

Synthesis and Characterization of pH-Sensitive Hydrogels Based on Chitosan and D,L-Lactic Acid

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ABSTRACT: Novel pH-sensitive physically crosslinked hydrogels were synthesized by grafting D,L-lactic acid (LA) onto the amino groups in chitosan (CS) without a catalyst. The structures of these graft copolymers were analyzed by FTIR and X-ray measurements. Degree of substitution, side-chain length, and yield of copolymers were evaluated from $^1\text{H-NMR}$, salicylaldehyde, and elemental analysis. The crystallinity of chitosan gradually decreased after grafting, since the side chains substitute the $-\text{NH}_2$ groups of chitosan randomly along the chain and destroy the regularity of packing between chitosan chains. Water uptake of the hydrogels was investigated as a function of side-chain length and degree of substitution. The influence of pH and salt concentration on the swelling behavior of the hydrogels was determined and interpreted. © 1999 John Wiley & Sons, Inc. *J Appl Polym Sci* 74: 3193–3202, 1999

Key words: chitosan; D,L-lactic acid; graft copolymer; pH-sensitivity; hydrogel

INTRODUCTION

Chitin and chitosan (CS) and their modified analogs have shown many applications in medicine, cosmetics, agriculture, and biotechnology.^{1–3} Recently, much research on the preparation of CS gels and their usage in drug-delivery systems and biospecific chromatography as well as enzyme immobilization and metal-ion separation has been carried out.^{4,5} The preparation of a hydrogel from a naturally occurring polymer is interesting because it is a bioactive material and also biodegradable. Among such hydrogels, pH-sensitive polymer gels have potential use in the delivery of drugs to specific regions of the gastrointestinal (GI) tract due to pH variations throughout the GI tract.^{6–8} Homo- and copolymers based on lactic acid have also been extensively studied. They have already been widely used in sutures and drug-release systems, and their biocompatibility and biodegradability have been demonstrated.^{9–11}

The use of the high molecular weight poly(lactic acid) is limited due to the presence of impurities, such as residues of the catalyst, and to difficulties encountered in the preparation of the delivery system using heat or organic solvents.^{12,13}

Graft copolymerization might be one of the best methods to bring together synthetic and natural polymers in order to retain the good properties of natural polymers such as biodegradation and bioactivity. In the literature, chemical modification of CS to import advantageous properties has attracted the attention of many researchers.^{14,15} A variety of graft copolymers of CS were synthesized and evaluated as flocculants, ion-exchangers, controlled-release systems, etc.^{16–18} The preparation and usage of CS lactate as a film-forming product for fruit and seed conservation has been reported, the conserving effect being due, in part, to the antimicrobial properties of the polysaccharide.¹ *N*-Formylchitosan has been made by heating a solution of CS in 100% formic acid at 90°C.¹⁹ The *N*-acetylation of CS was performed in 20 vol % acetic acid.²⁰ Also, amide formation was obtained by casting a film from a mixture of citric acid and CS to make a novel

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polyampholyte.²¹ However, no publication has appeared concerning the grafting of poly(lactic acid) (PLA) onto CS and the application for such hydrogels.

In this work, novel pH-sensitive physically crosslinked hydrogels were synthesized by directly reacting D,L-lactic acid (LA) and the primary amino group of CS in the solution. LA and CS with a degree of deacetylation (DD) of 88% were used to synthesize the graft copolymers with hydrophobic, synthetic side chains, and hydrophilic, natural main chains. Graft copolymers, which form pH-sensitive hydrogels in aqueous solution, are of general interest for biomedical applications, such as artificial muscles or switches, in biochemical separation systems, and in controlled-release systems.

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 copolymers. Acetic acid and sodium phosphate for the preparation of buffers were purchased from Merck (Darmstadt, Germany). The degree of deacetylation (DD = 88%) of CS was determined by the IR spectroscopy method.²² All these chemicals were used as delivered, without further purification. Deionized water with additional purification was obtained from the Millipore Synthesis Unit.

Preparation of CS Hydrogels

CS powder (1 g) dispersed in water (40 mL) was dissolved by adding predetermined amounts of LA (pH around 3–5). Each solution was poured onto a Teflon dish (15.0-cm diameter) and dried first at 80°C for 4 h to avoid evaporation of the monomer during the polymerization of LA. The CS lactate films formed were then put under a vacuum at 80°C for 3 h to promote dehydration of the CS copolymer salts with formation of the corresponding amide linkages. To remove the homopolymers or salts formed during the grafting reaction, all samples were extracted with methanol or chloroform in a Soxhlet apparatus for 24 h. The thickness of the membranes used in this work was 0.17 ± 0.02 mm.

Analyses

The Fourier-transform infrared (FTIR) transmission spectra were obtained from the film samples

on a Perkin–Elmer FTIR 1725X spectrometer. Wide-angle X-ray diffraction (WAXD) measurements were carried out at room temperature using a D/MAX-YA diffractometer with a CuK α tube, 40 kV, 100 mA, made by Rigaku Co., Japan. The diffraction patterns were determined over a range of the diffraction angle $2\theta = 3^\circ$ – 40° .

