

A Novel Amphoteric, pH-Sensitive, Biodegradable Poly[chitosan-g-(L-lactic-co-citric) acid] Hydrogel

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ABSTRACT: The novel amphoteric, pH-sensitive, biodegradable poly([chitosan-g-(L-lactic-co-citric) acid]) hydrogel (CLC) was synthesized through the reaction of chitosan (CS) with poly(L-lactic-co-citric acid) (PLCA). The structure of CLC was characterized by Fourier transform infrared spectroscopy, elemental analysis, and wide-angle X-ray diffraction measurement. The degree of substitution of CS amino groups was evaluated from salicylaldehyde analysis. The swelling behavior of CLC film in an aqueous solution with various pHs and the apparent swelling kinetics were stud-

ied. The swelling mechanisms of CLC film in acidic and alkaline mediums are discussed. The results showed that CLC hydrogel had a higher degree of swelling in the pH range of $4 > \text{pH} > 8$ and that the swelling rate order in different buffers was neutral $>$ acidic $>$ basic mediums. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 89: 3850–3854, 2003

Key words: chitosan; L-lactic acid; citric acid; hydrogel; pH sensitive

INTRODUCTION

A hydrogel is often a multicomponent system consisting of a hydrophilic polymer swollen with water. Incorporation of a multitude of comonomers having specific functional groups into network structures open many application potentials, e.g. solute recovery, affinity separation, environmentally sensitive membranes.^{1–2} Hydrogels can be tailor-synthesized to possess some responsive moieties showing selectivity for proteins,³ enzymes,⁴ biomolecules⁵ and dyes,⁶ pigments, or some anions and cations.⁷

Chitin and chitosan (CS) and their modified analogs have shown many applications in medicine, cosmetics, agriculture, and biotechnology. We studied a series of hydrogels based on chitosan, such as polyelectrolyte complexes (PECs)^{8–9} of chitosan and natural polymers, semi-IPN hydrogels prepared by chitosan, and polyether.^{10–11}

The family of poly(lactic acid)s and their copolymers have attracted much attention in medical and pharmaceutical interests because of their biocompatibility and biodegradability. Qu¹² synthesized a series of pH-sensitive hydrogels based on chitosan and D,L-

lactic acid and investigated their swelling behavior and thermal degradation kinetics.¹³

To combine the advantages of synthetic and natural polymers and at the same time maintain the favorite properties of natural polymers such as biodegradation and bioactivity, in this work a novel amphoteric hydrogel was prepared by the reaction of CS with the oligomer of citric acid and L-lactic acid. Here the oligomer took the effects not only of its carboxyl group supplier but also of the crosslinker. The swelling mechanisms in various pH buffers were investigated. Fourier transformer infrared (FTIR) spectroscopy was used to characterize the structural change of the hydrogel in different pH solutions. Moreover, a mechanism of ionization and deionization is suggested based on the changes observed by FTIR spectroscopy.

EXPERIMENTAL

Materials

Chitosan (CS) ($\bar{M}_w = 1.5 \times 10^5$, degree of deacetylation, DD = 90%) from Yuhuan Ocean Biochemical Co. (Zhejiang, China) was used for the preparation of the graft copolymer. The degree of deacetylation (DD = 90%) was determined by FTIR spectroscopy. L-Lactic acid (88% aqueous solution) was obtained from Purac Biochem (Holland) and used directly. Citric acid was purchased from Tianjin Chemicals Co. (China); all other chemicals were of analytical pure grade and used as delivered, without further purification.

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Copolymerization of L-lactic acid and citric acid

An 88% aqueous solution of L-lactic acid and citric acid (the mole ratio of L-lactic acid to citric acid was 8:1) was dehydrated at 140°C–150°C, first at atmospheric pressure for 3 h, then at a reduced pressure of 100 mm Hg for 1 h, and finally under 30 mm Hg of pressure for another 1 h. A viscous liquid of poly(L-lactic-co-citric acid) (PLCA) was obtained. The carboxyl terminal groups of PLCA were determined by titration in a 1:1 methanol and dichloromethane solution of the samples with a KOH concentration of 0.05 mol/L in ethanol, using bromothymol blue as the indicator. The result was 7.98×10^{-3} mol/g.

