Journal of Applied Polymer Science

Synthesis and Solution Behavior of Hydrophobically Associating Polyacrylamide Containing Capsaicin-Like Moieties

Lanni Jia, Liangmin Yu, Ru Li, Xuefeng Yan, Zhiming Zhang

Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, People's Republic of China

Correspondence to: L. M. Yu (E-mail: yuyan@ouc.edu.cn)

ABSTRACT: A novel hydrophobically associating polyacrylamide (PAAHO) of acrylamide, 2-acrylamide-2-methylpropane sulfonate, capsaicin-like monomer *N*-(4-hydroxy-3-methoxy-benzyl) acrylamide (HMBA), and hydrophobic monomer octadecyl acrylate was synthesized by micellar copolymerization and characterized by fourier transform infrared spectrum, ultraviolet-visible spectrum, and thermogravimetric analysis, respectively. By adjusting various factors, a series of PAAHOs with different block structures were prepared. The hydrophobic association properties were then studied by viscometry and fluorescence spectrometry. Solution behavior was examined with respect to polymer concentration, ionic strength, and temperature. Also, the antibacterial activity was investigated. Results show that initiator amount influenced not only the molecular weight but also the yield and composition of resulting copolymers. PAAHOs possess strong thickening capability, and the hydrophobic association is enhanced as hydrophobe content increases. PAAHOs have excellent viscosity retention in brine solution and exhibit unique rheological behavior including a salt-thickening response. Incorporation of HMBA greatly improves the thermal stability of PAAHO. Moreover, PAAHO is endowed with considerable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 1794–1804, 2013

KEYWORDS: hydrophobically associating; solution behavior; hydrophobe content; antibacterial activity; aromatic group

Received 17 January 2013; accepted 30 March 2013; Published online 6 May 2013

DOI: 10.1002/app.39350

INTRODUCTION

Over the past three decades, synthetic water-soluble polymers have been extensively developed and used as viscosity modifiers even at very low concentrations in various water-based fluids due to their enormous hydrodynamic size.[1] However, these polymers could exhibit a loss of their viscosifying properties when subjected to strong mechanical deformations, increasing ionic strengths, or high temperature.[2,3] To overcome these drawbacks, hydrophobically modified water-soluble polymers have been widely studied in both academic and industrial laboratories. In particular, hydrophobically associating polyacrylamides (HAPAMs) that contain a relatively small amount of hydrophobic groups attached along the acrylamide (AM) backbones have received increasing attention on account of their unique rheological characteristics.[4,5]

In aqueous solutions, the hydrophobic groups tend to associate together through intra- and intermolecular interactions to minimize their exposure to the solvent. Above a critical concentration (C^*), intermolecular associations prevail and lead to the formation of a transient network structure of polymer chains causing a rapid increase in viscosity.[6,7] HAPAMs have better stability with respect to salts than unmodified ones, because the enhancement of solution polarity could strengthen the hydrophobic interactions. Moreover, it is possible for HAPAMs to decrease irreversible mechanical degradation because the physical links are disrupted under increasing shear rates but re-form with decreasing shear. All these unusual properties enable us to consider HAPAMs as a new family of promising candidates as rheology modifiers in a number of commercial applications, ranging from latex paints, drag reduction, and water treatment, to oil production processes including oilwell drilling, water shutoff, and particularly enhanced oil recovery (EOR).[8–11]

Up to now, however, the extensive applications of HAPAMs have still been constrained by some unsolved problems. For example, the critical association concentration (C^*) is a little bit high, and the viscosities below C^* are not high enough to be used in practice. In addition, they have poor thermal stability at elevated temperatures especially when they are used in petroleum industry. The amide pendant side in the molecule is readily hydrolyzed, and the apparent viscosity reduces abruptly above 50°C, though the solution exhibits the heat-thickening at low temperature.[12,13] Recently, various types of processes have been used to improve the temperature-tolerance property of

© 2013 Wiley Periodicals, Inc.

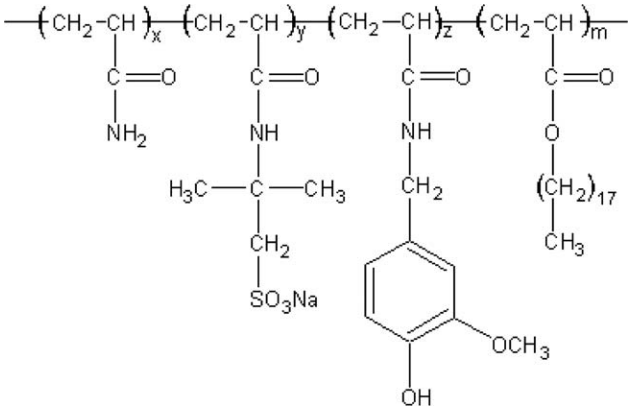

Figure 1. Schematic representation of the chemical structure of PAAHO.

