Structural Change and Swelling Mechanism of pH-Sensitive Hydrogels Based on Chitosan and D,L-Lactic Acid

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ABSTRACT: Graft copolymerization of D,L-lactic acid (LA) onto chitosan (CS) was attempted without using a catalyst. pH-sensitive hydrogels were obtained which are based on two different components: a natural polymer and a synthetic polymer. These polyester substituents provide the basis for hydrophobic interactions that contribute to the formation of hydrogels. The swelling mechanisms in enzyme-free simulated gastric fluid (SGF, pH 2.2) or simulated intestinal fluid (SIF, pH 7.4) at 37°C were investigated. Meanwhile, structural changes of the graft copolymers in the different pH buffers were studied by FTIR, and these are discussed together with the swelling mechanisms. The effect of pH on the water uptake of hydrogel was investigated by using McIlvaine buffer with the same ionic strength. The morphological change of hydrogels in different aqueous solutions is investigated by scanning electron microscopy (SEM). © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 3186–3192, 1999

Key words: chitosan; D,L-lactic acid; hydrogel; pH-sensitivity; structural change; swelling mechanism

INTRODUCTION

Hydrogels are three-dimensional networks of hydrophilic polymers held together by crosslinks of covalent bonds or ionic bonds and secondary forces in the form of hydrogen bonds or hydrophobic interactions.[1,2] They are used for a variety of applications such as contact lenses, wound dressings, absorbents, monolithic drug-delivery systems, membrane materials, and chromatographic packing materials. Hydrogels containing ionic and/or hydrophobic moieties show a sudden or gradual change in their dynamic and equilibrium swelling properties as the external environmental conditions are changed. These environmental conditions include pH, ionic strength, solvent composition, temperature, pressure, electromagnetic radiation and/or photoelectric stimulus.[3–5]

Recently, attention has been focused on employing natural polymers such as cellulose,[6] starch,[7] gelatin,[8,9] and chitosan[10,11] to compose hydrogels with a specific response to a biological environment. Chitosan derived by *N*-deacetylation of chitin is a biodegradable and biocompatible polymer. It has been shown recently that partially deacetylated chitin can be hydrolyzed by enzymes.[12,13] The enzymatic digestibility depends on the degree of *N*-deacetylation and the method of preparation. Moreover, chitosan itself has some pharmaceutical activities such as antacid, antibacterial, hypocholesterolemic activity, and suppression of growth of tumor cells. These excellent properties have attracted many investigators to work in this field.[14,15]

We prepared hydrogels based on grafting chitosan (CS) with D,L-lactic acid (LA), and investigated their structure, pH-sensitivity, and swelling properties.[16] In this article, we investigated the sample prepared with an LA/CS feed ratio of 2 in regard to structural changes and swelling

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mechanism in various pH buffers. FTIR was used to characterize the structural change of the hydrogel in different pH solutions. Moreover, a mechanism is suggested based upon the changes observed by FTIR spectroscopy.

EXPERIMENTAL

Materials

CS (M_w = 70,000) from Fluka (Buchs, Switzerland) and LA (99%) from Lancaster (Lancashire, England) were used for the preparation of graft copolymer. The degree of deacetylation (DD = 88%) of CS was determined by IR spectroscopy. Acetic acid (100%), methanol, and chloroform from Merck (Darmstadt, Germany) were used for buffers and extraction. All chemicals were used as delivered, without further purification. Deionized water (Milli Q) was used in all aqueous solutions.

Preparation of CS Hydrogels

The synthesis of the hydrogel was carried out by direct grafting of LA onto CS in the absence of catalysts according to the method already reported.[16] In short, CS powder (1 g) dispersed in water was dissolved by adding LA (2 g). The solutions were poured onto a Teflon dish with a 15.0-cm diameter and dried at 80°C, followed by extraction with methanol in a Soxhlet apparatus for 24 h.