Preparation of CLC hydrogel

CS powder was dissolved in the aqueous solution of PLCA. The solution was poured into a frame mold and maintained at 65°C for 5 h for film formation. Then the film was treated at 80°C–90°C under a pressure of 5–10 mm Hg for 3 h and under the same pressure for another 2 h to promote dehydration of the CS copolymer salts with formation of the corresponding amide linkages. To remove the unreacted PLCA and ammonium salts, the sample was extracted with chloroform and methanol separately in a Soxhlet apparatus for 48 h. The CLC film was about 0.07 mm thick, with a nitrogen content of 4.03%.

Swelling behavior of the CLC hydrogel

Dried hydrogels (0.5 × 1.5 cm) were left to swell in a solution of the desired pH (1.74–12.80) and an ionic strength of $I = 0.5$ mol/L at 37°C. Swollen gels removed from the solution at regular intervals were dried superficially with filter paper, weighed, and replaced in the same bath. The measurements were continued until a constant weight was reached for each sample. The degree of swelling, W , is expressed as the amount of absorbed water per gram of dry polymer during a definite time interval.

$$W = (W_s - W_0) / W_0$$

where W_s and W_0 are the weights of the samples in the swelled and dry states, respectively.

Characterization

The Fourier transform infrared (FTIR) transmission spectra were obtained from the film sample on a BIO-RAD FT3000 spectrometer. Wide-angle X-ray diffraction investigation of the hydrogels was carried out on film samples at room temperature with nickel-filtered Cu K α radiation. The scan width was 3°–60°, and the step length was 0.02°. Analysis of the C, H, and N

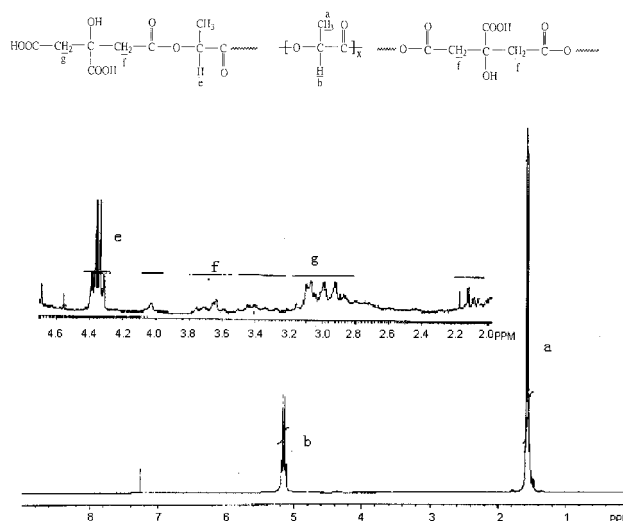


Figure 1 $^1\text{H-NMR}$ spectrum of PLCA.

content of CLC hydrogel was carried out on a PE-2400 elemental analysis instrument.

The $^1\text{H-NMR}$ spectrum of PLCA was recorded with a Varian Mercury VX300 spectrometer in CDCl_3 that contained 1 vol % tetramethylsilane as an internal reference.

The degree of substitution of CS amino groups in CLC was determined by the formation of *N*-salicylidene CS. An accurately weighed sample was immersed for 48 h in 100 mL of 0.02M salicylaldehyde in a methanol/1% acetic acid aqueous solution (80:20 v/v). After 48 h a portion of the filtrate was diluted 625 times, and the UV absorbance at 255 nm was measured to determine the residual concentration of salicylaldehyde (RSA).