HAPAMs, such as incorporating more thermal stable or rigid monomers into the copolymer backbone to minimize excessive hydrolysis,[6,14] or inserting lateral chains which present a loss of water solubility above a critical temperature, giving rise to a viscosity enhancement.[2,3]

N-(4-hydroxy-3-methoxy-benzyl) acrylamide (HMBA), a unique functionalized AM monomer derived from capsaicin, was designed and synthesized in our laboratory. A detailed description of the synthesis of HMBA has been previously reported.[15] The polymerizable vinyl double bond in the structure of HMBA enables it to be involved in the copolymerization process. Then the phenyl groups on HMBA can act as spacer increasing the rigidity of the polymer chains which can consequently improve the temperature-tolerance property of the polymer.[6] Moreover, sharing congeneric structure with capsaicin, HMBA possesses properties similar to those of capsaicins which are heat-stable, as well as broad-spectrum antibacterial, antifouling, and environmentally friendly.[16,17] Prior efforts of our laboratory[18–20] have demonstrated that the incorporation of HMBA to acrylic resin could impart its antimicrobial and antifouling activity to such resin. In this light, there appears a reasonable possibility that HAPAMs could be rendered antibacterial by the incorporation of HMBA, and thus, it is believed that the application fields of HAPAMs could be further broadened and deepened, particularly in EOR where the abundant microorganism in oilfield could cause serious performance deterioration of HAPAMs.[21–23] However, to the best of our knowledge, research dealing with antimicrobial activities of HAPAMs is yet very scarce so far in the literature.

To obtain a hydrophobically associating polymer with good solution properties, such as high viscosity, salt-thickening, thermal resistance, and antibacterial activity, in this study, we synthesized a novel hydrophobically associating AM-based quadripolymer PAAHO with 2-acrylamide-2-methylpropane sulfonate (NaAMPS), HMBA, and hydrophobic monomer octadecyl acrylate (OA) by micellar copolymerization. The copolymer was characterized by means of fourier transform infrared spectrum (FTIR), ultraviolet-visible (UV-Vis) spectrum , and thermogravimetric analysis (TGA), respectively. In the copolymerization, by adjusting various factors, we prepared a series of PAAHOs with different block structures. Effects of hydrophobe content on the hydrophobic association properties were examined by using viscometry and fluorescence probe technique. Also, the solution behavior was examined as a function of polymer concentration, ionic strength, and temperature. Finally, due to the incorporation of functional monomer HMBA, we present some results about the effects of HMBA on the temperature-tolerance property and the antibacterial activity of PAAHO copolymer.

EXPERIMENTAL

Materials

AM (Zibo Dongpu Chem. Co.) was recrystallized from acetone and stored in dark until required. HMBA was synthesized according to the method described previously.[15] OA, 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AIBA), and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) were obtained from Tianjin Tianjiao Chem. Co. and used as received. Pyrene (Aldrich) was recrystallized twice by ethanol prior to use. Other reagents were of analytical grade and used without further purification. *Escherichia coli* and *Staphylococcus aureus* bacteria were provided by the lab of microorganism in the Ocean University of China.

Synthesis of Copolymers

PAAHO copolymers were prepared by micellar free radical copolymerization[24,25] using sodium dodecyl sulfate (SDS) as the surfactant and AIBA as the initiator. A 500 mL, three-necked, round-bottomed flask was equipped with a mechanical stirrer and a nitrogen inlet and outlet. AM and AMPS were dissolved into deionized water, and the mixture solution was then placed in the flask. NaOH was used to control the pH value of the reaction solution between 6 and 7. HMBA was then added into the flask, after which the mixture was heated to 55°C in a tempering kettle and stirred for 10 min to allow HMBA dissolved completely. SDS and OA were then added respectively into the flask. The mixture was stirred under N_2 until a clear homogeneous mixture was observed. Then the AIBA solution was added to the reactant solution to initiate the copolymerization. After the copolymerization proceeded under continuous stirring for 6–7 h, the polymer mixture was diluted with deionized water and precipitated into an excess of acetone. Subsequently, the polymers were dipped and washed many times with ethanol to remove unreacted monomers and surfactants. Finally, the polymers were dried under vacuum at 50°C for 24 h and conserved in a desiccator. The chemical structure of the quadripolymer PAAHO can be seen in Figure 1.

