Poly(vinyl alcohol) Films Crosslinked by Glutaraldehyde Under Mild Conditions

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ABSTRACT: The use of mild conditions to perform the entrapment of biomolecules in polymeric matrices is a crucial step in a broad range of applications as biosensors, biocarrier-mediated facilitated transport membranes, and drug-controlled release devices. In this study, we investigated the crosslinking of poly(vinyl alcohol) (PVA) by glutaraldehyde in the absence of an acid catalyst and organic solvents to improve the water resistance of the hydrophilic biocompatible polymer. Glutaraldehyde was chosen as the crosslinking agent because it favors the intermolecular reaction with PVA and is able to bind nonspecifically to proteins. The effects of the temperature and glutaraldehyde content on the thermal and structural properties of the PVA films were examined. Membranes prepared at 40°C showed a maximum crosslinking density for low glutaraldehyde content namely, 0.04 wt % in the spreading solution. Higher amounts of the crosslinker led to the branching of PVA. The increase in membrane thermal properties and reduction in crystallinity were ascribed to the crosslinking treatment, which was confirmed by Fourier transform infrared analysis. The oxygen permeability of the films was reduced up to 2.7 times, which indicated that the crosslinking of the polymer was successfully accomplished. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 3074–3080, 2009

Key words: crosslinking; films; gelation; sensors; thermal properties

INTRODUCTION

The immobilization of biomolecules and cells in a hydrophilic biocompatible support is the Achilles' heel of a broad range of biotechnological processes because the technique must consider the operational constraints, namely, the pH, temperature, and concentration of chemicals, to preserve the structure of such sensitive substances. Hence, the investigation of new procedures that aim to fixate the biomolecules in polymeric matrices is a breakthrough that can improve the efficiency of the method and decrease costs, which may lead to economically viable routes and the launch of new products.[1]

Poly(vinyl alcohol) (PVA) is a synthetic water-soluble biocompatible polymer. It is produced by the alcoholysis of poly(vinyl acetate), which can impart different physical properties to the polymer, such as hydrophilicity, mainly related to the hydrolysis degree and also to the polymerization extent.[2] This versatility makes feasible the widespread application of PVA in areas such as membranes for pervaporation,[3] a process that can be used to remove water from organic mixtures; gas permeation;[4] and facilitated transport,[5] as well as in biosensors[6] and drug-controlled release devices.[7] In the separation field, the PVA film-forming ability and mechanical resistance are used to separate hydrophilic substances from a feed stream or to immobilize biocarriers to produce facilitated transport membranes.[8] In the biotechnological and pharmaceutical areas, the softness and high water content of PVA hydrogels have been explored to incorporate biologically active substances because of the similarity to living tissues. For instance, the water-swelling degree and hydrophilicity of modified PVA can be adjusted to control the drug-delivery rate in the body and, thus, prevent overdosing and rejection problems. Biomolecules and cells may be immobilized in these life-resembling systems to exploit their physiological function in nonliving environments.[9]

Unmodified PVA hydrogels have poor stability in water, which can damage the integrity of the films and diminish their performance. Therefore, by crosslinking PVA, it is possible for one to decrease the membrane hydrophilicity and provide a suitable network to entrap biomolecules. The major techniques used to accomplish the polymer partial insolubilization in aqueous media are based on heat, physical,

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or chemical treatments. When PVA is heat treated, the supplied energy is used to modify the spatial organization of the chains and to establish stronger hydrogen bonding among hydroxyl groups, which leads to a higher crystallinity content. The physical aging of PVA aqueous solutions may cause network formation because of chain entanglements, especially when the system undergoes freezing/thawing cycles consecutively. On the other hand, chemically crosslinked films are based on the reaction between the crosslinker and the high amount of hydroxyl groups of PVA. There are a wide variety of crosslinking agents for PVA, such as maleic acid, formaldehyde, and glutaraldehyde (GA). Intramolecular or intermolecular reaction modes can be adjusted by specific crosslinker agents and/or operational conditions.[10]

In recent years, GA has gained increasing attention as a PVA crosslinking agent because of the absence of thermal treatment needed to drive the reaction. Additionally, it is also well known that GA can bind nonspecifically to biomolecules, such as proteins. The polymerization of hemoglobin for the production of artificial blood is an example of GA's ability to join the polypeptide backbone.[11] Because the crosslinker has two active sites, it can be successfully used to bind proteins and PVA together. This feature enables the development of tailored structures to be used in biosensors once the constraints imposed by the biomolecules are considered.