RESULTS AND DISCUSSION

$^1\text{H-NMR}$ analysis of PLCA

Figure 1 shows the $^1\text{H-NMR}$ spectrum of PLCA together with the signal assignment. In addition to the large signals of the LA [poly(lactic acid)] units at $\delta 1.58$ (a) and $\delta 5.16$ (b) ppm, small signals, g and f, were detected at $\delta 3.0$ and $\delta 3.65$ ppm, respectively, as shown in the expanded signals. These were assigned to the methylene protons of citric acid unit. The quaternary peaks at $\delta 4.35$ ppm (e) were attributed to the methane of lactyl combined with citric acid.

Reaction of CS with PLCA

CS was dissolved in an aqueous PLCA solution to make a homogeneous, viscous polyelectrolyte solution. By heating the solution, dehydration of CS ammonium salt will occur, forming amide groups between CS and PLCA. The freshly prepared CLC film

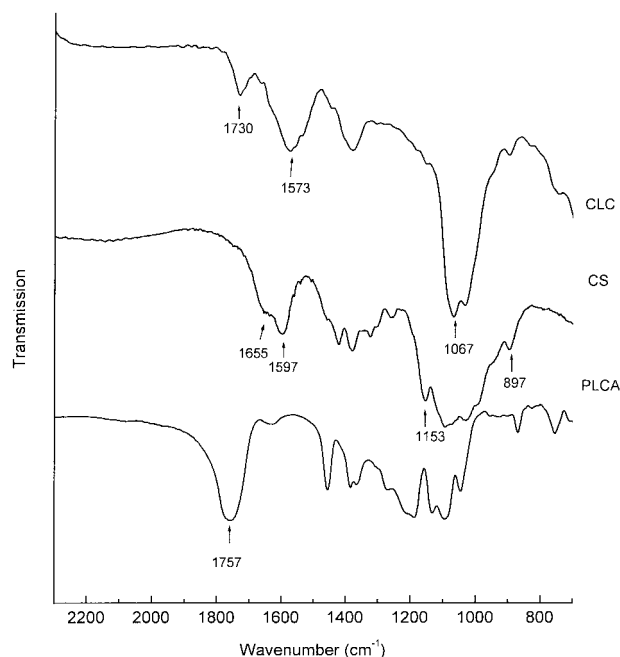


Figure 2 FTIR spectra of CLC, CS, and PLCA.

was brittle and transparent. When exposed to air, it absorbed moisture slowly and became soft through plasticization. After extracting with chloroform and methanol separately, it appeared to have better tensile strength and flexibility than did the initial CS and as-prepared samples. The tensile strength of the extracted film was 39.79 MPa, the elongation at break was about 19.6%, and the substituted degree of the amino groups of CS was 38.6%.

IR analysis of CLC hydrogel

Figure 2 shows the IR spectra of PLCA, CS, and CLC, respectively. The IR spectrum of CS shows peaks assigned to the saccharine structure at 897 and 1153 cm^{-1} and a strong amino characteristic peak at around 1597 cm^{-1} . The shoulder peak at 1655 cm^{-1} is characteristic of the amide I band of N-acylated chitosan. The IR spectrum of PLCA shows peaks assigned to the carbonyl group at around 1757 cm^{-1} .

Compared to the IR spectra of CS and PLCA, CLC has a new peak appearing around 1730 cm^{-1} , corresponding to the ester or carboxylic groups of the PLCA side chains. An obvious shift to a lower wave number in comparison with PLCA can be observed and is attributed to the formation of hydrogen bonds between the ester groups of PLCA and amino or hydroxyl groups of CS. The new absorption band at around 1573 cm^{-1} is a result of the overlapping of the peaks from the amide I bands and the amino groups of CS with the peaks from the salt links that conjoin CS with PLCA. The change of the amide I absorption of CLC at around 1655 cm^{-1} is not a very obvious con-

trast to CS. The shift of the amino group absorption from 1597 cm^{-1} of CS to 1574 cm^{-1} of CLC means that PLCA linked with CS both through amide bonding and static electron interaction. A distinct ether band at around 1067 cm^{-1} can be observed in the CLC spectrum compared with CS, so a chemical crosslink reaction between the polyfunctional PLCA and CS happened along with the formation of amide.

