Preparation and Characterization of Crosslinked and Heat-Treated PVA–MA Films

FENG JYH LIOU and YNG JIIN WANG*

Institute of Biomedical Engineering, National Yang Ming University, Shih Pai, Taipei, Taiwan, Republic of China

SYNOPSIS

Two kinds of water-insoluble PVA–MA hydrogel films were prepared from PVA–MA, an esterification product of poly(vinyl alcohol) and maleic anhydride, by heat treatment and a crosslinking reaction, respectively. Both films changed their dimensions upon environmental pH changes. The crosslinked gel expanded to approximately 230% of its original length, with most changes occurring in the pH range of 2–7. The heat-treated PVA–MA film swelled stepwisely, with about 45% of the total expansion occurring at pH 2–7 and the remaining 55% at pH 9–12. Results from IR spectra analysis and acid–base titration suggest that the ionization of carboxylic acid accounts for the pH-induced gel swelling irrespective of the differences in the swelling behavior of these two gels. The pK_a values of the heat-treated PVA–MA gel increased from 4 to 10 while the degree of dissociation varied from 0 to 80%, whereas that of the crosslinked PVA–MA film is limited in a range of 3.3–4.2. The permeability of glucose across the crosslinked PVA–MA film increased when pH was raised from 2 to 7. No significant change of permeability was noticed between pH 7 and 12. For the heat-treated PVA–MA film, glucose permeability increased when pH was changed from 2 to 7 and from 7 to 12. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Hydrogels are well known for their excellent biocompatibility in biomedical applications. Of the various biomedical hydrogels, poly(vinyl alcohol) (PVA) and its derivatives are of special interest.[1] PVA is commercially available with different molecular weight distributions and various saponification extents. As a hydrogel, PVA contains a significant amount of water but still retains fair mechanical strength.[2] PVA has been used as the material for artificial tendon and artificial larynx. In addition, membranes for kidney dialysis and wound coverings can be prepared from PVA.[3] A crosslinked PVA gel was commercialized under the trade name of Ivalon®. This material was, however, found to cause adverse effects *in vivo* due to calcification. Heparin-bounded PVA[4,5] has been recently evaluated as a material used in blood contacting.

Hydrogels that contain ionizable groups change their dimensions upon changing the solution pH. Several pH-sensitive hydrogels have been investigated for their possible applications in controlled drug release.[6] Among the commonly studied pH-sensitive polymeric materials are the derivatives of methacrylate and acrylamide.[7,8]

PVA does not contain any ionizable group. It therefore is incapable of alternating its dimensions upon pH changes. Nevertheless, since PVA has a high content of the hydroxyl group, it can be esterified with dicarboxylic acid to form anionic macromolecules.[9–11] By employing 1,1,1-trimethyloyl propane trimethacrylate (TMPTMA) as a crosslinker, we previously prepared a copolymer of PVA and 2-hydroxyethyl methacrylate (HEMA). The poly(HEMA-*co*-[PVA–MA]) (MA = maleic anhydride) film thus formed was found undergo swelling and dehydration in response to pH change.[12] Since PVA–MA contains multiple maleic acid residues, it may have functioned as a macrocrosslinker in the copolymerization reaction of PVA–MA and HEMA. It is conceivable that PVA–MA molecules by them-

* To whom correspondence should be addressed.

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selves can form a crosslinked hydrogel network. We thus prepared the crosslinked PVA–MA hydrogel and its swelling characteristics are discuss in this article. In addition, a water-insoluble, pH-sensitive PVA–MA hydrogel is fabricated by a heat-treatment procedure similar to that employed in making insoluble PVA films. The properties of these two PVA–MA hydrogels are discussed here.

EXPERIMENTAL

Materials

Poly (vinyl alcohol) (PVA), maleic anhydride (MA), and dimethyl sulfoxide (DMSO), all of reagent grade, were purchased from Merck-Schuchardt (Germany). PVA DP = 1700, with a 98.5–99.2% degree of saponification and a molecular weight of 72,000 was used throughout this study. 2,2-Diethoxyacetophenone (DEAP) from TCI (Kasei Co., Tokyo, Japan) was used as the initiator for the crosslinking reaction. The glucose concentration in the solution was measured using a glucose analysis kit (Unison Biotech. Co., Taiwan). Methanol, acetone, and other organic solvents were used without further purification.

