

# Preparation and Characterization of Alginate Hydrogel Membranes Crosslinked Using a Water-Soluble Carbodiimide

J. B. Xu, J. P. Bartley, R. A. Johnson

Centre for Instrumental and Developmental Chemistry, Queensland University of Technology, 2 George Street, Brisbane 4001, Australia

Received 24 September 2002; accepted 30 December 2002

**ABSTRACT:** The preparation of alginate hydrogel membranes by the film immersion method was optimized for maximum crosslinking using swelling measurements as an indicator of the degree of crosslinking. The variables investigated were the concentration of the nonsolvent (ethanol) for sodium alginate, water-soluble carbodiimide (WSC) concentration, and pH of the crosslinking medium. Optimum conditions resulted when the crosslinking medium contained 60 vol % ethanol and 100 mM WSC at pH 4. Membranes prepared using different ethanol concentrations (100 mM WSC, pH 4) and different WSC concentrations (60 vol % ethanol, pH 4) were investigated using infrared spectroscopy. The spectra showed the characteristic ester linkage (crosslinking) band at  $1698\text{ cm}^{-1}$  in cases where swelling measurements indicated that crosslinking had occurred. Differential scanning calorimetry of noncrosslinked and

crosslinked membranes indicated that crosslinking increased the crystallinity of the membrane. Durability trials showed that membranes crosslinked using the optimum conditions determined in this work retained all weight when immersed in water for 32 days. Membranes prepared using these conditions possessed the characteristics required for use in the pervaporation separation of ethanol–water mixtures. These membranes also have potential as protective coatings for hydrophobic, microporous membranes in the membrane distillation and osmotic distillation concentration of feeds containing surface active components. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 90: 747–753, 2003

**Key words:** hydrophilic polymers; FTIR; membranes; hydrogels; crosslinking

## INTRODUCTION

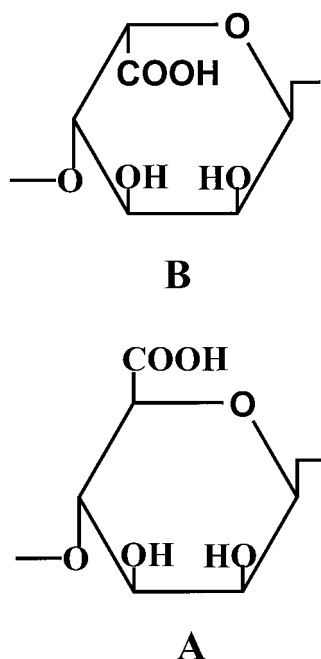
Hydrogels are hydrophilic polymeric materials such as polysaccharides that are swollen by, but do not dissolve in, water.<sup>1</sup> Alginate is an anionic, hydrophilic polysaccharide that is used in a wide range of industrial and medical applications in both the non-crosslinked and hydrogel forms. This species is a copolymer of  $\beta$ -D-mannuronic acid and  $\alpha$ -L-guluronic acid linked together in varying proportions by 1-4-linkages.<sup>2</sup> The structures of these units are shown in Figure 1. Alginate has been extracted commercially from brown seaweeds such as *Laminaria digitata*. It has also been extracted from fermentation broths of bacteria such as *Azotobacter vinelandii*.<sup>3</sup>

Industrially, alginate has been used as a stabilizing, thickening, and gelling agent in the food industry,<sup>4</sup> a surface uniformity enhancer in the paper manufacturing industry, and a color brightness enhancer in the textile industry.<sup>2</sup> Also, the natural origin of alginate has resulted in considerable research into medical applications. In that regard, alginate–chitosan–polyeth-

ylene glycol microcapsules have provided an immunosolative membrane to entrap islets of Langerhans in a bioartificial pancreas.<sup>5</sup> Alginate has also been used in combination with hyaluronate for cartilage repair<sup>6</sup> and in combination with gelatin for use as a wound dressing material and as a drug delivering agent.<sup>7</sup> Alginic acid, the protonated form of alginate, has been combined with various heterocyclic and aromatic amines to facilitate gene transfer.<sup>8</sup>