Wide-angle X-ray diffraction analysis

X-ray diffraction profiles of PLCA, CS, and CLC are shown in Figure 3. CS had an orthorhombic unit cell with $a = 0.824$ nm, $b = 1.039$ nm, and $c = 1.648$ nm. The peaks appearing at around $2\theta = 10^\circ$ were assigned to (001) and (100), whereas the peaks around $2\theta = 20^\circ$ were assigned to (020) and (200).¹⁴ PLCA, the same as poly(L-lactic acid) (PLLA), crystallized in a pseudo-orthorhombic unit cell (dimensions: $a = 1.07$ nm, $b = 0.595$ nm, and $c = 2.78$ nm), which contained two 10_3 helices (α form). The main peaks in the X-ray diffraction profile appeared at 2θ values of 15° , 17° , and 19° .¹⁵

The WAXD pattern of CLC showed an obvious decrease corresponding to the peaks of CS moiety,

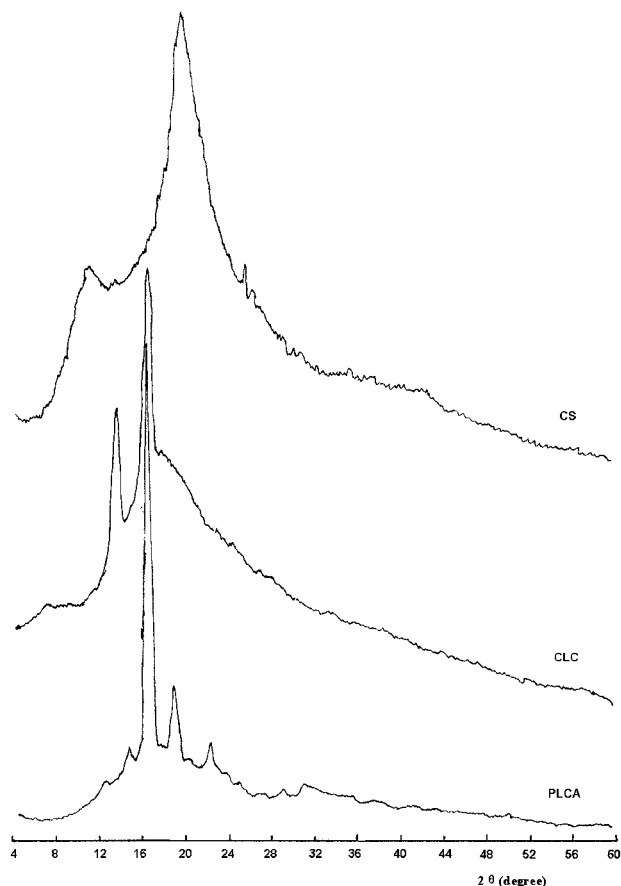


Figure 3 X-ray diffraction profile of CS, CLC, and PLCA.

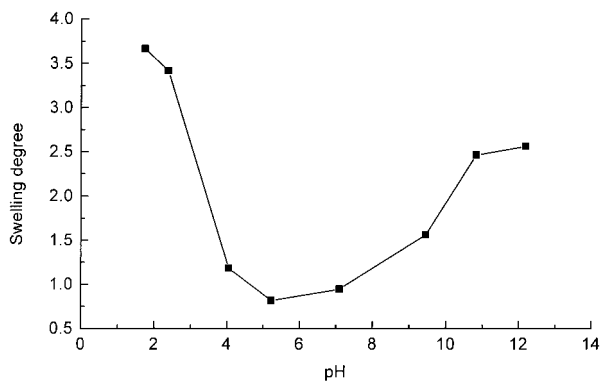


Figure 4 Equilibrium swelling degree of CLC hydrogel as a function of pH.

meaning that the CS in CLC was almost amorphous. However, the side chains of PLCA were crystallizable.

