Preparation and Characterization of Novel Hyperbranched Poly(amine-ester) Films Crosslinked by Glutaraldehyde

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ABSTRACT: Novel films based on hydroxyl terminated hyperbranched poly (amine-ester) (HPAE-OH) of different generation were prepared by crosslinking the terminal hydroxyl groups of HPAE with glutaraldehyde (GA). The progress of crosslinking reaction was characterized by Fourier transform infrared (FTIR) and viscosity measurement. The surface morphology of the crosslinked HPAE films was characterized by field emission scanning electron microscope (FE-SEM) and atomic force microscopy. The results suggested that the films have homogenously dense interior matrices and smooth surface. The hydrophilicity/hydrophobicity of the crosslinked HPAE films was charac-

terized by the water contact angle measurement. Variable swelling behavior in different solvents was also studied. The *in vitro* biocompatibility of the film was investigated by the bovine hemoglobin (Hb) adsorption measurement. And these results showed that these crosslinked HPAE films had excellent hydrophilicity, variable swelling behavior in different solvents, and relative low protein absorption. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 3613–3621, 2008

Key words: hyperbranched; hydrophilic polymers; films; swelling; protein

INTRODUCTION

Dendritic polymers are a kind of emerging polymers comprising dendrimers and hyperbranched polymers (HBPs).[1] Unlike their linear analogues, the dendritic polymers have monomolecular sphere-like structure, low solution or melt viscosity, high solubility in various solvents etc.[2] In comparison, dendrimers have more defined and ordered branching structure than HBPs. However, the practical applications of dendrimers are limited owning to the difficulty of large-scale synthesis and purification. On the contrary, HBPs could be synthesized very efficiently on a large-scale through a one-step polycondensation process of AB_x type monomers.[3] Although the three-dimensional architectures of HBPs are not so perfect as those of dendrimers, HBPs still have research interests in ultra-thin functional coatings, resin additives,[4–6] encapsulated catalysts,[7] and organic nanoparticles.[8] Therefore, increasing efforts are

turned to the synthesis and functional modification of HBPs.[2]

The main disadvantage of such branched macromolecules accompanied with a variety of functional groups and high reactivity is the lower tensile strength. Combining polymers with complementary properties have drawn increasing attention, which is usually done either by blending or crosslinking. Blending leads to a material with one phase dispersed in another, while crosslinking leads to the formation of network structure. The chemical crosslinking is a good way to improve the capability of the polymer and a well-studied process of incalculable practical importance. Recently, the corresponding investigation of crosslinking macromolecules like HBPs have been mainly focused on the irradiation curing of HBPs containing double bonds,[9–14] improving the surface properties of other polymers (polypropylene, polyethylene etc.),[15–22] the reversible crosslinking[23] and the crosslinking options of HBPs.[24] Although the advantages of high functionality, broad variety of functional groups make HBPs ideally outlined for film forming applications by crosslinking the end groups, the fabrication of HBPs into a larger matrix material (e.g., in thicker films or plates) with a certain function was rarely taken into account.[14,16,25]

In the present work, the crosslinked hyperbranched poly(amine-ester) (HPAE) films with ace-

tal/hemiacetal linkages were prepared by crosslinking the hydroxyl groups of HPAE-OH with glutaraldehyde (GA). The influences of HPAE-OH molecular weight on the structure, hydrophilicity, swelling behavior, and protein absorption of the resultant films were studied.

EXPERIMENTAL

Materials

1,1,1-trimethylolpropane (TMP), diethanolamine (DEA), p-toluene sulfonic acid (p-TsOH), glutaraldehyde (GA), and bovine hemoglobin (Hb) were purchased from Aldrich. Methanol, methyl acrylate (MA), and N,N-dimethylacetamide (DMAc) were distilled just prior to use. All chemicals used were analytical grade. Deionized water was used throughout the experiments.

Synthesis and characterization of hydroxyl-terminated hyperbanched poly(amine-ester)

Hydroxyl-terminated hyperranched poly(amine-ester) (HPAE-OH) were synthesized via a pseudo one-step alcoholysis reaction in the melt at 120°C using TMP (as a molecular core) and N,N-diethylol-3-amine methylpropionate (as an AB$_2$ monomer) with p-TsOH as the catalyst.[26] N,N-diethylol-3-amine methylpropionate was synthesized via Michael additon using the same mole ratio of methyl acrylate and diethanolamine at 25°C under a nitrogen atmosphere. The generation of HPAE-OH (Gn, n = 1–6) was increased by repeatedly adding AB$_2$ monomer to the former generation product (Scheme 1). The third-generation HPAE-OH (G3) was obtained by repeating the process twice. Gn present the generation of the HPAE-OH and n present the repeating times of alcoholysis reaction. The molecular weight (M_n) and distribution were determined by gel permeation chromatography (GPC) (Waters 515 with Styragel® columns HT3, HT4, and HT5) with tetrahydrofuran (THF) as eluent and using polystyrene as calibration standards. The hydroxyl values of HPAE were determined from a standard titration method.[27] The morphology of HPAE particles was imaged on transmission electron microscope (TEM JEM200CX). The isoelectric point (IEP) was determined by turbidimetry/spectrometry method.[28]