In the field of membrane technology, alginate membranes have attracted considerable interest for use in the pervaporation separation of ethanol–water mixtures.<sup>9–11</sup> These membranes have provided larger water fluxes and separation factors than those cast from hydrogels that have been established in this application, for example, poly(vinyl alcohol) crosslinked with amic acid.<sup>12</sup> Despite this superior performance, however, noncrosslinked alginate membranes have been found to be unsuitable for applications other than dehydration applications because of their water-solubility and mechanical weakness. Membranes crosslinked by cations such as  $\text{Ca}^{2+}$  were also found to be unsuitable because of a loss of mechanical properties with time attributed to the loss of cations.<sup>13</sup> Alginate membranes covalently crosslinked with glutaraldehyde<sup>14,15</sup> were found to be water-insoluble and to

Correspondence to: R. Johnson (ra.johnson@qut.edu.au).



**Figure 1** Structural units of alginic acid. (A)  $\beta$ -D-mannuronic acid. (B)  $\alpha$ -L-guluronic acid.

possess stable mechanical properties. However, glutaraldehyde is known to be toxic when released as a result of biodegradation<sup>7</sup> and is therefore unsuitable for use in food and pharmaceutical separation applications.

Several methods for membrane fabrication using alginate in combination with other polymers have also been reported. For example, an alginate top layer has been crosslinked to an aminated polyacrylonitrile<sup>16</sup> sublayer using heat curing to effect amide bond formation. Alginate has also been blended with cellulose cuoxam,<sup>17</sup> with intermolecular hydrogen bonding as the only crosslinking interaction, and with poly(vinyl alcohol),<sup>18</sup> using glutaraldehyde as the crosslinking agent.

The present work involved the fabrication and characterization of a single-polymer, crosslinked alginate membrane that was nontoxic, water-insoluble, and durable. In addition to pervaporation applications, a hydrogel membrane having these characteristics has potential for use as a protective coating for microporous, hydrophobic membranes in membrane distillation and osmotic distillation applications. It is envisaged that the hydrogel coating will prevent wet-out of the hydrophobic substrate by surface-active feed components such as oils, fats, and detergents.

A water-soluble, nontoxic<sup>7</sup> carbodiimide [WSC; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] has been shown to be effective in the crosslinking of protein. WSC facilitates the formation of an amide linkage from a carboxylic acid and an amine group in aqueous media without itself remain-

ing part of the linkage.<sup>19,20</sup> WSC was also shown to facilitate the formation of an ester linkage between a carboxylic acid and a hydroxyl group of hyaluronic acid.<sup>21</sup> Alginate also contains carboxylic acid and hydroxyl groups and hence WSC was used as the crosslinking agent in the present work. The crosslinking process was optimized in terms of film nonsolvent (ethanol) concentration, WSC concentration, pH, and membrane durability. Membranes were characterized using FTIR, differential scanning calorimetry (DSC), swelling measurements, and durability testing.

## EXPERIMENTAL

### Materials

Sodium alginate (Manugel GMB) was donated by Germantown International Limited (Sydney, Australia). WSC was purchased from Sigma-Aldrich (Sydney, Australia). Ethanol (spectrophotometric grade) was purchased from Aldrich Chemical Co. (Sydney, Australia). Hydrochloric acid (32% content, AR grade) was purchased from BDH Chemicals (Melbourne, Australia). Potassium hydroxide (AR grade) was purchased from AJAX Chemicals (Sydney, Australia). Deionized water was used in all experiments. All chemicals were used without further purification.