The ¹H-nuclear magnetic resonance (NMR) spectrum was recorded with a Bruker AC-400 FT-NMR spectrometer at 400 MHz. The chloroform-extracted graft copolymers were slowly dissolved in D₂O with stirring for 3 days. The operating temperature was 27°C.

The degree of substitution of the CS amino groups was determined by formation of *N*-salicylidene CS³ for methanol-extracted samples. An accurately weighed sample was immersed for 24 h in a 100 mL 0.02M solution of salicylaldehyde in a methanol/1% acetic acid aqueous solution (80/20, v/v). After 24 h, the mixture was filtered, a portion of the filtrate diluted 400 times, and the UV absorbance at 255 nm measured to determine the residual concentration of salicylaldehyde (RSA).

Elemental Analysis

The content of C, H, and N was determined for virgin and methanol-extracted grafted CS samples. The total nitrogen content was determined according Dumas with a Carlo Erba NA 1500 instrument. All analyses were performed by Mikro Kemi AB, Uppsala Sweden.

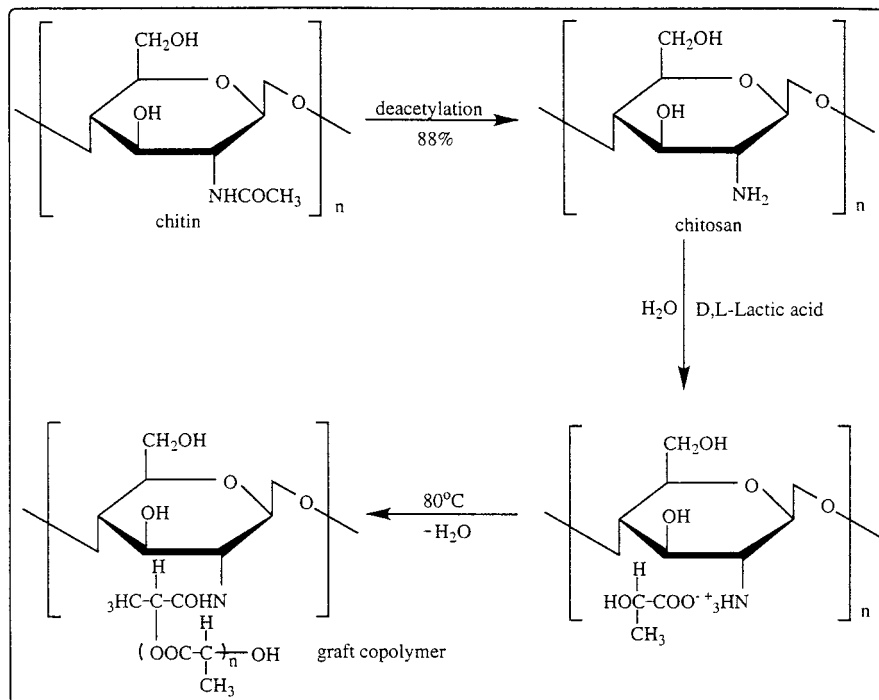
Swelling of Methanol-Extracted Hydrogels

All swelling experiments were carried out on the methanol-extracted samples. To determine the effect of pH on hydrogel swelling, McIlvaine buffer¹⁴ with the same ionic strength, $I = 0.5M$, and various pH's was used in this work [citric acid/Na₂HPO₄ (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 or water uptake of the hydrogels was expressed by the following equation:

Specific solution content (water uptake) (W)

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

where W_d and W_s are the weights of the samples in the dry (W_d) and swelled states (W_s).



Scheme 1 Graft copolymerization of LA onto CS.

To study the effect of salt concentration on the equilibrium swelling of the graft copolymers, the samples (1×1 cm) were immersed for 3 h in 50 mL of aqueous 1% acetic acid (HAc) solution in which the NaCl or CaCl_2 concentrations were from 0 to 3 wt %. The swelling of the gels in the salt-containing solutions in relation to the swelling in the 1% HAc solution was determined gravimetrically after removing excess liquid from the gel surface with filter paper:

$$\text{Swelling ratio (SR)} = (W_x - W_d)/(W_w - W_d)$$

where W_x , W_w , and W_d are the weights of the samples after swelling in the salt solutions (W_x), 1% HAc solution (W_w), and dry states (W_d). (x is the wt % salt concentration in the 1% HAc solution.)

RESULTS AND DISCUSSION

Graft Copolymerization

CS was dissolved in an aqueous LA solution to give a homogeneous viscous polyelectrolyte solution. As shown in Scheme 1, dehydration of the CS amino lactate salt will occur to form amide groups between the CS and LA by heating the

solution, the polycondensation of LA occurring at the same time.¹¹ Normally, the polyesterification of LA follows a well-known acid-catalyzed reaction involving protonation of the carboxylic group, followed by reaction of the protonated species with the hydroxylic functional group to yield the ester linkage. In the absence of an externally added acid, LA acts as its own catalyst for the esterification reaction. Transparent films were obtained after the reaction between CS and LA as described in the Experimental section. Both amide and salt links could exist in the graft copolymers due to the difficulty of removing water from the solid state in the amide forming dehydration step.

