periodically between higher temperature and lower temperature. Furthermore, the less MC in the hydrogel, the more obvious the thermosensitive effect, and the greater the range of reversibilities. Figure 5 (b) shows the effect of periodic change of pH on the EWC of these ionic networks. The pH of the buffer solution was changed from 2 to 7.4, and the same cycle was repeated. In this case, the more MC in the hydrogel, the more obvious the pH effect, and the greater the range of reversibilities.

Conclusion

Maleilated chitosan was synthesized by the reaction of chitosan with maleic anhydride. A series of hydrogels which exhibited pH-temperature sensitivity could be prepared from MC and NIPAAm. Increase of pH in buffer solution caused the increase of EWC due to the increase of ionic repulsion in the hydrogels. In addition, the higher the MC content in the hydrogels, the greater the hydrophilicity of the hydrogels after the ionization of carboxyl groups of MC and then the higher the EWC of the hydrogels. For the hydrogels with higher than 20 mol % carboxyl groups the

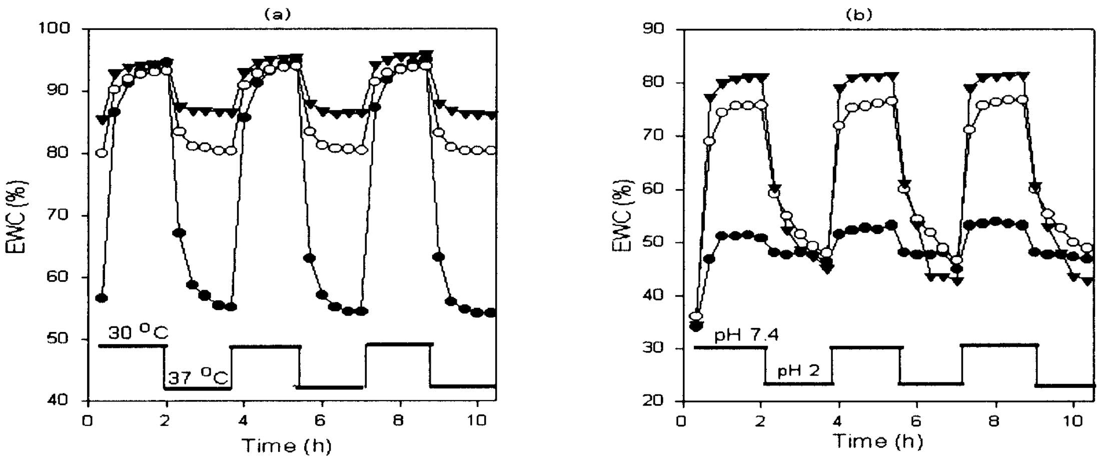


Figure 5. Swelling reversibility of MC-NIPAAm hydrogels (**a**) at pH 7.4 (**b**) at 37°C (● : MC100-NIPAAm, ○ : MC350-NIPAAm, ▼ : MC500-NIPAAm)

LCST still appeared in high pH buffer solution. This result indicates that carboxyl groups in MC-NIPAAm crosslinked hydrogels behave differently from those in random copolymer of NIPAAm and AAc. The hydrogels also showed a typical of reversibility depending on pH or temperature.

Acknowledgements.
This work was supported by the Brain Korea 21 Project.

References

1. Christopher SB, Pappas NA. (1996) J Controlled Rel 39:57
2. Okano T, Bae YH, Jacobs H, Kim SW (1990) J Controlled Rel 11: 255
3. Peppas NA, Khare AR (1993) Adv Drug Delivery Rev 11:1
4. Hyon SH, Cha W, Ikda Y, Kita M, Ogura Y (1994) J Biomater Sci Polym 5:397
5. Woerly S, (1993) Biomaterials, 14:1056
6. Vazquez B, Gurruchaga M, Goni I, Narvarte E, Roman, JS (1995) Polymer 17:3327
7. Kataoka K, Koyo H, Tsuruta T (1995) Macromolecules 28:3336
8. Bae YH, Okano T, Kim SW (1990) J Polym Sci Polym Phys 28:923
9. Yu H, Grainger DW (1994) Macromolecules 27:4554
10. Eisenberg SR, Grodzinski AJ (1984) J Membr Sci 19:173.
11. Ricka J, Tanaka T (1984) Macromolecules 17:2916
12.Bae YH, Okano T, Sakurai Y, Kim SW (1991) Pharm Res 4:223
13. MK, Sung YK, Lee YM, Cho CS (2000) Polymer 41:57141
14 Yoo. Chen G, Hoffman AS (1995) Nature 373(5):49
15 Ebara M, Aoyagi T. Sakai K, Okano T (2000) Macromolecules 33:8312
16. Brazel CS, Peppas NA (1995) Macromolecules 28:8016
17. Amiji MM (1995) Biomaterials 16:593
18. Shantha KL, Bala U, Rao KP (1995) Eur Polym J 31:377
19. Berkovich LA, Tsyurupa MP, Davankov VA (1983) J Polym Sci Part A 21:1281
20. Dong LC, Hoffman AS (1991) J Controlled Rel 15:141