By adjusting various factors, a series of PAAHOs with different microstructures were obtained. The characteristics of the copolymers prepared are listed in Tables I and II. For comparison, under polymerization conditions identical to those for hydrophobically modified PAAHO copolymers, unmodified copolymer HPAM without OA comonomer and P (AM–NaAMPS–OA) (PAAO) copolymer without HMBA comonomer were prepared. For all reactions, the total monomer concentration was constant at 10 wt %, and the conversion was determined gravimetrically.

Characterization of the Structure of the Copolymers

The molecular structure of the copolymers was characterized by FTIR and UV–Vis spectroscopy. The FTIR spectrum was recorded using an IFS-113 FTIR instrument and the UV–Vis

Table I. Conditions for the Synthesis and Characteristics of the PAAHO Copolymers

Copolymer	Feed ratio (mol %) AM : NaAMPS : HMBA : OA	[SDS][a] (mol L^{-1})	N_H	Initiator[b] (mol %)	Yield (wt %)	[η] (dL g^{-1})
OA-0.3	76.7 : 20 : 3.0 : 0.3	0.0335	8	0.55	80.2	6.12
OA-0.6	76.4 : 20 : 3.0 : 0.6	0.0574	8	0.55	79.7	6.16
OA-0.9	76.1 : 20 : 3.0 : 0.9	0.0809	8	0.55	78.5	6.07
OA-1.2	75.8 : 20 : 3.0 : 1.2	0.1041	8	0.55	81.4	5.94

[a] The surfactant concentration during polymerization.

[b] Mole fraction of initiator AIBA in the total feed.

spectroscopy was measured by Hitachi UV3100 UV-Vis spectrophotometer. The thermal stability of the copolymers was characterized by a STA 409 synchronal thermal analyzer at a heating rate of 10°C min^{-1} under nitrogen flow of 20 mL min^{-1} from room temperature to 800°C.

Solution Apparent Viscosity and Intrinsic Viscosity Measurements

Polymer solutions were prepared by dissolution of purified polymers in deionized water or NaCl aqueous solution at room temperature. All the solutions were kept standing overnight for equilibrium prior to measurements. The apparent viscosities of polymer solutions were measured with a Brookfield DV-I+ viscometer at a shear rate of 6 s^{-1} at 25°C without special statement. The intrinsic viscosities [η] were measured with a 0.6 mm Ubbelohde capillary viscometer in a 1 mol L^{-1} sodium chloride solution at 30.0 ± 0.1°C through dilution extrapolation method, and [η] were used to represent the molecular weights of the copolymers relatively.

Fluorescence Spectroscopy

Fluorescence measurements were performed on a Hitachi F-4600 fluorescence spectrophotometer using pyrene as a free probe at 25°C. Copolymers were dissolved in pyrene-saturated aqueous solution (the concentration of pyrene was 2×10^{-6} mol L^{-1}), and then these solutions were diluted with the pyrene-saturated aqueous solution. Finally, polymer solutions containing pyrene probe and with different concentrations were prepared. The steady-state fluorescence spectra of the pyrene probe were determined with excitation at 335 nm and slit widths of 2.5 nm for both excitation and emission at a scanning speed of 60 nm min^{-1}.

Antibacterial Assessment

Organism and Growth Conditions. The Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* were grown in nutrient agar medium, and a loopful of each culture was spread to give single colonies on nutrient agar and incubated at 37°C for 24 h. Two representative colonies were selected with a wire loop, subcultured to 5 mL fluid nutrient medium, and then incubated overnight at 37°C at 130 rpm. At this stage, the culture of *S. aureus* contained about 10^9 CFU mL^{-1}, and that of *E. coli* 10^{10} CFU mL^{-1}. By diluting with sterile deionized water, culture of *E. coli* containing 10^9 CFU mL^{-1} was prepared and used as test bacteria.

Inhibition Zone Test. The copolymers stored dry at room temperature were dissolved in sterile deionized water. The agar medium that had been autoclaved was poured into Petri dishes and air-dried. Then the Petri dishes were inoculated with 200 μL bacterial suspension spread throughout the Petri dishes uniformly with a sterile glass spreader. To each disk the test samples were added with a sterile micropipette. Disks containing solvent (sterile deionized water) served as control. The plates were then incubated at 37°C for 24 h. Inhibition was recorded by measuring the diameter of the inhibition zone. All the experiments were repeated thrice, and the average values are presented.

