Polymer 50 (2009) 3670–3679

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Organic compatible polyacrylamide hydrogel fibers

Ping Lu, You-Lo Hsieh*

Fiber and Polymer Science, University of California, One Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 10 March 2009
Received in revised form
14 May 2009
Accepted 19 May 2009
Available online 27 May 2009

Keywords:
Polyacrylamide hydrogel fibers
Crosslinking
β-Galactosidase

ABSTRACT

Ultra-fine fibrous PAAm hydrogel membranes were fabricated by electrospinning of aqueous solutions, followed by reaction with glutaraldehyde. A wide range of fiber diameters (267 nm to 2.8 μm) and morphologies (beaded, round, branched and ribbon) could be achieved by PAAm molecular weight and solution properties. The optimally glutaraldehyde-crosslinked membranes were highly stable in water as well as in organic solvents (methanol, ethanol, acetone, chloroform, DMF and cyclohexane). Glutaraldehyde formed imine with PAAm amide side groups in the form of crosslinking bridges as well as grafts with hydrolyzed carboxylic end groups. The fibrous membrane showed excellent thermal stability, hydrophilicity, super water absorbency and exceptional tensile strength. The fibrous membrane exhibited excellent ability to entrap β-galactosidase enzyme while allowing efficient diffusion of the substrate from and released products into surrounding media. The dual organic solvent and aqueous media compatible fibrous membranes have the promise for solid supported catalysis and diagnostic as well as a wide range of biomedical and industrial applications.

1. Introduction

Hydrogels are three-dimensional hydrophilic macromolecular networks that can absorb water at many times of their dry mass and to expand significantly in their volume [1]. The ability of hydrogels to undergo substantial swelling and collapsing in response to the presence and absence of water allows their potential use as actuators, sensors, artificial muscles, drug delivery, and protein separation and immobilization [2]. Hydrogels have been made into various forms of rods, blocks and films as well as particles and fibers [3]. At the common micrometer range dimensions, particles and fibers are superior forms for hydrogels because of their high specific surface allowing quick response to external stimulus while minimizing steric hindrance. Fibrous membranes offer further advantage over particles because of their continuous and porous matrixes that provide reasonable mechanical and handling properties [4,5]. Fibrous membranes with further reduced fiber diameters can further enhance their responsiveness and potential application properties.

Electrospinning, in which polymer solutions or melts are charged with a high voltage, can generate micrometer to nanometer scaled fibers in an interconnected web [6]. In our laboratory, ultra-fine hydrogel fibers with diameters in the range of 270 nm to 1.2 μm have been successfully generated by electrospinning of binary mixtures of poly(acrylic acid), a polyelectrolyte, with poly(vinyl alcohol) [4] or β-cyclodextrin [5] in water, and poly(*N*-isopropylacrylamide) in DMF [7] followed by subsequent heat-induced crosslinking.

Polyacrylamide hydrogels are highly hydrophilic 3-D polymer networks and have found wide applications, including gel electrophoresis for protein separation [8] and immobilization [9]. PAAm ($M_w = 9 \times 10^6$ g/mol) has been electrospun alone from DMF or DMF/H_2O mixtures to various forms of beads, beaded fibers, ribbons and fibers [10,11] as well as mixtures with *N*-carboxyethylchitosan to facilitate fiber formation [10]. These electrospun PAAm fibers are, however, water-soluble, and in fact, completely lose fibrous structure even in the presence of a trace amount of moisture. To maintain fibrous forms and to function like hydrogels in aqueous environment, PAAm fibers must be chemically crosslinked. To our best knowledge, crosslinking electrospun PAAm fibers into hydrogel fibers has not been reported.

The objective of this research was to study the feasibility to electrospun PAAm from aqueous solutions into fibrous membranes and crosslink into fibrous hydrogels for enzyme entrapment. Efficient fabrication of uniform PAAm fibers was investigated first by optimizing the polymer concentration, molecular weight, viscosity and surfactant. Chemical crosslinking linear PAAm is challenging mainly due to the inertness of the saturated carbon backbone and the stable amide group of the PAAm structure [12]. Crosslinking of linear PAAm has been reported by using the gamma rays [13] and with formaldehyde [14]. However, both were carried out in aqueous solutions which would dissolve PAAm fibers and not suitable as means to crosslink. In this work, glutaraldehyde (GA) was chosen as

* Corresponding author. Tel.: +1 530 752 0843.
E-mail address: ylhsieh@ucdavis.edu (Y.-L. Hsieh).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.05.040

the crosslinker. Aldehydes are very reactive and have been used for crosslinking polymers such as chitosan [15] and poly(vinyl alcohol) [16]. The crosslinking reaction was performed in ethanol media because it effectively dissolves GA and acid catalyst but not the PAAm fibers. The effects of crosslinking conditions such as acid concentration, reaction temperature, and reaction time on the morphology, strength, packing, swelling, hydrophilicity and stability of the hydrogel fibers were studied. The successfully crosslinked PAAm hydrogel fibrous membranes were used for the entrapment of β-galactosidase, a large, bulky enzyme (540 kDa) that catalyzes the breakdown of lactose sugar.

2. Experimental section

2.1. Materials

Polyacrylamide (PAAm) ($M_n = 5–6 \times 10^6$ Da, non-ionic) was purchased from Acros Organics. PAAm ($M_n = 10{,}000$ Da, non-ionic, 50 wt% aqueous solution) and glutaraldehyde (GA) (50% aqueous solution) were obtained from Sigma–Aldrich Chemical Company. Hydrochloric acid (36.5–38.0% aqueous solution, GR ACS), ethyl alcohol (anhydrous, denatured), Triton X-100, and pH 7 buffer solution, all from EMD Chemicals, were used as received without further purification. β-Galactosidase from *Escherichia coli* (Grade X, 504 U/mg protein) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) were received from Sigma. All aqueous solutions were prepared with purified water (Millipore Milli-Q plus water purification system).

2.2. Electrospinning of PAAm fibrous membrane

Aqueous solutions of the 5000–6000 kDa PAAm with concentrations from 0.5 wt% to 3.5 wt% were prepared at 25 °C under constant stirring for at least 12 h. Aqueous solutions of the 10 kDa PAAm with concentrations from 10 wt% to 50 wt% were prepared from dilutions of the 50 wt% stock solution. Aqueous mixtures of the 10 kDa and 5000–6000 kDa PAAm were prepared at selected weight concentrations. The electrospinning setup consisted of a syringe with a flat-end metal needle (24 gauge, Popper and Sons), a syringe pump (KDS 200, KD Scientific Inc.), a high-voltage DC power supply (ES 30-0.1P, Gamma High Voltage Research Inc.) with the positive electrode connected to the syringe and ground to the aluminum foil collector. In a typical electrospinning experiment, PAAm solution placed in a plastic syringe (20 mL) was delivered by the syringe pump at a constant 1 mL/h feeding rate. An 8 kV positive voltage was applied to charge the solution jet toward the grounded target placed at a 25-cm distance under the ambient condition. The fibrous web collected on an aluminum foil was dried under vacuum and detached for further experiments.

2.3. Crosslinking with glutaraldehyde

The electrospun PAAm fibrous membranes were immersed in glutaraldehyde solutions of specific concentrations by diluting 50% aqueous GA with ethanol for 2 min. Each saturated membrane was placed in between two glass plates to prevent shrinking and heated at 120 °C for 1 h. The fibrous membranes were rinsed in ethanol then water, each several times, to remove any residue GA and residue HCl, respectively, and then dried at ambient temperature under vacuum for 24 h.

2.4. Enzyme entrapment

The β-galactosidase enzyme was dissolved in 1 mL pH 7 buffer to reach 2 mg/mL concentration. 50 mg crosslinked PAAm membrane was immersed in this enzyme solution at 4 °C for 24 h. The membrane was thoroughly rinsed with pH 7 buffer and deionized water for 20 min each to remove the surface bound enzymes. The washing solutions were monitored until no enzyme was detected and then the membrane was dried at ambient temperature under vacuum for 5 h.