Swelling of CS Hydrogels

To determine the effect of pH on hydrogel swelling, McIlvaine buffer[17] with the same ionic strength, I = 0.5M, and various pH's was used in this work [citric acid/Na_2HPO_4 (pH 2.2–7.4), and 0.5M NaOH/KCl (pH 12.0)]. The samples (approximately 0.05 g) were immersed into 200 mL of the buffer solution, and the weight of the solution absorbed by the gels was calculated from the weights of the gel before and after vacuum-drying at room temperature. The specific solution content and weight increase or decrease of the hydrogels were expressed by the following equations:

Specific solution content (water uptake) (W)

$$= (W_s - W_d)/W_d$$

Weight increase/decrease (%) = $(W_d - W_0)/W_0$

where W_0, W_d, and W_s are the weights of the samples dried after methanol extraction (W_0), dried after exposure to buffer solutions (W_d), and in the swollen states (W_s).

Kinetic swelling experiments were conducted in enzyme-free simulated gastric fluid (SGF, pH 2.2, I = 0.1M) or simulated intestinal fluid (SIF, pH 7.4, I = 0.1M) at 37°C. SGF and SIF were prepared from acetic acid/KCl buffer and a phosphate buffer solution according to the literature.[18] The specific solution content of the samples was also calculated according to the above equation.

Fourier-transform infrared (FTIR) spectra were recorded on a Perkin–Elmer FTIR 1725X spectrometer with thin films. In an attempt to investigate the structural changes of the samples related to the solution pH, the films, which had been swollen for predetermined time periods, were dried directly from buffer solution to constant weight in vacuum at 25°C.

Morphological changes were studied using a scanning electron microscope (SEM) JEOL JSM-5400. Samples were mounted onto metal stubs and sputter-coated with gold–palladium (Denton Vacuum Desc II).

RESULTS AND DISCUSSION

IR Spectra Analysis

To investigate the pH response of the CS hydrogels, the structural change of the gel that had been exposed to different buffer solutions was measured by FTIR after vacuum drying. The main band positions, types, and assignments of the sample are listed in Table I. The IR spectrum of CS [Fig. 1(A)] shows peaks assigned to the saccharide structure at 897 and 1153 cm^{-1} and a strong amino characteristic peak at around 1591 cm^{-1}. The absorption bands at 1655 and 1325 cm^{-1} are characteristic of N-acetylated chitin and were reported to be the amide I and III bands, respectively. The sharp band at 1377 cm^{-1} was assigned to the CH$_3$ symmetrical deformation mode. The methanol extracted sample before exposure to any buffer is shown in Figure 1(B). The peak at 1735 cm^{-1} could be assigned to the ester group in the PLA side chains. Two peaks at 1655 and 1597 cm^{-1} corresponding to N-acetylated chitin units and amino groups of chitosan units still appeared. Increase of the amide I peak at 1655 cm^{-1} indicated increase of N-acetylation by

Table I   Main Infrared Band Assignments of CS and Graft Copolymer

Sample State	Band Position (cm^{-1})	Band Type	Assignment
CS	1655	Medium peak	Amide I (C=O)
	1591	Strong peak	C—N absorption or NH_2 deformation
	1420	Medium peak	OH and CH deformation (ring)
	1377	Strong peak	CH_3 sym deformation (bend)
	1325	Weak peak	OH and CH deformation (ring)
	1263	Weak peak	CH wag (ring)
Graft copolymer CS/LA = $\frac{1}{2}$	1735	Weak peak	Carbonyl group (C=O) in side chains
	1655	Strong peak	Amide link between side chain and CS, amide I
	1597	Strong peak	C—N absorption or NH_2 deformation
	1454	Weak shoulder	CH_3 deformation of side chains
Dry sample after 0.5 h pH 7.4 buffer	1735	Strong peak	Ester group (C=O) in side chains
	1651	Strong peak	Amide I (C=O) and amide linkage
	1597	Strong peak	C—N absorption or NH_2 deformation
	1574	Medium shoulder	NH_3^+ deformation
Dry sample after 0.5 h in pH 2.2 buffer	1735	Strong peak	C=O in salt links and side chains
	1625	Strong peak	NH_3^+ deformation
	1521	Strong peak	NH_3^+ deformation

reacting CS with LA, while the peak of the remaining amino groups that shifted from 1591 to 1597 cm^{-1} indicated the hydrogen bonding between carbonyl groups of side chains and amino groups of CS. No peak corresponding to ether groups from reaction between the hydroxyl groups was found in the IR spectrum, so it can be supposed that chemical crosslinking does not occur at the relatively mild graft copolymerization conditions used.