Methods

Esterification of PVA with MA

MA (11.2 g) was mixed with 5 g PVA in 50 mL DMSO and stirred with a magnet in a 250 mL reaction flask. The esterification reaction was performed at 60°C for 5 h; after that, the reaction mixture was poured into water to form a fibrous PVA–MA product. The PVA–MA was further purified by repeated solubilization in methanol and precipitation in water according to the method of Liou et al.[12]

Characterization of PVA–MA

PVA–MA precipitated from water was freeze-dried to form powders. These powders were then mixed with KBr and pressed into a disc for IR analysis with a Perkin-Elmer 983 spectrometer.

Both acid–base titration and NMR analysis were used to determine the esterification extent of PVA–MA. The acid content was estimated by the signal ratio of the carboxyl proton (—COOH) to the hydroxyl proton (—OH) from NMR spectra. Alternatively, the esterification extent (E) can be calculated by the following equation using the data derived from acid–base titration:

$$E = m \times M/\text{wt} \quad (1)$$

where m is the moles of NaOH consumed in titrating the PVA–MA solution to pH 7.0; wt, the dry weight (g) of PVA–MA; and M, the molecular weight of PVA–MA. The molecular weight of PVA–MA is related to the extent of esterification, E, by the following equation:

$$M = 72000 + 99 \times E - E \quad (2)$$

where 72,000 is the average molecular weight of PVA and 99 is the molecular weight of the maleic acyl group, i.e., —(CO)CHCHCOOH.

Preparation of PVA–MA Films

The water-insoluble PVA–MA films were prepared in two forms of gels by heat treatment and crosslinking. The heat-treated PVA–MA film was fabricated by spreading a methanol solution of PVA–MA on a coverslip followed by evaporating the solvent in an oven preheated to 130°C. After 24 h, the film was immersed in water and peeled off from the glass surface.

To form the crosslinked PVA–MA film, DEAP (final concentration 0.4%) was added to the PVA–MA methanol solution as the reaction initiator. The reaction mixture was spread on a coverslip and then loaded in a chamber which was purged with N_2 gas. The crosslinking reaction was performed by irradiation for 30 min using a light source (365 nm, Model B-100, Black-ray, U.S.A.) 25 cm away from the cover glass. The film was peeled off from the glass and washed with distilled water three times. It was immersed and stored in water until use.

Characterization of PVA–MA Films

The gel content of the PVA–MA film was determined from the weight of the dried gel before and after solvent extraction. Since both methanol and water (pH 7.0) are good solvents for PVA–MA, they were used to extract the unreacted residual polymer molecules from the film. All films were dried at 100°C in oven overnight before being weighed. The gel content (G) of the polymeric film is calculated according to the following equation:

$$G = (w - w_0)/w_0 \quad (3)$$

where w is the dry weight of the gel before solvent extraction, and w_0, the dry weight of the gel after solvent extraction.

For infrared spectra analysis, PVA–MA films were peeled off the glass, immersed in water, and adjusted to the designated pH with sodium hydroxide solution. The films were then dried in air for IR analysis.

For pH titration, the PVA–MA films were cut into small pieces and suspended in 0.2% NaCl solution. The mixture was then titrated with 0.1N NaOH with a Radiometer® TTT80 pH titrator. Titration of the 0.2% NaCl solution in the absence of PVA–MA was taken as a blank value.

Swelling of PVA–MA Films

PVA–MA film immersed in water (about 25 μm thick) was cut with a razor blade to 1 × 0.2 mm in dimension. The gel was then immersed in saline and adjusted to the designated pH with 0.1 N NaOH. The rate of gel expansion is determined by measuring the change in gel length at various time intervals after pH changed from 7 to 2. The dimension change was usually completed within 2–3 min. The equilibrium gel length at each pH was measured 30 min after the pH change. The swelling ratio of hydrogel at equilibrium, ESR, is defined as follows:

$$\mathrm{ESR} = (L - Lo)/Lo \quad (4)$$

where Lo is the original gel length, and L, is the equilibrium gel length at the designated pH.

Permeability of Glucose Through the PVA–MA Membranes

The transfer of glucose through PVA–MA membranes were measured using a mass transfer apparatus. The apparatus consists of a diffusion chamber partitioned in the middle by a PVA–MA membrane into two cells. One of these cells was filled with 500 mg/mL glucose in 0.2% NaCl, while another cell contained 0.2% NaCl. At various time intervals, aliquots of 10 μL were withdrawn from each cell and the concentration of glucose in the sample was determined by enzyme assay. The permeability of glucose across the membrane was determined using the following equation[13]:

$$\mathrm{Ln}[(1 - C_2/C_1)/(1 + V_2 \times C_2/V_1 \times C_1)] = -[P \times A \times ((1/V_1) + (1/V_2))/L] \times t \quad (5)$$

where C_1 and C_2 are the glucose concentrations in compartments 1 and 2, respectively; V_1 and V_2 the volume of compartments 1 and 2, respectively; L and A, the thickness of the membrane and the contact area between two compartments, respectively; and P, the permeability of glucose across the membrane.