2.5. Measurement and characterization

The viscosities of PAAm aqueous solutions were measured at 40 °C according to ASTM D 445 using a Cannon–Fenske Routine Viscometer (Cannon Instrument Company, USA). The kinetic viscosity (ν, mm^2/s) was calculated from the flow time (t, s) taken for the meniscus of the liquid to flow between two marks on the tube using Eq. (1) [17]:

$$\nu = C \times t \tag{1}$$

where C (mm^2/s^2) is a constant characteristic of the specific viscometer. The viscosity (η) in mPa s or cP was calculated as:

$$\eta = \nu \times \rho \tag{2}$$

where ρ is the density of polymer solution (g/mL). The specific viscosity (η_{sp}) was derived with the solvent (water) viscosity (η_0) as:

$$\eta_{sp} = (\eta - \eta_0)/\eta_0 \tag{3}$$

The swelling and the mass loss of the crosslinked PAAm fibrous membranes in water were determined gravimetrically and the swelling degree (q) and the mass loss (ΔW, %) were calculated:

$$q = (W_s - W_d)/W_d$$

$$\Delta W = (W_0 - W_d)/W_0 \times 100$$

where the original electrospun membrane was weighed (W_0) and immersed in water for 24 h, dabbed dry, and weighted immediately for W_s; the final dry mass (W_d) was obtained after drying under vacuum at ambient temperature for 24 h.

The water contact angles of crosslinked PAAm membranes were measured by a tensiometer (K14, KRÜSS, USA) using a previously reported method [18]. The electrospun PAAm fibrous membranes was sputter coated with gold for 2 min and examined using a scanning electron microscopy (SEM) (XL 30-SFEG, FEI/Philips, USA) at a 5 kV accelerating voltage. The average fiber diameters were calculated from 100 measurements of individual fibers using an analySIS FIVE (Olympus) image processing software. The thermal properties of the electrospun PAAm fibrous membranes were measured by differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Japan) and thermogravimetric analysis (TGA) (TGA-50, Shimadzu, Japan), in N_2 from 30 to 500 °C at 10 °C/min. The DSC sample (about 5 mg) was sealed in an aluminum pan whereas the TGA sample (about 3 mg) was placed in a platinum pan. The tensile properties of the electrospun PAAm fibrous membranes were measured using an Instron tensile tester (4465, Illinois Tool Works Inc., USA) at a 10-mm gauge length and a 5 mm/min cross-head speed at 21 °C and 65% relative humidity. The specimens were cut along the same direction perpendicular to the needle with a typical size of 20 mm (length) × 5 mm (width) and a thickness of about 100 μm. The activity of the entrapped β-galactosidase was assayed with X-gal at 20 mg/mL concentration in DMF. Briefly, one 10 mg blank (crosslinked PAAm without β-galactosidase) and one 10 mg sample (crosslinked PAAm with entrapped β-galactosidase) were added to two bottles both containing 1 mL pH 7 buffer solution. Several drops of the X-gal solution were added into the above two bottles. The blank and sample solutions were then incubated at 37 °C for 24 h. The

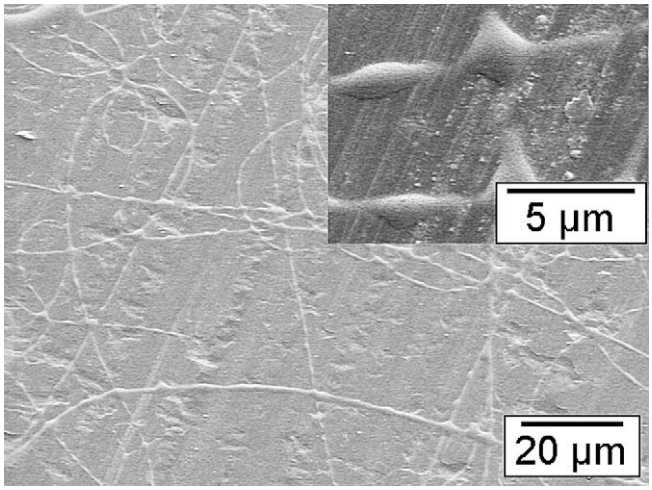

Fig. 1. SEM of electrospun product from 50 wt% 10 kDa PAAm aqueous solution.

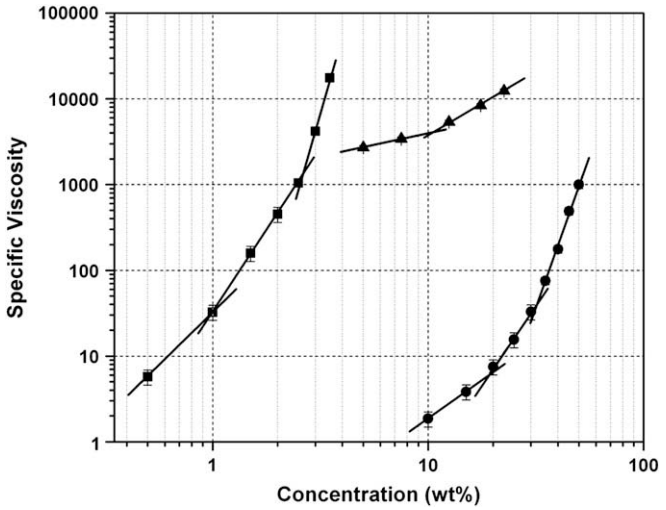

Fig. 3. Specific viscosities of aqueous solutions of PAAm: 5000–6000 kDa (■), 10 kDa (•), and their mixtures (▲) containing constant 2.5 wt% 5000–6000 kDa PAAm and 10 kDa PAAm at varying concentrations of 2.5, 5.0, 10.0, 15.0, and 20.0 wt%.

colorless X-gal is a galactose sugar with a glycosidic linkage to a chromophore which turns blue when cleaved by β-galactosidase and oxidized into an insoluble blue colored 5,5′-dibromo-4,4′-dichloro-indigo. Presence and distribution of β-galactosidase was further verified by sulfur mapping, performed on carbon sputter coated sample under vacuum at a 7 kV accelerating voltage with a scanning area of 50 μm × 50 μm for 60 min using a Cameca SX-100 electron microprobe (AMETEK Inc., Gennevilliers, France).

3. Results and discussion

3.1. Fabrication of PAAm fibers

The 10 kDa PAAm was highly water-soluble to reach concentrations up to 50 wt%. Electrospinning of the aqueous solutions at up to 40 wt% failed to produce observable jets under voltages ranging from 2 to 25 kV. The 50 wt% PAAm solution was too viscous to maintain a continuous jet, generating some large (~3 μm diameter) and irregular fiber segments (Fig. 1). The fibers were brittle and broke easily, likely due to insufficient entanglement and weak cohesion among the short chains.

The 5000–6000 kDa PAAm was also readily soluble in water, but could only reach a much lower maximum concentration of 3.0 wt%. Electrospinning of 0.5–2.5 wt% PAAm solutions produced fibers with increasing uniformity as well as diameters with increasing concentrations. Fragmented thin fibers interspaced with beads were produced from the high molecular weight PAAm at below