The freshly prepared grafted CS films are brittle and transparent. When exposed to air, they easily absorb moisture and become sticky and soft. Chloroform could wash out the unbound oligomers of LA from the sample; however, the chloroform extracted films, which still contain salt-bound LA and a trace of monomeric LA, show similar properties to those of the unextracted samples: The films could swell extensively and the salt structure is hydrolyzed in aqueous solutions. Methanol was found to be a more efficient extractant than was chloroform. It could dissolve not only unreacted monomers and oligomers but

Table I Comparison of Sample Weight Remains, Structures, and Property After Extracted with CCl_3H and/or CH_3OH

LA/CS = 2	Weight Remains	Structure Contains	Characteristics
Original grafted CS	100%	LA oligomers, CS, amide, and salt-linked LA side chains	Sticky, brittle, highly swellable in water
First extracted with CCl_3H	96%	CS, amide, and salt-linked LA side chains	Sticky, brittle, highly swellable in water
Then extracted with CH_3OH	45%	CS, amide-linked side chains	Stiff, flexible, swellable in water
Only extracted with CH_3OH	46%	CS, amide-linked side chains	Stiff, flexible, swellable in water

also salt-bound LA. The methanol-extracted hydrogels have low swellability at all pH ranges investigated and appear to have better tensile strength and flexibility than those of the initial CS and chloroform-extracted samples. A comparative investigation of these two kinds of samples regarding structure provides complimentary data in the characterization of these physically crosslinked hydrogels. The composition and some characteristics of the extracted CS graft copolymers are shown in Table I. The structures contained in the hydrogels will be further analyzed below.

Characterization of CS Hydrogels

Figure 1 shows the IR spectra of CS and chloroform or the methanol-extracted graft copolymer CS/LA = 2 (wt/wt). The IR spectrum of CS (Fig. 1, curve 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 have been reported to be the amide I and III bands, respectively.⁷ The sharp band at 1377 cm^{-1} has been assigned to the CH_3 symmetrical deformation mode. The degree of deacetylation of chitosan is calculated to be about 88% from this IR spectra, the amide I absorption band is measured using the baseline proposed by Miya et al.,¹⁹ and the *N*-acetyl content is calculated from the equation %*N*-acetylation = $(A_{1655}/A_{3450}) \times 115$, which was determined from analysis of a number of fully *N*-acetylated samples.³

Compared to the IR spectra of CS, the chloroform-extracted graft copolymer (Fig. 1, curve B) has a new peak appearing around 1580 cm^{-1} . This is due to the overlapping of the peaks from the amide I and amino bands of CS with the peaks

from the amide and salt links which conjoin CS with the LA oligomers. The peak at 1735 cm^{-1} could be assigned to the ester or carboxylic groups in the LA side chains. After the sample was extracted with methanol (Fig. 1, curve C), the peak at 1580 cm^{-1} disappeared and the two peaks corresponding to the *N*-acetylated and free amino groups of CS appeared again. The increase of the amide I peak (1655 cm^{-1}) indicated increase of the amidation by reacting CS with LA. The peak of the amino groups shifted slightly from 1591 to 1597 cm^{-1} , while the peak at 1735 cm^{-1} decreased after the methanol extraction. These changes show that the salt-linked LA monomer and oligomers have been washed out and that only amide-linked LA side chains remained in the samples. No peak corresponding to ether groups from the reaction between the hydroxyl groups of CS and LA 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. However, the reaction between the hydroxylic groups of CS and the carboxylic groups of LA may occur to some extent under the present reaction conditions according to the literature.^{1,3}

Figure 2 shows the IR spectra of CS and methanol-extracted graft copolymers with different ratios. The small peak corresponding to the amide group of the grafted CS (1656 cm^{-1}) shows an initial increase with increase of the LA/CS ratio up to LA/CS = 2. Over this ratio, the amide peak seemingly remains constant. This corresponds well with the degree of substitution (DS) of the samples measured by RSA and elemental analysis (EA) (see Table II).