After 0.5 h in pH 7.4 buffer, the two peaks that have similar positions in the spectrum of the original sample still existed at 1651 and 1597 cm^{-1} [Fig. 1(C)]. A new shoulder that could be assigned to the deformation of NH_3^+ groups in CS appeared at 1574 cm^{-1}. The residual —NH_2 group of the grafted copolymers would be partly ionized in the pH 7.4 buffer. Since the pK_b for CS is reported to be in the range of 6.5–6.7 depending on the degree of acetylation, a major part of the amino groups would not be protonated in the pH 7.4 buffer. After 0.5 h in a pH 2.2 buffer, two new and strong peaks (1625 and 1521 cm^{-1}), which are related to the deformation of NH_3^+ groups in CS, appeared in the spectrum [Fig. 1(D)]. The peak of the carbonyl groups at 1735 cm^{-1} significantly

increases, which indicates that the gel had formed an ionic complex with the acid in the buffer. The carbonyl group of the acid has a strong absorption in the IR spectrum. These results indicate that the structure of the graft copolymers would change according to the pH value of the buffer solution as shown in Scheme 1.

Effect of pH on Hydrogels Swelling

The synthesized graft copolymers still contain unreacted amino groups of CS, which would be ionized by protonation in acid solutions. This ionization is reversible and leads to hydrogels that will respond to the environmental pH. Graft copolymers after methanol extraction were allowed to imbibe buffer solutions with pH varying from 2.2 to 12.0. To separate the influence of pH, the ionic strength of the buffers were kept constant ($I = 0.5M$), since it will largely affect the swellability of hydrogels as mentioned in a previous article.[16] As seen in Figure 2, the hydrogels show a lower specific solution content at basic pH as compared to acidic pH. It is well known that a high concentration of charged ionic groups in the gel increases swelling due to osmosis and charge re-
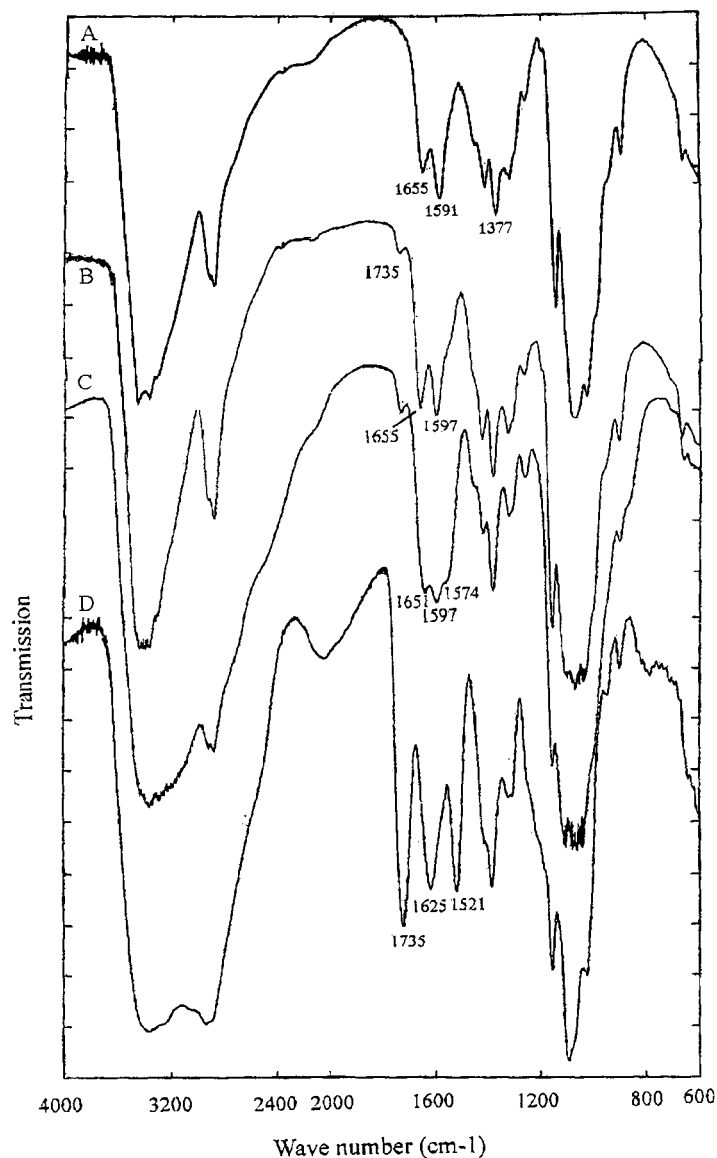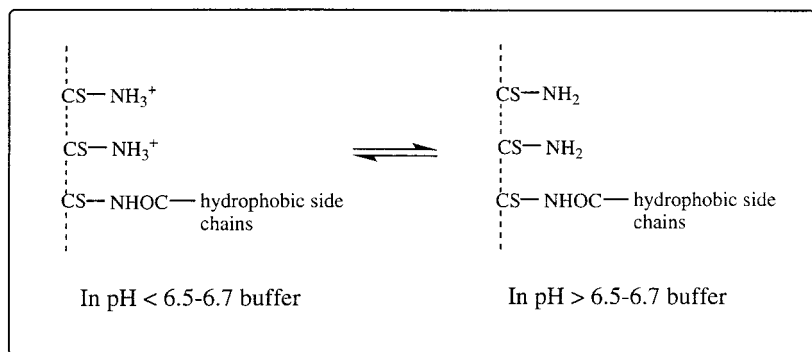
Figure 1 FTIR spectra of (A) chitosan, (B) hydrogel in dry state, (C) swollen in pH 7.4 buffer and dried, and (D) swollen in pH 2.2 buffer and dried.