Preparation of crosslinked hyperbranched poly(amine-ester) films

The preparation and structure of crosslinked HPAE film is also given in Scheme 1. The reaction progress and crosslinking condition were determined by monitoring the viscosity (Ubbelohde viscometer, 0.2 wt
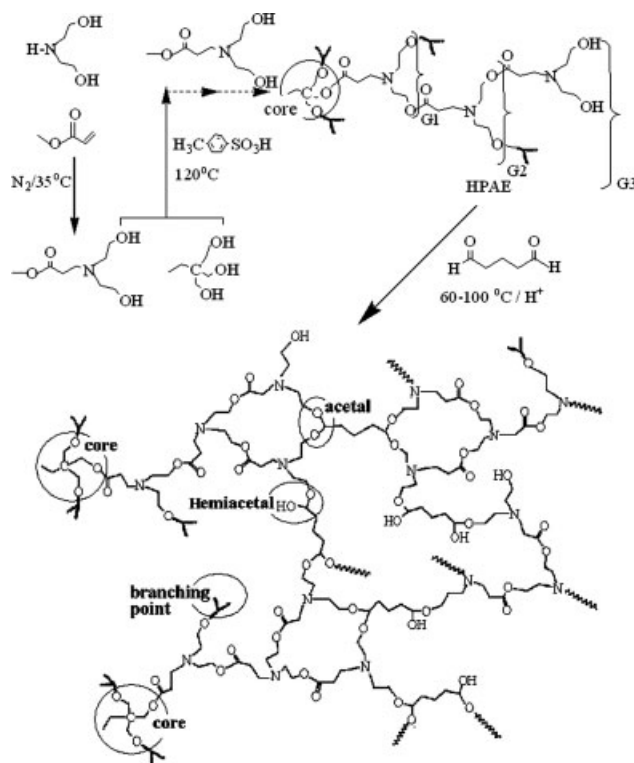
Scheme 1 Synthesis of G3 HPAE-OH and preparation of HPAE crosslinked film.

%, 30°C) changes of HPAE-OH/GA/DMAc solution (containing trace quantity of sulfuric acid as catalyst) after being heated to various temperatures for different periods of time. For fabricating a crosslinked HPAE film, the sticky HPAE-OH was dissolved in DMAc to form a 40 wt % solution. Then, glutaraldehyde/water solution (75 wt % in water, the moles ratio of aldehyde groups/hydroxyl groups = 1 : 1) and the trace quantity catalyst of concentrated sulfuric acid were then added. The mixture was slowly stirred at 65°C for 4 h and then cast onto glass plate, thus forming a solution film with a thickness of about 150 μm. After removing the solvent at 100°C for 4 h, the film on the glass plate was further treated at 100°C under vacuum for 6 h to yield a solid crosslinked film with a thickness of 60–80 μm.

Characterization of the crosslinked films

Fourier transform infrared (FTIR, Bruker Vector 22 FTIR) was used to characterize the chemical changes in formation of crosslinked films. The surface morphology and roughness parameter (S_a) were obtained from atomic force microscopy (AFM, SPA3800) measurements in the tapping mode. The interior structures of the films were imaged on a field emission scanning electron microscope (FE-SEM, JEOL, JSM-5510LV, Japan). The mechanical properties were characterized on a tensile tester (Shi-

TABLE I
Parameters of Different Generation HPAE-OHs

HPAE generation	M_z	M_W	M_n	M_n^t	M_W/Mn	Hydroxyl value (Theoretical)	Hydroxyl value (Experimental)
G3	1028	945	873	3044	1.08	441	469
G4	1298	1184	1074	6860	1.10	391	408
G5	1207	1121	1038	14492	1.09	370	384
G6	1130	1074	998	29756	1.03	361	372

madzu AG-1) using a stretching rate of 10 mm/min at room temperature. The reported data for a specific sample were taken as an average of eight experiments. The water static contact angles of films surfaces were measured on OCA20 (Data Physics) instrument. The swelling ratios (Q_t) of film were taken as $Q_t = (w_t - w_0)/w_0 \times 100\%$, where w_0, w_t represent the weight of the dry film and the film soaked in different solvents for certain time at 25°C respectively. To characterize the protein absorption properties, the film specimen (3.5×4.0 cm^2) was immersed in the Hb/phosphate buffered saline (PBS) solution (10 mL, pH = 7.4) at 25 ± 0.5°C for 24 h to reach the absorption equilibrium. After the film was taken out, it was rinsed with 5 mL of PBS buffer solution. The amount of the adsorbed protein was determined spectrophotometrically (Shimadzu model UV-240) by measuring Hb concentration changes in the solution before and after absorbing.[29]

