Characterization of the Water State of Hyaluronic Acid and Poly(vinyl alcohol) Interpenetrating Polymer Networks

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ABSTRACT: Interpenetrating polymer networks (IPNs) composed of hyaluronic acid and poly(vinyl alcohol) hydrogels were prepared, and the influence of water and the drying kinetics were investigated. The IPN hydrogels were characterized with thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The glass-transition temperatures of the IPN hydrogels decreased with increasing water content. The bound water was the sum of the nonfreezing bound water and freezing bound water. From the DSC melting curves, the values of the total water and freezing bound water were evaluated for IPN hydrogels containing large amounts of water. At the same time, the bound water value was estimated with TGA. In the TGA curves, one-step and two-step weight losses, corresponding to free water and nonfreezing bound water, were observed. The bound water of the hydrophilic polymers broke the hydrogen bonding between the hydroxyl groups of the polymers. The swollen IPN hydrogels exhibited relatively high bound water contents (43.04–59.17%) by DSC and TGA. The bound water contents of the dry IPN hydrogel films were 10.2–15.29% by TGA. The drying reaction rate constant of the IPN hydrogel increased with increasing temperature. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 1467–1472, 2004

Key words: hydrogels; thermal properties

INTRODUCTION

An interpenetrating polymer network (IPN) is defined as a combination of two polymers, and it has the two following characteristics: first, one of the polymers must be synthesized, or crosslinked, in the immediate presence of the other, and second, the combination must provide the possibility of effectively producing advanced multicomponent polymeric systems, with new property profiles.[1–4] Recently, IPNs have gained widespread acceptance in industrial applications, and newer IPNs, showing the possibility of a wider range of applications, are emerging day by day.[5] Studies of hydrogels are interesting not only from a chemical point of view; they are also being used in chemical engineering, pharmaceuticals, food processing, biochemistry, biology, and medicine.

There are many kinds of natural polyelectrolytes, such as proteins and polysaccharides. One of these, 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 bonds (see Fig. 1).[6,7] HA is a weak polyacid, with a very low charge density, as only one charge can be present for each of the two aforementioned residues. HA, a component of the extracellular matrix of all higher animals, has a high capacity for lubrication, water sorption, and water retention, and it influences several cellular functions, such as migration, adhesion, and proliferation.[8] Recent biomedical applications of HA include ophthalmic surgery, arthritis treatment, scaffolds for wound healing, tissue engineering, and components for implant materials, such as cartilage and eye vitreous.[9–12]

Poly(vinyl alcohol) (PVA) is a water-soluble polyhydroxy polymer that is used in practical applications because of its easy preparation, excellent chemical resistance, and physical properties; it is also completely biodegradable.[13] Chemically crosslinked PVA hydrogels have received increasing attention in biomedical and biochemical applications because of their permeability, biocompatibility, and biodegradability.[14–16] PVA hydrogels are also suitable for ion sensing and can be applied as solid supports for optical sensors because of their ability to form very homogeneous films of high quality.[17]

In the presence of excess water, a polymer may become swollen, exhibiting major changes in its mechanical and chemical properties. Because of the various natural and synthetic polymers that contain hydrophilic groups, such as hydroxyls, carboxyls, carbonyls, and sulfonates, they can have either strong or
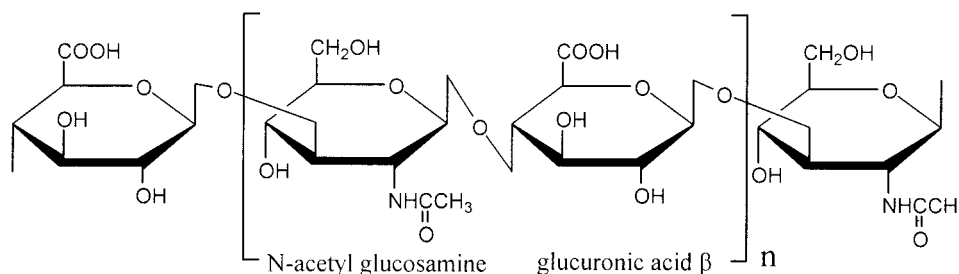
Figure 1 Chemical structure of HA.