Methanol-extracted samples could not dissolve in water, while chloroform-extracted samples free of oligomers but containing salt-bound side

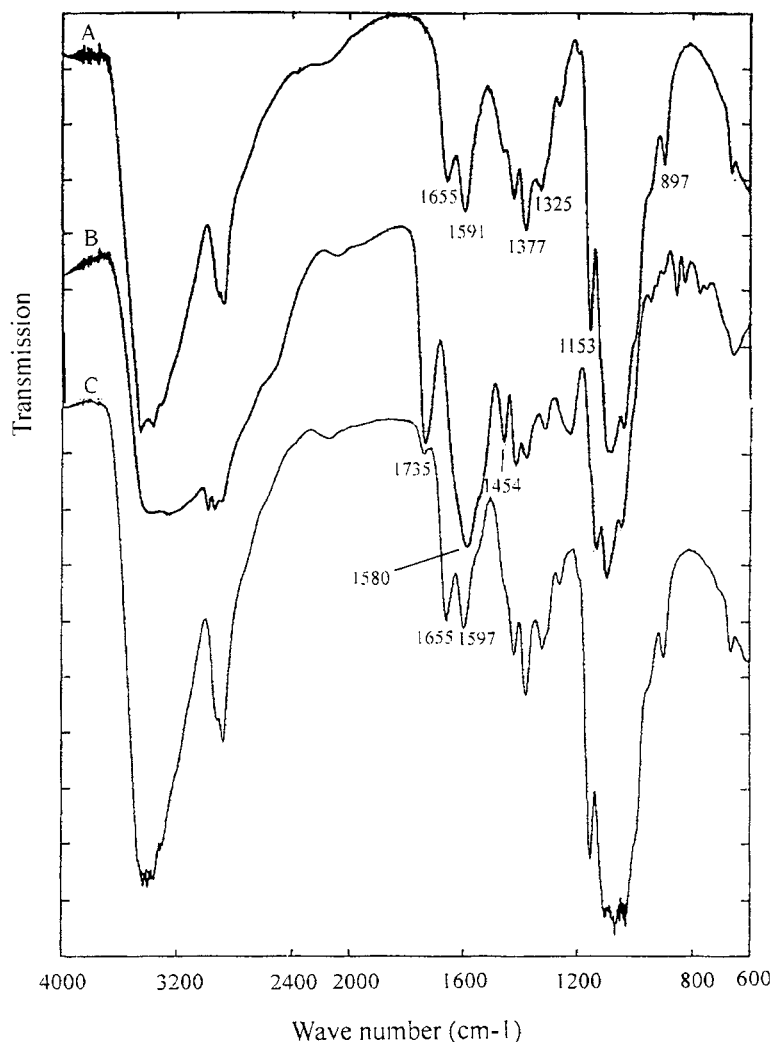


Figure 1 FTIR spectra of (A) CS and (B) chloroform-extracted and (C) methanol-extracted hydrogel LA/CS = 2.

chains could dissolve in water after several days of stirring. It allows the LA side-chain length to be measured by $^1\text{H-NMR}$ while assuming that the salt- and amide-linked side chains have the same length. As seen in Figure 3, curve A, the resonance of methyl protons in lactyl units arising from amide- and salt-linked main chain units (1.4–1.3 ppm) and of hydroxylated lactyl end units (1.3–1.2 ppm) were well resolved, while a trace of LA, which could not be extracted by chloroform, gave small peaks at 1.5 and 4.4 ppm as compared to the spectrum of LA [Fig. 3(B)]. Meanwhile, some oligomers also exist in the LA which give the peaks at around 5.1, 4.4, and 1.4 ppm in Figure 3(B).

The results of EA for CS and its derivatives are shown in Table III. C wt % increases and N wt % decreases after grafting LA. By EA and DP values

computed from $^1\text{H-NMR}$, the DS of the available CS amino groups was calculated, which increases with the LA to CS ratio, approaching a saturation level of about 16–17% for LA/CS ≥ 2 (Table II). In addition, the DS could be measured by the residual salicylaldehyde (SA) method, which gave similar results as those of the EA method. Thus, the assumption about the DP values measured by $^1\text{H-NMR}$ is reasonable.

Increasing the reaction time and temperature in the amide-forming dehydration step as long as undesirable degradation of the CS–PLA graft copolymers does not occur can possibly attain a higher DS. Meanwhile, the film thickness will also influence the graft reaction. Higher DS can be attained for the thinner films, since the water can evaporate more easily during the dehydration step.