### Membrane preparation

An aqueous solution of sodium alginate (2.0 wt %) was used for membrane casting. To prevent alginate lumping and incomplete dissolution during solution preparation, 1–2 mL of ethanol was first added to disperse the alginate powder (0.6 g). Water (30 mL) was then added with vigorous stirring. The solution was then heated to  $60 \pm 5^\circ\text{C}$  with constant stirring until a homogeneous solution was obtained ( $\sim 30$  min). The clear solution was then poured into polystyrene petri dishes (diameter 5 cm), taking care that all air bubbles had first been allowed to escape from the viscous medium. This resulted in a solution depth of 0.3 mm. The cast solution was then allowed to dry in air for 2 days to form a membrane. The thickness of this membrane was 30–40  $\mu\text{m}$ . More dilute sodium alginate solutions (1.0 wt %) were also used for membrane preparation to obtain samples with a suitable thickness for examination by transmission infrared spectroscopy. The thickness of these membranes was 15–20  $\mu\text{m}$ .

For crosslinking purposes, the dried sodium alginate membranes were cut into small pieces ( $1.5 \times 1.5$  cm) and immersed in an ethanol–water mixture (5 mL) to which WSC powder and hydrochloric acid (for pH adjustment) had been added. The ethanol–water mixture composition and the WSC and hydrochloric acid concentrations were varied for the purpose of method

optimization. The crosslinking reaction was allowed to continue for 24 h at  $24 \pm 2^\circ\text{C}$ . The crosslinked membranes were removed from the reaction solution, soaked in water with stirring for 1 min to remove residual WSC and acid, and then rinsed with ethanol and air-dried.

### Measurement of membrane swelling

Measurements of membrane swelling in water were carried out to determine the degree of crosslinking achieved in the optimization experiments. A lower degree of swelling was attributed to a higher degree of crosslinking.<sup>21</sup> Membrane swelling was also determined for water-soluble, noncrosslinked membranes using ethanol–water mixtures as the swelling medium. The latter experiment was undertaken to determine the optimum ethanol concentration for use in subsequent crosslinking reactions.

All membranes used in this work were allowed to establish their equilibrium moisture content in air at  $24 \pm 2^\circ\text{C}$  before being placed in the swelling medium. Equilibrium was taken to correspond to the establishment of constant weight in the constant humidity environment. Dry (air-equilibrated) membranes were weighed and then immersed in water or ethanol–water mixtures of various compositions for 24 h at  $24 \pm 2^\circ\text{C}$ . The swollen membranes were then placed between two dry filter papers to remove residual liquid from the membrane surface and then weighed. The water or ethanol–water mixture content of the membrane was calculated using the following equation:

$$\text{Water or mixture content (\%)} = \frac{W_s - W_d}{W_s} \times 100 \quad (1)$$

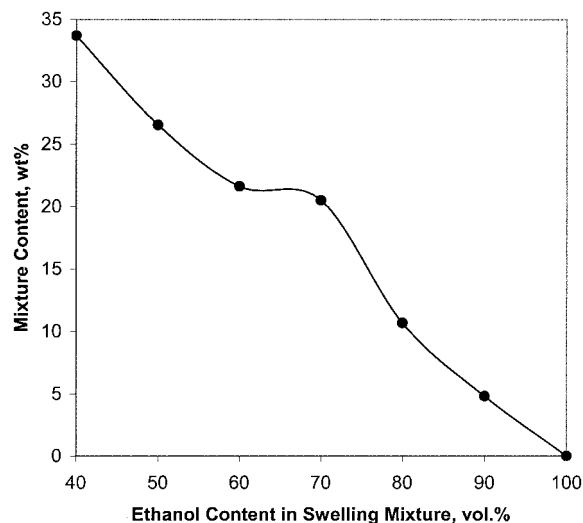
where  $W_s$  and  $W_d$  are the weights of swollen and dry membranes, respectively.

### Infrared spectroscopy

Infrared spectra of crosslinked and noncrosslinked sodium alginate membranes and of an alginic acid membrane were obtained using a Perkin–Elmer FT-IR 1000 spectrophotometer (Perkin Elmer Cetus Instruments, Norwalk, CT). The spectra were used to confirm the presence and type of crosslinking. Spectra were obtained in the transmission mode using thin films prepared as described above.

