

Synthesis and Characteristics of Polyelectrolyte Complexes Composed of Chitosan and Hyaluronic Acid

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ABSTRACT: Polyelectrolyte complexes (PEC) composed of various weight ratios of chitosan and hyaluronic acid (HA) were prepared. The PECs were formed by a reaction of the oppositely charged polymers. The characterizations of the PEC films were investigated by using Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). The PECs were found to swell rapidly, reaching equilibrium within 30 min, and exhibited relatively high swelling ratios of 250–325% at room temperature. The swelling ratio increased when the pH of the buffer was below pH

6, as a result of the dissociation of the ionic bonds, and with increments of temperature. Therefore, the swelling ratios of the films were pH and temperature dependent. DSC was used to determine the amount of free water in the PEC films, which increased with increasing chitosan content. © 2004 Wiley Periodicals, Inc. *J Appl Polym Sci* 91: 2908–2913, 2004

Key words: polyelectrolyte; chitosan; hyaluronic acid; swelling

INTRODUCTION

Generally, polymer complexes can be classified as hydrogen-bonding complexes, polyelectrolyte complexes (PEC), stereocomplexes, and charged-transfer complexes.¹ In particular, PEC is formed by a reaction of oppositely charged polymers. The formation and properties of polymer complexes depend on the charge ratio of the anionic-to-cationic polymers, the degree of neutralization, the ionic strength, and the valences of the simple ions in the electrolyte solution.^{2,3} Therefore, a strong polyelectrolyte complex is obtained if the anions and cations in the polymers contain strong acids and bases, or if polyions attain their fully ionized forms. Conversely, a weak polyelectrolyte complex is formed in both weak acids and bases.⁴ As an important class of polymer materials, they are widely used in many applications, such as membranes, medical prosthetics, antistatic coatings, environmental signals to the sensors, drug delivery systems, and for protein separation, etc.^{5,6} A PEC with a high tendency of aggregation usually results when both the polyions have strong ionic groups and a good match of charges. The PECs prepared from natural polymers, such as polysaccharides, have the addi-

tional advantage of being nontoxic and bioabsorbable.⁷

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide with a high molecular weight. It has a repeating disaccharide structure, consisting of 2-acetamide-2-deoxy- β -D-glucose and β -D-glucuronic acid residues, linked by alternating (1–3) and (1–4) glycoside bonding.^{8,9} HA is a weak polyacid with a very low charge density as only one charge can be present for every two residues.⁴ HA, a component of the extracellular matrix of all higher animals, has high capacities for lubrication, water sorption, and water retention, which influences several cellular functions, such as migration, adhesion, and proliferation.^{10,11} Recent biomedical applications of HA have included ophthalmic surgery, arthritis treatment, scaffolds for wound healing, tissue engineering, and utilization as a component in implant materials.^{12–14}

Chitosan, obtained from deacetylation of chitin, appears to be more useful in biomedical applications and the dehydrations of aqueous solutions than chitin as it has both hydroxyl and amino groups that can be easily modified.^{15,16} For these uses, chitosan's key properties are its biocompatibility, bioactivity, nonantigenicity, nontoxicity (its degradation products are known natural metabolites), the ability to improve wound healing and/or blood clotting, the ability to absorb liquids and form protective films and coatings, and its selective binding of liquids, which has been used to lower serum cholesterol levels.^{17,18} Chitosan is a weak base, with an intrinsic pKa near 6.5, and a low charge density.⁴

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Chitosan has a cationic characteristic, with many other useful features, such as hydrophilicity, biocompatibility, and good mechanical strength. HA is an anionic natural polymer. It is that one of the components of the extracellular matrix has the water sorption and water retention. Much attention has been paid to the improvement of the chemical and physical properties of hydrogels in the swollen state. A new hydrogel can be prepared by complexing the useful properties of the anionic HA and cationic chitosan.

In this article, the studies on the reaction between two ionic polysaccharides, chitosan and HA, are reported, as well as an estimate of the degree of complexation between the oppositely charged ionic groups. The purpose of this work was to prepare HA/chitosan PECs at various pHs and to investigate the swelling properties of their insoluble forms. The characterizations of the PEC films were also investigated by using Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC).

