

pH-Sensitivity of Hydrogels Based on Complex Forming Chitosan: Polyether Interpenetrating Polymer Network

KANG DE YAO,¹ and TAO PENG,¹ M. F. A. GOOSEN,² JI MEI MIN,³ and YU YIN HE³

¹Department of Materials Science and Engineering, Tianjin University (300072), Tianjin, People's Republic of China, ²Department of Chemical Engineering, Queen's University, Kingston Canada K7L 3N6, and ³National Laboratory of Natural and Biomimetic Drugs, Beijing Medical University, P.O. Box 216, Beijing (100083), People's Republic of China

SYNOPSIS

This article deals with a novel, semi-interpenetrating polymer network (semi-IPN), composed of crosslinked chitosan (cr-CS) with glutaraldehyde (GA) and polyether. The pH responsibility of swelling data shows that swelling reaches a maximum at pH = 3.19 and a minimum at pH = 13. The concentration of chitosan acetic acid solution, the amount of crosslinking agent, and polyether, have effects on the swelling behavior of the network. Stimulating-response of the semi-IPN, induced by abrupt changes of pH between 1 and 13, is discussed as well. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Considerable attention has been drawn to various stimulative responsive polymer gels, such as pH-sensitive hydrogels for low-mol wt and protein drug delivery systems (DDS),¹⁻³ temperature sensitive DDS for indomethacin,⁴ and a microcapsulate self-regulating release system for insulin, depending on glucose concentration in blood.⁵ Meanwhile, chitin, chitosan, and their derivatives have become useful polysaccharides in biomedical areas, for example, chitosan aspirin ester is stable in a dilute acidic medium, while aspirin can be detached from the ester in the case of alkaline medium and chitosan can be digested by bacteria in intestines. So chitosan is an effective carrier for the drug target organ. Moreover, chitosan can be used as a matrix for DDS as well. The oral tablet of indomethacin/chitosan exhibits delayed drug releasing behavior, due to salt formation between carboxy of indomethacin and amino of chitosan.⁶

Therefore, a pH-sensitive semi-IPN hydrogel of crosslinked chitosan (cr-CS) and polyether for a

carrier of DDS has been researched and developed, which is superior to chitosan gel in reversible responsibility of swelling and deswelling in acid and alkali, respectively, and in flexibility of the IPN gel.

This article deals with the synthesis and pH dependence of the swelling behavior of the semi-IPN. The effects of the concentration of chitosan acetic acid solution, the amount of crosslinking agent, and polyether on swelling were investigated as well.

EXPERIMENTAL

Materials

Chitosan was kindly provided by the Department of Applied Chemistry, Tianjin University. Its viscosity-average mol wt was 1.17×10^6 , calculated by the Mark-Houwink equation⁷: $[\eta] = K_m M^a$, where $K_m = 1.81 \times 10^{-3}$, $a = 0.93$, and the *N*-deacetylation degree was 63%, respectively.

Poly(oxypropylene glycol), polyether N330 was obtained from Jinling Petroleum Co. The average mol wt was 3000 ± 100 and the hydroxyl number was 56 ± 4 mg KOH/g. Polyether N330 has three functional hydroxyl groups. Glutaraldehyde and acetic acid (all chemical grade) were used without further purification.

* To whom correspondence should be addressed.

Synthesis of Chitosan/Polyether Semi-IPN

Chitosan was dissolved in 0.25 N acetic acid and was mixed with a certain amount of polyether N330 in a container. The appropriate quantity of glutaraldehyde solution was added under agitation. The mixture was maintained at 30°C for more than 6 h. The semi-IPN obtained was then washed with distilled water and was dried at 35°C, under reduced pressure of about 0.01 MPa, until weight constancy. The composition of the mixture used for synthesizing IPN is shown in Table I.

IR Spectra of Chitosan and Semi-IPN

The samples were first swollen in the distilled water or pH = 1 and were then ground to the suitable size of powder. After being dried completely under 35°C, the powder could be used for IR analysis. Infrared spectra of the powder samples were obtained with a Perkin-Elmer 983 spectrometer.

UV Spectra of Chitosan

The chitosan hydrochloric acid solution was prepared after chitosan was dissolved in hydrochloric acid (pH = 1) and filtered with an Ace Buchner tunnel with glass frit G2. The other solution was prepared after sample 7 was swollen in pH = 1 hydrochloric acid to equilibrium and the solution was filtered with the Ace Buchner tunnel with glass frit G2. The UV spectra of the above two solution samples were observed with a DMS-200 UV visible spectrophotometer.

Swelling of Semi-IPN

Swelling samples were cut into disks, 16.18 mm in diameter, 0.27 ± 0.02 mm in thickness. The semi-IPN samples were swollen in sodium acetate and potassium dihydrogen phosphate buffer solutions with the same ionic strength at various pH values at 25°C. The degree of swelling was calculated from the following expression:

$$(W_{\infty} - W_0)/W_0$$

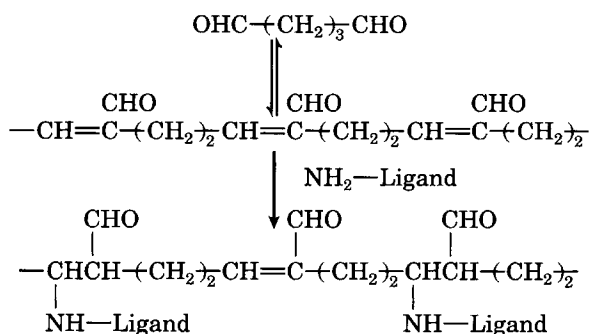
where W_{∞} and W_0 is the weight of sample for equilibrium swelling state and dry state, respectively.

The reversibility of swelling was also determined. Sample 7 was first swollen in a solution of pH = 1 and the degree of swelling was measured vs. time. After a certain time, the gel was transferred to a solution of pH = 13 and the swelling vs. time was determined again.

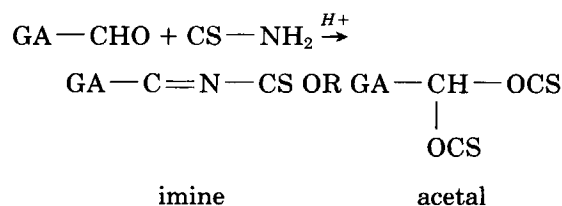
RESULTS AND DISCUSSION

Chitosan-Polyether Semi-Interpenetrating Polymer Network (Semi-IPN)

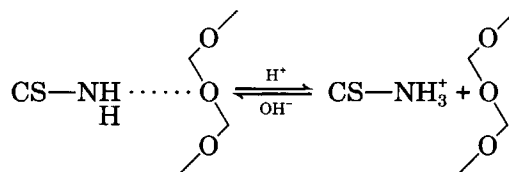
The crosslinking reaction between glutaraldehyde and chitosan is complex. C. M. Sturgen⁸ described that the formation of Schiff's base is not a straightforward reaction of aldehyde and amino groups and the linkage obtained is resistant to extremes of pH and temperature. The mechanism of this reaction is not well characterized.