RESULTS

Characterization of PVA–MA

The IR spectra of PVA and PVA–MA are shown in Figure 1. The absorption pattern of PVA in the IR spectra is very similar to that reported by Murahashi et al.[14] Stronger absorption between 3000 and 3500 cm^{-1} was observed for PVA–MA as compared to PVA, indicating the presence of extensive inter-and intrahydrogen bonds between the hydroxyl groups and carboxylic acids. Two additional strong absorption peaks at 1640 and 1720 cm^{-1} were noted in the spectra of PVA–MA. These resulted from the stretching of —C=C— and —C=O bonds of the maleic ester, respectively. The extent of esterification was estimated to be about 41% from the pH titration experiment, which is comparable with a value of 43% calculated from NMR spectroscopy analysis.

Properties of PVA–MA Films

As shown in Figure 1, the IR spectra of heat-treated PVA–MA film is almost identical to that of powdered PVA–MA, indicating that heating at 130°C did not elicit any chemical reaction. On the other hand, the 1640 cm^{-1} stretching peak disappeared in the spectra of crosslinked PVA–MA, suggesting that almost all of the —C=C— groups were involved in the crosslinking reaction.

When immersed in phosphate-buffered saline (PBS, pH 7.4), heat-treated and crosslinked PVA–MA films remained insoluble but absorbed water to about 77 and 76% of total weight, respectively. Instead, the PVA–MA film prepared without heat treatment dissolved completely in PBS at room temperature. Extensive washing with a solvent did not extract any significant amounts of polymer from either one of the two hydrogel networks (Table I).

pH-Induced Hydrogel Swelling of PVA–MA Films

The hydrogels based on PVA–MA changed dimension in response to the changing of the solution pH.

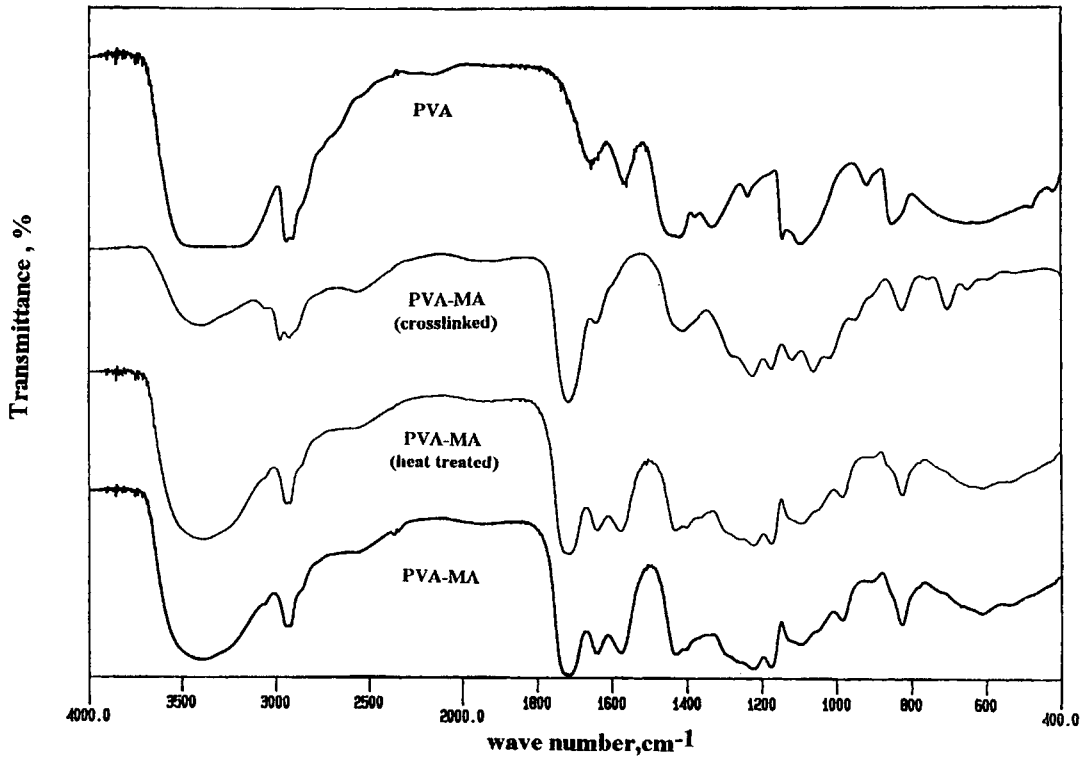

Figure 1 The IR spectra of PVA and PVA–MA films.