The procedure commonly used to crosslink PVA membranes with GA is based on the immersion of the film in an alcoholic solution containing the crosslinking agent and a mineral acid, such as HCl or H_2SO_4.[12] The alcoholic medium is used to swell the membrane, which allows the diffusion of the dialdehyde molecules and the protonic ions, which are used to catalyze the reaction. The mechanism proposed for the reaction indicates that there is an optimum ratio between the reactants that favors the crosslinking of the polymer, whereas higher amounts of the aldehyde can lead to the branching of PVA, as illustrated in Figure 1.[13] In the case of biosensors, the low pH can damage the structure of biomolecules, and other routes have been proposed, such as a decrease in acidity or a time-controlled exposition of modified PVA to the crosslinking solution. However, we did not know of an acid-free reaction between PVA and GA.

Our goal in this study was to investigate the crosslinking of PVA by GA in the absence of an acid catalyst and organic solvents, with the aim of the further entrapment of proteins in this support. The effect of temperature, limited to 40°C, and GA content was varied, and the reaction was performed simultaneously with the membrane production to simplify the procedure. Characterization tests included water-swelling degree tests, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Fourier transform infrared (FTIR) analysis to obtain information about membrane thermal and chemical changes. The selection of operational conditions milder than the ones observed in the literature to promote the insolubilization of PVA by GA with no damage to the physiological activity of proteins is of utmost importance to the development of biosensors and facilitated transport membranes containing biocarriers.

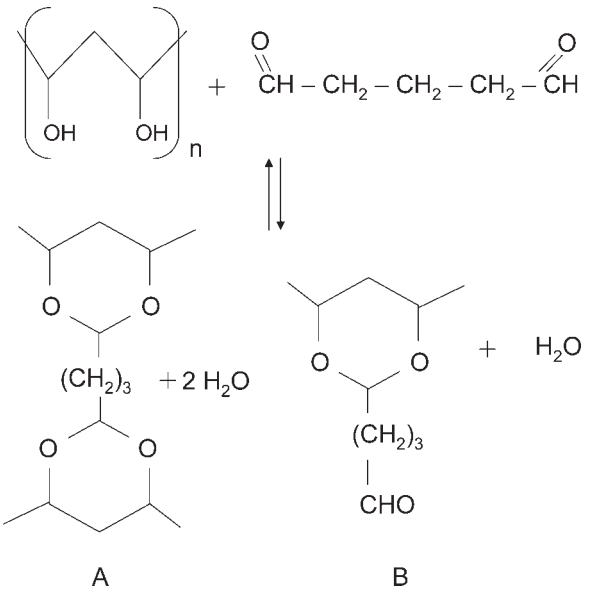

Figure 1 Mechanism of the reaction between PVA and GA.

EXPERIMENTAL

PVA (Aldrich, Milwaukee, WI, molecular weight = 85,000–146,000, hydrolysis degree = 99.5%) was dissolved in distilled water by heating the solution at 100°C under stirring. After the solution was cooled to room temperature, GA (Aldrich, aqueous solution, 50 wt %) was added to the polymer solution at the desired ratio, with the PVA final content maintained at 4 wt %. The final pH of this solution was 6.2. The reaction mixture was spread in Petri dishes and dried at a specified temperature to produce flat, dense membranes.

The effects of GA content, referred to as the GA/PVA mass ratio, and the temperature of the reaction mixture on the membrane properties and structure were examined. The GA/PVA mass ratio was varied from 0.005 to 1, whereas the temperatures used were 10, 25, and 40°C. For the preparation of membranes at 10°C, the Petri dishes with the spreading solution were transferred to a desiccator *in vacuo*. The recipient was placed in a refrigerator under controlled temperature. The silica was changed every 2 days to remove water. In the case of the membranes dried at

room temperature, the Petri dishes were left in a fume hood until film formation was accomplished. The membranes dried at 40°C were placed in an incubator (Nova Etica, Shaker 430 RDB) (Vargem Grande, Paulista, Brazil).

Water swelling, TGA, DSC, and FTIR tests were performed to characterize the membrane properties, including crosslinking density, thermal stability, glass-transition temperature, melting enthalpy, and chemical structure.