weak interactions with water.[12,18] In addition, the hydrophilic groups tend to be soluble in water. The behavior of water can be transformed in the presence of a polymer, depending on the degree of chemical or physical association between the water- and polymer-rich phases. Through these interactions, the physical properties, such as the thermal properties of both the polymers and water, are markedly influenced. Many researchers have reported interactions between water and natural polysaccharide. Previously, we reported on the swelling behavior of PV- and HA-based IPN hydrogels.[19,20] Takigami et al.[21] reported that HA retained a large amount of water and was compatible with biopolymers and hydrophilic polymers. Quinn et al.[22] and Yoshida and Takahashi[23] reported on the annealing effect in the HA hydrogel. Yoshida et al.[24] reported that the structure of nonfreezing bound water changed according to the surroundings. Tatsuko et al.[25] reported on the determination of the bound water contents in polymers by thermal methods.

This article reports on the characterization of the water state of HA/PVA IPN hydrogels with differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). We also studied the drying kinetics and activation energy of HA/PVA IPN hydrogels.

EXPERIMENTAL

Materials

PVA, with an average molecular weight of 8.50×10^4 to 1.46×10^5, was purchased from Aldrich Chemical Co. (United States). HA (sodium salt; viscosity-average molecular weight = 1.70×10^6) was provided by Pacific Chemical Co., Ltd. (Korea). The molecular configuration and structure of the repeating disaccharide unit of the HA is shown in Figure 1. Glutaraldehyde (GA; 25 wt % solution in water) and hydrochloric acid (HCl) were purchased from Yakuri Chem Co. (Japan). 1-Ethyl-(3,3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Sigma Chemical Co. (United States). All other chemicals were reagent-grade and were used without further purification.

Preparation of the HA/PVA IPN hydrogels

The crosslinked HA/PVA IPN hydrogels were prepared with the following two steps with the sequential IPN method. First, HA and PVA powders were dissolved in deionized water at 50°C for 12 h. Each solution was mixed at the specified weight ratios (Table I), and the mixed solution was stirred at room temperature for 6 h. The mixed solution was heated at 80°C for 2 h, and this produced a 5 wt % aqueous solution; PVA was crosslinked in the presence of HA, with GA and HCl as the crosslinking agent and catalyst, respectively. The compositions and designations of the HA/PVA IPN hydrogels are listed in Table I. The mixed solution was poured into a petri dish and dried at room temperature for 2 days. Second, for the crosslinking of HA with EDC, the prepared films (3 cm × 3 cm) were immersed in acetone/water mixtures (9:1 v/v) containing a given concentration of EDC (30 wt % HA in each sample). For good crosslinking, the samples were shaken slowly at room temperature for 48 h. The dry films were washed with distilled water to remove any nonreactive materials not incorporated into the network. The films were dried in a 40°C in a vacuum oven for 1 week.

Measurements

The equilibrium water content (EWC) was the total water, and it was calculated as follows:

$$EWC(\%) = [(W_e - W_d)/W_e] \times 100 \tag{1}$$

TABLE I
Designation in Accordance with the Composition and T_g of the IPN Hydrogels

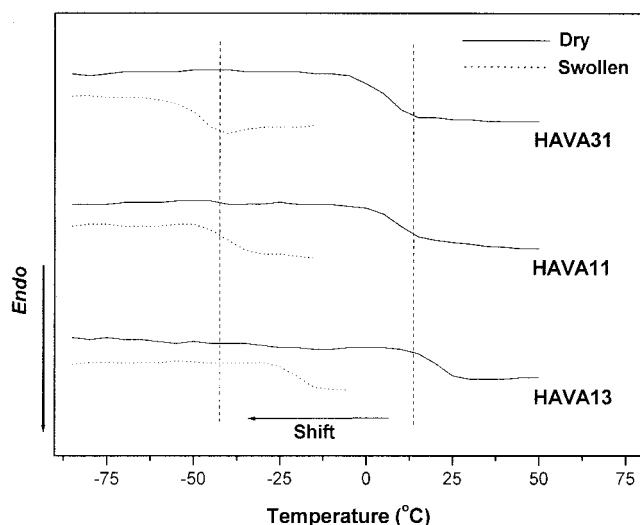| IPN hydrogel film	HA/PVA (w/w)	T_g (°C)	
		Dry film	Swollen film
HAVA31	3:1	4.5	−46.2
HAVA11	1:1	8.7	−39.9
HAVA13	1:3	19.8	−19.3