RESULTS AND DISCUSSION

Characterization of HPAE-OHs

It is well known that the sphere-like structured hyperbranched polymer have smaller hydrodynamic volume and viscosity in solution than their linear analogues with comparable molecular weight.[30] However, the relationship between the hydrodynamic volume and molecular mass of the HBPs was different from that of linear polymers. So, the traditional size exclusion-based GPC measurements calibrated against polystyrene standards demonstrated that experimental molecular weight of branched polymers was smaller than that of HPAE-OH. The measured molecular weight and distribution is listed in Table I. The results indicated the observed molecular weights of all HPAE-OH samples were much smaller than calculated ones, but the polydispersity (M_w/M_n) was relative narrow (smaller than 1.15) as expected. This result showed an excellent agreement with reference.[31–34] The experimental and theoretical hydroxyl values (HV) of the HPAE-OH are also listed in Table I. The experimental HV were larger than the theoretical values, which could infer that the molecular weights of HPAE-OH were smaller than the calculated ones. This might be caused by

the fact that the molecular mass determined was a little smaller than the theoretical one. The HV decreases gradually with increasing of generation, reasonably indicating the increase in HPAE-OH's molecular weight.

A method to evaluate the change tendency of molecular weight is to compare the values of intrinsic viscosity. As shown in Figure 1, the intrinsic viscosity of HPAE-OH in DMAc first increases then decreases with the increasing of HPAE generation. These intrinsic viscosity behaviors of HPAE are similar to those of dendritic polymers rather than those of the linear polymers behavior. As we all known, the intrinsic viscosity of linear polymers increases with molecular weight following the Mark–Houwink equation,[35] while dendrimers display a viscosity maximum at approximately generation three. The molecular shape of HPAE changes from open to spherical[1] with the generation, which lead the intrinsic viscosity to rise through a maximum at a given third generation. The low viscosity value shown in Figure 1 is attributed to the compact morphology hampering chain entanglements of HPAE and indicates the compactness of the HPAE-OH molecules. According to the results of Hammond et al.,[36] such a maximum indicated that the structure of HPAE was branched.
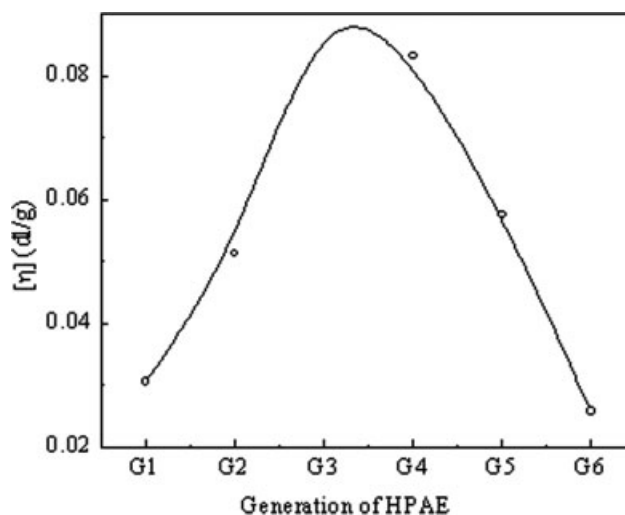

Figure 1 The change of the intrinsic viscosity with the generation of HPAE.
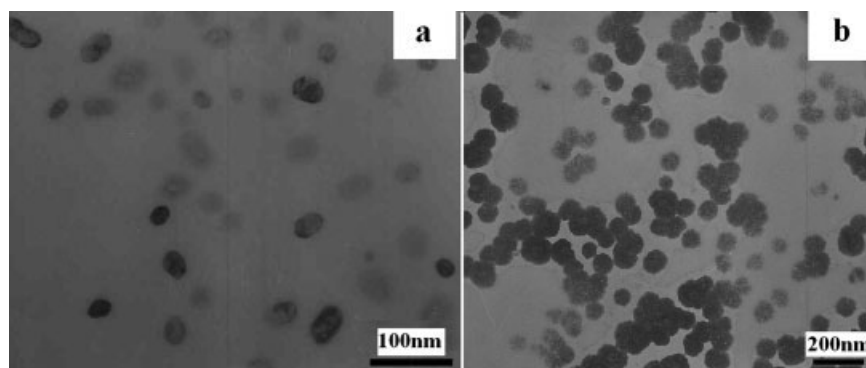
Figure 2 TEM images of (a) G3 and (b) G6 HPAE-OH.