In contrast to this reaction, Katsumaa Tsurugai⁹ used the Schiff's bases reaction and acetal reaction mechanisms to describe the crosslinking process, but this has not been proved.



In our case, the IR spectra of the semi-IPN was shown in Figure 1. However, it was confirmed that the semi-IPN is formed via crosslinking reaction of amino groups on chitosan and aldehyde groups on glutaraldehyde in the presence of polyether and intermolecular association through hydrogen bonding between amino hydrogen and oxygen from polyether.



In addition, the polyether in network can enhance the flexibility of semi-IPN.

Table I Details of Synthesis of Chitosan–Polyether Semi-IPN

Samples	Chitosan (CS)		Polyether (g)	Crosslinker GA mmol $\times 10^{-3}$	GA-CHO (mol)/CS-NH ₂ (mol) $\times 10^{-3}$	Polyether (g)/Chitosan (g)
	Concentration by Weight	Amino (mmol)				
1	1.22	1.71	0.67	5.65	6.61	1.67
2	1.95	1.71	0.67	5.65	6.61	1.67
3	3.17	3.43	0.67	3.79	2.21	0.70
4	3.17	3.43	0.67	7.51	4.38	0.70
5	1.59	1.71	0.45	5.65	6.61	0.94
6	1.59	1.71	0.88	5.65	6.61	2.37
7	3.90	3.43	0.45	3.79	2.21	0.47
8	3.90	1.71	0.45	7.51	8.78	0.94
9	3.90	1.71	0.00	7.51	8.78	0.00

IR Spectrum Analysis of Chitosan and Semi-IPN

The IR spectrum of chitosan [Fig. 1(A)] shows around 905 cm^{-1} and 1153 cm^{-1} peaks of assigned saccharide structure and a weaker amino characteristic peak at around 1590 cm^{-1} .¹⁰ It was noticed that, in spite of disappearance of the amide II band at 1554 cm^{-1} , there still were stronger absorption bands at 1649 cm^{-1} and 1322 cm^{-1} , which are characteristic of chitin and have been reported to be the amide I and III bands, respectively.¹¹ The sharp band at 1383 cm^{-1} has been assigned to the CH₃ symmetrical deformation mode.¹² These may be due to the fact that the degree of deacetylation of chitosan is not high and there are many amide groups present in the molecule.^{13,14}

Figure 1(B), obtained from sample 7, does not show a qualitative difference from the spectrum of chitosan, although it was insoluble in acid. This may be due to the low density of crosslinking in the semi-IPN.

Figure 1(C), obtained from sample 8, shows a significant peak at 1573 cm^{-1} , which can be attributed to the characteristic peak of C—N,^{11,15} forming from the crosslinking reaction between CS and GA. It cannot be confirmed, however, from bands at 1270 cm^{-1} and 1110 cm^{-1} , which are due to the hydroxyl group on chitosan,¹⁶ whether the reaction of alcoholic groups on CS with aldehyde groups on GA exists.

Complex Formation between Crosslinked Chitosan (cr-CS) and Polyether

The type of hydrogen bonding within the semi-IPN may be complicated, because there are several groups that can form a hydrogen bond in chitosan.¹¹ Fur-

thermore, polyether may form a new type of hydrogen bonding with these groups competitively. Here, however, the focus is on the evidence of the formation of hydrogen bonding between amino hydrogen and polyether oxygen by IR spectroscopic analysis. Figure 2 shows IR spectra of chitosan, polyether N330, two kinds of sample 8, one swollen in distilled water and the other in pH = 1. In the IR spectra of polyether N330 [Fig. 2(B)], the typical absorption peak, due to C—O stretching, was observed at 1112 cm^{-1} .¹⁷ The relative peak can be seen at 1101 cm^{-1} and 1107 cm^{-1} in the IR spectra of Figures 2(C) and (D), which is assigned to the sum of C—O stretching vibrations in the ether bond of polyether and the hydroxyl groups in chitosan, respectively.^{11,17} Figure 2(D) plots two absorption peaks at 1631 cm^{-1} and 1516 cm^{-1} , which can confirm the —NH₃⁺ formation when the semi-IPN was swollen in acid medium.¹¹

In comparison to Figure 2(C), the wavenumber of C—O stretching in Figure 2(D) shifts to 6 cm^{-1} higher frequency after the sample was swollen in pH = 1 to form —NH₃⁺, which may lead to the disassociation of the hydrogen bonding between amino and other groups in the network and an increase of the frequency of C—O stretching. Moreover, by comparing Figure 2(C) with Figure 2(D), it was also noticed from the 1260 cm^{-1} band, due to hydroxyl groups in chitosan,¹⁷ that there is no shift of the peak that resulted from the —NH₃⁺ formation, which can result in the disassociation of hydrogen bonding between amino and hydroxy. This can confirm that the hydrogen bonding between amino groups and hydroxyl groups does not exist and that the shift of the frequency of C—O stretching, due to the sum of polyether and chitosan, should be attributed to the disassociation of the hydrogen bond-