Figure 2 T_g's of dry and swollen HA/PVA IPN hydrogel films.

where W_e is the weight of the swollen state at equilibrium. A DSC instrument (2010, TA Instruments) was used to examine the state of the water in the swollen hydrogels with different water contents. The samples, sealed in aluminum pans, were cooled to −70°C and then heated to 35°C, at a heating rate of 5°C/min, under a 60 cc/min nitrogen gas flow. The glass-transition temperatures (T_g's) were observed with DSC. The bound water was determined with a thermogravimetric analyzer (SDT 2960 simultaneous DSC–TGA instrument, TA Instruments) through the heating of the samples from room temperature to 300°C, at a heating rate of 5°C/min, under nitrogen. When a sample containing water was measured, with an open aluminum pan, it was first frozen in liquid nitrogen to avoid vaporization during the handling.

RESULTS AND DISCUSSION

T_g's of the prepared samples, in the presence of water, were measured via DSC. Figure 2 shows T_g's of the HA/PVA IPN hydrogels, comparing dry and swollen IPN hydrogel films. T_g of PVA itself was 80°C according to a DSC analysis in our previous work.[26] Figure 2 shows that T_g of the IPNs was lower than that of PVA and shifted down because of the low T_g of HA, which was not detected by DSC.[27] The overall T_g's of IPNs were observed between −46.2 and 19.8°C. T_g of the dry films was observed at 4.5, 8.7, and 19.8°C for HAVA31, HAVA11, and HAVA13, respectively. However, T_g of the swollen films was observed at −46.2, −39.9, and −19.3°C. T_g's of the swollen films were markedly decreased in comparison with those of the dry films. This result indicates that T_g is dependent on the contained water of an HA/PVA IPN hydrogel film. HA and PVA, which have hydrophilic groups,

such as hydroxyl and carboxyl groups, have strong interactions with water. Through this interaction, T_g of a swollen film decreases, because of the partially hydrolyzed HA and PVA, due to the breakage of the hydrogen bonds with the entrapped water (or sorption water) of the HA/PVA IPN hydrogel. T_g's of the HA/PVA IPN hydrogels are listed in Table I.

Figure 3 shows the DSC thermograms of fully swollen HA/PVA IPN hydrogels. The states of water in the polymer hydrogel can be categorized as free water, freezing bound water, and nonfreezing bound water. Free water is the water that does not take part in hydrogen bonding with the polymer molecules. Freezing bound water, or intermediate water, is the water that interacts weakly with the polymer molecules. These have transition temperatures, enthalpies, and DSC curves similar to those of pure water. Nonfreezing bound water shows no endothermic peak in the temperature range of −70 to 0°C, as it consists of molecules bound to polymer molecules through hydrogen bonding. In Figure 3, two melting peaks can be seen in the DSC curves for the IPN hydrogels, indicating that free water and freezing bound water exist in the IPN hydrogels.