EXPERIMENTAL

Materials

The chitosan, with an average molecular weight of 2.0×10^5 and a 76% degree of deacetylation, was obtained from Jakwang Co. (Korea). The sodium form of HA, with an average molecular weight of 1.7×10^6 , was obtained from Pacific Chemical Co., Ltd. (Seoul, Korea). The formic acid was purchased from Kanto Chemical Co., Inc. (Japan), and the sodium hydroxide (NaOH) was acquired from Duksan Pure Chemical Co., Ltd. (Japan). The water used in the experiments was distilled and deionized by a Milli-Q System (Millipore, MA).

Preparation of polyelectrolyte complexes

The HA was dissolved in distilled water, and the chitosan was distilled in 1 wt % acetic acid solution. Mixtures of HA and chitosan were prepared by the dissolving various ratios of the two components in 50 wt % aqueous formic acid solution. The concentrations of the chitosan and HA in the solutions were both 1 wt %. The chitosan and HA solutions were obtained by mechanical stirring for 24 h. The detailed composition and designation of the HA and chitosan PECs are listed in Table I. The solutions were filtered by use of a glass filter. The mixed solutions were poured into Petri dishes and dried in an oven for 48 h at 50°C. The Petri dishes were then dipped into 0.1M NaOH aqueous solution to remove any remaining formic acid not participating in the PECs formation. The products obtained were washed with distilled water to remove any chitosan and HA not participating in the PECs

TABLE I
Water States of the HA and Chitosan PEC Films, as Estimated by DSC Analysis

| Sample | Weight ratio [chitosan/HA] | EWC (%) | Free water (%) | Bound water (%) |
|----------|----------------------------|---------|----------------|-----------------|
| Chitosan | — | 87.50 | 69.65 | 17.85 |
| CSHA11 | 1 : 1 | 72.05 | 60.25 | 11.80 |
| CSHA31 | 3 : 1 | 74.73 | 62.37 | 12.36 |
| CSHA51 | 5 : 1 | 76.46 | 64.05 | 12.41 |

formation. The PEC films were dried for 1 week in a vacuum oven at 40°C.

Characterizations

FTIR spectroscopy (Nicolet Model Magna IR 550) was used to confirm the chemical structure of the polyelectrolyte complexes of HA and chitosan. The spectra were obtained by using the KBr pellet technique.

The dried gels were immersed in deionized water at 27°C. The swelling ratios were obtained by weighing the initial and swollen samples at various time intervals, temperatures, and pHs. To measure the swelling ratios, preweighed dry samples were immersed in deionized water. After the excess surface water had been removed with filter paper, the swollen samples were weighed at various pHs, temperatures, and time intervals, and the swelling ratios were determined according to the following equation:

$$\text{Swelling ratio (\%)} = [(W_s - W_d)/W_d] \times 100 \quad (1)$$

where W_s and W_d represent the weights of swollen and dry state samples, respectively.

The equilibrium water content (EWC) was calculated from the following equation:

$$\text{EWC (\%)} = [(W_e - W_d)/W_e] \times 100 \quad (2)$$

where W_e represents the weight of the swollen state at equilibrium.

The state of water in the hydrogels was investigated by DSC (DuPont Instruments DSC910), in the temperature range of -30 to 20°C , with a heating rate of $10^\circ\text{C}/\text{min}$, under a N_2 flow. To investigate the melting endothermic properties of the PEC film, the amounts of free and bound water were calculated from the melting enthalpies. The following equation assumes that the heat of fusion of free water in the hydrogel (Q_{endo}) was the same as that of ice (Q_f ; 79.9 cal/g):

$$W_b(\%) = W_t - (W_f + W_{fb}) = W_t - (Q_{\text{endo}}/Q_f) \times 100 \quad (3)$$

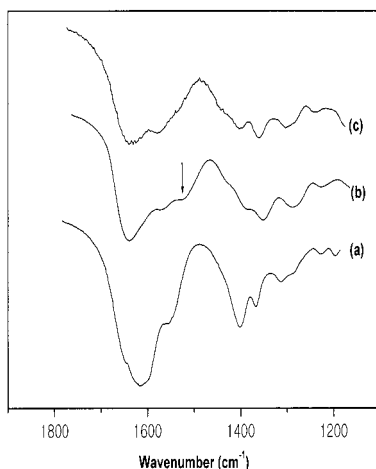


Figure 1 FTIR spectra of chitosan, HA, and the PEC film: (a) HA; (b) CSHA11; (c) chitosan.