As shown in Figure 2, the length of crosslinked PVA–MA film decreased with time after the medium pH decreased from 7 to 2. The change is almost completed within 2 min after pH changes. Both crosslinked and heat-treated PVA–MA films had about the same shrinking rates. The extent of this pH-induced gel shrinkage is independent of the original gel length. It is worth mentioning that the crosslinked PVA–MA film shrank by 60%, which is about twofold the shrinkage observed for heat-treated PVA–MA film by changing the pH of the hydrogel from 7 to 2.

The effect of pH on the equilibrium swellability of the crosslinked PVA–MA hydrogel is shown in Figure 3. As can be seen, a major dimensional change occurred between pH 2 and 4. The equilibrium gel length reached a maximum at pH 7 and remained constant thereafter up to pH 12. The extent of gel expansion is reduced by increasing NaCl concentration. However, the pH at which 50% of the max-

Table I Gel Contents of PVA–MA films

	Gel Content (% Wt)	
PVA–MA films	Water Extraction	Methanol Extraction
Crosslinked	95.3 ± 0.5	96 ± 2
Heat-treated	95.8 ± 0.2	97 ± 2

Extraction of each film was performed in triplicate. Water extraction was conducted at pH 7.0.

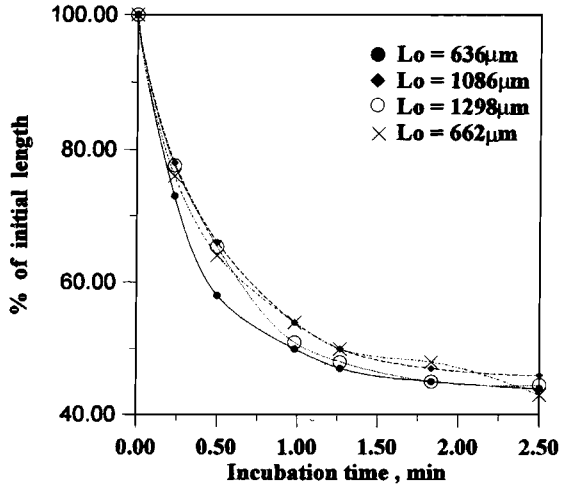

Figure 2 The dimensional change of crosslinked PVA–MA hydrogels as induced by a pH change from pH 7 to 2.

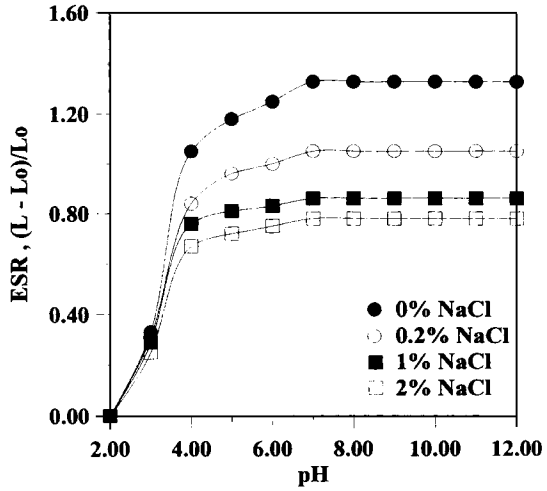

Figure 3 The effects of solution pH on the equilibrium swelling ratio of crosslinked PVA–MA hydrogel.

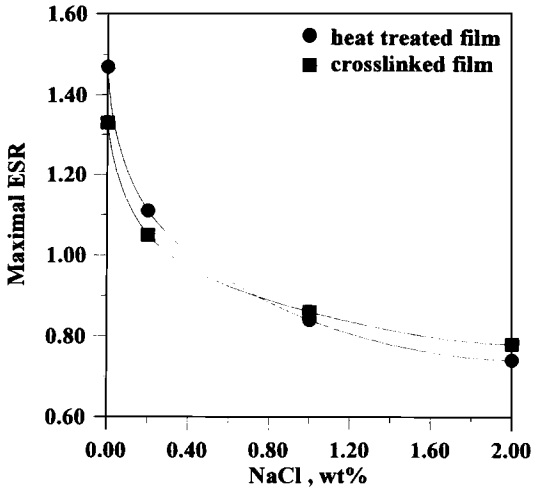

Figure 5 The effects of NaCl concentration on the equilibrium swelling ratio of PVA–MA films. The maximal ESR is defined as the equilibrium swelling ratio of hydrogel attained by increasing the solution pH from 2 to 12.

imal equilibrium gel expansion being attained remained unchanged at about 3.3. This value is between the first pK_a values of maleic acid (2.0) and succinic acid (4.1).