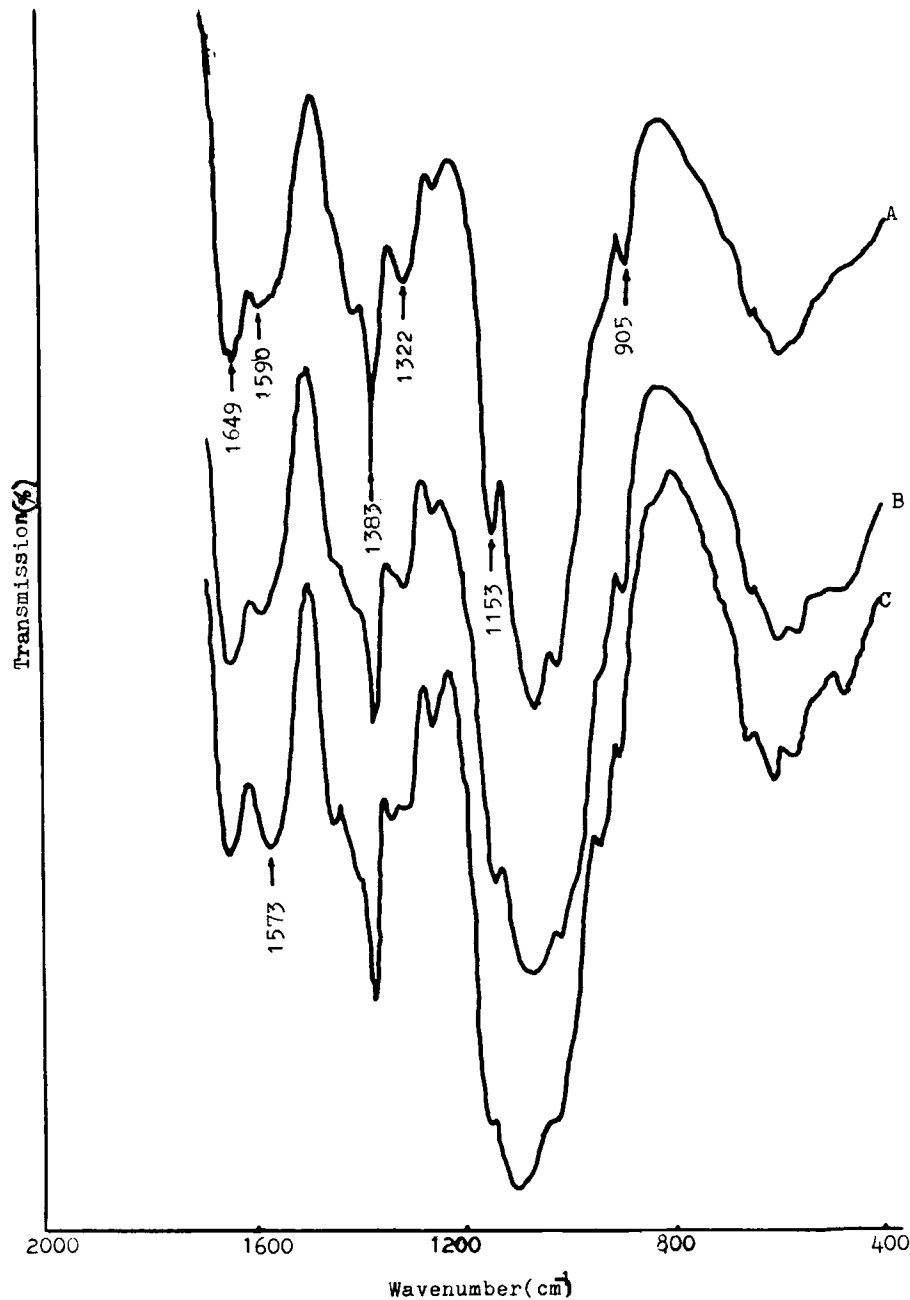


Figure 1 IR spectra of chitosan (A) and semi-IPN (B and C).

ing between amino hydrogen and polyether oxygen. On the other hand, there are more peaks relative to the polyether shown in Figure 2(D), for example, the peak at 1451 cm^{-1} , which is due to C—O symmetric deformation of methylene groups from polyether,¹⁷ was also in contrast with Figure 2(C). This can be explained by the fact that, because of the —NH_3^+ formation in $\text{pH} = 1$ and disassociation of hydrogen bonding between amino hydrogen and ox-

ygen in polyether, the complex collapses and the peak due to polyether appears in the IR spectra.

UV Spectrum Analysis

The UV spectrum of chitosan hydrochloric acid solution [Fig. 3(A)] shows a peak at 201.3 nm assigned to C—O in the amide of chitosan.^{18,19} There was also a peak at 201 nm in the UV spectra [Fig. 3(B)] of

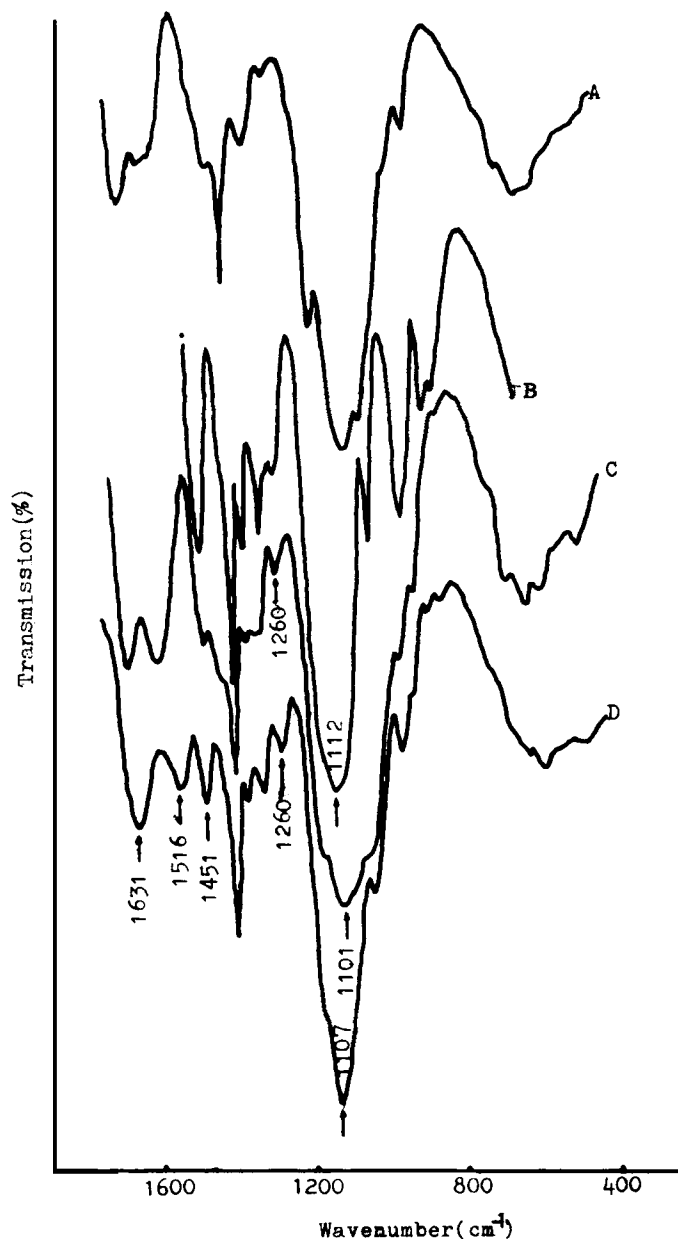


Figure 2 IR spectra of chitosan (A), polyether (B), chitosan-polyether semi-IPN (C), and IPN swollen in pH = 1 medium (D).

the solution left after sample 7 had been swollen in pH = 1 hydrochloric acid solution. This can confirm that when sample 7 was swollen in pH = 1, the uncrosslinked chitosan could dissolved out.