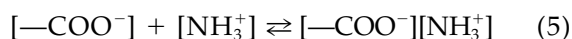
where W_b is the amount of bound water (%); W_f and W_{fb} are the amounts of free and freezing bound water, respectively, and W_t is the EWC (%).

RESULTS AND DISCUSSION

Polyelectrolyte complexes are formed when the polycations and polyanions of two oppositely charged polymers interact. When the polyelectrolyte was formed below the pKa of HA, the interaction between the polyions competed with the protonation of HA.⁴ In this case, the reactions occurred in two steps; first, the deprotonation of carboxyl groups in HA



and second, the reaction between the ammonium salt groups of the chitosan with the carboxyl groups of the HA



Consequently, the formation of PECs is possible below the pKa of HA. The PECs of chitosan and HA were shown to be insoluble in water.

Figure 1 shows the FTIR spectra of chitosan, HA, and the PEC films. Characteristic peaks of chitosan appeared at 3500–3450 cm^{-1} , stretching peaks of the hydroxyl groups, and at 1640 and 1560 cm^{-1} , due to the amide I and II bands, respectively.¹⁹ Characteristic peaks of the sodium form of HA were shown at 1620 and 1560 cm^{-1} . The PEC films have a small absorption peak at around 1520 cm^{-1} , which did not appear in

either the spectra of chitosan or the HA. This peak was due to a $-\text{NH}_3^+$ group of the chitosan after the formation of the polyelectrolyte complexes.²⁰

Figure 2 shows the water swelling kinetics of the PEC films measured at various time intervals and temperatures. The samples swelled rapidly, reaching equilibrium within 30 min. The swelling ratio of PEC films ranged between 250 and 325% and changed in relation to the chitosan content of the network. The swelling ratio also increased with increases in the temperature. All the PEC films swelled rapidly, reaching equilibrium as the temperature was increased. In all cases, the swelling ratio of the CSHA51 was higher than that of both the CSHA11 and the CSHA31 films and it was observed that an increase in the chitosan fraction of the films led to an increase in the equilibrium swelling ratio. This was attributed to the hydrophilic characteristic of the chitosan. This behavior may be due not only to the degree of chitosan and HA complexation of the network, but also to the chitosan content having a large number of water-binding sites.²¹

To characterize the response of PEC films to changes in the external pH conditions, PEC film samples were allowed to swell to equilibrium in various aqueous swelling media, at pHs 4, 7, 9, and 10, at room temperature. In Figure 3, the swelling ratio of various samples is plotted as a function of the pH. It is clear that the PEC films are most stable at pH values around neutrality and are more labile in acidic than basic conditions. The swelling ratio of the films increased when the pH of the buffer was below pH 6, which was caused by the dissociation of the ionic bonds in the PEC films. In this case, the pH of the buffer solutions brings about the dissociation of ionic bonds as most of the carboxyl groups are charged, and change into their protonated form. In extremely low pH conditions, most of the carboxyl groups of the HA are in the COOH form, and most of the amine groups of the chitosan are in the NH_3^+ form. On the other hand, at high pHs, most of the amine groups of the chitosan are in the $-\text{NH}_2$ form and most of the carboxyl groups of the HA are in the $-\text{COO}^-$ form.

The states of the water in PEC films were investigated using DCS, and the results are listed in Table I. To further elucidate the swelling behavior of the chitosan/HA PEC films, the free and bound water contents were measured by using the DSC melting thermograms of the swollen film and calculated according to eq. (3). The heat of melting of the freezing water (intermediate and free water) was determined from the area under the endothermic curve and was calibrated by using pure distilled water as a standard, as described by Mansor and Malcolm.²² Figure 4 shows the DSC thermograms of the fully swollen IPN hydrogels. The endothermic peak of swollen gel appears between -3 and 3°C . The fraction of free water was