TEM images of HPAE-OH are shown in Figure 2. The observed domains of G3 and G6 HPAE-OH were 20–40 nm and 50–90 nm respectively. These sizes were much larger than the perfectly structured dendrimers with the comparable components and molecular weight. For instance, the diameter was 6.9 nm for the perfect G6 dendrimer with the same components.[37] This phenomenon was resulted from the aggregation of HPAE-OHs molecules, caused by hydrogen bonds formed among the peripheric hydroxyl groups of HPAE-OHs. During TEM measuring, the HPAE-OH molecules flattening on the supported carbon were still in the aggregation state.

Reaction betweens HPAE-OH and glutaraldehyde

To investigate the crosslinking reaction condition, the HPAE-OH/GA/DMAc mixtures were treated with two steps at different temperatures for variable times. The progress of the crosslinking reaction was detected through the measurements of viscosity of reaction solutions and FTIR spectra of the resultant solid films. In this work, the temperature of the HPAE-OH and GA solution was kept at 65°C for 19 h and then raised to 100°C for another 35 h. Figure 3 presents the time dependent changes in viscosity for systems with different generation. It was found that the viscosity of all samples increased gradually to a maximum value with time, and then decreased slightly. This phenomenon could be regarded as an indication of crosslinking among HPAE molecules. This trend briefly reflected the molecular weight change of HPAE-OH and the crosslinking speed. The maximum viscosity meant the highest crosslinking degree could occur in aqueous solution. The viscosity decreased slightly after high temperature treatment for long period of time. This phenomenon could be attributed to the reversibility of the acetal/hemiacetal formation. The acetal and hemiacetal groups could decompose partially in the presence of H_2O and acid after the solution was kept at 100°C for over than 12–13 h. Although the decomposition

might be disadvantageous for the mechanical strength of the resultant crosslinked solid materials of HPAE, this degradation might be interesting in other fields, e.g., biodegradable materials. Another interesting information inferred in Figure 3 was that the higher the generation of HPAE-OH systems was, the lower viscosity would be. This phenomenon might be resulted from the reactions. As the generation increased, the number of hydrophilic hydroxyl groups increased almost exponentially and the molecular shape of HPAE changed from opening to sphere,[1] which would lead the enhancement of intra-HPAE-OH crosslinking. As indicated in ref,[36] the intrinsic viscosity is the sensitive and reliable technique for determining the hydrodynamic size of the polymer chains in solution. Since the compact morphology may hamper the entanglement of HPAE chain, low viscosity value was therefore observed as shown in Figure 3, which in turn indicated the compactness of the crosslinked HPAE-OH molecules.
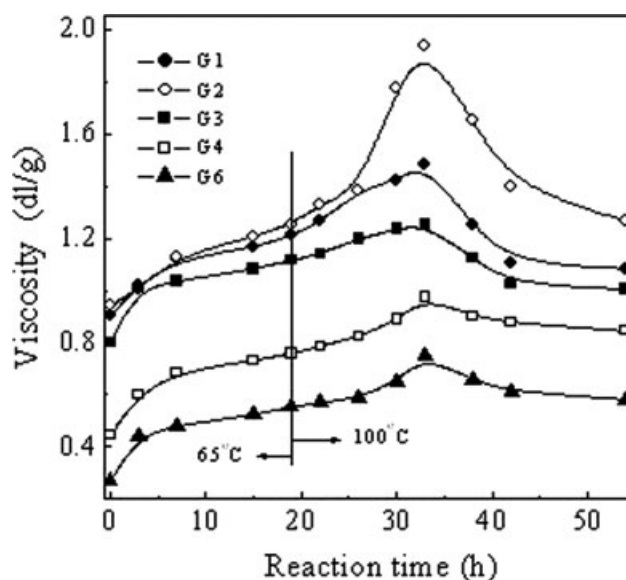

Figure 3 Viscosity change with reaction time for different generation HPAE/GA/DMAc solutions.
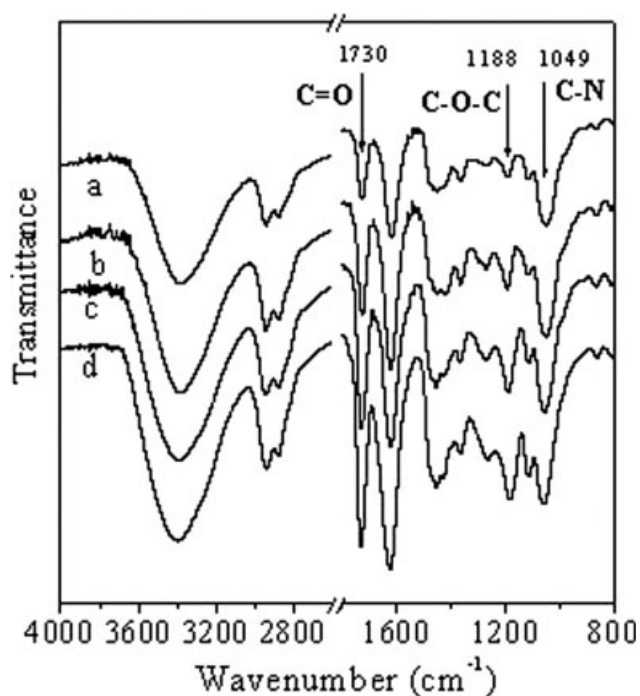
Figure 4 FTIR spectra of (a) G3 HPAE-OH and cross-linked samples formed from (b) 65°C for 4 h, (c) 100°C for 4 h, (d) 100°C for 6 h in vacuum.