pulsion. Thus, when the degree of ionization of gel-bound groups is decreased, swelling decreases. At high pH, since the aggregation and intermolecular interactions and the protonation of amino groups have already reached their maximum (pH > pK_b), the swellability of the hydrogel becomes unchanged when the buffer pH is higher than 7. This pH-sensitive behavior is typical of ionic hydrogels.[19]

Swelling Mechanism Studies

Figure 3 presents the specific solution content of methanol-extracted samples during the first 10 min of swelling. In the pH 7.4 buffer, the gel swelled fast during the first 5 min and then leveled off gradually at a value of 1.4. As compared to this, the gel has higher swellability in acidic buffer. A specific solution content of 8.5 was obtained in the pH 2.2 buffer. After the swelling tests in the respective buffers, the gels were completely dried in a vacuum and weighed. As shown in Figure 4, the weight of the dried gels decreased 3% in the pH 7.4 buffer, while they first decreased by 2% and then increased by 15% in the pH 2.2 buffer. During the swelling process, water-soluble impurities (about 3%) inside the hydrogel could

$CS—NH_3^+$ $CS—NH_2$

$CS—NH_3^+$ $CS—NH_2$

$CS—NHOC—$ hydrophobic side $CS—NHOC—$ hydrophobic side
                          chains   chains

In pH < 6.5-6.7 buffer   In pH > 6.5-6.7 buffer

Scheme 1   Structure change of CS graft copolymer in acidic and alkaline buffers.

be dissolved in the pH 7.4 buffer. Since the swelling process of gels involves the ionization of amino groups by the acid in the pH 2.2 buffer (Scheme 1), the acid would be attached to the gels by the ionic bonds. Therefore, the weight of the gels increased in the pH 2.2 buffer. The initial weight decrease of 2% at pH 2.2 is again related to water-soluble impurities being simultaneously compensated by the weight gain from the acid.

The swellability of the methanol-extracted sample within 7 h is shown in Figure 5. In the pH 2.2 buffer, the hydrogel absorbs the buffer solution fast during the first 0.5 h; then, the value shows only a small increase until it reaches equilibrium after about 1 h. As compared to the swelling in the pH 2.2 buffer, the process is even faster in the pH 7.4 buffer although at a much lower level of specific solution content. The equilibrium value of 2.1 could be obtained in the first 0.5 h. These results would further confirm the conclusion from the data of FTIR about structural changes of the hydrogels. The study about swell-

ing kinetics will be reported in detail in next article.