Swelling Behavior of Semi-IPN

The equilibrium degrees of swelling at different pH for six kinds of samples are shown in Figures 4–6, respectively. They all indicate that the degree of swelling drops sharply in high pH (pH \geq 7). This

can be explained by the fact that the network is dissociated in an acidic medium, which is due to amino groups on chitosan that ionized, but not as much as in an alkaline medium. On the other hand, Figures 4–6 also show that the equilibrium degree of swelling reaches a maximum at pH = 3.19 and a minimum at pH = 13, among all the pH values chosen.

Because of the Donnan equilibrium²⁰ between the IPN, which carries fixed $-\text{NH}_3^+$ in low pH and the exterior solution phase, the difference in the total

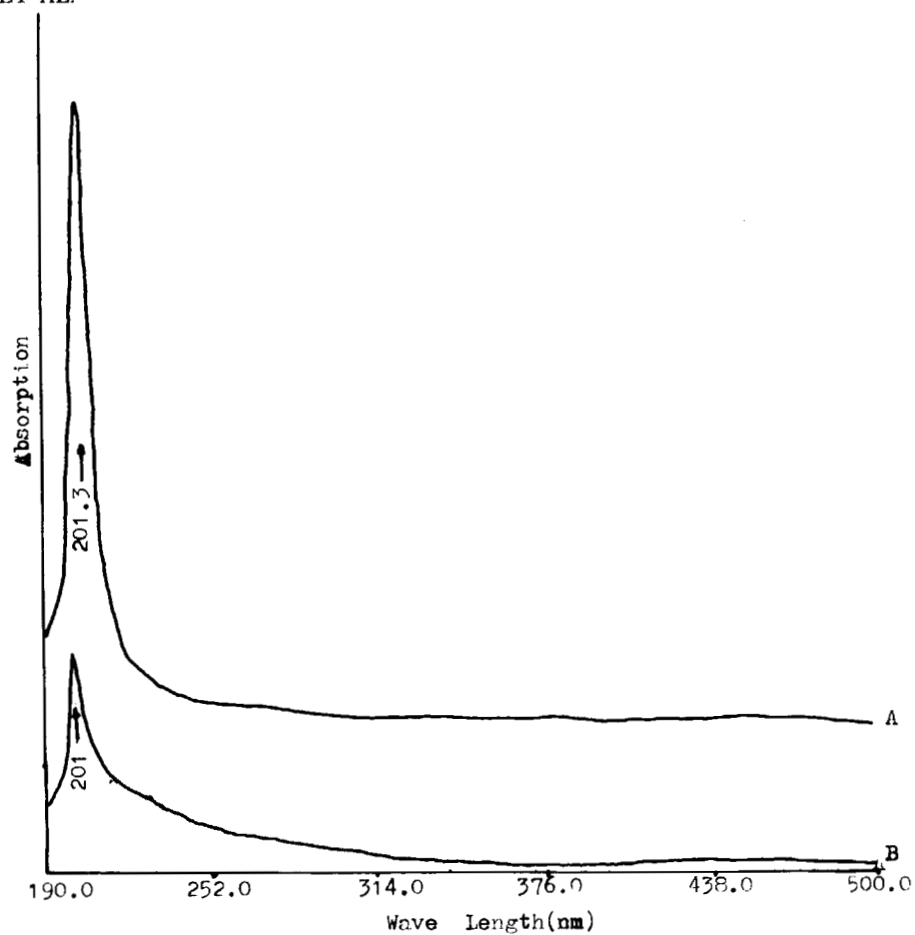


Figure 3 UV spectra of chitosan solution (A) and its swelling solution (B).

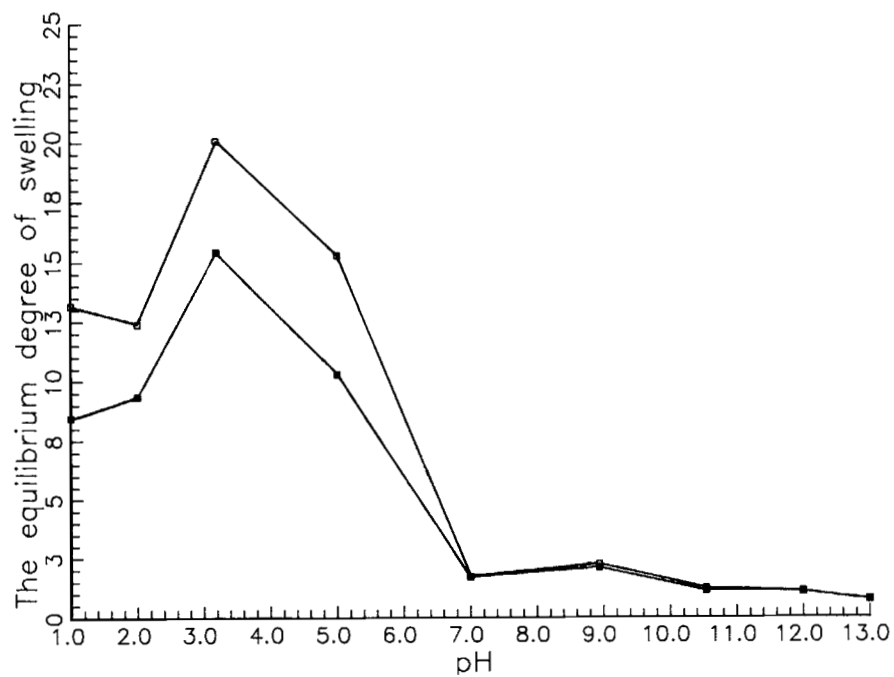


Figure 4 The equilibrium degree of swelling as a function of pH for the semi-IPN synthesized from different concentrations of chitosan solution. Sample 1 (□), sample 2 (■). For composition of the semi-IPN, see Table I.