Figure 4 shows the FT-IR spectra of solid cross-linked HPAE films after various treatments. The bands at 1730, 1188, and 1049 cm^{-1} were attributed to stretching vibrations of C=O, C—O—C, and C—N groups respectively. The broad bands centered at 3383 cm^{-1}, which should be due to the stretching vibrations of hydroxyl groups in hydrogen-bonded state.[38] Because the adopted molar ratios of hydroxyl/aldehyde group were 1 : 1, the theoretical and perfect linkages in solid HPAE films should be hemiacetal units. However, all the reactions could not be precisely controlled via one hydroxyl to one aldehyde process and only yield hemiacetal linkages. It was unavoidable to form acetal linkages between two adjacent hydroxyls and one aldehyde group. Consequently, the linkages among the HPAE cores should include both hemiacetal and acetal groups.

Concerning the concentration of C—N unit was stable and the concentration of C—O—C unit increased while the proceeding of crosslinking reaction, the examination on the absorbance change at 1188 and 1049 cm^{-1} could monitor the reaction extent (RE) or crosslinking degree indirectly.[39] In some quantitative degree, the RE or crosslinking degree could be properly expressed as eq. (1).

$$RE_t(\%) = [(A_{1188}/A_{1049})_t - (A_{1188}/A_{1049})_{t_0} /$$
$$(A_{1188}/A_{1049})_{t_0}] \times 100\% \quad (1)$$

where, A_{1188} and A_{1049} is the absorption intensity at 1188 and 1049 cm^{-1} respectively, while t_0 and t rep-

resents the reaction beginning time (just after removing the solvent from mixture) and the reaction lasting time respectively.

As shown in Figure 5, the extent of crosslinking reaction increased greatly with the reaction time. The crosslinking reaction rate slowed down after the sample was treated at 100°C for 5 h. Because the crosslinking reaction can also occur during evaporating DMAc from HPAE-OH/GA/DMAc mixture, and t_0 is set as the time at which DMAc is removed, it is acceptable that the found RE value is lower than the theoretically expected one.

Morphology of crosslinked HPAE films

After crosslinking of the terminal hydroxyl groups of HPAE-OH with glutaraldehyde, the transparent pale-yellow thin films were obtained. SEM images revealed that the films were dense and homogenous (Fig. 6). This result primarily indicated that sphere-like HPAE molecules could be crosslinked into a larger network throughout the film without obvious phase separation. Additionally, as revealed by AFM images in Figure 7, the surfaces of obtained films were smooth. The roughness parameter showed in Table II increased gradually with the generation. In fact, the chemical crosslinking of hydroxyl group and glutaraldehyde could happen among inter- or intra- HPAE-OH molecules. However, with the generation increasing, the molecular structure of HPAE changed from open to sphere, the number of hydroxyl group content increased almost exponentially and the length of branch arm increased. The increase of branch arm length restricted the motility of HPAE molecule. At the same time, the increasing of hydroxyl group content induced more glutaraldehyde to react with the intramolecular hydroxyl
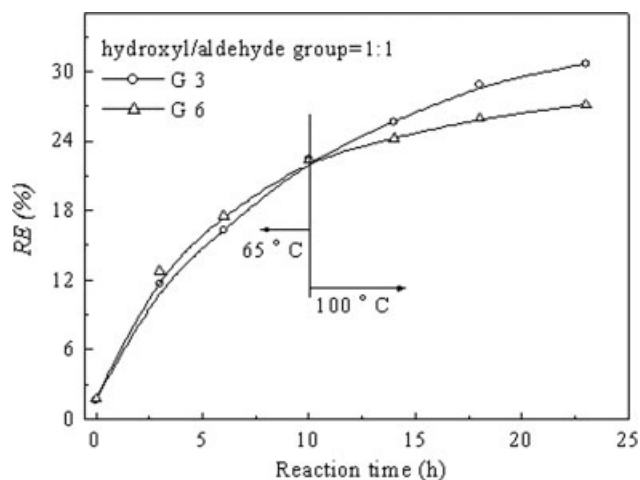