### Differential Scanning Calorimetry (DSC)

The heats of fusion and melting points of crosslinked and noncrosslinked membranes were measured using a Thermal Advantage DSC Q 100-0026 calorimeter



**Figure 2** Variation of the degree of swelling of non-crosslinked sodium alginate membranes with ethanol content of the swelling medium.

(TA Instruments-Waters, New Castle, DE). These were obtained to determine the effect of crosslinking on membrane structure. Membrane samples ( $2.1\text{--}2.8 \pm 0.1$  mg) were hermetically sealed in aluminum sample pans and heated at  $5^\circ\text{C min}^{-1}$  from 25 to  $300^\circ\text{C}$ . The system was purged with dry nitrogen at  $50$  mL  $\text{min}^{-1}$ .

### Membrane durability

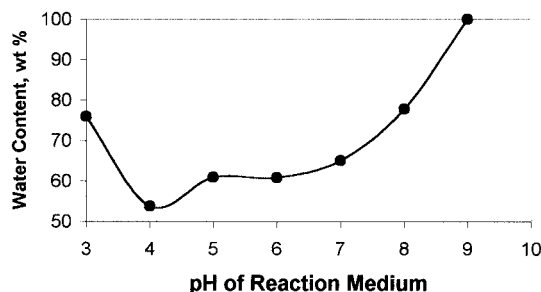
Crosslinked membranes were weighed and immersed in water for a total of 32 days. Each membrane was periodically removed, rinsed in ethanol, air-dried, and reweighed. A reduction in weight was taken to be indicative of membrane dissolution.

## RESULTS AND DISCUSSION

### Swelling measurements of noncrosslinked membranes

The water solubility of sodium alginate necessitated the use of a sufficiently high concentration of nonsolvent (ethanol) for this species to prevent dissolution of the membrane during the crosslinking process. On the other hand, the poor solubility of WSC in ethanol<sup>21</sup> necessitated the use of a sufficiently high water concentration to prevent precipitation of this species from solution.

The variation of the ethanol–water mixture content of the swollen membrane with mixture composition is shown in Figure 2. The figure shows that there was an apparent two-stage decline in the mixture content of the membrane with increasing ethanol content, the first between 40 and 60 and the second between 70 and 100 vol % ethanol. The apparent similarity in the mix-



**Figure 3** Variation of the degree of membrane swelling with pH of the crosslinking solution (60 vol % ethanol, 100 mM WSC).

ture content at 60 and 70 vol % ethanol was observed in quadruplicate experiments. The decline to an insignificant mixture content of the membrane at 100 vol % ethanol was also reported by Yeom and Lee<sup>15</sup> for sodium alginate membranes exposed to ethanol-water mixtures in the 70–100 vol % ethanol range.

The general trend to reduced swelling with increasing ethanol concentration over the entire mixture composition range was attributed to the increasing osmotic pressure of the mixture. Accordingly, this resulted in a progressive reduction in the quantity of water entering the polymer matrix. Ethanol was expected to make only a minor contribution to the mixture content of the membrane, despite the possibility of coupling sorption<sup>15</sup> between water and ethanol at lower ethanol concentrations. Indeed, pervaporation experiments on the dehydration of ethanol-water mixtures with 70–95 vol % ethanol using non-crosslinked sodium alginate membranes have given separation factors in the 500–700 range.<sup>11</sup> This corresponded to a maximum ethanol content of 3 wt % in the permeate, thus highlighting the high selectivity of the membrane for water.