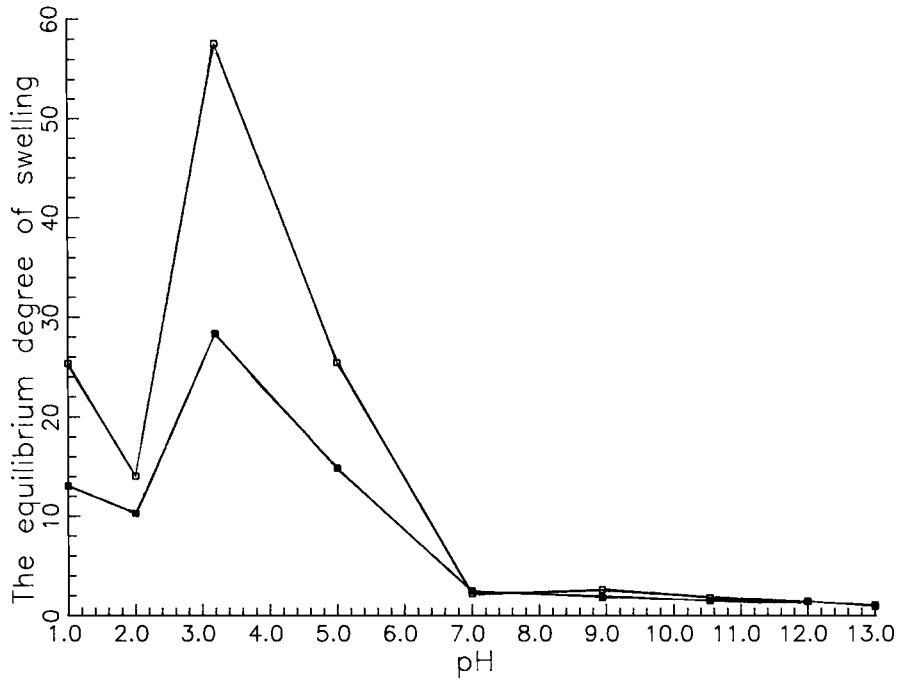


Figure 5 The equilibrium degree of swelling vs. pH for the semi-IPN, synthesized from different amounts of crosslinking agent. Sample 3 (□), sample 4 (■). For composition of the semi-IPN, see Table I.

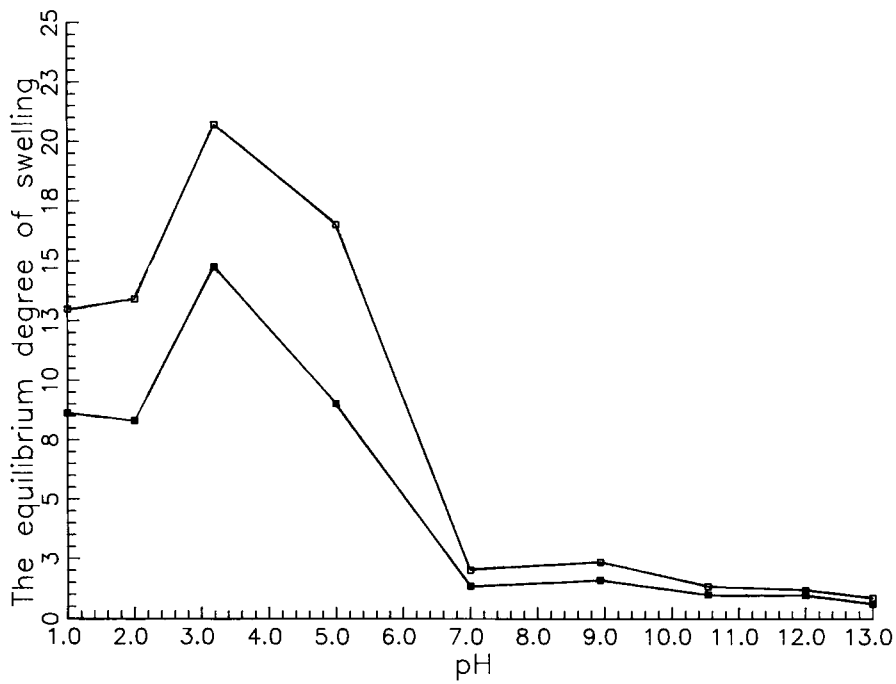


Figure 6 The pH dependence of the equilibrium degree of swelling for the semi-IPN containing different amounts of polyether N330. Sample 5 (□), sample 6 (■). For composition of the semi-IPN, see Table I.

ion concentration between the two phases becomes maximum at $\text{pH} = 3.19$ and decreases with further increase in pH , which, in turn, induces deswelling in the region of pH below 3.19.

Factors Influencing the Swelling of Semi-IPN

In order to enable us to design a desired IPN, we studied the factors affecting the pH -dependent swelling of the semi-IPN.

Three factors were considered, one being the concentration of the chitosan acetic acid solution. Figure 4 shows the relationship between the equilibrium degree of swelling and pH for the semi-IPN synthesized from different concentrations of chitosan acetic acid solution. As the concentration decreases, the equilibrium degree of swelling increases and the hydrogel becomes more pH -sensitive. At high pH ($\text{pH} \geq 7$), where the hydrogen bonding forms, the equilibrium degree of swelling is almost the same for both semi-IPNs. This can be explained by the fact that in the dilute solution, the intermolecular crosslinking reaction with GA decreases, while the intramolecular crosslinking reaction increases. Moreover, the crosslinking reactions involve the acetal reaction of CS with GA, the schiff's base reaction, and the reaction between the GA themselves. The lower the concentration, the more the

reaction in the latter two cases.⁹ With the decrease of crosslink density of the semi-IPN, its equilibrium degree of swelling increases and the semi-IPN becomes more pH -sensitive.

Figure 5 shows the dependence of the equilibrium degree of swelling on the amount of crosslinker GA. As the amount of GA increases, the crosslink density of semi-IPN increases and the equilibrium degree of swelling becomes more restricted. But, in high pH ($\text{pH} \geq 7$), where amino groups are not ionized and hydrogen bonding associates, there is almost no effect of the amount of crosslinking agent on its equilibrium degree of swelling and pH sensitivity.

Figure 6 shows that, as semi-IPN contains a greater amount of polyether, the equilibrium degree of swelling decreases and IPN becomes less sensitive. This is due to the intensification of hydrogen bonding between chitosan and polyether in semi-IPN. It was noticed that in high pH ($\text{pH} \geq 7$), the equilibrium degree of swelling for a semi-IPN composed of more polyether is still smaller, which is different from the two former cases. The reason for this may be that the amount of polyether in semi-IPN affects the intensity of hydrogen bonding in the network, while the concentration of chitosan solution and the amount of crosslinker affect the crosslink density of chitosan and have little effect on hydrogen bonding. Therefore, only at low pH ($\text{pH} < 7$), the degree of swelling is dependent on crosslinking density.