The amounts of free water and bound water were calculated from the melting enthalpies. The following equation assumes that the heat of fusion of free water in the hydrogel is the same as that for ice:[19]

$$W_{nf}(\%) = W_t - (W_f + W_{fb}) = W_t - (Q_{endo}/Q_f) \times 100 \tag{2}$$

where W_t is the EWC (%) obtained by weighing; W_{nf} is the amount of nonfreezing bound water (%); and W_f and W_{fb} are the amounts of free water and freezing
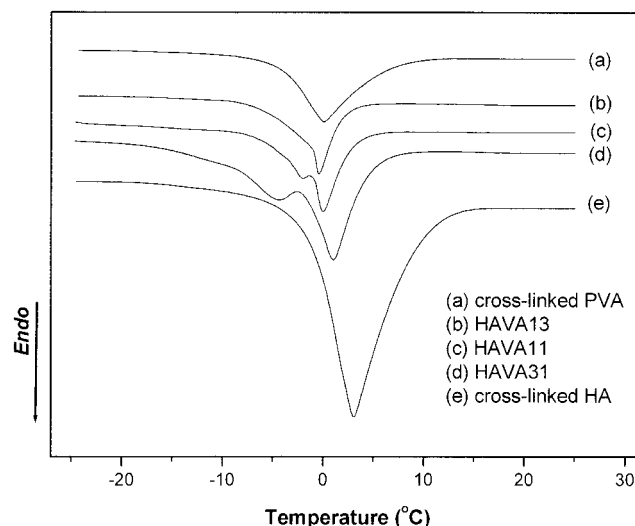

Figure 3 DSC thermograms of fully swollen HA/PVA IPN hydrogels.

TABLE II
Water State of the IPN Hydrogels Estimated
with TGA and DSC Analysis

Sample	Total water[a] (%)	Free water[a] (%)	Nonfreezing bound water (%)		
				TGA	
			DSC	Swollen film	dry film
HAVA31	81.6	38.56	43.04	50.82	10.2
HAVA11	78.9	25.96	52.94	65.3	20.48
HAVA13	74.7	23.89	50.81	59.17	15.29

[a] The total water and free water (including freezing bound water) were measured by DSC.

bound water, respectively. Q_{endo} and Q_f are the heats of fusion of free water in the IPN hydrogel and of ice (334 J/g), respectively. The fraction of free water in the total water was approximately calculated as the ratio of the endothermic peak area for the water-swollen hydrogel to the melting endothermic heat of fusion for pure water. W_f increased with increasing HA contents in the IPN hydrogels because of the increasing number of hydrophilic groups. The endothermic peak of the swollen gel appeared between −4 and 3°C. The EWC values, and W_f and W_{nf}, were calculated, and they are listed in Table II. The bound water contents of the HA/PVA hydrogel samples ranged from 43.04 to 52.94% of the total water. The W_f values, including W_{fb}, were 38.56, 25.96, and 23.89% for compositions HAVA31, HAVA11, and HAVA13, respectively, at room temperature. HAVA13 showed the lowest EWC and W_f. Because HA/PVA IPN hydrogels have a crosslinking polyelectrolyte structure, the degree of crosslinking should have an influence on the EWC of HA/PVA IPN hydrogels. It is thought that the crosslinking polyelectrolyte captures the hydrophilic group, producing a tight and ionically bonded structure.

Generally, bound water (W_b) consists mostly of nonfreezing bound water (W_{nf}); W_{fb} is only a few percent. With the DSC method, it is difficult to separate W_{nf} from W_b. However, the TGA method shows the curve for their separation in swollen or dry films. Figure 4(a) shows a typical TGA curve for the swollen films. Line A indicates the total weight of the IPN hydrogel film (the weight of only the sample + the weight of the water). Line B shows the weight loss of water in the second step, which is W_{nf}. Line C shows the weight, with the exception of the water of the IPN hydrogel film (the weight of only the dry film). Line D shows the weight loss for the free water and freezing bound water occurring in the one step. Line D includes the weight loss during the IPN hydrogel film handling. However, TGA was used to measure the nonfreezing bound water, which is indicated by line B. W_{nf} was obtained from the TGA curve and was calculated as follows:[25]