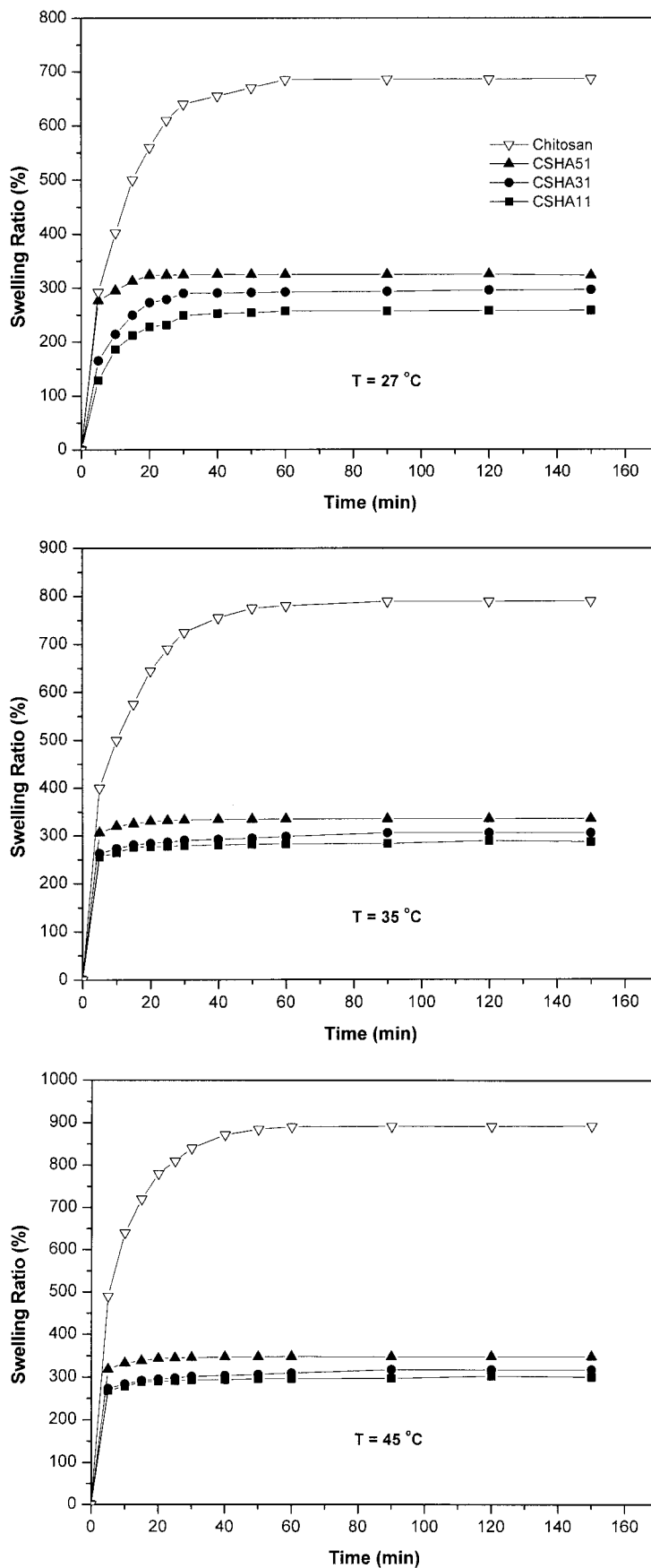


Figure 2 Swelling kinetics of the chitosan/HA PEC films at 27, 35, and 45°C: (a) CSHA51; (b) CSHA31; (c) CSHA11.