We determined the water-swelling degree by immersing 2 × 2 cm^2 strips into water at 40°C for 48 h, until equilibrium was reached. Then, the swollen size (l_w) was recorded, and the strips were placed in a desiccator at room temperature and *in vacuo* for 3 days, when the dried membrane size (l_d) was measured again. The water-swelling degree (R) was calculated as follows:

$$R = \frac{(l_w - l_d) \times 100}{l_d} \quad (1)$$

TGA and DSC analysis of membranes were performed to characterize membrane thermal properties. For the TGA tests (PerkinElmer, TGA 7, Norwalk, CT), 8 mg of each sample was heated from 50 to 600°C at 10°C/min. We obtained DSC (PerkinElmer, DSC7) curves by heating 15 mg of the films from 30 to 250°C at 10°C/min under a nitrogen flow of 22.5 mL/min. Then, the samples were cooled to 30°C at 200°C/min; this was followed by a second heating step, from 30 to 250°C, at 10°C/min.

The functional groups were determined by FTIR analysis (PerkinElmer, 1720X) of the membranes. Each sample was scanned 20 times at a resolution of 2 cm^{-1}. The relative number of groups was calculated to provide an assessment of the membrane structure.

The oxygen permeability in the liquid phase was addressed by means of a dialysis cell. Air was continuously bubbled on the feed side. The permeate stream was previously deoxygenated. The detection of the dissolved oxygen was performed by a specific sensor (YSI model 58, 5739 DO, Yellow Springs, OH). The PVA membranes were placed between two microfiltration membranes (cellulose acetate, Millipore, Bedford, MA) with a mean pore diameter of 0.45 μm to improve the mechanical resistance.

RESULTS AND DISCUSSION

The strategy used to investigate the acid-free reaction between PVA and GA was based on the evaluation of the temperature and the crosslinker content in the properties of the membrane. The first stage of the research was devoted to an exploratory study of the range of the selected variables to determine whether the reaction could be completed under these mild conditions. Water-swelling tests were used to infer the crosslinking density of the films. The results are presented on Table I. All of the tests were performed in triplicate. The membrane mean thickness was around 100 μm.

TABLE I
Water-Swelling Degree of PVA Membranes Crosslinked with GA

Temperature (°C)	GA/PVA mass ratio	Water-swelling degree (%)
10	0.01	Soluble
	0.1	Soluble
	1	Soluble
25	0.01	>300
	0.1	Soluble
	1	Soluble
40	0.005	54 ± 6
	0.01	44 ± 5
	0.03	53 ± 4
	0.1	Soluble
	1	Soluble

Membrane formation and PVA crosslinking occurred simultaneously; hence, the reaction time refers to the total time in which the solution was evaporated from the Petri dish. The corresponding values were 45, 110, and 170 h for the membranes prepared at 40, 25, and 10°C, respectively.

The membranes dried at 10°C were soluble in water, which indicated that there was no reaction between PVA and GA. This result was probably due to the fact that the reactants did not have enough activation energy to react at such a low temperature. The films had high water content, which suggested the physical gelation of the polymer instead of the reaction. Almost the same profile was observed for the films prepared at room temperature, except for the sample with a GA/PVA mass ratio equal to 0.01, in which the membrane was not soluble, but the swelling degree was still very high for our purposes.

In the case of the reaction conducted at 40°C, the trend shown at 25°C was confirmed: a low GA content led to higher crosslinking densities. The existence of a GA concentration in which the crosslinking density reached a maximum can be explained by the mechanism of the reaction proposed in the literature.[13] For a very low GA content, the few molecules of the dialdehyde were not able to promote a suitable network to prevent solubility in water, and the membrane had a high water-swelling value. The addition of more crosslinker to the polymeric solution favored the reaction in both edges of the GA molecule and reduced the free volume of the material, which improved the water resistance of the films. The highest crosslinking density was observed for the GA/PVA mass ratio of 0.01. Further increase

in GA content led to the branching of PVA instead of crosslinking, possibly because the solution was more viscous than the diluted ones, which compromised the diffusivity of the molecules. The reaction was limited by the diffusivity of the reactants in the whole range of GA content because the water was evaporated and the viscosity of the spreading solution increased. The branching of the PVA increased the space among the chains and led to a higher solubility in water because the packing ability was lost.

An inspection of Table I reveals that better results were obtained for the films prepared at 40°C and a GA/PVA mass ratio around 0.01. These conditions were milder than the ones used in the literature to stabilize PVA in aqueous media.[14] With respect to the synthesis of biosensors and biocarrier-mediated facilitated transport membranes, these operational parameters may preserve the native folding of biomolecules once the temperature is slightly higher than 37°C and the GA content is 10 times lower compared to the value used in the production of hemoglobin-containing PVA membranes,[15] as an example.