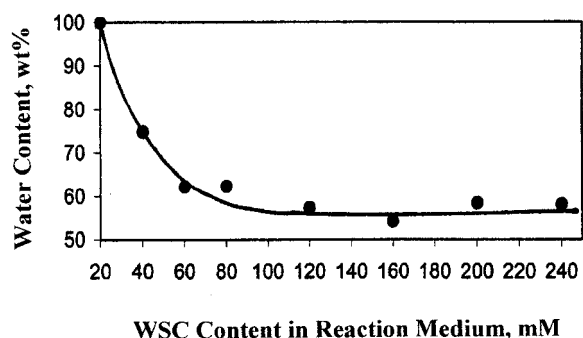
The apparent two-stage decline in membrane swelling was attributed to the exposure of additional hydration sites as polymer strands partially separate in a preliminary dissolution step at ethanol concentrations below 60 vol % ethanol. This would be expected to result in a greater than expected weight loss on drying, with a break in the smooth continuity of the plot. The partial separation of polymer strands below 60 vol % ethanol is likely to result in reduced crosslinking. Based on these observations, it was concluded that a swelling medium containing 60 vol % ethanol was suitable for use in subsequent crosslinking procedures. In addition to the requirement for WSC solubility, the high degree of swelling associated with this condition was required to ensure that membrane penetration by WSC was sufficient to provide the degree of crosslinking required for membrane stability.

### Effect of pH on the degree of crosslinking

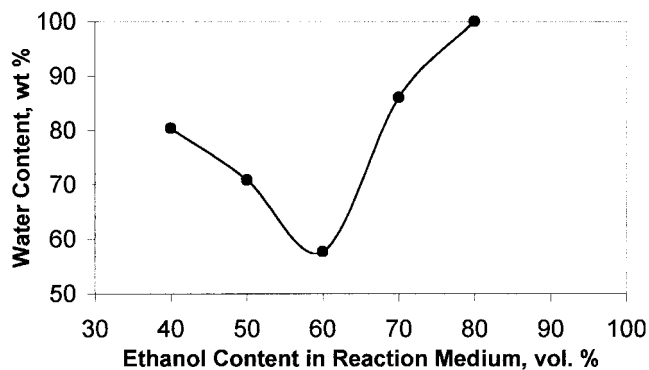
The effect of pH on the degree of crosslinking was investigated by conducting the crosslinking reaction in 60 vol % ethanol solution using 100 mM WSC and a range of pH values. The membranes were then subjected to swelling measurements. The variation of the water content of the swollen membranes with the pH of the reaction medium is shown in Figure 3. The figure shows that the water content had a minimum value at pH 4. These results are consistent with the reaction mechanism proposed for WSC and carboxylic acids.<sup>19,21</sup> Accordingly, WSC reacts with a carboxylic acid group to form the acid anhydride, which in turn reacts with a hydroxyl group to form an ester linkage. In the present work, low pH values increased the concentration of the protonated form of the acid groups, thereby increasing the extent of anhydride and hence ester formation. The higher water content at pH 3 was attributed to the increased instability of WSC below pH 4<sup>19</sup> and a consequent reduction in WSC concentration and ester linkage formation. Therefore, it was concluded that pH 4 was optimum for the crosslinking of sodium alginate using WSC.

### Effect of WSC concentration on the degree of crosslinking

The effect of WSC concentration on the degree of crosslinking was determined by carrying out the crosslinking reaction in 60 vol % ethanol at pH 4 using a range of WSC concentrations. The membranes were then subjected to swelling measurements. The variation of the water content of the swollen membranes with WSC concentration is shown in Figure 4. The results showed that the degree of crosslinking increased with increasing WSC concentration up to 60–100 mM and then remained approximately constant at higher concentrations. The results suggested that crosslinking could be assumed to be complete when 100 mM WSC was used and hence this concentration



**Figure 4** Variation of the degree of swelling with WSC concentration of the crosslinking solution (60 vol % ethanol, pH 4).



**Figure 5** Variation of membrane swelling with ethanol content of the crosslinking solution using 100 mM WSC and pH 4.

was selected for use in subsequent crosslinking experiments.