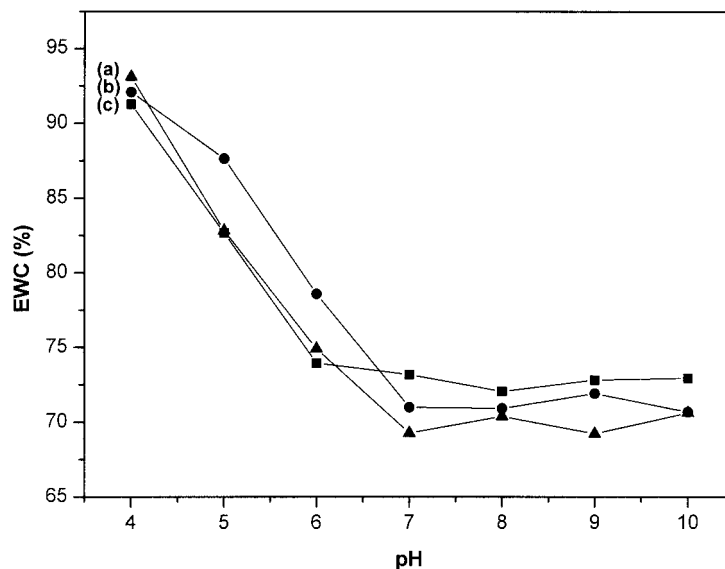


Figure 3 pH-dependent swelling behavior of the chitosan/HA PEC films at 27°C: (a) CSHA51; (b) CSHA31; (c) CSHA11.

estimated by the ratio of the endothermic peak, integrated between these ranges, to the melting endothermic peak of the heat of fusion for pure water. The amount of bound water was expressed as the difference between total and free waters. The EWC values, and the free and bound water contents, were calculated and are listed in Table I. The free water had no interaction with the polymer chains, whereas the bound water was involved in hydrogen bonding with polymers. The bound water content of the chitosan/hyaluronic acid PEC film samples ranged between 13.19 and 56.35% of the total water. As in Table I, CSPA-1 showed the lowest swelling ratio and free water content. The free water contents in the CSHA11,

CSHA31, and CSHA51 PEC films at 27°C were 60.25, 62.37 and 64.05%, respectively. CSHA11 shows the lowest EWC and amount of free water. Because PEC films have polyelectrolyte complex structures, composed of chitosan, the degree of complexation should influence their swelling ratios. The polyelectrolyte complexes are considered to capture the hydrophilic group, producing the tight and ionic bonded structure.

CONCLUSION

The PECs of chitosan and HA were prepared by mixing various weight ratios of two oppositely charged

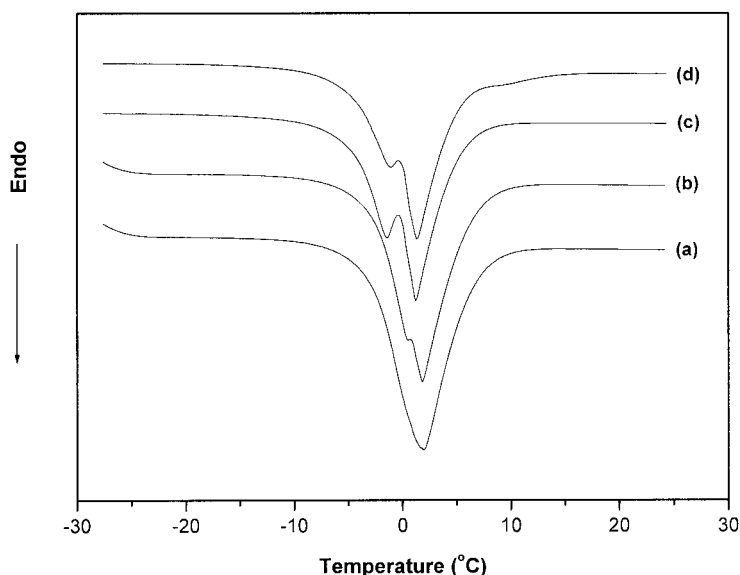


Figure 4 DSC thermograms of the swollen chitosan/HA PEC films: (a) chitosan; (b) CSHA31; (c) CSHA11,

polymers at various pHs. From the FTIR measurements, the PECs had a small absorption peak at 1520 cm^{-1} (ionized amine group), which did not appear in either of the spectra for chitosan or HA. The PEC films swelled rapidly, reaching equilibrium within 30 min, with equilibrium swelling ratios from 250 to 325%. The PEC films exhibited swelling changes in response to external stimuli, such as pH and temperature. The swelling behaviors in the various buffer solutions were investigated. The PECs exhibited relatively high swelling ratios as the chitosan content was increased. The CSHA-3, the sample containing the highest chitosan content, showed the highest pH and temperature-dependent swelling ratios. DSC was used to quantitatively determine the amounts of freezing and nonfreezing water. The free water in the PEC films increased with increasing chitosan content. Therefore, the chitosan and HA PEC films of this study could be useful for artificial organ components, such as biosensors and switches, soft tissues, and tissue engineering application due to their good biocompatibility and relatively high water content.

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