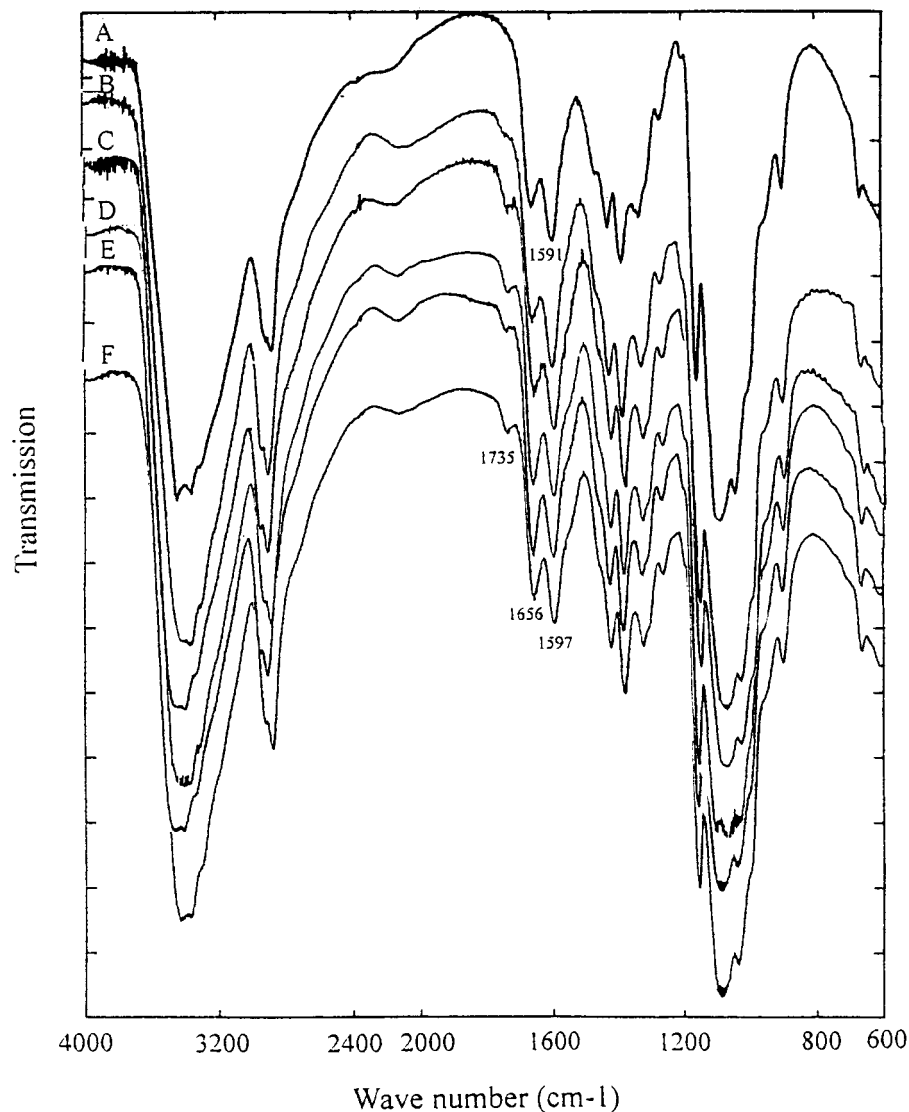


Figure 2 FTIR spectra of (A) chitosan, (B) LA/CS = 0.5, (C) LA/CS = 1, (D) LA/CS = 2, (E) LA/CS = 3, and (F) LA/CS = 4 graft copolymers.

Figure 4 shows the X-ray patterns of CS and methanol-extracted graft copolymers with different ratios. According to Ogawa's analysis of X-ray patterns for CS film, CS has an orthorhombic unit cell with $a = 8.24 \text{ \AA}$, $b = 10.39 \text{ \AA}$, and $c = 16.48 \text{ \AA}$.^{23,24} The peaks appearing at around $2\theta = 10^\circ$ are assigned to (001) and (100), while the peaks around $2\theta = 20^\circ$ are assigned to (020) and (200). These WAXD patterns also show that the grafting decreases the intensity at both peaks. When the feed ratio reaches LA/CS = 2 [Fig. 4(F)], the graft-polymerized samples became almost amorphous. Since LA reacts with CS in a homogeneous solution, the grafting by PLA will take place at random along the chain, giving rise to a random copolymer. This will efficiently destroy the regu-

larity of the packing of the original CS chains, which results in the formation of almost amorphous copolymers.

As mentioned earlier, chemical crosslinking is not possible to occur in this grafting reaction. However, the samples could form pH-sensitive hydrogels in aqueous solutions. So, we can assume that the swellability but insolubility of the graft copolymers is due to the physical crosslinking through hydrophobic side-chain aggregation and/or intermolecular interactions through hydrogen bonds with hydrophilic main chains, which would lead to a corresponding decrease of the polymer chain mobility (see Fig. 5). We have noticed in the literature that by grafting different amounts of hydrophobic monomers with a natural

Table II Graft Copolymerization of LA onto CS

CS (g) ^a	LA (g)	LA/NH ₂ (mol/mol)	Chloroform-extracted Samples		Methanol-extracted Samples			
			Yield (g)	DP of Side Chains ^b	Yield (g)	DS (%)		DP of Side Chains ^b
						SA ^c	EA ^d	EA ^d
1.0	0.5	1.05	1.35	1.35	0.86	6	2.3	0.96
1.0	1.0	2.10	1.67	1.54	0.85	12	13.1	1.81
1.0	2.0	4.19	2.20	2.30	0.94	14	17.6	4.03
1.0	3.0	6.29	2.78	3.82	0.93	17	16.8	3.87
1.0	4.0	8.38	3.12	4.27	0.87	16	16.2	4.41

^a Water-soluble CS with 88% deacetylation was used.

^b DP_{PLA} determined by ¹H-NMR from the integration ratio of the CH₃ groups within PLA blocks versus those of the hydroxylated lactyl end units.

^c Degree of substitution as measured by residual salicylaldehyde.

^d Measured by elemental analysis.