Measurement of Viable Cell Numbers After Contacting with the Copolymers. A desired amount of the copolymers was dissolved in 20 mL sterile deionized water in a 50 mL Erlenmeyer flask. Then 0.1 mL of the cell suspension was added to the flask, which was shaken continually at 37°C at 130 rpm. At prescribed time intervals, 0.5 mL of the cell suspension was pipetted out

Table II. Initiator Amount, Yield, and Viscosity of PAAHO (OA-0.9)[a]

Copolymer	Initiator amount (mol %)	Yield (%)	Apparent viscosity in pure water (mPa s)	Viscosity retention in 3.3 wt % NaCl (%)	Intrinsic viscosity (dL g^{-1})
PAAHO-0.50	0.50	57.3	180	31.3	6.65
PAAHO-0.55	0.55	78.5	526	60.3	6.07
PAAHO-0.60	0.60	87.1	487	27.7	6.11
PAAHO-0.65	0.65	92.8	304	20.4	5.14

[a] Polymer concentration: 0.7 g dL^{-1}.

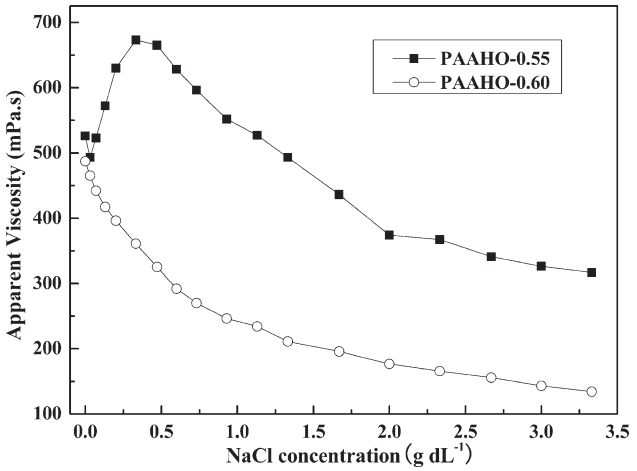

Figure 2. Effect of electrolytes on apparent viscosity of PAAHO copolymers obtained at different initiator amounts (copolymer concentration: 0.7 g dL^{-1}).

from the container and quickly mixed with 4.5 mL of sterile deionized water, and then decimal serial dilutions were prepared. From these dilutions, the surviving bacteria were counted by the spread plate method. After inoculation, the plates were incubated at 37°C for 24 h, and the number of viable cells was calculated from those colonies that formed on the plates. Blank test was finished by the same means without copolymer. The counting was done in triplicate every time.

RESULTS AND DISCUSSION

Micellar Copolymerization

In case of HAPAMs prepared by micellar copolymerization, the key parameters controlling the hydrophobic association properties are the content and length of the hydrophobic microblock and the molecular weight of the copolymer.[26] In the present work, to study the influence of hydrophobe content on the rheological properties, we prepared a series of PAAHOs which have varying hydrophobe contents but basically identical hydrophobic microblock length and molecular weight.

The hydrophobe content is dependent on the proportion of OA in monomer feed and the hydrophobic microblock length equals roughly to the number of hydrophobes per micelle, N_H, which can be calculated from the following relationship[8,27]:

$$N_{\mathrm{H}} = ([\mathrm{H}]\times N_{\mathrm{agg}})/([\mathrm{SDS}]-\mathrm{CMC}) \quad (1)$$

where [H], [SDS], CMC, and N_{agg} are, respectively, the initial molar concentration of hydrophobic monomer, the molar concentration of the surfactant, its critical micellar concentration, and aggregation number (the CMC of SDS was taken to be 9.2×10^{-3} mol dm^{-3}, and $N_{agg}=60$ was assumed at the temperature of 55°C). By adjusting the hydrophobe/surfactant molar ratio adequately, the desired N_H was obtained. The copolymerization parameters and copolymer characteristics are given in Table I. The molecular weight of the copolymer is decided by the amount of initiator. Therefore, the initiator amount for the copolymerization was constant to obtain copolymers with basically identical molecular weight (Table I). In addition, it was found that the initiator amount influenced not only the molecular weight of the copolymers but also the degree of conversion and the rheological properties (Table II).