Swelling behavior of CLC hydrogel in aqueous solution of various pHs

In Figure 4 the equilibrium degree of swelling of CLC hydrogel is represented as a function of pH. The ionic strength of the buffers was kept constant ($I = 0.5$ mol/L). The synthesized CLC network contained both unreacted amino groups of CS and carboxyl groups of PLCA. The ionization of these groups led to hydrogel that would respond to changes in environmental pH. Because of the protonation of amino units, the degree of swelling increased sharply for $\text{pH} < 4$ and reached a minimum at $\text{pH} = 5.20$. After that, the degree of swelling increased rapidly again with $\text{pH} > 8.0$ because of the ionization of carboxyl groups in the alkaline medium and reached a maximum at $\text{pH} 10.82$. When the pH of the buffer was between 4.0 and 8.0, both amino and carboxyl groups could be ionized, but the degree of ionization was very low, in the form of some electrostatic ion pairs, and the swelling of the hydrogel was very low and was almost unchanged in this range.

From Figure 4 it can be seen that the swelling degree of CLC in the acid medium was higher than in the basic medium. This implied that the number of carboxyl groups on the CLC hydrogel was less than the number of amino groups. On the other hand, in the alkaline medium the hydrogen bond between amino and ester or hydroxyl groups could form again, so the elastic free energy resulting from the resilient force of the hydrogel increased, and the equilibrium swelling degree decreased compared with that in the acid medium.

Apparent swelling kinetics

Figure 5 displays plots of swelling degree versus time for CLC hydrogel in buffer solutions. The films ab-

sorbed water along the time until a maximum swelling was achieved; after that they maintained their equilibrium value. The order of swelling rate values in different buffers was: neutral $>$ acidic $>$ basic. The swelling of the CLC film mainly involved water diffusion, ionization of amino or carboxyl groups, dissociation of hydrogen and ionic bonds, dissociation of hydrophobic bonds between side chains, polymer relaxation, and ion exchange outside and inside the hydrogel film. The swelling of CLC hydrogel in pH 1.74 buffer mainly involved water diffusion, protonation of amino groups, and a polymer-relaxation process. Initially, as the solution penetrated from the polymer surface, the amino groups were ionized, leading to a partial dissociation of the hydrophobic bonds between side chains, dissociation of the hydrogen bonding between amino groups and hydroxyl or carboxyl groups. Thus, a sharply moving boundary between the unpenetrated polymer region and the swollen gel phase was formed. As the diffusion process continued, the front boundary moved forward until the entire sample had reached its equilibrium swelling. Meanwhile, the mechanical relaxation of the swollen gel and the redistribution of mobile ions occurred between the gel interior and external solution. In the pH 10.82 buffer, the swelling process of CLC hydrogel was very similar to that in the pH 1.74 buffer, except that the ionization group was carboxyl. Hydrogen bonding between the amino and hydroxyl groups with the ester groups still existed in the alkaline medium, so the obstruction of the polymer relaxation was stronger, and that resulted in a slower moving of the osmotic boundary. In the pH 7.10 buffer, the swelling of CLC film involves mainly water diffusion and polymer relaxation. The swelling process is much faster and needs less time to reach its equilibrium.

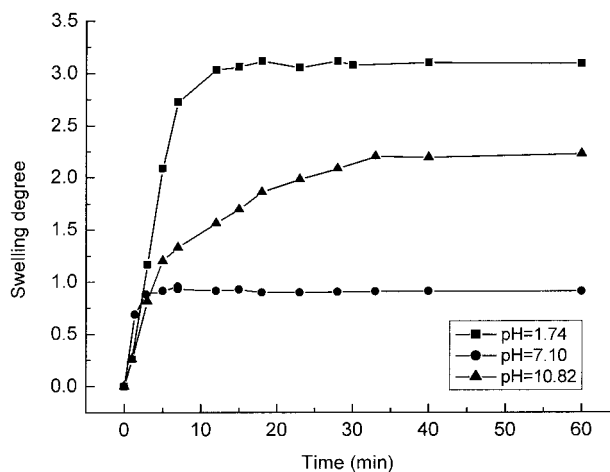


Figure 5 Swelling degree of CLC hydrogel as a function of time in pH 1.74, 7.10, and 10.82.