Figure 5 The time dependence of reaction extent for different generation HPAE/GA systems.
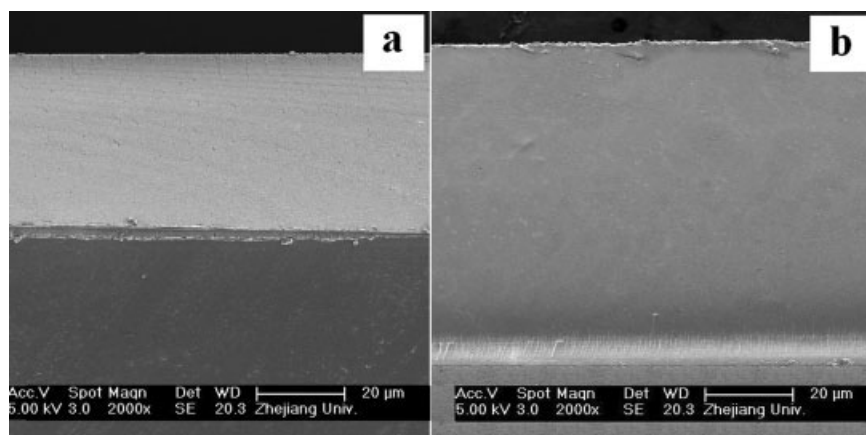
Figure 6   SEM images of cross section for crosslinked (a) G3 and (b) G6 HPAE films.

group. What's more, with the generation increase, some part-crosslinked HPAE molecules may be attached on the surface of the crosslinked HPAE film. More intramolecular hemiacetal units and some part-crosslinked HPAE molecules attached on the surface led the roughness of crosslinked HPAE film surface increased with the generation increasing.

Mechanical properties of crosslinked HPAE films

As listed in Table II, when the generation was increased the tensile strength of the crosslinked film decreased, while the elongation at break increased concomitantly. Such performances were similar with the effect of crosslinking on the rubber's property. The elongation at break of crosslinked film of G6 HPAE is almost 3.5 times of that of G1. On the con-

trary, the tensile strength of the former is only about one quarter lower than that of the latter. The mechanical properties of corsslinked HPAE flims were affected on the three-dimensional structures and the length of the branch arms. The relative weight content of hydroxyl groups in HPAE decreased from 16.7% of the G1-HPAE to 10.7% of the G6-HPAE and the higher generation HPAE-OH increased with longer branched arms. The phenomenon contributed to the longer consistent chains and lower crosslinking density in the final HPAE crosslinked films. Moreover, the spherical structure of the HPAE-OH restricted the deformations. Since the tensile strength and break elongation was larger than 7.0 MPa and 9.0% respectively, for the films based on HPAE over G3-HPAE, the crosslinked HPAE films should have the performance sufficient for most of the functional materials.
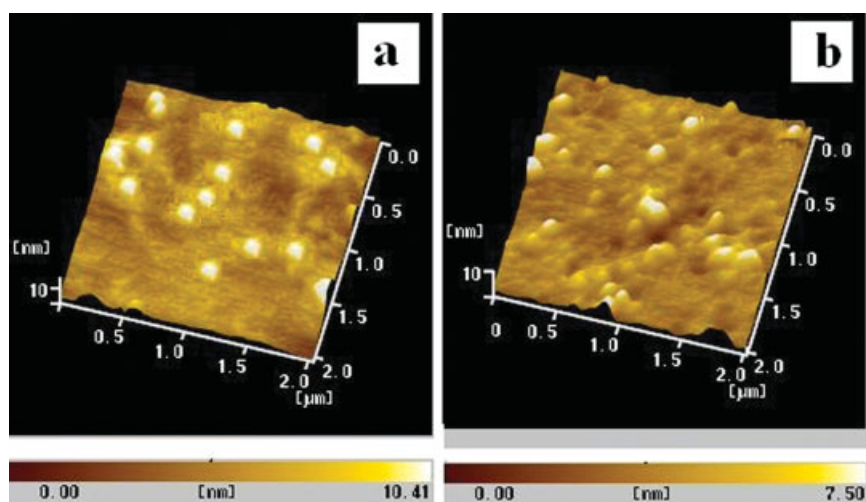

Figure 7   AFM images of surface for crosslinked (a) G3 and (b) G6 HPAE films. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The Typical Characteristic of Crosslinked HPAE Films

Generation	Roughness parameter, S_a (nm)	Tensile strength (MPa)	Elongation at break (%)	Water contact Angle (°)
G1	0.97	12.3	5.1	41.7
G3	1.04	10.6	9.2	21.8
G4	–	8.9	11.2	30.8
G6	1.64	7.45	17.1	35.3

Hydrophilicity and swelling behavior of crosslinked film

The hydrophilic properties of the crosslinked films were evaluated by the water static contact angle on the upper surface, and the results are listed in Table II. All the samples have excellent hydrophilicity because all the water contact angles are less than 42°. Since the hydroxyl groups were the most hydrophilic constituents in HPAE films, the higher degree residue of hydroxyl groups led to a better hydrophilicity of the films. Because the molar ratio of

hydroxyl/aldehyde units was controlled at 1 : 1, most hydroxyls in HPAE-OH were converted to hemiacetal units. The existing hydroxyls in hemiacetal units also contributed to the hydrophilicity of the final films. With exception of G1 HPAE film, the hydrophilicity decreased with generation. The reasons might lay in the lower concentration of hydroxyl groups and the formation of lower hydrophilic acetal units.