The thermal analysis of the films prepared at 40°C was used to compare the differences in the polymeric arrangements of the samples. The thermogravimetry (TG) and differential thermogravimetry (DTG) curves are presented in Figure 2. The unmodified PVA had three main degradation steps: the first one, around 100°C, was ascribed to the release of volatile compounds, mainly water, which were present in the hydrophilic material. The second stage, at 270°C, was regarded as the elimination of acetic acid to form a polyene, whereas the third step, around 450°C, corresponded to the breakage of the main chain.[16]

The TG curves for the GA/PVA mass ratios of 0.01 and 0.1 also showed three main degradation steps. In comparison to the unmodified PVA, it was clear that the first stage of weight loss was reduced, which indicated a decrease in membrane hydrophilicity. Also, the degradation events happened at higher temperatures, namely, 290 and 470°C, which denoted an increase in the membrane thermal stability as a result of the crosslinking of PVA by GA.

The increase of the GA/PVA mass ratio to 1 changed the TG profile. The weight loss was continuous, and there was no marked difference in the degradation stages. At high temperatures, above 300°C, the stability was even greater than in the other samples with lower GA contents. This behavior was attributed to the gelation of the branched PVA, which has a broad range of composition because of the different structures that GA can form in aqueous solution.[17]

The DSC curves of the GA-modified PVA films are presented in Figure 3. The samples with GA/PVA mass ratios of 0.005 and 0.03 were included to establish a detailed behavior of the organization of polymeric chains. Figures 4 and 5 show the glass-transition temperature and melting enthalpy as a function of the GA content. For the former parameter, there seemed to be a maximum that corresponded to a GA/PVA mass ratio of 0.03. This implied that the highest crosslinking density was related to a limited mobility of the chains, which was quite reasonable. On the other hand, the melting enthalpy decreased with increasing GA content. This property was related to the membrane crystallinity. The addition of GA to the polymeric solution increased the distance between the chains, regardless of whether the polymer had been crosslinked or branched, which made the organization of PVA in crystalline lattices difficult. These results corroborated the results of TGA and the water-swelling degree of the films.

The changes in the PVA chemical structure were addressed by FTIR spectra. The results are presented in Figure 6, and the typical PVA pattern was

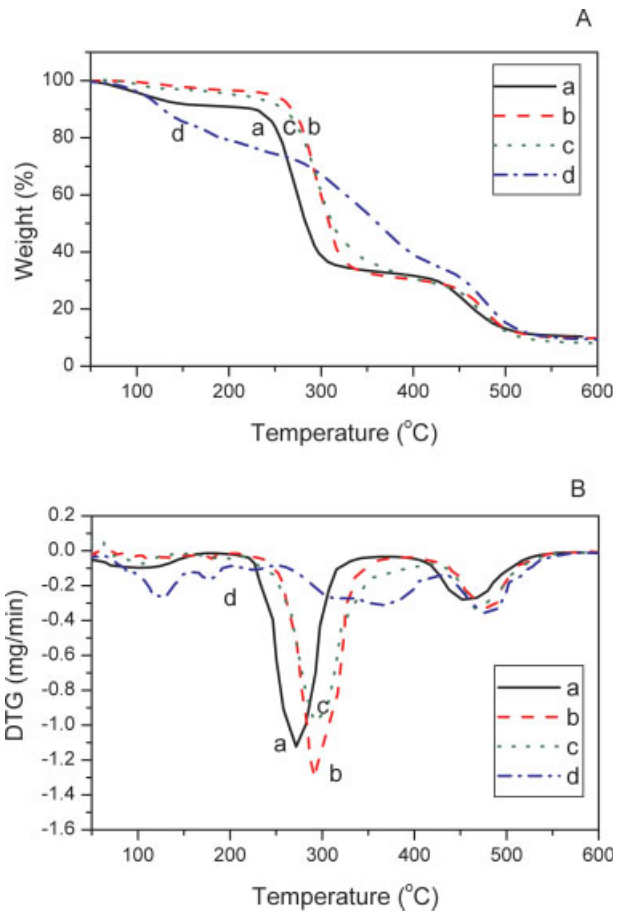

Figure 2 (A) TG and (B) DTG curves of PVA membranes prepared at 40°C: (a) PVA, (b) GA/PVA = 0.01, (c) GA/PVA = 0.1, and (d) GA/PVA = 1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