The gel swelling behavior of the heat-treated PVA–MA film is different from that of the crosslinked film (Fig. 4). In addition to the expansion that occurred at pH 2–7, another major dimensional change took place at pH 9–12. This delayed swelling accounted for at least 50% of the total gel expansion. Despite their different swelling patterns, high salt was shown to dampen the extent of gel swelling for both of the crosslinked and heat-treated PVA–MA films. Both gels also had about the same extent of equilibrium gel swelling by increasing the pH from 2 to 12. In addition, the effect of ionic strength on the maximal equilibrium gel length is similar for both gels (Fig. 5).

Relationship of Acid Dissociation and Gel Swelling

The driving force for swelling of the PVA–MA films can be attributed to the dissociation of pendant maleic acid. The extent of acid dissociation can be monitored by the amounts of base added in the

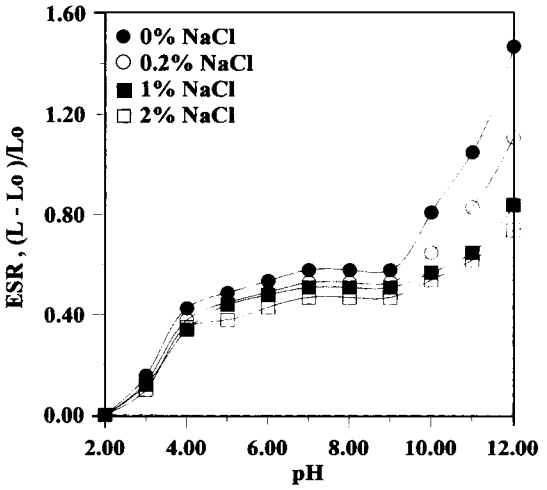

Figure 4 The effects of solution pH on the equilibrium swelling ratio of heat-treated PVA–MA hydrogel.

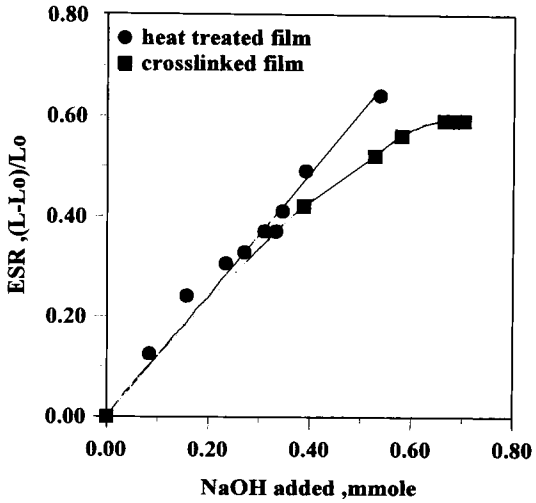

Figure 6 The relation between the equilibrium swelling ratio and the amounts of NaOH consumed.

course of pH titration. Figure 6 shows the relationship of ESR and the volume of NaOH consumed during titration. As indicated, gel lengths were shown to be proportional to the degree of acid dissociation.

Further evidence supporting acid dissociation came from IR spectra analysis of PVA–MA films at various pHs. The stretching of the acid carbonyl group typically gives an absorption peak at around 1720 cm^{-1}. Dissociation of acid resulted in the resonance of the carboxylate ion and shifted the stretching absorption to a lower frequency of 1580 cm^{-1}. As shown in Figure 7, when the pH was increased from 2 to 7, a strong absorption peak appeared at 1580 cm^{-1}, indicating the dissociation of carboxylic acid. The pendant maleic acid of the crosslinked PVA–MA appeared to be fully dissociated at pH 7 since a further increase of the pH to 12 did not increase the intensity at 1580 cm^{-1}. By contrast, a significant portion of the pendant maleic acid of PVA–MA in the heat-treated film was dissociated at high pH. As indicated in Figure 8, the absorption of carboxylate ions at 1580 cm^{-1} increased consecutively as the pH was raised from 2 to 7 and from 7 to 12. Therefore, results from both acid–base titration and IR analyses indicate that the delayed gel swelling correlated well with that significant amounts of the pendant maleic acids of the heat-treated PVA–MA film remained undissociated at neutral pH.