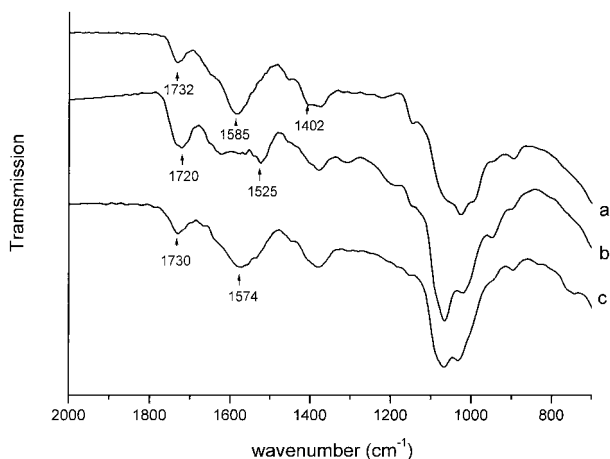


Figure 6 FTIR spectra of CLC and its swollen samples in buffer solutions: (a) pH 10.82, (b) pH 1.74, (c) CLC hydrogel.

Swelling mechanism

As described above, there are amino and carboxylic groups in the CLC network. These —NH_2 and —COOH function groups can form hydrogen bonds with hydroxyl and ester groups. When CLC was in an acidic medium, the —NH_2 groups protonized to —NH_3^+ . This partly induced the dissociation of the hydrogen bonding concerning the —NH_2 groups. Similarly, when the CLC was in an alkaline medium, the —COOH groups dissociated to form —COO^- anions. This induced the partial dissociation of the hydrogen bonds concerning the —COOH groups. Meanwhile, the hydrophobic bonds between the side chains would be dissociated in a strong acidic or basic medium. Then the intermolecular forces would weaken, and the CLC matrix would be loosened up and able to swell.

This mechanism can be proved by changes in FTIR spectra (Fig. 6) of the CLC and its swollen samples in the acidic (pH = 1.74; curve b), and alkaline (pH 10.82; curve a) mediums. Comparing spectra b and c, it can be seen from curve b that the C=O stretching vibration of the ester groups of PLCA at 1730 cm^{-1} became stronger and split into two components attributable to the ester groups of PLCA and the carboxyl groups of citric acid in buffer that joined with the amino groups of CS; meanwhile, the peak around 1574 cm^{-1} of CLC became obviously weaker, and a new peak around 1525 cm^{-1} appeared, which was assigned to the ab-

sorption of —NH_3^+ . In Figure 6, curve a, it can be observed that a new absorption appeared at around 1402 cm^{-1} , which was attributed to —COO^- ; moreover, the absorption of CLC at around 1573 cm^{-1} became sharper and shifted to 1585 cm^{-1} , implying dissociation of the $\text{—NH}_3^+\text{—OOC—}$. This also means the linking between CS and PLCA occurred not only through amide, but through ionic bonds as well. This explains why at pH 1.74 —NH_2 groups protonized to —NH_3^+ and —COOH did not dissociate, whereas at pH 10.82 —COOH groups dissociated to —COO^- .

CONCLUSION

The novel pH-sensitive amphoteric biodegradable hydrogel (CLC) was synthesized by the reaction of CS and poly(L-lactic-co-citric acid) (PLCA). The crystallinity of CS moiety showed an obvious decrease after network formation, whereas PLCA was still crystallizable. The CLC hydrogel had a higher degree of swelling below pH 4 and above pH 8, and order of the swelling rates in different buffers was: neutral > acidic > basic mediums. The structure and swelling mechanisms were different in acidic and alkaline mediums. The structural change of CLC hydrogel was characterized by FTIR, which further confirmed the swelling mechanism proposed.

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