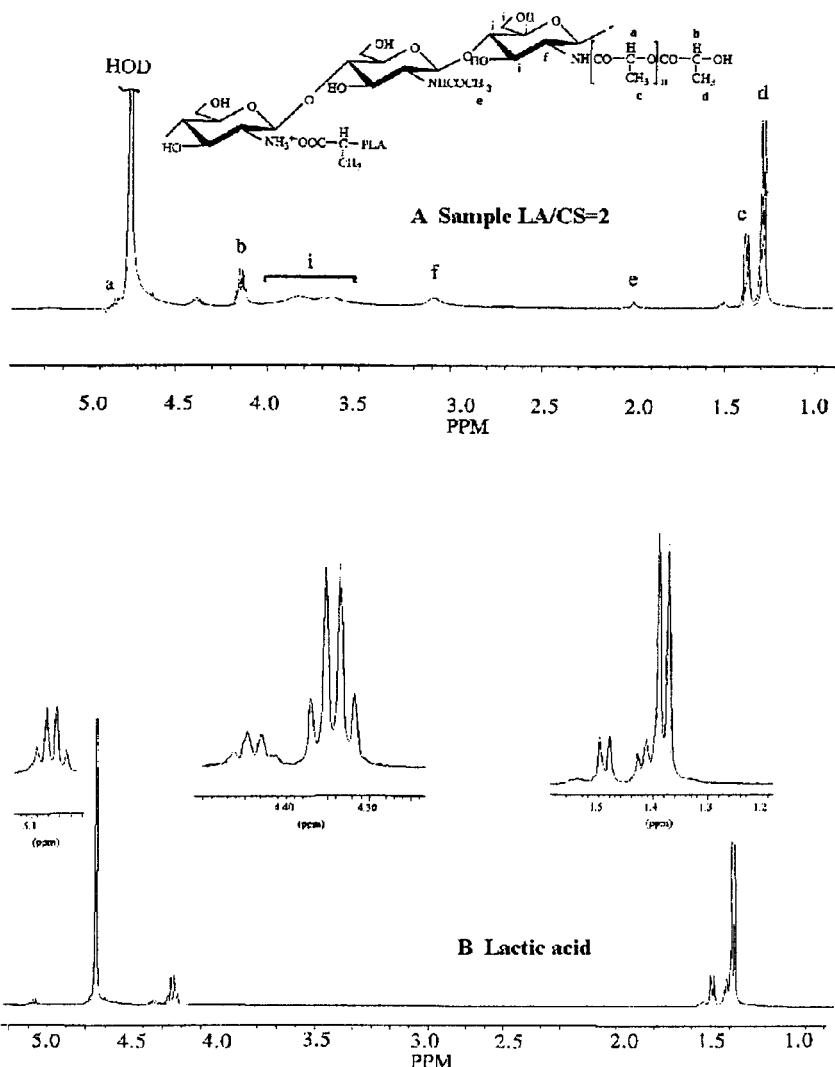


Figure 3 ¹H-NMR spectrum of (A) CS graft copolymer and (B) LA.

Table III Element Analysis of CS and Its Derivatives

	CS	LA/CS = 0.5	LA/CS = 1	LA/CS = 2	LA/CS = 3	LA/CS = 4
C	40.9%	40.7%	41.7%	42.0%	41.9%	41.9%
N	7.6%	7.5%	7.3%	7.1%	6.9%	6.9%
H	7.0%	7.0%	7.2%	7.3%	7.1%	7.1%

substrate such as cellulose and gelatin²⁵ pH-sensitive hydrogels with different degrees of swelling were obtained. These characteristics offer systems with a potential as support for controlled drug-delivery formulations. Meanwhile, Desbrières et al.²⁶ reported that CS which is substituted with alkyl chains having a minimum of six carbon atoms demonstrates hydrophobic interactions in solution.

Swelling Behavior of Hydrogels in Aqueous Solution of Various pHs

Figure 6 shows the effect of the LA/CS feed ratio and pH value on the equilibrium water uptake of the hydrogels. The ionic strength of the buffers was kept constant ($I = 0.5M$), since it will largely affect the swellability of the hydrogels. With increase of the buffer pH, the concentration of the charged ionic groups in the hydrogels also in-

creases. The swelling of the samples will increase due to increase of the osmotic pressure and charge repulsion. While at higher pH, the degree of ionization is reduced due to the deprotonation of the amino units of CS and the swelling of the hydrogels is decreased. In addition, we assume that hydrophobic side-chain aggregation and hydrogen bonds in the hydrogels are much stronger, which will also lead to lower swellability of the samples. This pH-sensitive behavior is typical of ionic hydrogels.