Normally, the swelling behavior of polymeric gels is dependent on the relative magnitudes of water diffusion and polymer-relaxation times. But, in our case, the swelling mechanisms become more complex because of the ionization of CS amino groups in the acidic buffer. An increase in the degree of ionization contributes to the electrostatic repulsion between adjacent ionized amino groups, leading to chain expansion, which finally affects macromolecular chain relaxation. The swelling of a chitosan hydrogel in the pH 2.2 buffer involves mainly water diffusion, the ionization of amino groups, and a polymer-relaxation process. Initially, as the solution penetrates from the polymer surface, the amino groups are ionized, leading to a partial dissociation of the hydrophobic bonds between side chains. Thus, a sharp moving boundary between the unpenetrated polymer region and the swollen gel phase is formed. As the diffusion process continues, the
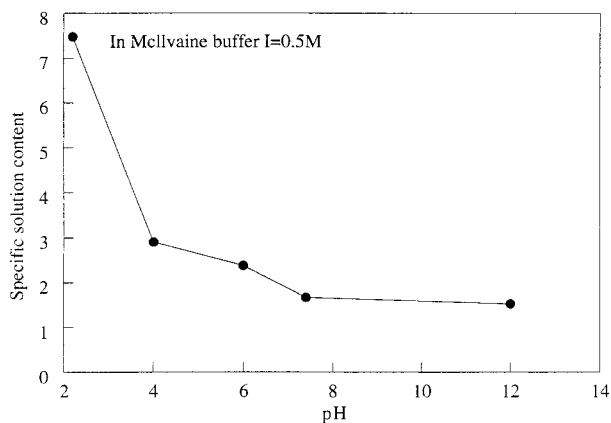

Figure 2   Specific solution content of hydrogel (LA/CS = 2) as a function of pH.
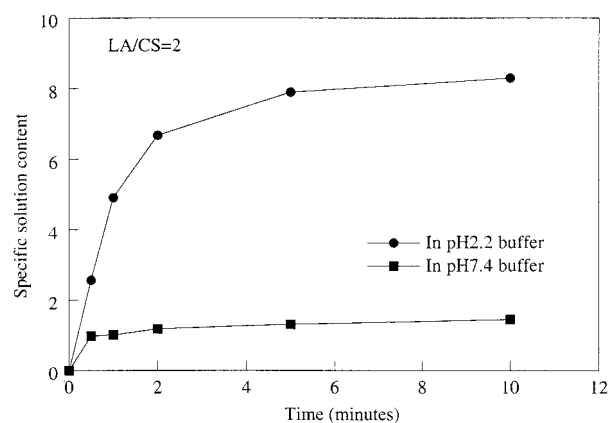

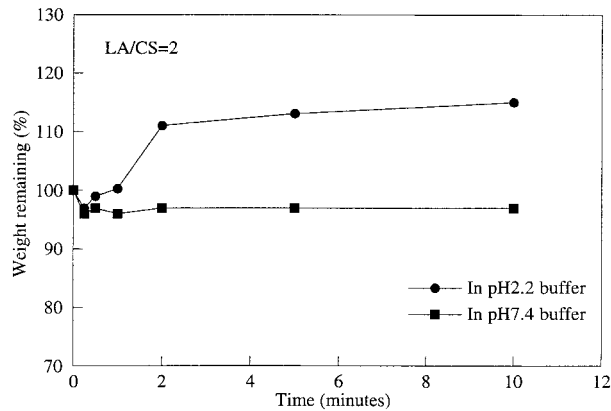**Figure 3**   Specific solution content of hydrogel as function of time in pH 2.2 and 7.4 buffers.

Figure 4 Weight loss of hydrogel as function of time in pH 2.2 and 7.4 buffers.

front boundary moves forward until the entire sample has reached its equilibrium swelling. Meanwhile, the mechanical relaxation of the swollen gel and the redistribution of mobile ions occurs between the gel interior and external solution. In the pH 7.4 buffer, the swelling of the sample involves mainly water diffusion and polymer relaxation. The swelling process is much faster and needs less time to reach its equilibrium.