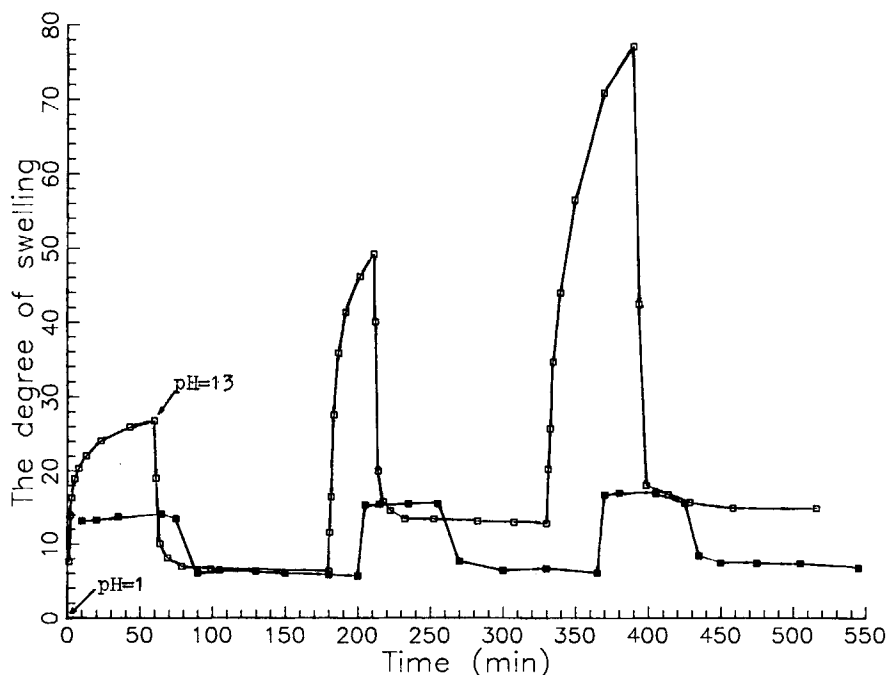


Figure 7 The degree of swelling as a function of time for sample 8 (■), sample 9 (□) after repeated abrupt changes of pH between 1 and 13. For composition, see Table I.

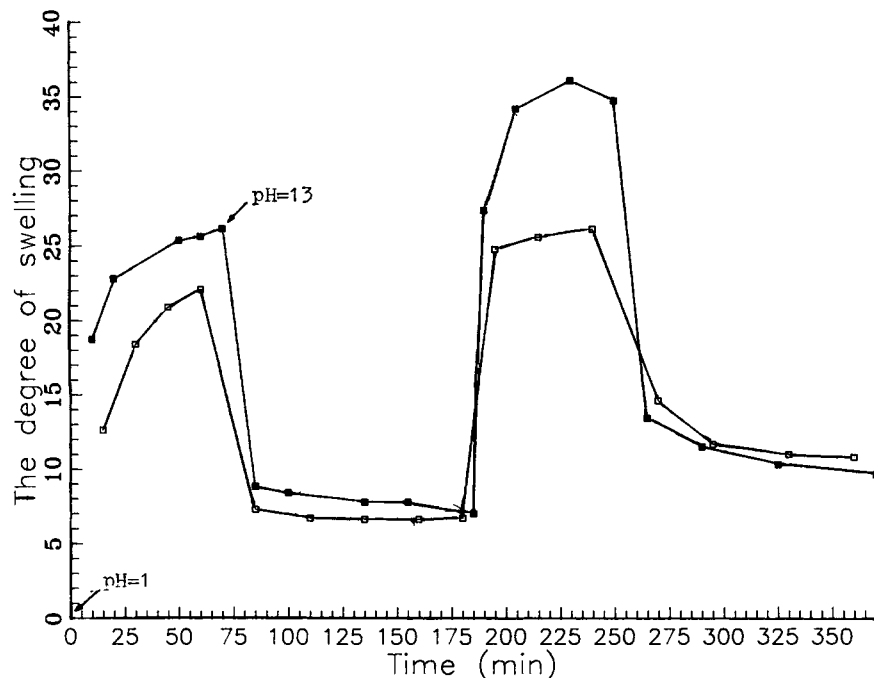


Figure 8 The degree of swelling against time for two kinds of sample 7 [(□) not swollen in pH = 1; (■) swollen in pH = 1] after repeated abrupt changes of pH between 1 and 13. For composition, see Table I.

Reversibility of pH Dependent Swelling

The contrasting reversibilities of the swelling of samples 8 and 9 are shown in Figure 7, where sample

9 is a control of 100% chitosan crosslinked with GA according to the composition of sample 8 without polyether. It was observed that the degree of swelling of the 100% chitosan gel was higher than that of

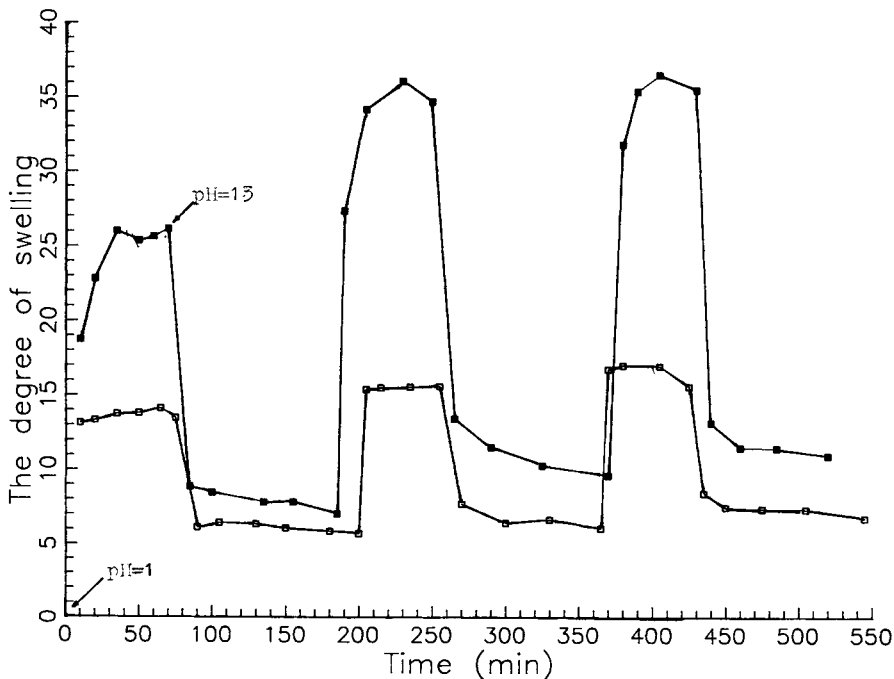


Figure 9 The degree of swelling as a function of time for sample 7 (■) and sample 8 (□) after abrupt changes of pH between 1 and 13. For composition, see Table I.

sample 8 after the same time interval, but that the reversibility of swelling of the gel was not, as well as the swelling and reversibility of sample 8, which was due to the semi-IPN structure providing crosslinks at low pH, while the hydrogen bonds within the network acted as physical crosslinks to suppress the swelling of the semi-IPN at high pH.