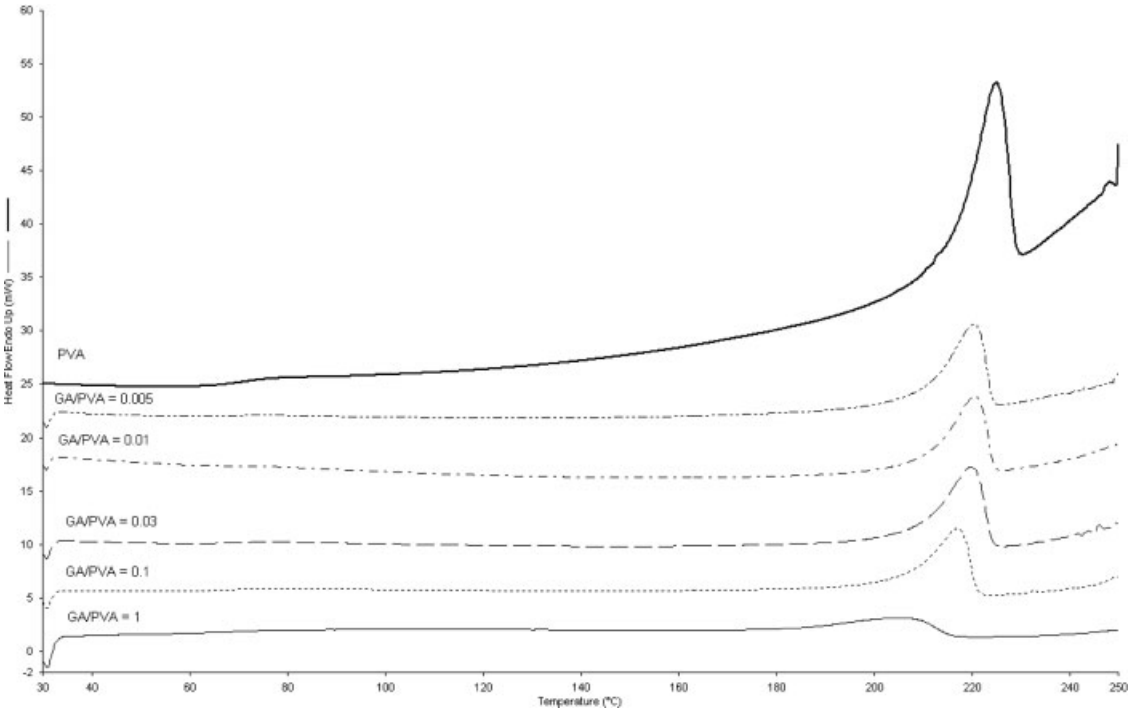

Figure 3 DSC curves of PVA membranes prepared at 40°C.

recognized: a broad band around 3315 cm^{-1} related to O—H stretching, two peaks at 2940 and 2898 cm^{-1} corresponding to the asymmetrical and symmetrical stretching of methylene groups, respectively, and another band at 1090 cm^{-1} due to C—O stretching.[3]

A semiquantitative analysis of the FTIR data was performed to confirm the effectiveness of the reaction between PVA and GA and the structure of the products. The absorbances of the samples at 3315 cm^{-1} (hydroxyl groups), 1718 cm^{-1} (aldehyde groups), and 1097 cm^{-1} (acetal groups) relative to the ones at 2940 (methylene groups) cm^{-1} were plotted as a function of the GA/PVA mass ratio. The graphics are illustrated in Figures 7–9.

We observed a relative decrease in the amount of hydroxyl groups with the addition of GA to the spreading solution, which indicated that the —OH was consumed in the reaction. In the case of the aldehyde groups, presented in Figure 8, there was first an increase in the relative absorbance (GA/PVA = 0.01), followed by a decrease in GA/PVA of 0.1 and another increase in GA/PVA of 1, which corroborated the reaction mechanism shown in Figure 1.[13] The decrease in the aldehyde groups from a GA/PVA value of 0.01 to 0.1 corresponded to the