#### Confirmation of optimum ethanol concentration for crosslinking reaction

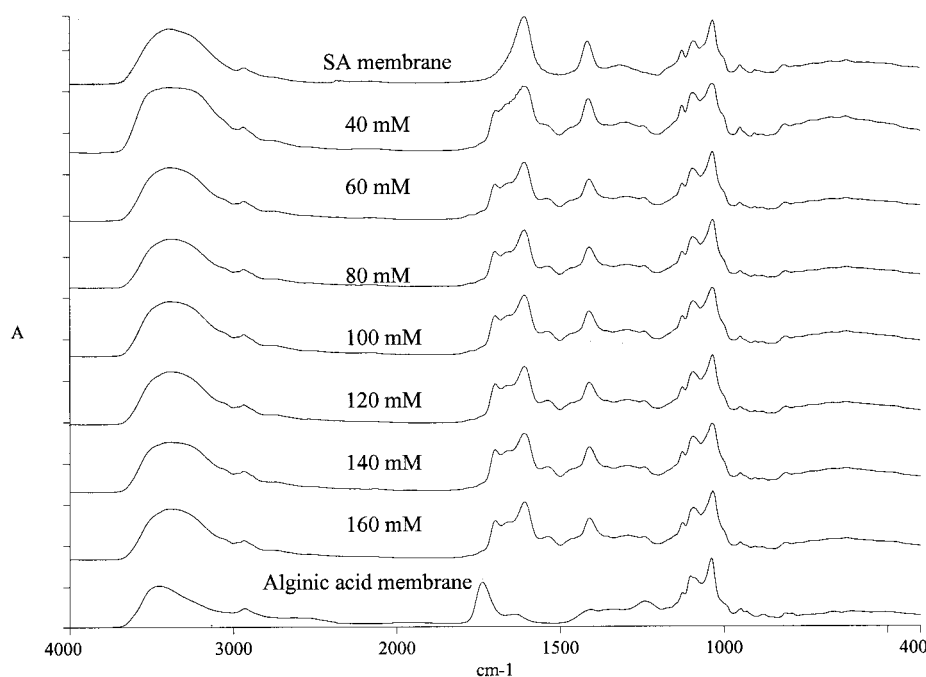
The ethanol concentration of 60 vol % selected as optimal on the basis of swelling measurements on noncrosslinked membranes required experimental confirmation under the crosslinking reaction conditions determined as described above. The variation of the water content of the swollen membranes with ethanol concentration when crosslinked using 100 mM WSC at pH 4 is shown in Figure 5. The results indicated that membranes crosslinked in a medium containing 60 vol % ethanol had the lowest water content

and hence the highest degree of crosslinking. The membrane crosslinked in a medium containing 80 vol % ethanol underwent complete dissolution when immersed in water because of insufficient crosslinking to maintain the hydrogel structure.