The reversibilities of swelling of samples 7 and 8 are shown in Figures 8 and 9. In Figure 8, the sample (\square) was prepared according to what has been mentioned above. The sample (\blacksquare) was first swollen to

equilibrium in pH = 1 at 25°C and then was washed in distilled water and was dried under the condition that has been mentioned above until weight constancy before use. For Figure 9, the samples were both first swollen to equilibrium at 25°C and then washed in distilled water and dried.

Figures 8 and 9 reveal that the swelling is reversible. In pH = 1, amino groups on chitosan are ionized, which leads to the dissociation of hydrogen bonding in the network, whereas in pH = 13, ionized amino groups can revert to amino groups, which re-

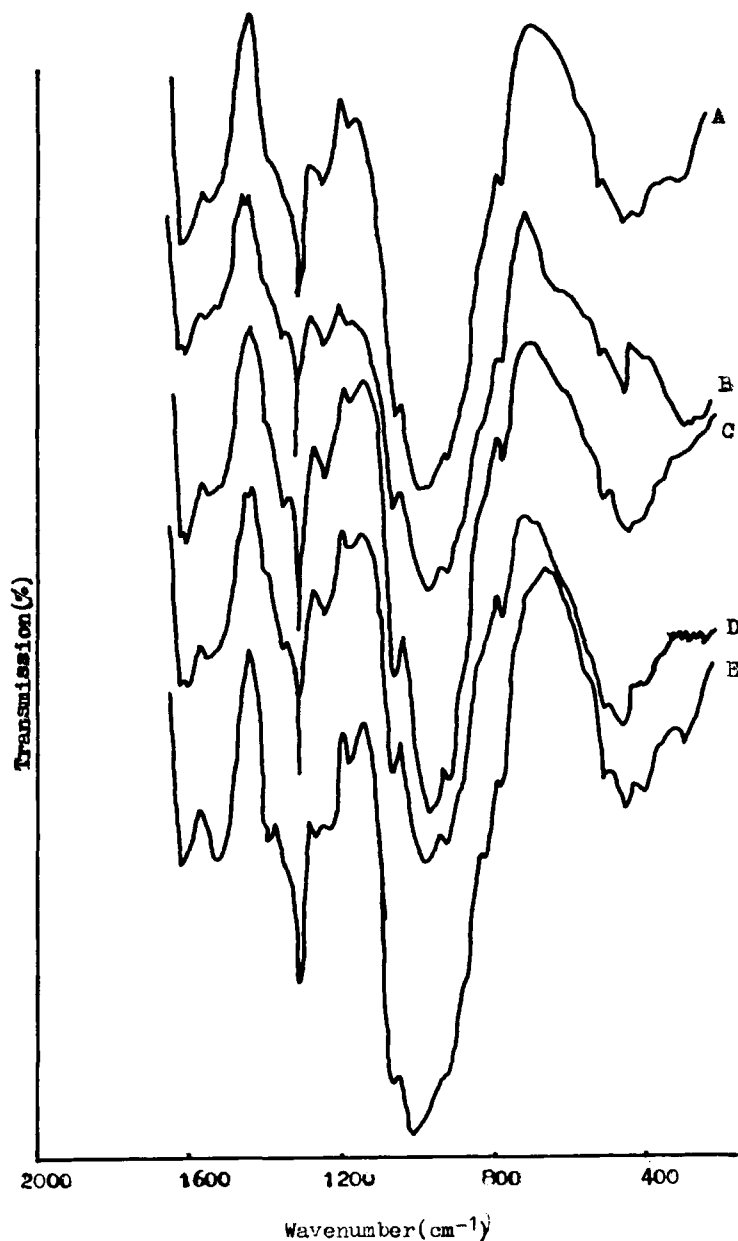


Figure 10 IR spectra of chitosan (C), chitosan/polyether semi-IPN (A, E), and their corresponding swollen IPN (B, D).

sults in the association of hydrogen bonding in the network. The process is reversible.

Figure 8 shows that the swelling and deswelling rate of the sample, first swollen to equilibrium in pH = 1, is faster than that of the sample that was not swollen in pH = 1. This may be attributed to the fact that when the sample was swollen in pH = 1, the uncrosslinked chitosan may have dissolved in the solution, which weakened the intensity of hydrogen bonding and enlarged the molecular pores in the network.

Figure 9 indicates that, because of lower crosslink density and weaker hydrogen bonding within the network, the sample 7 becomes more sensitive and the degree of swelling is higher than that of sample

8, both in pH = 1 and pH = 13, which is compatible with the above results.

IR Analysis of Samples 7 and 8, Swollen in pH = 1 and 13, Alternatively

After the reversibilities of the swelling of samples 7 and 8 were investigated, the samples were at last washed with distilled water, ground to powder, and dried at 35°C completely for IR spectra, which are shown in Figure 10. The IR spectra became similar to the IR spectra of chitosan, while different from the spectra of initiative samples 7 and 8, which can be explained by the dissolution of polyether and the change in imine bonds within the semi-IPN, which