The influence of the LA/CS feed ratio on the equilibrium water uptake of hydrogels is shown in Figure 7. In the pH 2.2 buffer, the sample with the ratio LA/CS = 0.5 has the highest specific solution content (W) value of 18, which decreases abruptly to 7 as LA/CS increases from 0.5 to 1. Then, the value slightly increases from 7 to 10 as the LA/CS ratio increases from 1 to 4. Because the

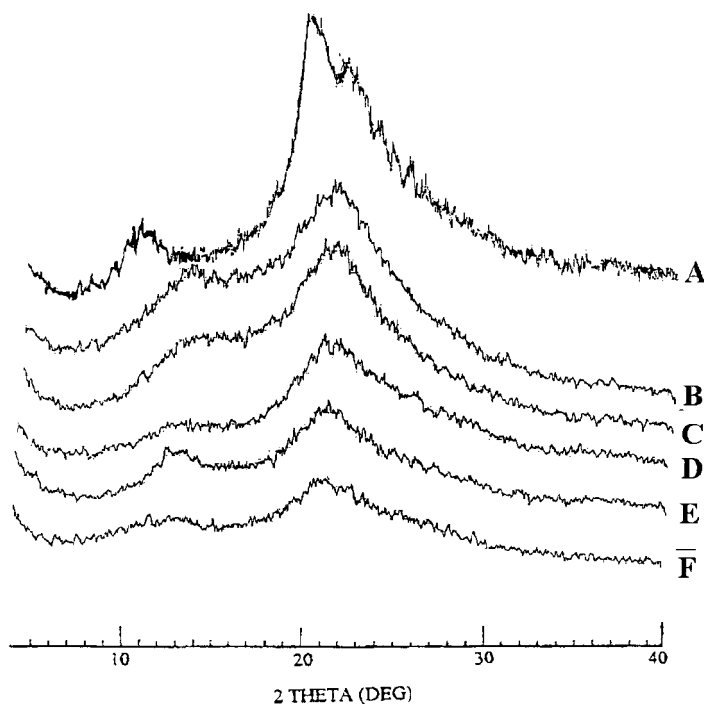


Figure 4 X-ray spectra of (A) CS and (B) LA/CS = 0.5, (C) LA/CS = 1, (D) LA/CS = 2, (E) LA/CS = 3, and (F) LA/CS = 4 methanol-extracted graft copolymers.

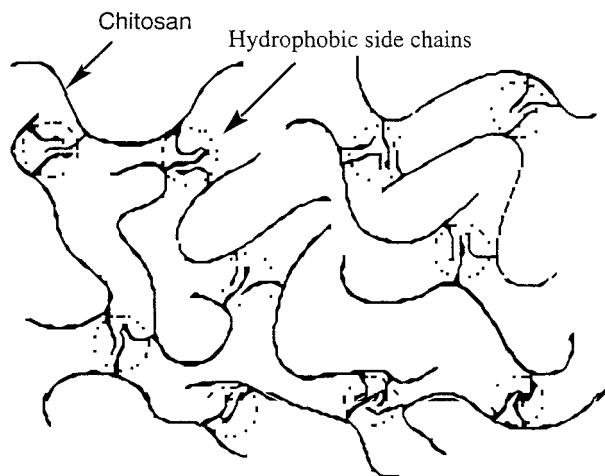


Figure 5 Structure of hydrogels in aqueous solutions.

sample LA/CS = 0.5 has the lowest degree of substitution (DS = 2.3–6%) and the shortest side-chain length (DP = 1.35) as shown in Table II, it will form a loosely physical crosslinked hydrogel and has more charge repulsion between the protonized amino groups. Thus, the sample LA/CS = 0.5 has the best swelling among the samples investigated. Moreover, at a ratio of LA/CS = 1 where the LA grafts are still short (DP = 1.54) but the degree of substitution significantly higher (DS = 12–13.1%), the water uptake is drastically reduced. The increase in the water uptake of the graft copolymers above an LA/CS ratio of 1 could be explained by the graft copolymer becoming more and more amorphous as shown by the X-ray spectra in Figure 4, so the water could more easily penetrate into the CS films. In fact, the crystallinity predominantly affects the swellability of hydrogels in the pH 7.4 buffer where the ioniza-

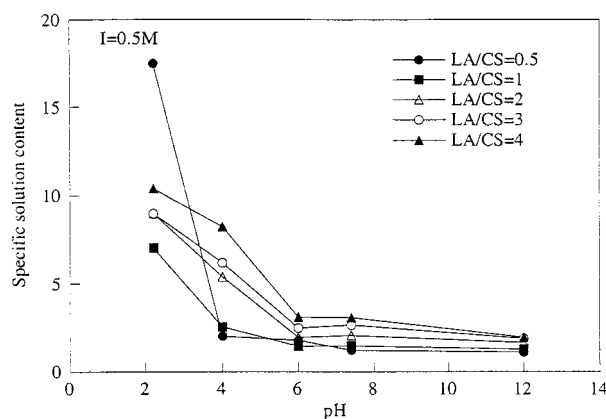


Figure 6 Equilibrium specific solution content of hydrogels as a function of buffer pH.