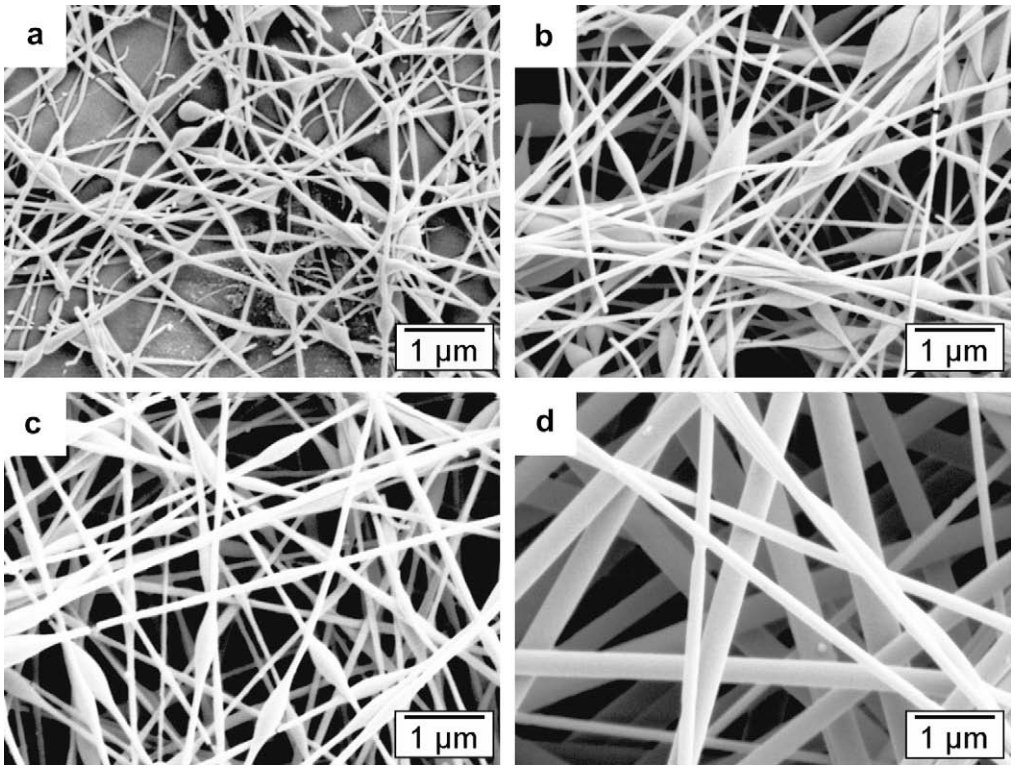

Fig. 2. SEM of fibers electrospun from aqueous solutions with varied PAAm (M_w: 5000–6000 kDa) concentrations: (a) 1.0 wt%, (b) 1.5 wt%, (c) 2.0 wt%, (d) 2.5 wt%.

1.0 wt% (Fig. 2a). As concentrations raised to 1.0 and 2.0 wt%, the fibers became continuous but with much larger and elongated beads (Fig. 2b and c). The 2.5 wt% PAAm solution generated smooth and continuous fibers at an average diameter around 267 nm (Fig. 2d). While no beads were observed, the fiber diameters vary considerably with a standard deviation (SD) of 105 nm or 39% coefficient of variation (CV). At concentrations 3.0 wt% and above, the PAAm solutions were gel-like and too viscous to be electrospun.

The specific viscosity of the 5000–6000 kDa PAAm solutions increased from 5.7 to 17,563 with increasing concentrations (C) from 0.5 wt% to 3.5 wt%. The η_{sp}–C log–log plot shows two slope changes at 1.0 wt% and 2.7 wt% (Fig. 3). The concentration dependence of viscosity of linear polymers in good solvents has been categorized into four concentrations of dilute, semidilute unentangled, semidilute entangled, and concentrated regimes [19–21]. The entanglement concentration C_e, the boundary between the semidilute unentangled and semidilute entangled regimes, is defined as the concentration at which significant overlap of the polymer chains topologically constrain the chain motions, causing entanglement couplings [22]. Fiber formation was observed at and above 1%, supporting the assignment of the 1.0 wt% as the entanglement concentration C_e and 2.7 wt% as the onset of the concentrated regime, respectively. The linear regressions showed that η_{sp} was proportional to $C^{2.51}$, $C^{3.64}$ and $C^{9.29}$ in the semidilute unentangled, semidilute entangled and concentrated regimes, respectively.

The specific viscosities of the 10 kDa PAAm solutions also increased with concentrations and the η_{sp}–C log–log plot showed two slope changes at 18.7 wt% and 32.9 wt%. These slopes were smaller and the slope changes were not as distinct as those for the 5000–6000 kDa PAAm solutions. Although the η_{sp} of 10 kDa PAAm above 32.9 wt% overlapped with those of 5000–6000 kDa PAAm above the C_e, the fact that only few non-continuous fibers were observed at 50 wt% pointed to low entanglement among short molecular chains.

Adding Triton X-100 surfactant to the 2.5 wt% solution of 5000–6000 kDa significantly reduced the SD to 38 nm at a similar mean fiber diameter of 263 nm. Lowering the solution surface tension greatly improved fiber size uniformity as indicated by the much reduced CV of 14%.

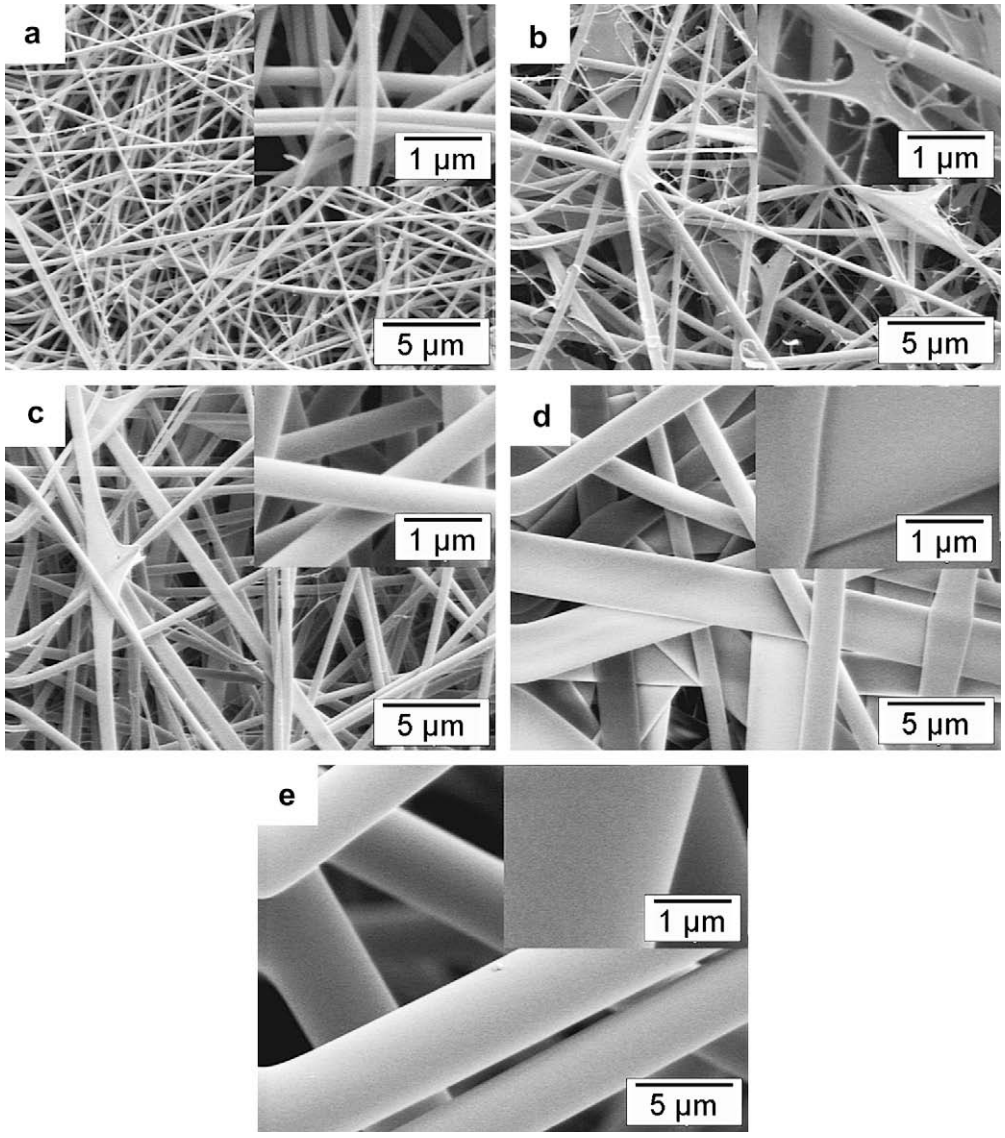

Fig. 4. SEM of fibers electrospun from aqueous PAAm mixtures at total concentrations of: (a) 5.0 wt%, (b) 7.5 wt%, (c) 12.5 wt%, (d) 17.5 wt%, and (e) 22.5 wt% (constant 2.5 wt% 5000–6000 kDa PAAm and varying 10 kDa PAAm concentrations of 2.5, 5.0, 10.0, 15.0, and 20.0 wt%).