Permeability of Glucose Across the Hydrogel Films

Mass transfers of glucose molecules across the crosslinked and heat-treated PVA–MA films were measured to evaluate the permeability of the films at various medium pH. Since PVA–MA gels expanded at higher pH, it is conceivable that the permeability of glucose molecules across the PVA–MA membrane will increase by raising the solution pH. As shown in Figure 9, permeability of glucose molecules across the crosslinked PVA–MA film increased when solution pH was raised from 2 to 7. No significant difference in permeability was observed between 7 and 12. For the heat-treated film (Fig. 10), increasing glucose permeability was seen when pH was changed from 2 to 7, and from 7 to 12. The permeabilities of glucose at various pHs are listed in Table II.

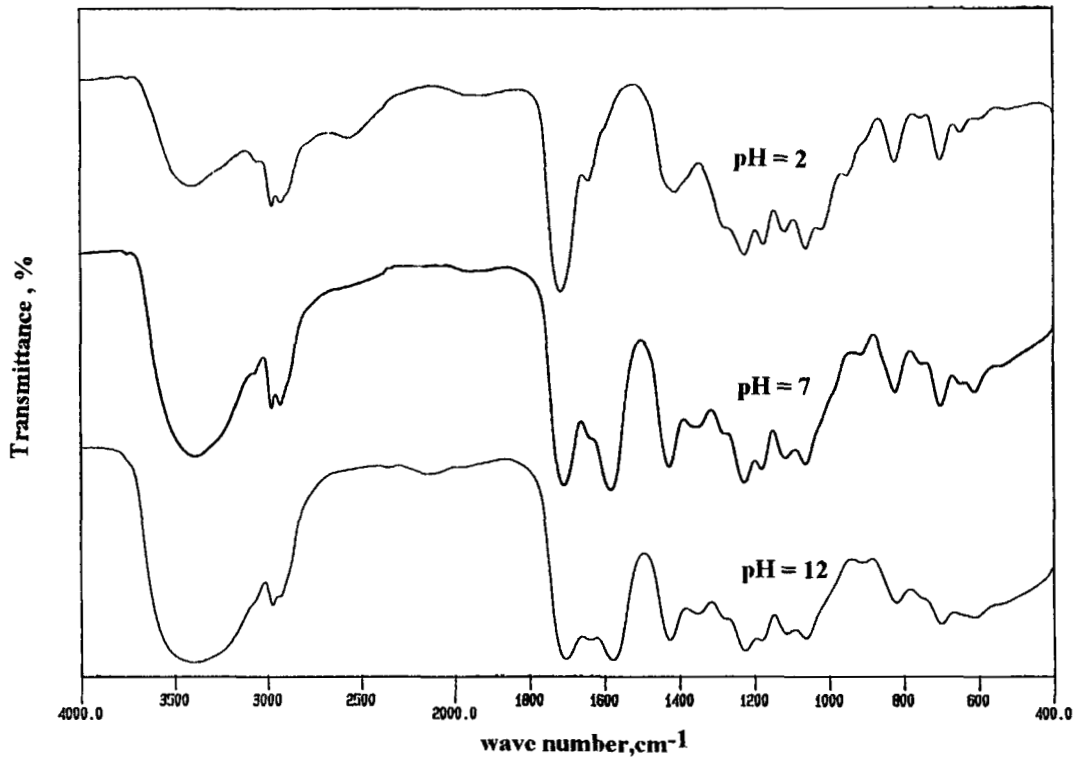

Figure 7 IR spectra of crosslinked PVA–MA films at pH 2, 7, and 12.