$$W_{nf} = \text{line } B/C \quad (3)$$

Figure 4(b) shows that the nonfreezing bound water was separated from the free and bound water. Curve a is the weight-loss curve for the swollen IPN hydrogel. In curve a, the second step weight loss corresponds to the nonfreezing bound water and the HA content in the IPN hydrogel, in the order HAVA31 > HAVA11 > HAVA13. Similar results were also obtained by DSC. Curve b is the weight-loss curve for the dry IPN film. This film was placed in an air-circulated oven, at 90°C for 12 h, to ensure the complete removal of the water, as the vaporization of the pure water was complete at 100°C, but water restrained by hydrophilic bonds in the IPN hydrogel
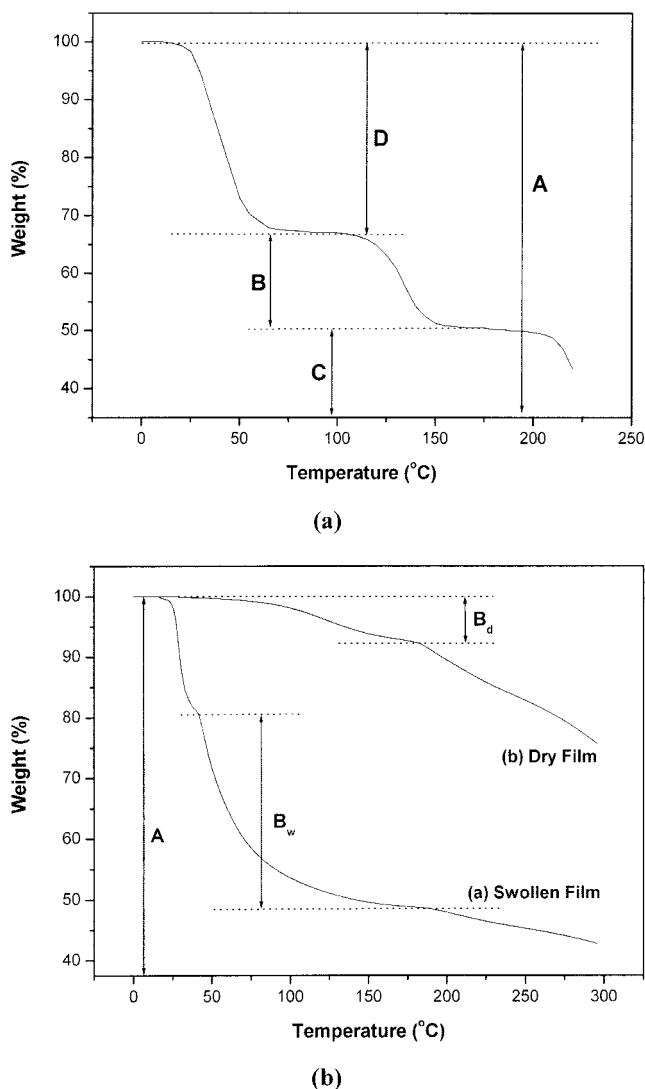

(a)

(b)

Figure 4 (a) Typical TGA curve of swollen films and (b) TGA curves of nonfreezing bound water separated from free water and freezing bound water. B_d is the amount of nonfreezing water of the dry film, and B_w is the amount of nonfreezing water of the swollen film.
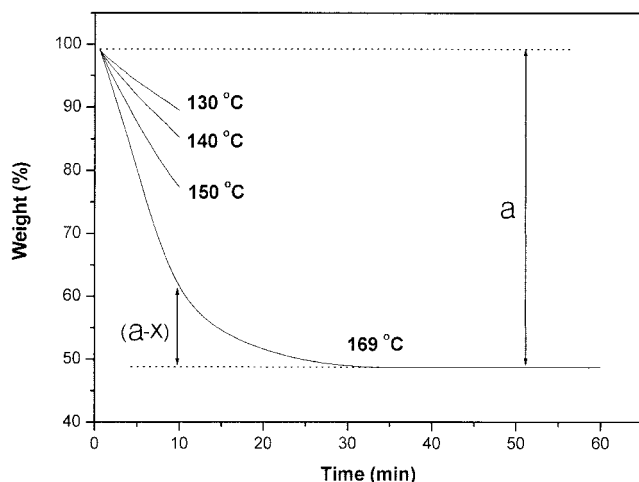
Figure 5 Weight-loss curves of HAVA11 at several isothermal temperatures.