In the attempt to further optimize the electrospinning process, varying concentrations of the 10 kDa PAAm (2.5, 5.0, 10.0, 15.0, 20.0 wt%) were added to a constant 2.5 wt% 5000–6000 kDa PAAm to yield mixtures with total PAAm concentrations of 5.0, 7.5, 12.5, 17.5, and 22.5 wt%. The 5.0 wt% PAAm mixture generated round fibers with an average diameter of 270 nm (SD 77) and few much finer fragmented fibers (Fig. 4a). With increasing concentrations from 7.5 to 12.5 and 17.5 wt%, the average fiber diameters also increased from 440 nm (SD 106) to 542 nm (SD 75) and 1109 nm (SD 375), respectively (Fig. 4b–d). Some fibers appeared flat ribbon-like. With the 22.5 wt% PAAm mixture, the fibers produced were mainly round, but very large with 2.84 μm average diameter (SD 824 nm) (Fig. 4e). The CV values of fiber diameters from the mixtures ranged from 14% to 34%, all lower than that of 2.5 wt% 5000–6000 kDa PAAm alone. Therefore, adding the shorter PAAm chains to the mix had the apparent effect of enlarging fiber dimensions and improving the electrospinning efficiency which is defined as the mass of fibers produced by electrospinning in a certain amount of time.

The η_{sp} of the mixtures increased from 2724 to 12,451 in the concentration range studied and exhibited a change in the slope at $C^{**} = 10.6$ wt%. Because all mixtures could be electrospun into fibers, the entire concentration range is considered to be in the entangled regimes. The concentrations below 10.6 wt% were assigned to the semidilute entangled regime and those above in the concentrated regime. The η_{sp} of 2.5 wt% 5000–6000 kDa PAAm alone was 2631 while that of 20 wt% 10 kDa PAAm was only 7.5. In fact, the η_{sp} of 10 kDa PAAm at below 20 wt% were so low and considered essentially the same. The addition of 10 kDa PAAm to 5000–6000 kDa PAAm did show synergistic effects on the η_{sp} of the mixtures (Fig. 3). The synergism became more significant with increasing 10 kDa PAAm contents. This synergistic effect on viscosity could be due to the higher degree of freedom of the shorter chains to allow better mixing and intermolecular hydrogen bonding. The stronger intermolecular cohesion explains the observed larger fiber sizes and is consistent with reduced instability in electrospinning. As observed earlier, however, it is the longer 5000–6000 kDa PAAm chains that provide the entanglement

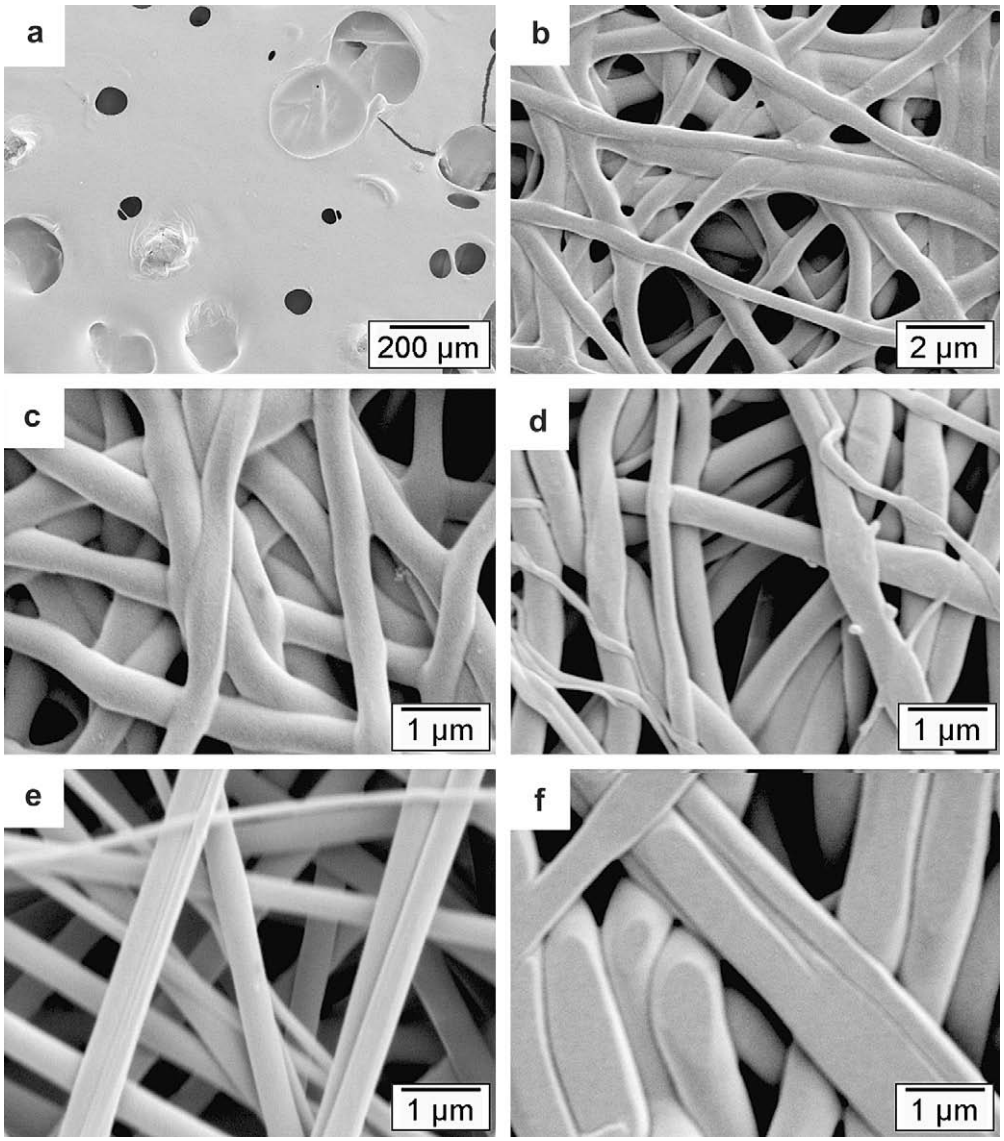

Fig. 5. SEM of glutaraldehyde-crosslinked PAAm fibrous membranes following 10-min water immersion: effect of catalyst (10 wt% GA for 2 h at ambient temperature) (a) 0 wt% HCl and (b) 4 wt% HCl; effect of reaction temperature (10 wt% GA with 4 wt% HCl for 60 min): (c) 60 °C; effect of reaction time (10 wt% GA with 4 wt% HCl at 120 °C): (d) 30 min and (e) 60 min; effect of glutaraldehyde concentration (4 wt% HCl for 60 min at 120 °C): (f) 15.0 wt%.

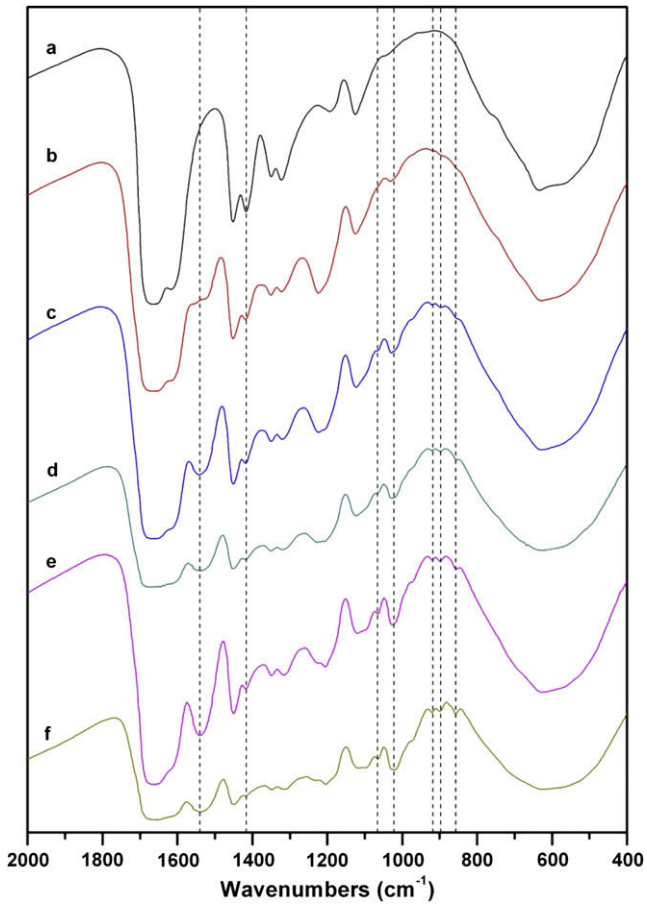

Fig. 6. FTIR of (a) original PAAm and crosslinked PAAm with glutaraldehyde at: (b) 2.5 wt%, (c) 5.0 wt%, (d) 7.5 wt%, (e) 10.0 wt%, (f) 12.5 wt% (in EtOH with 4 wt% HCl and heated for 60 min at 120 °C).

necessary for fiber formation in electrospinning. It should also be noted that increasing amount of the 10 kDa PAAm caused the fibers to become increasingly stiff and the fibrous membrane more brittle possibly due to the reduced chain entanglement.