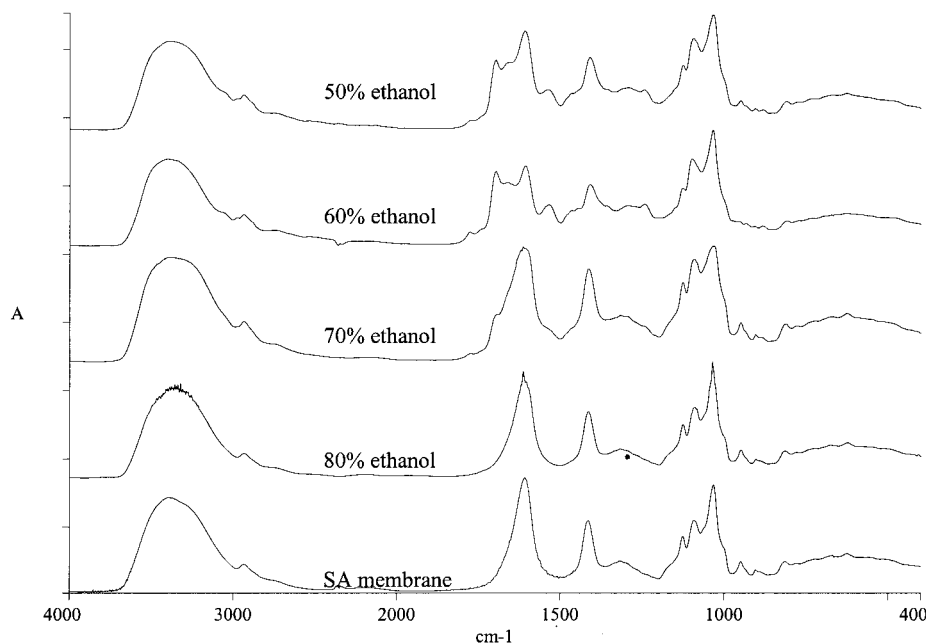
#### IR spectra

The infrared spectra of a noncrosslinked sodium alginate membrane and membranes crosslinked using a range of WSC concentrations are shown in Figure 6. The spectrum of alginic acid, the protonated form of alginate, is also shown. The figure shows the presence of a band at  $1698\text{ cm}^{-1}$  in the spectra of the crosslinked membranes only. This band, which has been assigned to a C—O stretching mode of an ester group,<sup>21</sup> had a similar intensity relative to the band at  $1608\text{ cm}^{-1}$  at all WSC concentrations. The latter band, which was also present in the spectrum of the noncrosslinked membrane, is characteristic of a carboxylate C—O stretching mode.<sup>9</sup> The alginic acid band at  $1736\text{ cm}^{-1}$  was absent from the spectra of the crosslinked membranes, indicating that extensive protonation did not occur at pH 4. These observations were consistent with the results of the membrane swelling measurements (Fig. 4), which indicated that the degree of crosslinking was similar for all WSC concentrations above 60–100 mM.

The infrared spectra of a noncrosslinked sodium alginate membrane and membranes crosslinked using a range of ethanol–water mixtures are shown in Figure 7. The figure shows that the ester band at  $1698\text{ cm}^{-1}$



**Figure 6** Infrared spectra of alginic acid, noncrosslinked sodium alginate (SA), and sodium alginate membranes crosslinked using various WSC concentrations (pH 4, 60 vol % ethanol). (A, absorbance).



**Figure 7** Infrared spectra of noncrosslinked sodium alginate (SA) and sodium alginate membranes crosslinked using crosslinking solutions of various ethanol concentrations (ph 4, 100 mM WSC). (A, absorbance).

had a maximum intensity relative to the band at  $1608\text{ cm}^{-1}$  at an ethanol concentration of 60 vol %. This result was consistent with the results of the membrane swelling measurements (Fig. 5), which indicated that maximum crosslinking occurred when a mixture of this composition was used.

### DSC

The heats of fusion ( $\Delta H_m$ ) and melting points ( $T_m$ ) of a noncrosslinked sodium alginate membrane and membranes crosslinked using various WSC concentrations are given in Table I. These measurements were carried out on the same membranes that were used for swelling measurements to ensure that the swelling behavior was representative of the structures on which these measurements were carried out. The membrane crosslinked using 40 mM WSC was omitted from the DSC study because some membrane dissolution occurred during the swelling test. The results showed that membranes crosslinked using different WSC concentrations had similar heats of fusion ( $\sim 500\text{--}508\text{ J}$

$\text{g}^{-1}$ ) and that these were considerably larger than that of the noncrosslinked membrane ( $398\text{ J g}^{-1}$ ). This indicated that crosslinking increased the crystallinity of the membrane. An interesting feature of these results was the similarity between the melting points of the noncrosslinked ( $93.8^\circ\text{C}$ ) and crosslinked membranes ( $93.7\text{--}101.7^\circ\text{C}$ ). This observation was attributed to a larger entropy change associated with the melting of the crosslinked material.

### Membrane durability

Membranes crosslinked using 60 and 80 mM WSC lost 17 and 15% respectively, of their original weight after 32 days. Membranes crosslinked using 100–160 mM WSC showed no significant weight reduction. This result was consistent with the conclusion based on swelling measurements that WSC concentrations above about 100 mM resulted in a similar degree of crosslinking.