The swelling properties of the film were evaluated by tracking the sample weight with the time in different solvents including water, ethanol, chloroform, tetrahydrofuran (THF), ethyl acetate and toluene. All the crosslinked films prepared with HPAE-OH of different generations showed the similar swelling trend (Fig. 8). The swelling speed was very quick initially. The film could be swollen over 50% degree of the swelling saturation (after 200 h) in less than 5 h after the film was immersed into the solvent. After that, the swelling speed slowed down and the swelling degree reached an equilibrium state. Kinetically, the swelling of crosslinked film in aqueous media
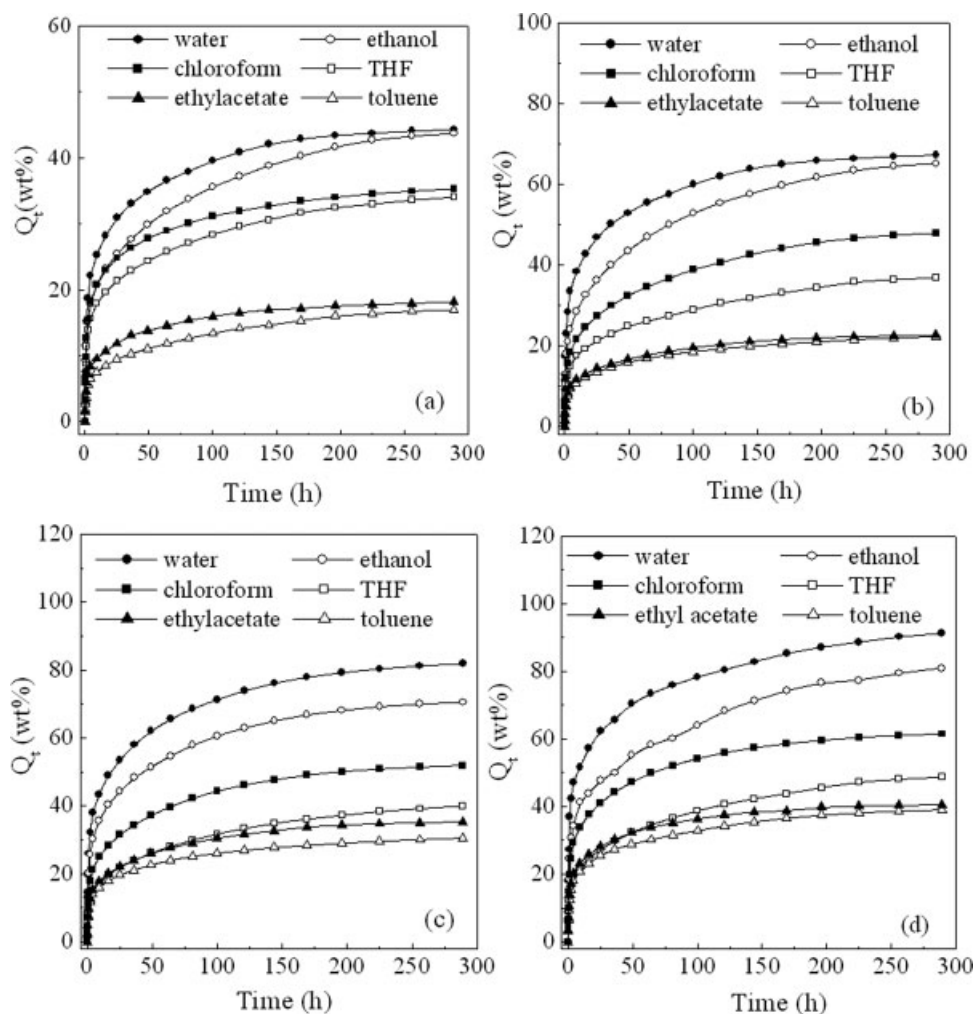

Figure 8 Swelling behaviors of the (a) G1, (b) G3, (c) G4, and (d) G6 crosslinked HPAE films.

followed a two-step mechanism.[25] The first was a fast step in which the solvent molecules penetrated into micro-voids[40] or free volume inside crosslinked film and interacted with the specific units therein via hydrogen bonds. The second was a slow relaxation process, during which the conformation changes of polymer chains allowed the additional solvent molecules to penetrate.