3.2. Crosslinking of PAAm fibers

Upon water contact, the electrospun PAAm fibrous membranes immediately turned clear and gelatinous, then dissolved rapidly. Crosslinking with glutaraldehyde (GA) was conducted on the PAAm fibrous membranes (electrospun from 2.5 wt% 5000–6000 kDa) under different conditions. The GA-crosslinked membranes were immersed in water for 10 min, dried, and then observed under SEM to determine if crosslinking was effective in rendering the membranes insoluble in water and retaining the best fibrous structure. PAAm membrane saturated with 10% GA and dried under ambient temperature for 2 h could not retain its fiber morphology (Fig. 5a), suggesting the need for a catalyst. The amide nitrogen is a very weak nucleophile because the unpaired electrons on the amide nitrogen are delocalized by its nearly parallel position to the carbonyl p orbitals. This prevents the lone pair from accepting hydrogen ions and acting as a base. Acid catalysts can protonate the carbonyl oxygen to allow reaction with GA carbonyl carbon (electrophile). By adding HCl, the fiber morphology was retained and the retention of fiber structure improved with increasing concentration from 1% to 4%. At above 4% HCl, the fibrous membranes became brittle. Therefore, 4 wt% HCl was the optimal catalyst concentration to achieve most water-insoluble and best shape retention fibrous membranes (Fig. 5b).

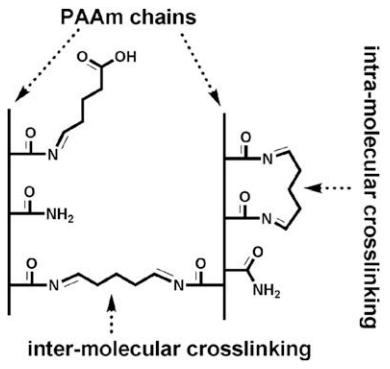

Fig. 7. Reactions between PAAm and glutaraldehyde: crosslinking and grafting.

To drive the reaction between PAAm and GA further, the time and temperature were further optimized at the same crosslinker (10 wt% GA) and catalyst (4 wt% HCl) concentrations. Reaction at 60 °C resulted in partial retention of the fibrous structure (Fig. 5e). Reactions conducted between 80 and 140 °C rendered the fibers water-insoluble and the membranes became easier to handle, especially in water, with increasing temperatures. Above 140 °C, the membrane turned yellow, showing signs of decomposition. At 160 °C, the membrane became soft and deformation of surface fibers from heating between the glass plates was observed. At 120 °C, the membranes crosslinked for 30 min showed some fiber deformation (Fig. 5c) while a reaction time of 1 h achieved full retention of fiber morphology (Fig. 5d). Longer crosslinking time of 2 h caused the membrane to turn yellow, rigid and brittle. Thus, 120 °C for 1 h is the optimal reaction condition for crosslinking PAAm with GA.

Heat is very effective in accelerating the reaction between PAAm and GA by one or more of the following: (1) enhancing the evaporation of ethanol and removal of the existing and newly formed water molecules; (2) raising HCl concentration from the evaporation of EtOH and water; (3) increasing chain movement to improve the diffusion of reactants and reagents and providing the energy for the endothermic crosslinking reaction.

GA concentration was then varied from 2.5 wt% to 15 wt% with the optimal 4 wt% HCl at 120 °C for 60 min heating condition. The membrane reacted with 2.5 wt% GA remained insoluble in water for 10 min, but became gel-like and hard to handle. Products from reactions with 5.0, 7.5, and 10.0 wt% GA exhibited excellent stability in water without any deformation and appeared similarly under SEM. Reaction with 12.5 wt% GA causes the fibrous membrane to shrink slightly while that in the reaction solution of 15.0 wt% GA led to instantaneous shrinkage and deformed fiber surfaces (Fig. 5f). The GA concentration can affect the reaction in two ways. While higher GA concentrations increase the available reagent for crosslinking, more water is also introduced from the original aqueous GA solution, imposing an adverse effect to the crosslinking reaction. All the above results showed that the reaction with 10 wt% GA in the presence of 4 wt% HCl at 120 °C for 1 h is the optimal condition to produce sufficiently crosslinked and water-insoluble PAAm membranes.

The reaction between PAAm amide and GA aldehyde forms an imine which was clearly evident by a new and strong adsorption peak of imine C=N at 1538 cm^{-1} in the GA-crosslinked membranes (Fig. 6). With the increase of GA concentrations, the imine peak became stronger, indicating increasing reaction between GA and the PAAm amide. The characteristic amide C–N peak at 1413 cm^{-1} reduced with increasing GA, which is consistent with the increasing conversion of the amide to imine observed. The reactions between

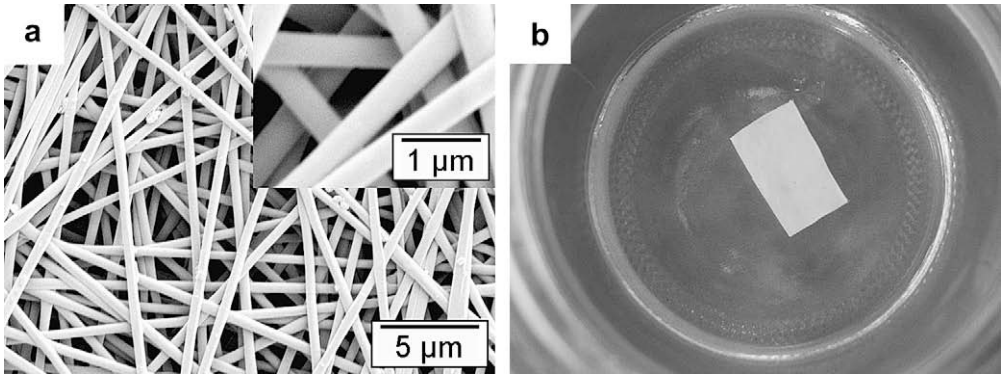

Fig. 8. Glutaraldehyde crosslinked PAAm (10 wt% GA with 4 wt% HCl at 120 °C for 60 min) after 72 h water immersion: (a) SEM of dried membrane and (b) image in water.