TABLE III
Rate Constants (*ks*) of HAVA11 at Several Temperatures

Temperature (°C)	Time (min)			
	2	5	9	11
169	0.071	0.071	0.071	0.078
150	0.050	0.050	0.047	0.048
140	0.037	0.037	0.028	0.025
130	0.027	0.027	0.019	0.018

mal weight-loss curves in Figure 5, k values were calculated according to the first-order kinetics at 5 min. The energy of activation was obtained from a linear least-squares analysis (Arrhenius plot) on the plot of ln k versus $1/T$. The slope of the resultant plot, when multiplied by the gas constant (8.314 J/mol k), revealed the energy of activation. The activation energy determined from this slope was 36.6 kJ/mol.

remained up to 180°C. The first step of vaporization was observed around 90–170°C. The weight-loss temperature, on the high-temperature side, depended on the chemical structure of the HA/PVA IPN hydrogel film. On the high-temperature side, the nonfreezing bound water was separated from the bound water. The calculated values of W_{nf} from the TGA curves are listed in Table II, along with those obtained by DSC.

Figure 5 shows the weight-loss curves of HAVA11, isothermally and at several temperatures. The 169°C curve was chosen for further evaluation because this temperature resulted in a completely dried IPN hydrogel film (−51% water) in a reasonable experimental time. This general low rate of drying can describe the first-order reaction kinetics:[28]

$$dC/dt = k(1 - C)^n \qquad (4)$$

where C is the fractional conversion with decreasing water content in a IPN hydrogel, t is the time, k is the rate constant (min^{-1}), and n is the reaction order. Therefore, the reaction rate for drying becomes

$$\frac{dx}{dt} = k(a - x) \Rightarrow k = \frac{1}{t}\ln\left[\frac{a}{a - x}\right] \qquad (5)$$

Calculated from the values of a, $a - x$, and t obtained from the experimental curve shown in Figure 5, the values of k are listed in Table III. Within the experimental accuracy, these data are consistent with first-order kinetics, at least up to $t = 9$ min. k of the IPN hydrogel increased with increasing temperature. The trend for k, which was temperature-dependent, was itself used for further analyses, such as the activation energy.

Figure 6 shows the activation energy for the drying reaction of HAVA11. With the data from the isother-

CONCLUSIONS

HA/PVA IPN hydrogels were prepared by a sequential method. DSC was used for the quantitative determination of T_g and the amounts of free water, including the freezing bound water. The amount of free water in the IPN hydrogels increased as the HA content in the HA/PVA IPN hydrogels increased. The IPN hydrogels exhibited relatively high bound water contents. The HAVA31 sample, which contained the highest HA content of the samples, also showed the highest total water content. The TGA curve showed the separation of the nonfreezing bound water from the bound water in the swollen and dry films. At the same time, the exact amount of restrained water in the dry samples was evaluated. The activation energy for the drying reaction of the hydrogels was calculated.
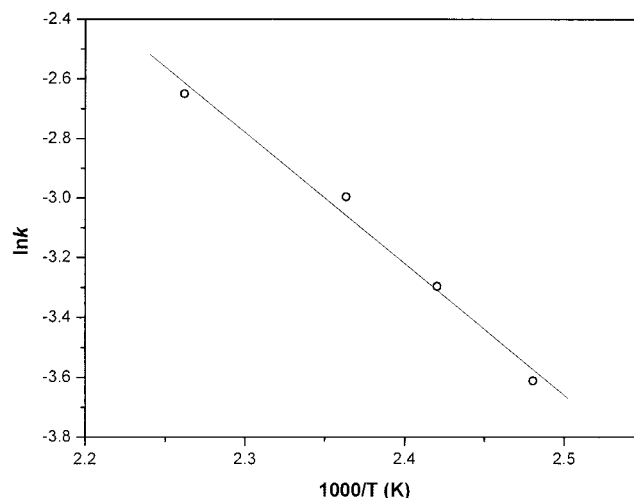

Figure 6 Activation energy for the drying reaction of HAVA11.

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