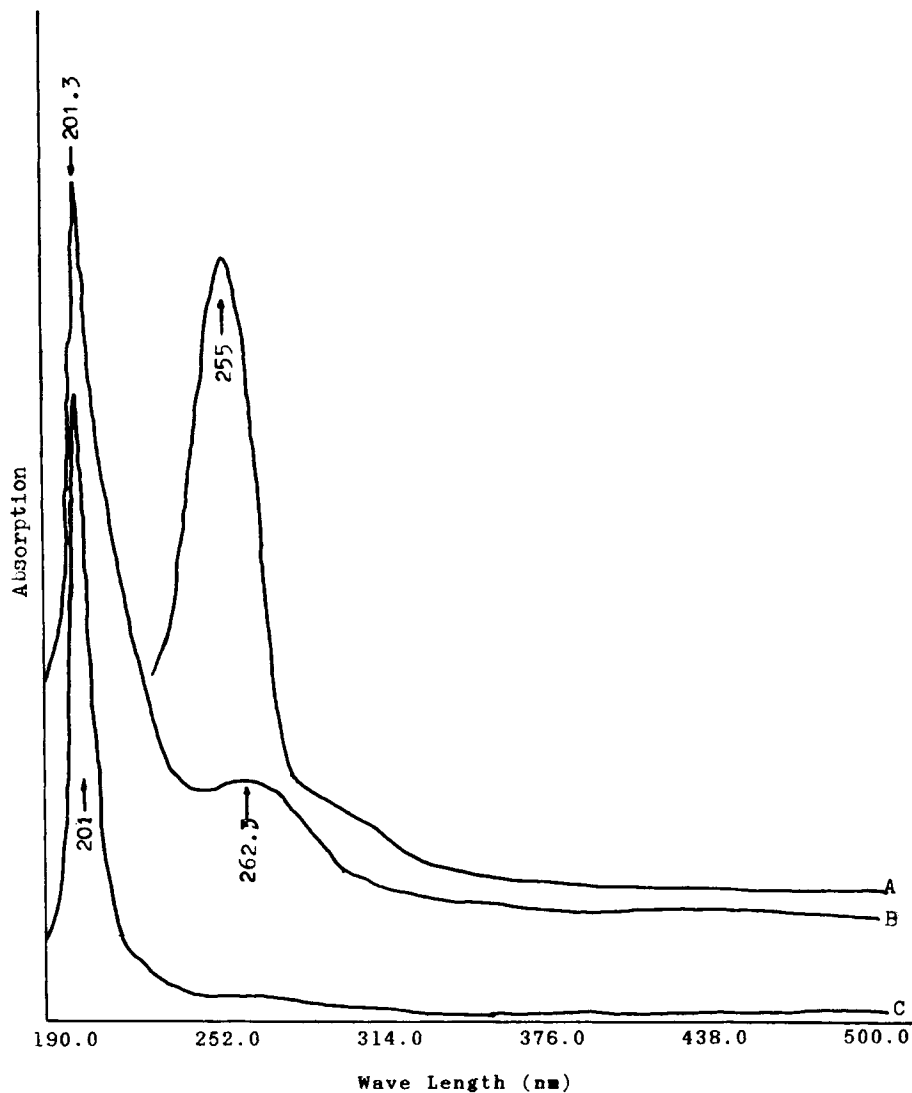


Figure 11 The UV spectra of chitosan/polyether IPN dissolved in pH = 1 hydrochloric acid for six days after acidic-alkaline exchange (B), vs. spectra of glutaraldehyde (A) and chitosan (C).

may be due to the cleavage of the imine bonds to result in amino and aldehyde groups.²¹



To test this assumption, we put a small amount of sample 7 in pH = 1 after the swelling reversibility experiment. It was found that after six days, the sample was dissolved almost completely. The solution left was filtered with the Ace Buchner funnel, with glass frit G2, and was used for estimating UV spectra. Two peaks were observed in Figure 11(B). The corresponding peaks were also noticed in the UV spectra of chitosan hydrochloric acid solution [Fig. 11(A)], and glutaraldehyde-hydrochloric acid solution [Fig. 11(C)], respectively. From these results, it was confirmed to a certain degree that, in our experiment, the crosslinking reaction between glutaraldehyde and chitosan may follow the Schiff's bases reaction mechanism. The effect of pH on the swelling behavior of the semi-IPN in the long period will be investigated further. It was observed, however, that the samples of chitosan/polyether IPN gels could be swollen to equilibrium in pH = 1 and the UV spectra for the solution left [Fig. 2(B)] did not show any peak relative to glutaraldehyde, as is the case in Figure 11(B), which indicated the gels had a certain stability.

CONCLUSIONS

A novel, pH-sensitive, semi-interpenetrating polymer network (semi-IPN) has been synthesized via crosslinking chitosan with glutaraldehyde using Schiff's bases reaction mechanism and interpenetrating polyether to form hydrogen bonding between the amino hydrogen in chitosan and polyether oxygen. Reaction parameters, for example, the concentration of chitosan acetic acid solution, the amount of crosslinking agent, and the amount of polyether, have obvious effects on the swelling behavior of the semi-IPN. The present semi-IPN exhibits reversible response to pH changes, because the formation and disassociation of hydrogen bonding can be controlled reversibly within the network.

REFERENCES

1. R. A. Siegel, M. Falamrizian, B. A. Firestone, and B. C. Moxley, *J. Contr. Rel.*, **8**, 179 (1988).
2. J. H. Kou, G. L. Amidon, and P. I. Lee, *Pharm. Res.*, **5**, 592 (1988).
3. K. Ishihara, M. Kobayashi, N. Isimaru, and I. Shinohara, *Polym. J.*, **16**, 625 (1984).
4. R. Yoshida, K. Sakai, T. Okano, and Y. Sakurai, *Jpn. Arti. Org.*, **20**, 465 (1991).
5. K. Makino, Eric J. Mack, T. Okano, and S. W. Kim, *J. Contr. Rel.*, **12**, 235 (1990).
6. S. Hirano, *Jpn. J. Med. Instru.*, **59**, 134 (1989).
7. G. A. F. Roberts and J. G. Domszy, *Int. J. Biol. Macromol.*, **4**, 374 (1982).
8. C. M. Sturgen, *The Synthesis of Polysaccharide Derivatives*, in "Carbohydrate Chemistry" John F. Kennedy, Ed., Clarendon, Oxford, 1988, p. 560.
9. K. Tsurugai, *Sen-i Kako*, **42**(8), 364 (1990).
10. A. Yoshikawa and K. Kaneko, *Sen-i Kako*, **42**(8), 351 (1990).
11. F. G. Pearson, R. H. Marchessault, and C. Y. Liang, *J. Polym. Sci.*, **43**, 101 (1960).
12. T. Sannan, K. Kurita, K. Ogura, and Y. Iwakura, *Polym.*, **19**, 458 (1978).
13. T. Sannan, K. Kurita, and Y. Iwakura, *Makomol. Chem.*, **177**, 3598 (1976).
14. M. Miya, R. I. Wamoto, K. Ohta, and S. Mima, *Kobunshi Ronbunshu*, **42**(3), 181 (1985).
15. L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Chapman and Hall, London, New York, 1980, p. 52.
16. K. Kaifu, N. Nishi, T. Komai, S. Tokura, and O. Somorin, *Polym. J.*, **13**(3), 241 (1981).
17. D. W. Brown, A. J. Floyd, and M. Sainbury, *Organic Spectroscopy*, Wiley, New York, 1988, p. 24-53.
18. S. Aiba, *Int. J. Biol. Macromol.*, **8**, 173 (1986).
19. T. N. Jankowska, K. Gorczynska, A. Michalik, and E. Wieteska, *Analytical Visible and Ultraviolet Spectrometry*, Elsevier, New York, 1986, p. 534-544.
20. F. G. Donnan, *Z. Elektrochem*, **17**, 572 (1991); S. Nishi and T. Kotaka, *Rep. Progr. Polym. Phys. Jpn.*, **26**, 215 (1983).
21. S. H. Pine, *Organic Chemistry* (Fifth Edition), McGraw-Hill, New York, 1987, p. 258.

Received February 11, 1992

Accepted July 7, 1992