PAAm amide and GA aldehyde can lead to two possible outcomes: both aldehydes of a GA molecule react with two PAAm amides to yield a crosslinking bridge with two imine links, or only one aldehyde end of GA reacts with a PAAm amide to form imine bond while the other aldehyde is hydrolyzed to carboxylic acid. The first case is supported by the significant reduction of the amide C–N at 1413 cm^{-1} and appearance of the imine C═N at 1538 cm^{-1}. The latter case is also confirmed with presence of the carboxylic acid

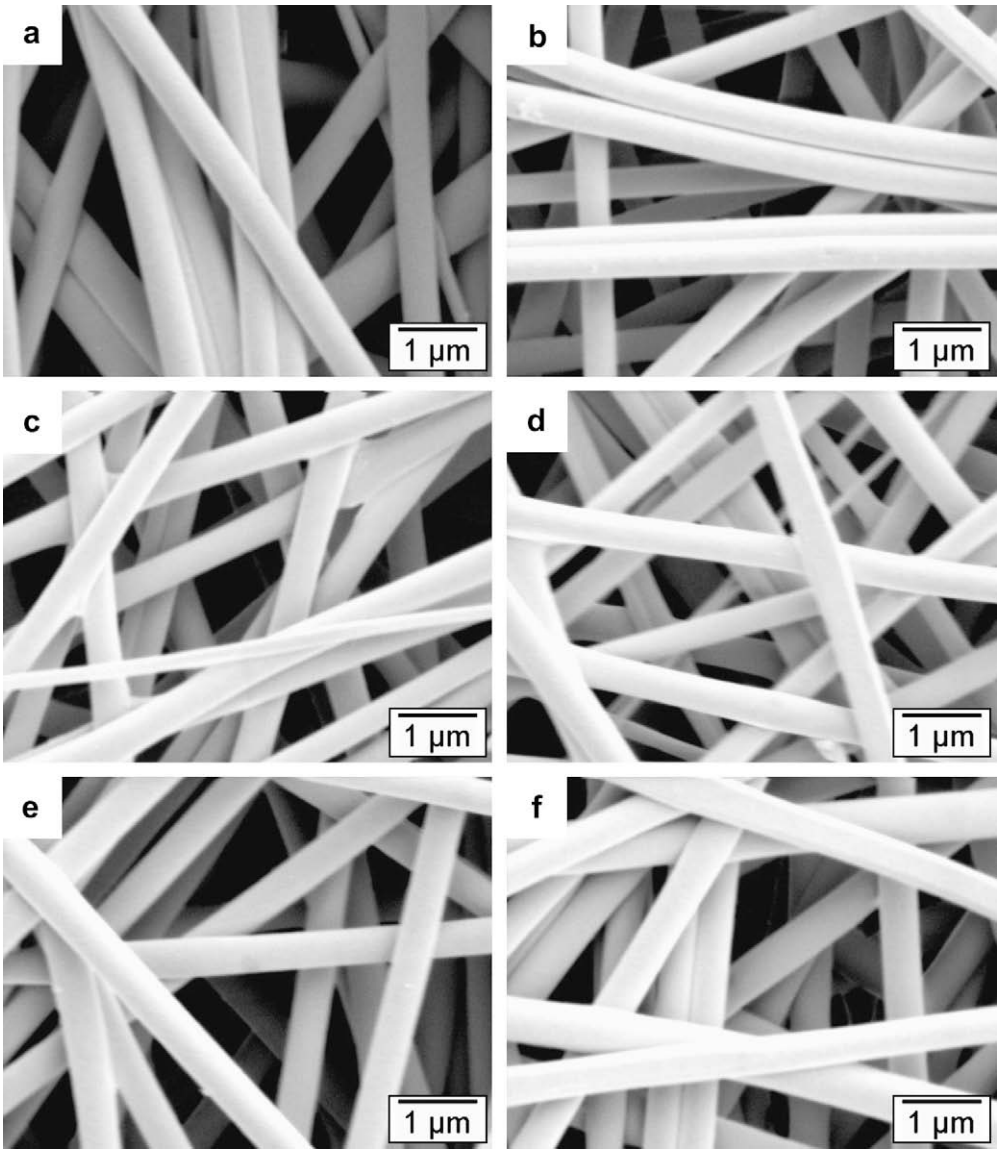

Fig. 9. Glutaraldehyde crosslinked PAAm (10 wt% GA with 4 wt% HCl at 120 °C for 60 min) following 72 h immersion in: (a) methanol, (b) ethanol, (c) acetone, (d) chloroform, (e) DMF, and (f) cyclohexane.

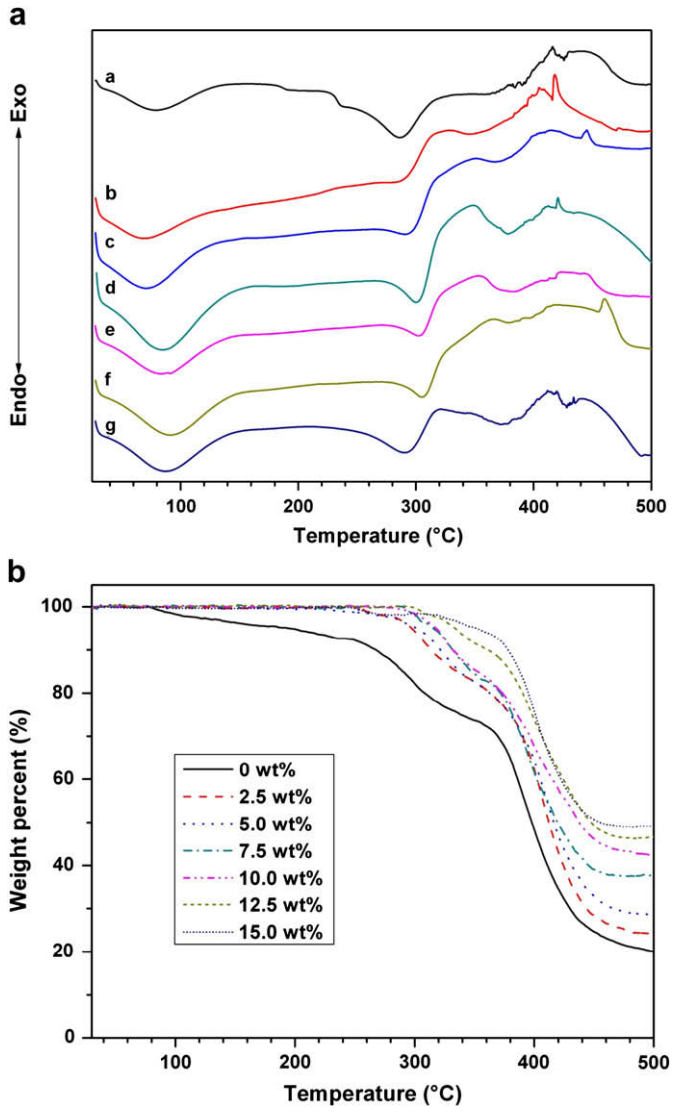

Fig. 10. DSC (A) and TGA (B) of (a) original PAAm and crosslinked PAAm with glutaraldehyde at: (b) 2.5 wt%, (c) 5.0 wt%, (d) 7.5 wt%, (e) 10.0 wt%, (f) 12.5 wt%, and (g) 15.0 (4 wt% HCl at 120 °C for 1 h).

peaks at 1065, 1020, 919, 900, and 855 cm^{-1}. With increasing GA, the increasing intensities of these peaks indicated that more GA were grafted onto rather than crosslinked with the PAAm side chains. While crosslinking of PAAm could be either inter- or intramolecular reactions, both could render PAAm membrane water-insoluble. Fig. 7 shows the possible reactions and products.

The optimally crosslinked (10 wt% GA in the presence of 4 wt% HCl at 120 °C for 60 min) PAAm membrane exhibited excellent stability in water following prolonged exposure up to 72 h (Fig. 8). The membrane could be easily handled in water as well as out of water. The dried membrane showed no observable structural change and retained essentially the same fiber morphology (Fig. 8a) as that immersed for only 10 min (Fig. 5d).

In addition to water stability, the crosslinked PAAm fibrous membranes showed excellent shape and morphology retention after extended 72 h immersion in methanol, ethanol, acetone, chloroform, DMF, and cyclohexane as shown in Fig. 9. The stability in such wide range of organic solvents further extends the potential of these crosslinked PAAm fibrous membranes in applications in diverse aqueous to organic media.