Reversibility and Morphological Change in Different pH Buffer

Figure 6 shows the reversibility of swelling of the methanol-extracted hydrogel between the pH 2.2 and 7.4 buffers. The film was brought into a swelling equilibrium at pH 7.4 for 3 h and then transferred to an acetic acid solution at pH 2.2 so that an abrupt swelling was ensured. After obtaining
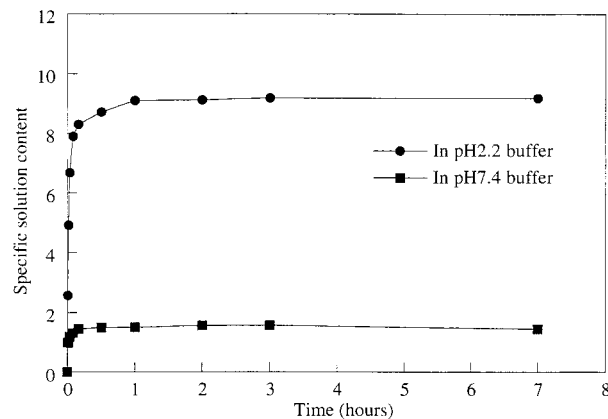

Figure 5 Specific solution content of hydrogel as function of time in pH 2.2 and 7.4 buffers.
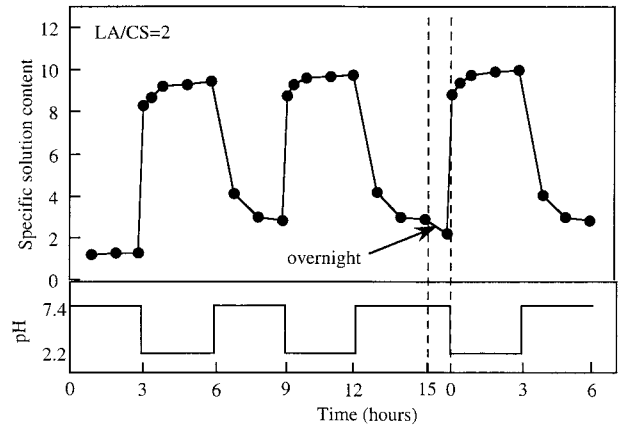

Figure 6 Specific solution content of hydrogel (LA/CS = 2) as a function of time under repeated abrupt change of pH between 7.4 and 2.2.

swelling equilibrium, the sample was placed back into the pH 7.4 buffer. The exchange of buffers was repeated at 3 h intervals and the specific solution content was measured as a function of time. The results demonstrated that the sample changes its ability to absorb the solution when the environmental pH is altered, and the time for swelling is much shorter than is the deswelling of the hydrogel. The reversibility toward the pH changes was retained even after prolonged treatment (overnight). This swelling transition is appealing since it implies that water permeability, which should increase with sample hydration, can also be converted in response to a change in environmental pH. It would be a desirable characteristic for a pH-sensitive controlled-release system.

The effect of pH value on the morphology of dried hydrogels was investigated by an SEM. As shown in Figure 7, CS hydrogel obtained from the pH 2.2 buffer solution has a highly porous structure. The pore size ranges from 1 to 10 μm depending on the swellability of the hydrogels, the pore size increasing with swellability. To the contrary, the CS hydrogel obtained from the pH 7.4 buffer only has a rough but nonporous surface. This change in morphology is reversible and pH-dependent.