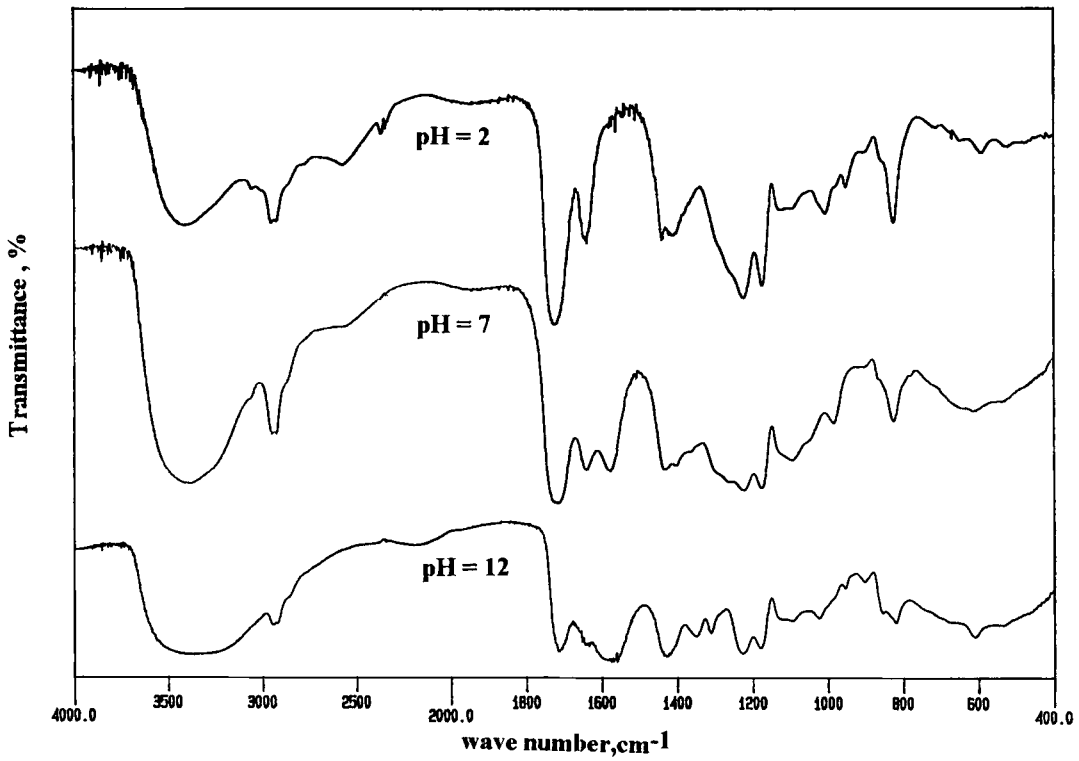

Figure 8 IR spectra of heat-treated PVA–MA films at pH 2, 7, and 12.

DISCUSSION

We previously reported that PVA–MA and HEMA could be crosslinked with TMPTMA to form a pH-sensitive poly(HEMA-*co*-[PVA–MA]) hydrogel. This copolymeric film expanded to about 120% of its initial length when pH was changed from 2 to 7.

We now report that the hydrogel, prepared by crosslinking PVA–MA molecules alone, had a greater swelling capacity. This PVA–MA gel expanded to about 230% of its original length in response to a change of pH from 2 to 7. This greater expansion potential is due to the presence of a higher content of carboxylic acid in hydrogels.

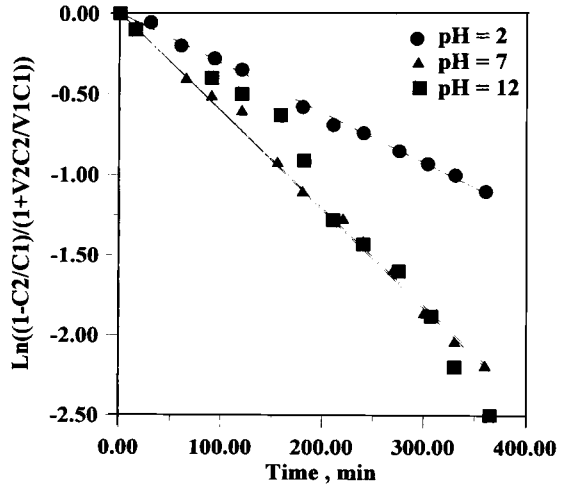

Figure 9 The semilog plot for glucose permeability across crosslinked PVA–MA membrane using eq. (5).

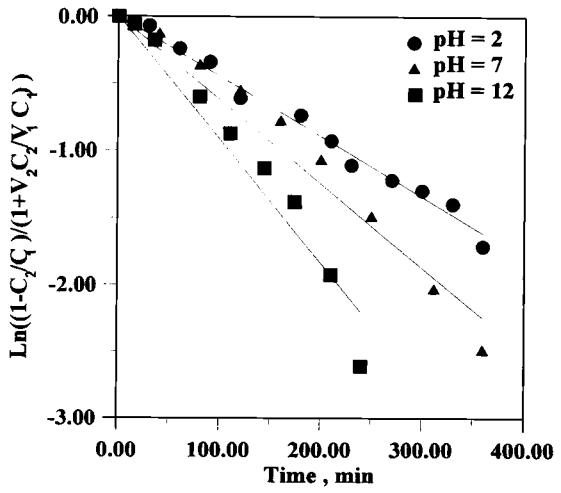

Figure 10 The semilog plot for glucose permeability across heat-treated PVA–MA membrane using eq. (5).