3.3. Property of crosslinked PAAm fibers

The DSC thermogram of the as-spun PAAm fibers exhibited a very broad endotherm from ambient temperature to ca. 130 °C associated with evaporation of the absorbed moisture (Fig. 10a). The base line shifted slightly around 180 °C then more distinctively at 218 °C before the major decomposition endothermic peak at 280 °C. The major differences in the DSC of the crosslinked samples were the disappearance of the base line shifts and the elevated decomposition peaks by up to 25 °C. In fact, the onset of decomposition was raised by at least 40 °C (from 218 °C to 260 °C or higher). The base line shifts are likely the glass transition of PAAm and their disappearance is clearly indicative of the reduced segmental motion expected from side group reactions and in particular from crosslinking, both consistent with FTIR and solubility results. The crosslinked membranes exhibited slightly elevated moisture evaporation endotherms with increasing GA concentrations. The TGA (Fig. 10b) thermograms showed onset the mass loss above 260 °C. Both of the DSC and TGA data clearly indicate the improved thermal stability of the GA-crosslinked PAAm fibrous membranes. New decomposition endotherms from

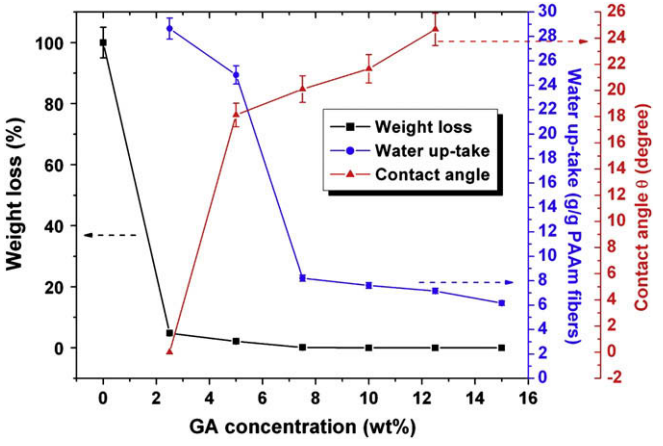

Fig. 11. Effects of glutaraldehyde concentrations (4 wt% HCl at 120 °C for 1 h) on the behavior of crosslinked PAAm: weight loss, water absorption and water contact angle.

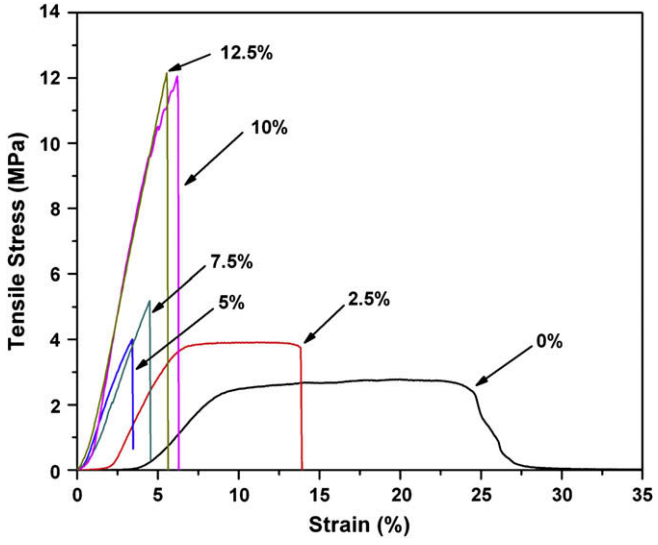

Fig. 12. Stress–strain curves of PAAm fibrous membranes crosslinked at varying glutaraldehyde concentrations (4 wt% HCl at 120 °C for 1 h).

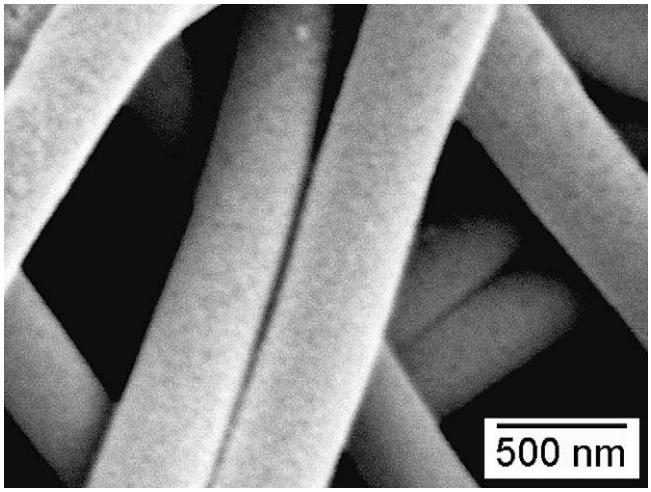

Fig. 13. SEM micrographs of β-galactosidase entrapped PAAm fibrous membranes (PAAm: ~50 mg; β-galactosidase: 2 mg/mL; 1 mL; pH 7; 4 °C for 24 h and washed thoroughly by buffer solution and deionized water until no enzyme leakage was detected).

mid to upper 300 °C also appeared upon crosslinking. Thermally induced intra- and intermolecular imidization of the PAAm amide side groups have been reported in the upper 200 °C [12]. The gradual mass loss of the as-spun PAAm observed in this temperature range on the TGA curve (8% at 250 °C) is consistent with the release of H_2O, NH_3 and CO_2 byproducts from imidization. Above 300 °C, the imides decompose to form nitriles and the polymeric main chain backbone formed hydrocarbons (exothermic). Reactions and crosslinking involving the amide side group are expected to reduce or prevent imidization, consequently alter the decomposition. The new endotherms and reduced exotherm in the upper 300 to over 400 °C range are consistent with the altered decomposition.

The extent of crosslinking on electrospun PAAm fibrous membrane was further observed by monitoring their mass changes from water immersion. Fig. 11 shows the weight losses and the water up-takes by the PAAm fibrous membrane as a function of the GA concentrations. The swelling, expressed as grams of water up-take per gram of PAAm membrane, decreased with increasing GA concentrations, indicating increasingly crosslinked PAAm structure. The as-spun PAAm membrane dissolved completely in water, i.e., losing 100% mass, whereas the one crosslinked with 10% GA retained essentially full mass. In fact, reaction with 5 wt% GA retained an impressive 95% mass, indicating efficient reaction between the PAAm amide side group and GA aldehyde. Furthermore, the water wetting contact angles of PAAm fibrous membranes increased slightly from 0 to 24.7° with the increase of GA concentrations. While all crosslinked PAAm membranes remained hydrophilicity with increased crosslinking, a slightly lowered work of adhesion or 9% decrease in the cosine contact is noted.

The tensile strength–strain curves of the crosslinked PAAm fibrous membranes showed completely altered behavior from the as-spun original, clear results of crosslinking (Fig. 12). The uncrosslinked membrane behaved more like an elastomer, i.e., high strain and low stress, and upon crosslinking, became more like stiff thermoset plastics with much higher stress and lower strain. In the increasingly crosslinked PAAm membranes, both the polymer chains and individual fibers were tightly connected to each other by covalent bonds and inter-fiber joints, whereby the sliding in chains and among fibers became more reduced leading to significantly improved overall mechanical properties.

3.4. Enzyme entrapment

The optimally crosslinked 5000–6000 kDa PAAm fibrous membrane also retained its morphology and structure after 24 h in the pH 7 buffer solution (Fig. 13). Along with the swelling process, β-galactosidase was driven into the PAAm hydrogel fibers by the concentration gradient. The membrane was thoroughly rinsed with pH 7 buffer and deionized water for 20 min each to remove the surface bound enzymes. The washing solutions were monitored until no enzyme leakage was detected and then the membrane was dried at ambient temperature under vacuum for 5 h. The dried PAAm membrane showed no enzyme aggregates and the fiber surfaces remained smooth. The microprobe elemental mapping of this membrane showed very strong and dense sulfur signals whereas that of the blank only had very small noises caused by the instrument limitations (Fig. 14). The sulfur signals of the sample clearly indicated large and even distribution of β-galactosidase in the membrane. The enzyme molecules are thought to be entrapped within the gel network inside the PAAm fibers. Since β-galactosidase releasing was not detected in the assay solution from this membrane, the enzyme molecules were entrapped in the crosslinked PAAm 3-D network structure. These pores in the crosslinked PAAm network open up from swell in the aqueous environment to allow the entry of β-galactosidase molecules. A combination of physical steric hindrance, abundant hydrogen bonds, electric attractions and other interactions was believed to jointly entrap the enzyme molecules in the PAAm structure during assay and applications.