### CONCLUSIONS

This work has shown that the maximum degree of alginate crosslinking using WSC and the film immersion method can be achieved using a nonsolvent (ethanol) concentration of 60 vol %, a WSC concentration of 100 mM, and pH 4. Infrared spectroscopy confirmed that crosslinking ester groups were formed when ethanol concentrations below about 80 vol % and WSC concentrations above 60 mM were used. Based on the results of DSC measurements, it was

**TABLE I**  
Results of DSC Measurements on a Noncrosslinked Membrane and Membranes Crosslinked Using Various WSC Concentrations (pH 4, 60 vol % ethanol)

Parameter	[WSC] (mM)						
	0	60	80	100	120	140	160
$\Delta H_m$ (J/g)	398	506	502	501	503	500	508
$T_m$ ( $^\circ\text{C}$ )	93.8	101.7	93.7	99.3	96.1	91.9	101.5

concluded that crosslinking increased the crystallinity of the membrane. Also, membranes prepared using these optimum conditions were found to have a durability of at least 32 days when immersed in water. Overall, this work has shown that sodium alginate can be used to produce a water-insoluble, nontoxic, durable membrane for use in the pervaporation separation of ethanol–water mixtures. This membrane also has potential for use as a protective coating for microporous, hydrophobic membranes of the types used in membrane distillation and osmotic distillation.

### References

1. Ramaraj, B.; Radhakrishnan, G. *J Appl Polym Sci* 1994, 51, 979.
2. Rehm, B. H.; Valla, A. S. *Appl Microbiol Biotechnol* 1997, 48, 281.
3. Saude, N.; Cheze-Lange, H.; Beunard, D.; Dhulster, P.; Guillochon, D.; Caze, A.-M.; Morcellet, M.; Junter, G.-A. *Proc Biochem* 2002, 38, 273.
4. Whistler, R. L.; BeMiller, J. N. *Industrial Gums, Polysaccharides, and Their Derivatives*; Academic Press: New York and London, 1973.
5. Chandy, T.; Mooradian, D. L.; Rao, G. H. R. *Artif Organs* 1999, 23, 894.
6. Pelletier, S.; Hubert, P.; Payan, E.; Marchal, P.; Choplin, L.; Dellacherie, E. *J Biomed Mater Res* 2001, 54, 102.
7. Choi, Y. S.; Hong, S. R.; Lee, Y. M.; Song, K. W.; Park, M. H.; Nam, Y. S. *Biomaterials* 1999, 20, 409.
8. Padmanabhan, K.; Smith, T. J. *Pharm Dev Technol* 2002, 7, 97.
9. Huang, R. Y. M.; Pal, R.; Moon, G. Y. *J Membr Sci* 1999, 160, 101.
10. Shi, Y.; Wang, X.; Chen, G. *J Appl Polym Sci* 1996, 61, 1387.
11. Yeom, C. K.; Jegal, J. G.; Lee, K. H. *J Appl Polym Sci* 1996, 62, 1561.
12. Huang, R. Y. M.; Yeom, C. K. *J Membr Sci* 1990, 51, 273.
13. Eiselt, P.; Rowley, J. A.; Mooney, D. J. *Mater Res Soc Symp Proc* 1998, 530, 37.
14. Yeom, C. K.; Lee, K.-H. *J Membr Sci* 1997, 135, 225.
15. Yeom, C. K.; Lee, K.-H. *J Appl Polym Sci* 1998, 67, 209.
16. Wang, X. P.; Li, N.; Wang, W. Z. *J Membr Sci* 2001, 193, 85.
17. Zhang, L.; Zhou, D.; Wang, H.; Cheng, S. *J Membr Sci* 1997, 124, 195.
18. Yeom, C. K.; Lee, K. H. *J Appl Polym Sci* 1998, 67, 949.
19. Nakajima, N.; Ikada, Y. *Bioconjugate Chem* 1995, 6, 123.
20. Park, S. N.; Park, J.-C.; Kim, H. O.; Song, M. J.; Suh, H. *Biomaterials* 2002, 23, 1205.
21. Tomihata, K.; Ikada, Y. *J Biomed Mater Res* 1997, 37, 243.