Similar to the heating procedure employed to form water-insoluble PVA film, PVA–MA can also be heat-treated to form a water-insoluble hydrogel. The heat-treated PVA–MA film obtained in this study is amorphous, as indicated by the absence of any peak in small-angle X-ray diffraction analysis.

Gel swelling is a consequence of the balance between an ionization-induced water influx into the gel network and the constraint imposed by the polymer chains. Brannon-Peppas and Peppas[15] demonstrated that the polyacid hydrogels swelled abruptly when the medium pH is raised above the pK_a value of the acid. In this study, we found that the crosslinked PVA–MA film swelled abruptly at pH 3.3, corresponding to the midpoint between the first pK_a values of maleic acid and succinic acid. The crosslinked gel was fully expanded at pH 7.0. In contrast, the heat-treated PVA–MA film was only half-expanded at neutral pH and attained its fully expanded form at pH 9–12. This stepwise gel expansion is probably due to the delayed acid dissociation of the pendant maleic acid of the heat-treated PVA–MA films.

The delayed acid dissociation of annealed PVA–MA film was demonstrated by plotting the pK_a values of polyelectrolyte vs. the degree of dissociation. Different from the well-defined pK_a of organic acids, polybasic acids may have apparent pK_a values extending over a wide pH range. This is because the dissociation of the first few acids affects the pK_a of the remaining pendant acids by shielding the residual acid units of the same polymer.[16] The degree of dissociation, α, affected the apparent dissociation constants of both the crosslinked and the heat-treated PVA–MA films (Fig. 11). The pK_a values of maleic acids in the annealed PVA–MA changed gradually from pH 4 to 10 as the degree of dissociation varied up to 80%. In contrast, pK_a values of the crosslinked PVA–MA were limited in a rather narrow range (pH 3.3 to 4.2) during the process of acid dissociation. These observations can fully explain the difference between the gel swelling behavior of crosslinked PVA–MA and heat-treated films. It is believed that the polymeric chains in the heat-treated PVA–MA film are more flexible than those in the crosslinked PVA–MA film. Therefore, the charged carboxylate group formed during the acid dissociation process may have a greater shielding effect on the remaining pendant maleic acid. This shielding effect, in turn, increased the pK_a of the residual maleic acid of PVA–MA. Work to elucidate the underlying mechanisms of the pH-induced expansion of these films is currently being undertaken.

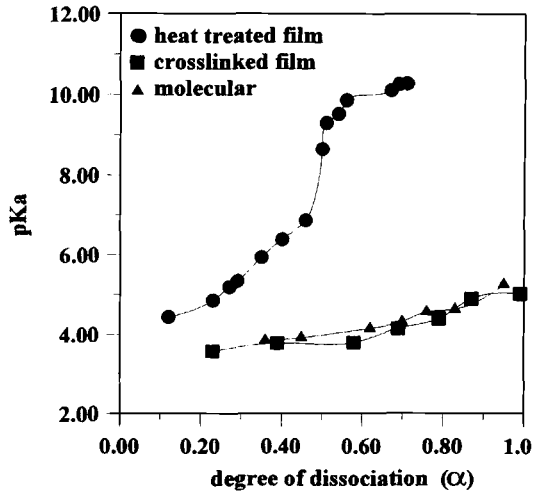

Figure 11 The effects of acid dissociation on apparent pK_a values of the PVA–MA macromolecules and two PVA–MA films.

Table II Permeability of Glucose Across PVA—MA Membranes at Various pH

PVA–MA membrane	Permeability ($\times 10^{-6}$ cm^2/s)		
	pH 2	pH 7	pH 12
Crosslinked	1.7	5.4	5.4
Heat-treated	2.5	4.0	8.2

In conclusion, we have successfully prepared two pH-sensitive water-insoluble hydrogels based on PVA–MA. These two hydrogel films exhibited a greater extent of pH-induced gel swelling as compared to that of the previously reported poly(HEMA-*co*-[PVA–MA]) films. The two PVA–MA films had distinct gel swelling behaviors which are closely correlated with the dissociation of the pendant maleic acids.

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