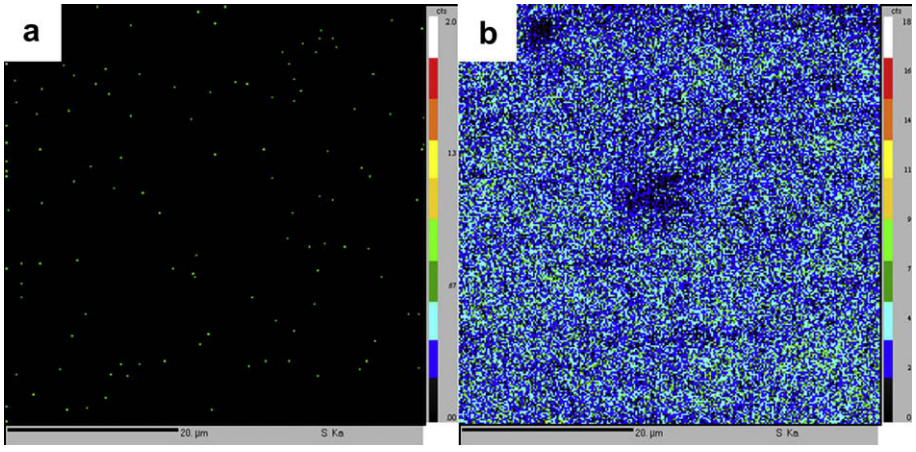

Fig. 14. Electron microprobe sulfur mapping of crosslinked PAAm (a) blank and (b) sample.

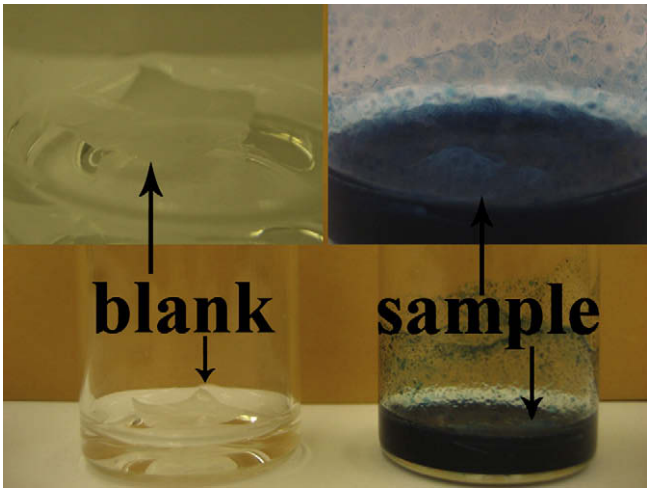

Fig. 15. Assay of β-galactosidase using X-gal (~10 mg membrane immersed in 1 mL pH 7 buffer solutions with drops of X-gal at 20 mg/mL and incubated at 37 °C for 24 h).

The activity of PAAm bound β-galactosidase was assayed using X-gal, a galactose sugar with a glycosidic linkage to a colorless chromophor. β-Galactosidase cleaves the glycosidic linkage of X-gal to yield galactose and 5-bromo-4-chloro-3-hydroxyindole which is oxidized into 5,5′-dibromo-4,4′-dichloro-indigo, a blue colored insoluble product. After 24 h incubation, the PAAm membrane containing the entrapped β-galactosidase turned blue while the blank still remained clear (Fig. 15). The intense blue color throughout the assay solution indicates the accessibility of the bound β-galactosidase to X-gal and the diffusion of blue product out of the PAAm fibers into the assay solution, confirming again the existence of β-galactosidase and its catalytic activity.

4. Conclusions

PAAm with molecular weight 5000–6000 kDa and 10 kDa has been successfully fabricated by electrospinning from aqueous media into ultra-fine fibrous membranes with a variety of diameters and morphologies. The fibrous morphologies include beaded, round and branched fibers, and flat ribbons. The 2.5 wt% 5000–6000 kDa PAAm alone generated continuous and smooth fibers with diameter 267 ± 105 nm. Adding Triton X improved the uniformity of fiber diameters (SD 35 nm) while adding the non-fiber-forming 10 KDa PAAm improved electrospinning efficiency, increased mean fiber sizes but also membrane stiffness and brittleness. Crosslinking with glutaraldehyde (GA) on the PAAm fibrous membranes (electrospun from 2.5 wt% 5000–6000 kDa) required an acid catalyst and the most water-insoluble and best shape retained fibrous membranes was achieved with 4 wt% HCl followed by heating at 120 °C for 1 h. The crosslinking reaction between PAAm amide and GA aldehyde was confirmed by the new and strong adsorption peak of imine C=N at 1538 cm^{-1} which became more intense with increasing GA concentrations. While crosslinking of PAAm could be either inter- or intra-molecular reactions, both render PAAm membrane water-insoluble. Furthermore, the carboxylic acid peaks appeared and became stronger with increasing GA concentrations, indicating increasing grafting of the PAAm amide side group with one GA aldehyde while the other hydrolyzing to carboxylic acid. The optimally crosslinked fibrous membrane showed excellent thermal stability up to 250 °C, super water absorbency of up to 28 g water/g PAAm membrane, high hydrophilicity with contact angle θ from 0 to 25°, and up to 6-fold increase in mechanical strength. The β-galactosidase enzyme was successfully entrapped into the inner microstructures of PAAm hydrogel fibers and the existence of the enzyme was confirmed by sulfur mapping. The entrapped β-galactosidase demonstrated catalytic activity by turning X-gal blue. Besides the entrapment of enzymes and proteins for solid supported catalysis and diagnosis, these highly aqueous and organic stable PAAm hydrogel fibrous membranes are potentially useful in a wide range of biomedical and industrial applications including controlled release and delivery, separation and recovery, etc.

Acknowledgements

This work was supported by a National Textile Center research grant (M02-CD05) and a Jastro-Shields Graduate Research Scholarship from University of California at Davis.

References

[1] Kopecek J. Biomaterials 2007;28(34):5185–92.
[2] Chaterji S, Kwon IK, Park K. Prog Polym Sci 2007;32(8–9):1083–122.
[3] Retama JR, Lopez-Ruiz B, Lopez-Cabarcos E. Biomaterials 2003;24(17):2965–73.
[4] Li L, Hsieh Y-L. Nanotechnology 2005;16(12):2852–60.
[5] Li L, Hsieh Y-L. Polymer 2005;46(14):5133–9.
[6] Huang Z-M, Zhang YZ, Kotaki M, Ramakrishna S. Compos Sci Technol 2003;63(15):2223–53.
[7] Chen H, Hsieh Y-L. J Polym Sci Part A Polym Chem 2004;42(24):6331–9.
[8] Zhang J, Tran NT, Weber J, Slim C, Viovy J-L, Taverna M. Electrophoresis 2006;27(15):3086–92.
[9] Burnham MR, Turner JN, Szarowski D, Martin DL. Biomaterials 2006;27(35):5883–91.
[10] Mincheva R, Manolova N, Paneva D, Rashkov I. J Bioact Compat Polym 2005;20(5):419–35.
[11] Zhao YY, Yang QB, Lu XF, Wang C, Wei Y. J Polym Sci Part B Polym Phys 2005;43(16):2190–5.
[12] Caulfield MJ, Qiao GG, Solomon DH. Chem Rev 2002;102(9):3067–84.
[13] Siyam T. Egypt J Chem 1995;38(1):91–8.
[14] Fong DW, Kowalski DJ. J Polym Sci Part A Polym Chem 1993;31(6):1625–7.
[15] Schiffman Jessica D, Schauer Caroline L. Biomacromolecules 2007;8(2):594–601.
[16] Ruckenstein E, Liang L. J Membr Sci 1996;110(1):99–107.
[17] Caulfield MJ, Hao X, Qiao GG, Solomon DH. Polymer 2003;44(5):1331–7.
[18] Hsieh Y-L. Textile Res J 1995;65(5):299–307.
[19] Colby RH, Fetters LJ, Funk WG, Graessley WW. Macromolecules 1991;24(13):3873–82.
[20] De Gennes PG. Scaling concepts in polymer physics; 1979.
[21] McKee MG, Wilkes GL, Colby RH, Long TE. Macromolecules 2004;37(5):1760–7.
[22] Graessley WW. Polymer 1980;21(3):258–62.