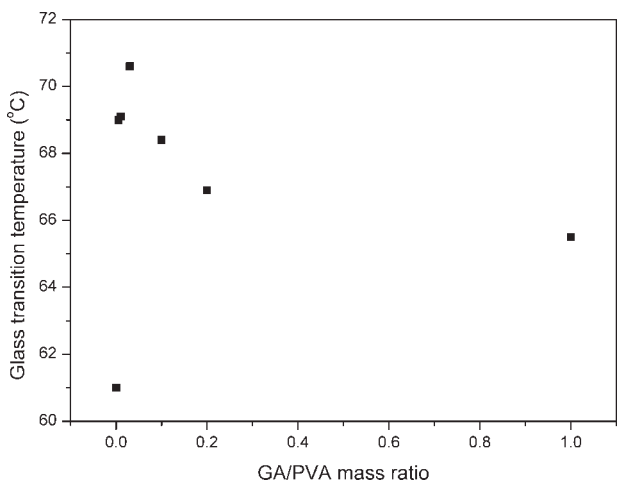

Figure 4 Glass-transition temperature as a function of the GA content in membranes prepared at 40°C.

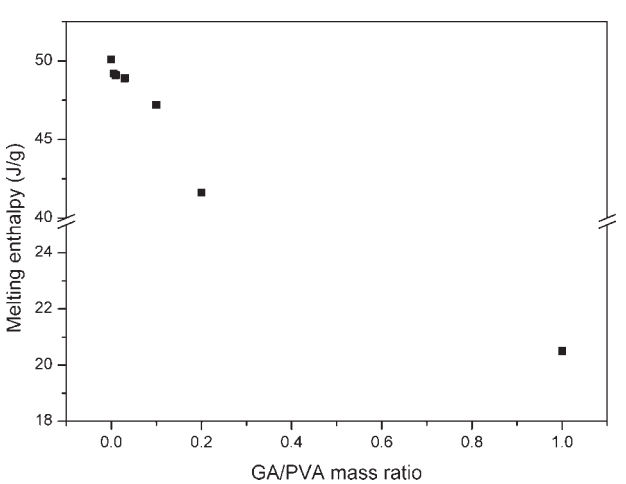

Figure 5 Melting enthalpy as a function of the GA content in membranes prepared at 40°C.

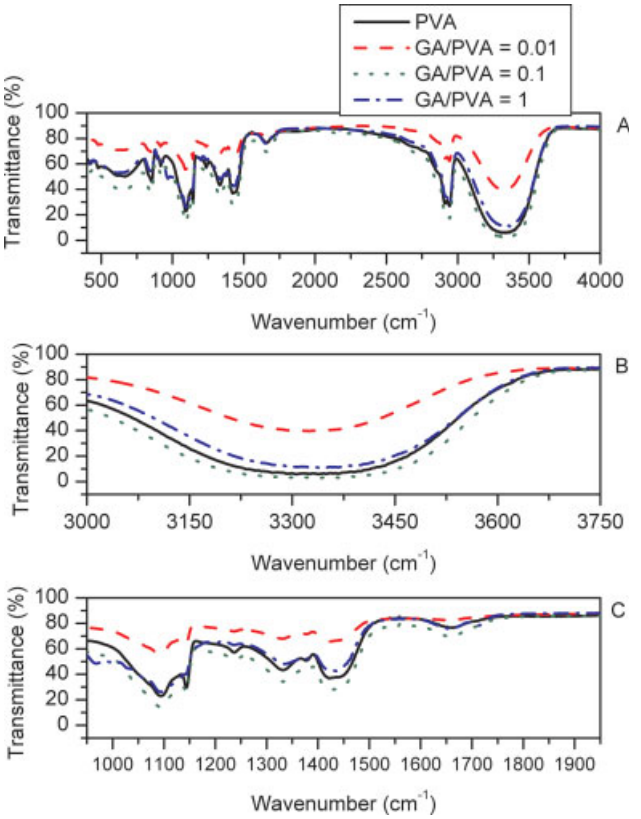

Figure 6 Infrared spectra of PVA membranes prepared at 40°C: (A) spectra from 400 to 4000 cm^{-1}, (B) the hydroxyl band, and (C) acetal and aldehyde peaks. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

increase in the crosslinking density, in which the bimodal reaction of GA occurred. For higher crosslinker contents, the free aldehyde corresponded to the branches in the PVA chain. The general trend for the relative absorbances at 1097 cm^{-1} showed an increase in the amount of acetal groups (C—O—C) at a GA/PVA ratio of 0.01, which confirmed the hypothesis of the structure of the products.