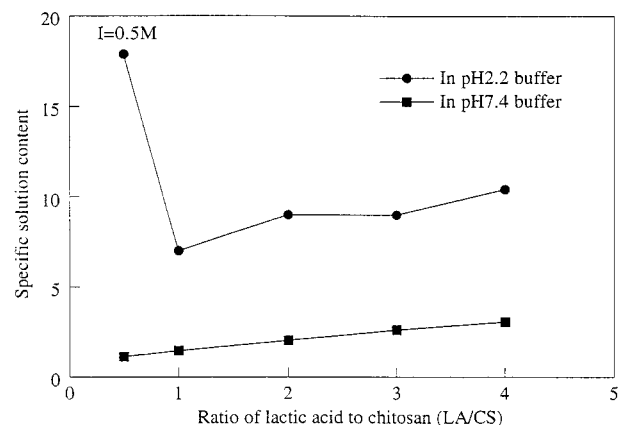


Figure 7 Equilibrium specific solution content of hydrogels with different LA/CS ratio in pH 2.2 and 7.4 ($I = 0.5M$) buffers.

tion of CS amino groups by citric acid in the buffer is absent, so the value increases continuously with the ratio of LA/CS. A more detailed study about the effect of pH values and swelling time on the degree of swelling, including the structural changes during the swelling, is in progress.

Effect of Salt Concentration on Swelling

With all other factors equal, the swelling of polyelectrolyte hydrogels depends mainly on the ionic strength of the solution and not on the size and nature of the ions. Figure 8(A) shows the change of the swelling ratio as a function of NaCl concentration in 1% acetic acid solution (pH is about 3.0). A marked volume decrease was observed in hydrogels with increasing salt concentration in the surrounding solution; the swelling ratio decreased abruptly from 1.0 in deionized water to 0.32 in 3 wt % NaCl solutions. When the gels were put in the 0.5 wt % CaCl_2 solution, the swelling ratio was decreased to about 0.5–0.6 as compared with that of 0.7–0.9 in 0.5 wt % monovalent salt ion solutions. It is well known that the Donnan effect is considered as the main driving force for the swelling of polyelectrolyte gels. Therefore, with increase in the salt concentration outside the gel, ionic swelling pressure is decreased, resulting in a decrease of the gel volume. Increasing ionic strength will strongly suppress swelling, and, thus, the higher the ion charge, the lower the concentration necessary to ensure the same degree of deswelling. Figure 8(B) shows the change of swelling ratio for samples as a function of ionic strength in salt solutions. The samples have similar swelling ratios independent of the salt spe-

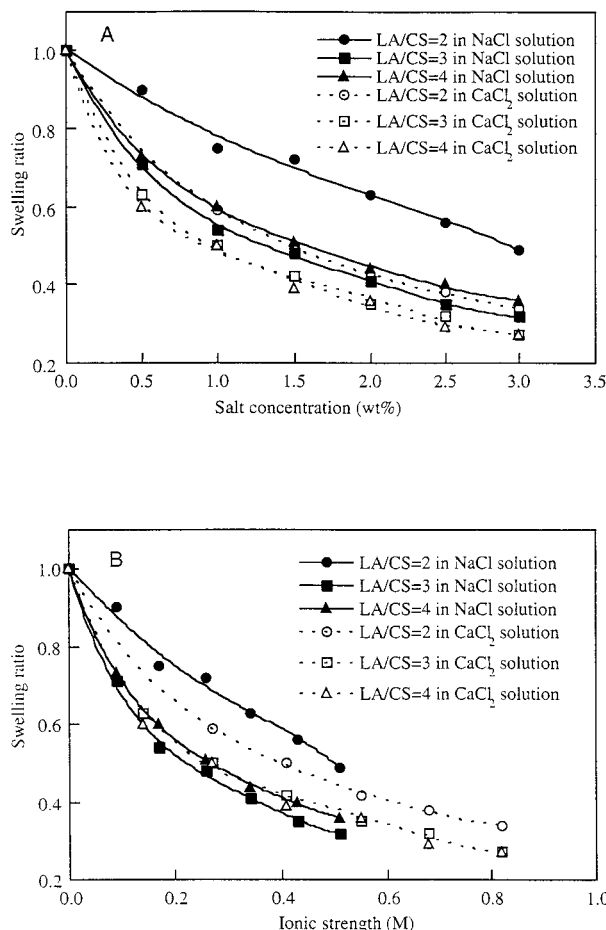


Figure 8 Swelling ratios of CS-PLA hydrogels in aqueous salt solutions as a function of (A) different concentrations, and (B) ionic strength.

cies, while all curves have the same trend of decrease as the ionic strength increases.

CONCLUSIONS

CS was grafted by LA without using catalysts. Methanol and chloroform were chosen to extract the samples. Only methanol extraction gives a pure amide-grafted CS. The crystallinity of CS gradually decreased after grafting because the side chains substitute the —NH_2 groups of CS randomly and destroy the regularity of packing between CS chains. In aqueous solutions, an LA-graft-CS copolymer could form a novel pH-sensitive hydrogel due to the aggregation of the hydrophobic side chains. The specific solution content of hydrogels decreased when the pH value and ionic

strength were increased. When increasing the salt concentration in the solutions outside the hydrogels, ionic swelling pressure is decreased, which results in a decrease of the gel volume.

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