CONCLUSIONS

The pH-sensitive and biodegradable hydrogels were synthesized by grafting LA to CS. The formation of hydrogels is explained by interactions
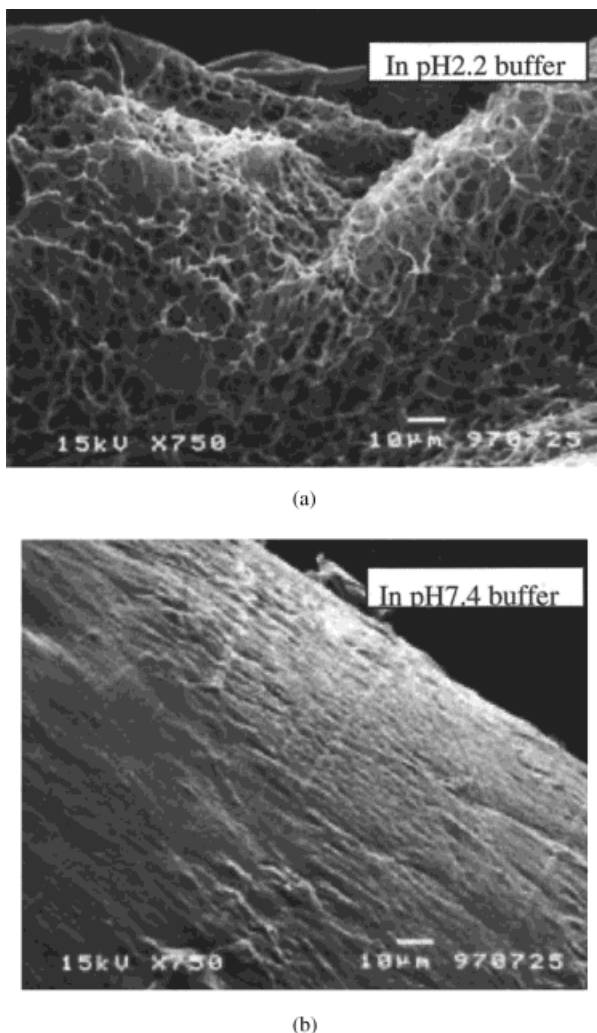
(a)

(b)

Figure 7 Scanning electron micrographs of vacuum-dried chitosan hydrogel after swelled in (a) pH 2.2 and (b) pH 7.4 buffer.

of the hydrophobic side chains and hydrogen bonding serving as pseudocrosslinks, which stabilize hydrogel-forming molecules against permanent deformation in the buffer. The structure and swelling mechanisms are different in acidic and alkaline media. The different structural changes of hydrogels were characterized by FTIR, which further confirmed the swelling mechanism proposed. This swellability and morphological change is pH-dependent and reversible according to the investigation.

REFERENCES

1. Mathur, A. M.; Moorjani, S. K.; Scranton, A. B. JMS—Rev Macromol Chem Phys C 1996, 36, 405.
2. Zdrahala, R. J. Macromol Symp 1996, 109, 135.
3. Fan, K.; Gonzales, D.; Sevoian, M. J Envir Polym Degrad 1996, 4, 253.
4. Paradossi, G.; Lisi, R.; Paci, M.; Crescenzi, V. J Polym Sci Part A Polym Chem 1996, 34, 3417.
5. Khare, A. R.; Peppas, N. A. Biomaterials 1995, 16, 559.
6. Oliveira, W. D.; Glasser, W. G. J Appl Polym Sci 1996, 61, 81.
7. Pascual, B.; Castellano, I.; Vazquez, B.; Gurruchaga, M.; Goni, I. Polymer 1996, 37, 1005.
8. Rathna, G. V. N.; Rao, D. V. M.; Chatterji, P. R. JMS—Pure Appl Chem A 1996, 33, 1199.
9. Vazquez, B.; Gurruchaga, M.; Goni, I. Polymer 1995, 36, 2311.
10. Guan, Y. L.; Shao, L.; Yao, K. D. J Appl Polym Sci 1996, 61, 2325.
11. Guan, Y. L.; Shao, L.; Liu, J.; Yao, K. D. J Appl Polym Sci 1996, 62, 1253.
12. Martin, G. P. JMS—Pure Appl Chem A 1995, 32, 629.
13. Muzzarelli, R. A. A. In Chitin: Pergamon: Oxford, 1977; p 135.
14. Roberts, G. A. F. In Chitin Chemistry; Macmillan: London, 1992; p 165.
15. Hong, X. Y.; Feng, H. B.; JMS—Pure Appl Chem A 1996, 33, 1459.
16. Qu, X.; Wirsén, A.; Albertsson, A. C., manuscript.
17. Elving, P. J.; Markowitz, J. M.; Rosenthal, I. Anal Chem 1956, 28, 1179.
18. Choi, H. J.; Yang, R.; Kunioka, M. J Appl Polym Sci 1995, 58, 807.
19. Allcock, H. R.; Ambrosio, A. M. A. Biomaterials 1996, 17, 2295.