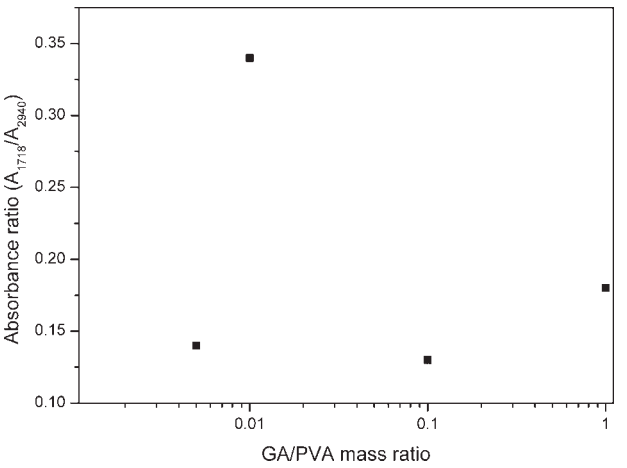

Figure 8 Relative amount of aldehyde groups with an increase in the GA content.

The oxygen fluxes though the membranes are presented in Table II. The condition of GA/PVA = 0.01 was selected because it showed the lower water-swelling degree. The average thickness of the membranes used in permeation tests was 10 μm. There was a decrease of 2.7-fold in the oxygen flux, which indicated that PVA was successfully crosslinked by GA at a pH of 6.2.

CONCLUSIONS

The acid-free reaction between PVA and GA was successfully accomplished. Membranes prepared at 40°C with a pH of 6.2 and a GA/PVA mass ratio of 0.01 had a swelling degree of 44%. The addition of more GA molecules caused PVA branching, which led to a decrease in the solubility of the films in water. TG and DSC tests showed an increase in the

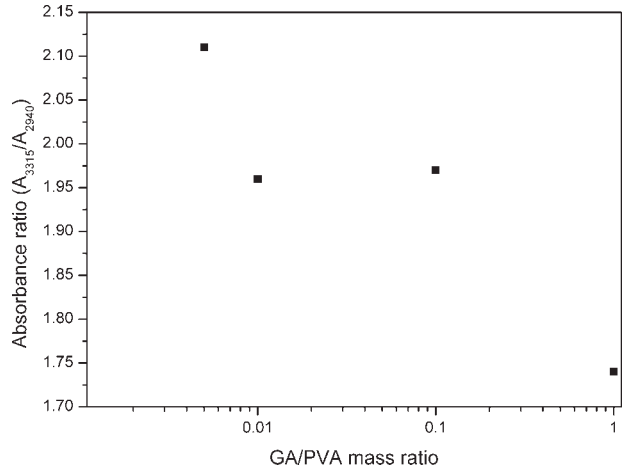

Figure 7 Relative amount of hydroxyl groups with an increase in the GA content.

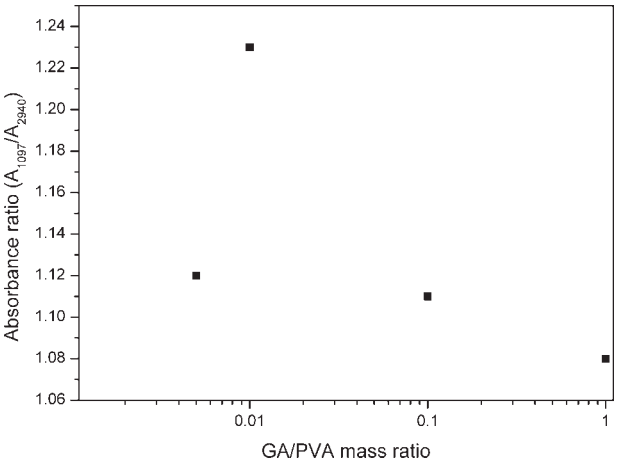

Figure 9 Relative amount of acetal groups with an increase in the GA content.

TABLE II
Oxygen Fluxes Through the Membranes in the Aqueous Phase

Membrane	O_2 flux (kg/h m^2)
Unmodified PVA	1.4×10^{-3}
GA/PVA = 0.01	5.1×10^{-4}

membrane thermal stability and a decrease in the polymeric crystallinity as a result of crosslinking. The use of such mild conditions make feasible the entrapment of biomolecules in the modified PVA matrix to prepare biosensors, drug-controlled release devices, and biocarrier-based facilitated transport membranes.

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