However, it must be noticed that the kinetic process can only affect the swelling rate, rather than the final swelling degree. In contrast, the thermodynamics of the system had the dominating effect on the saturated swelling degree of a specific crosslinked material and solvent pairs. As obviously displayed in Figure 8, the saturated swelling degree increased gradually with rising polarity of solvent, which offered another evidence of the films' polarity. Also, the swelling degree was greatly influenced by the generations of HPAE-OH. The higher the generation of HPAE-OH was, the higher of the saturated swelling degree would be. For example, the saturated swelling degree was 42, 65, 77, and 85 wt % respectively, when the crosslinked film prepared with G1, G3, G4, and G6 HPAE-OH were immersed in water. The dependence of swelling behavior on the generation of HPAE-OH and solvent polarity should be very notable in materialization HPAE based hyperbranched polymers. Above results indicated that these films should have different permeation performances, and hence a novel kind of membranes for liquid/liquid separation could be expected.

Protein adsorption on the surface of crosslinked films

Lower protein absorption is usually desirable in seeking biomaterials and membranes. To reduce protein adsorption, raising the surface hydrophilicity is frequently adopted via introducing the hydrophilic nonionic groups to the surface of a particular material.[41,42] The excellent hydrophilicity of crosslinked HPAE films showed in above results implied these films should have lower protein absorption properties.

The absorption behavior of bovine hemoglobin (Hb) on crosslinked HPAE films is showed in Figure 9. Bovine hemoglobin (Hb) is a natural polyampholyte with an isoelectric point (IEP) of 6.8.[43] When pH > IEP, the net charge of Hb is negative, and when pH < IEP, it has net positive charge.[44] The crosslinked HPAE films contained a large number of tertiary amine groups and have net positively charged at pH = 7.4.[45] Thus, the HPAE film might have a higher Hb absorption tendency at pH = 7.4 because of the charge–charge attraction between films and protein. The results showed in Figure 9 indicated Hb absorption on HPAE film was relative lower in com-
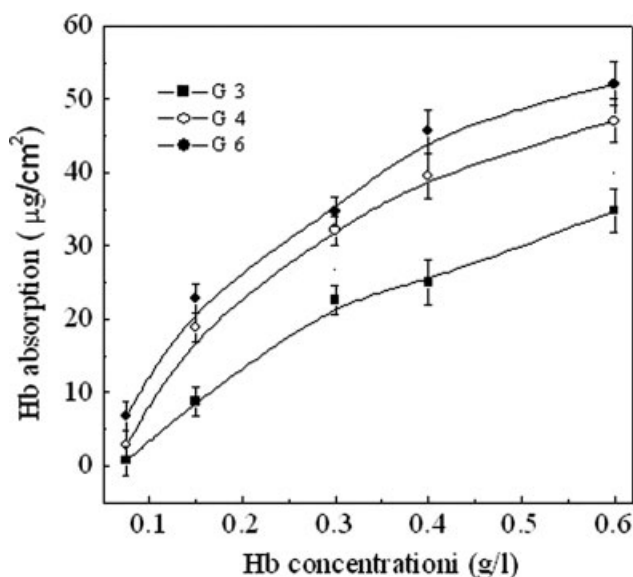

Figure 9 Adsorption of bovine hemoglobin (Hb) on different generation crosslinked HPAE films surface.

parison with the pure polyacrylonitrile (PAN) or chitosan- and heparin-modified PAN membranes.[46] These observations could provide a clue in exploring biocompatible materials.

Besides the adsorbed amount of Hb increased gradually with the Hb concentration, Hb adsorption increased with HPAE generation. This increase could be mainly ascribed as follows: firstly, the relative content of tertiary amine increased with the generation, which increased the electrostatic effect between the protein and the film; secondly, the amount of hydroxyl in film decreased and the hydrophilicity of film surface decreased while the HPAE molecules became larger. Both of them tend to increase the Hb adsorption. These results were consistent with those of the water contact angle value.

CONCLUSIONS

Different generation HPAE crosslinked films with acetal/hemiacetal linkages were successfully prepared using GA as crosslinking reagent. These films exhibited interesting properties. The films had good hydrophilicity (water contact angle was less than 42°), high solvents swelling degree (the swelling degree was higher than 42% in water), acceptable tensile strength (the tensile strength was higher than 7 Mpa) and low protein absorption (the absorbed Hb was smaller than 52 $\mu g/cm^2$). By changing the generation of HPAE, the properties of the crosslinked films could be controlled. In addition, the residual hydroxyl groups would provide the films with reactive surface. These results indicated that such crosslinked HPAE films might have applica-

tions as the novel biocompatible material, antifouling membrane, chemical sensors, and so on.

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