It is clear from Table II that with increasing initiator amount, the conversion of the copolymers keeps growing whereas the intrinsic viscosity [η] decreases gradually. In addition, the apparent viscosity in pure water increases first with the increasing initiator amount up to 0.55 mol % and then decreases with the further increase. Based on general kinetics, the rate of polymerization depends on the concentration of monomers and initiators for bimolecular termination. With increasing initiator amount, the number of free radicals in the system becomes larger, and then the chance to collide with reactive monomer is increased. As a result, the polymerization is accelerated and the conversion of comonomers is elevated. Meanwhile, the increased number of produced radicals leads to terminating step via bimolecular collision, resulting in the shortening of macromolecular chains[28] and the decrease of the copolymer molecular weight which is reflected by the decreasing [η]. On the other hand, as the initiator amount initially increases, although the copolymer molecular weight decreases, the conversion of hydrophobic monomers is increased, giving rise to the enhanced intermolecular hydrophobic associations and accordingly the enhancement of apparent viscosity. However, when the initiator amount exceeds 0.65 mol %, the copolymer molecular weight is too low for the copolymer chains to interact intermolecularly with each other, resulting in the decrease of apparent viscosity.

For PAAHO-0.60 obtained under 0.60 mol % initiator amount, both the conversion and solution viscosity are quite ideal, respectively, 87.1% and 487 mPa s. However, the viscosity retention in 3.3 g dL^{-1} NaCl aqueous solution is as low as 27.7%, far below that of PAAHO-0.55 which has excellent viscosity retention of 60.3% and high viscosity of 526 mPa s but a relatively lower conversion, i.e., 78.5%. Figure 2 shows the effect of electrolytes on the rheological behavior of the two copolymers. PAAHO-0.55 exhibits unique rheological behavior upon increasing NaCl concentration. A salt-thickening response occurs and a maximum viscosity appears. The enhancement of viscosity indicates that there exist strong intermolecular associations in the solution. However, for PAAHO-0.60, the apparent viscosity decreases dramatically by the addition of NaCl, and no salt-thickening process occurs.

It is well known that HAPAMs prepared via micellar copolymerization are characterized by blocky distribution of the hydrophobes and compositional inhomogeneity.[29] The solubilization of hydrophobic comonomers in micelles causes an increase in their apparent reactivity due to the local concentration effect of the micelles, and consequently, the hydrophobic comonomers are consumed more rapidly than other comonomers.[2] Thus, samples obtained at high conversion, e.g., PAAHO-0.60 and PAAHO-0.65, may contain an important proportion of polymer chains that possess no hydrophobe units, and then this heterogeneity will impede the formation of intermolecular associations and alter the rheological behavior.[30] The result agrees with the already reported results on HAPAM that the polymer sample

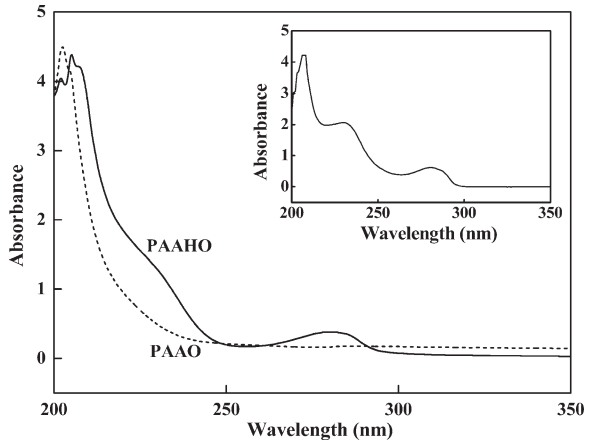

Figure 3. UV spectra of the PAAO and PAAHO copolymers. Inset: UV spectrum of HMBA comonomer.

with increased homogeneity shows strongly enhanced thickening properties, while the heterogeneous polymer does not exhibit significant associative properties.[31] One way to reduce the compositional heterogeneity of the sample is to stop the copolymerization at a relatively low conversion level, and thus, the suitable initiator amount in this study is 0.55 mol % relative to monomer feed.

Characterization

FTIR and UV–Vis spectra were used to characterize the molecular structure of the resulting copolymers. The characteristic FTIR absorption peaks (cm^{-1}) of PAAHO are as follows: —NH stretch, 3430; C=O stretch, 1658; —CH_3, —CH_2, —CH stretch, 2864, 2928, 2781; —CH_3, —CH_2, —CH bending, 1454, 1358, 1347; —COO in OA stretch, 1712; C—O—C stretch, 1180; =C—H in phenyl stretch, 3195; —C=C— in phenyl stretch, 1655 (overlap with C=O stretch), 1450 (overlap with —CH_3 bending); C—H in phenyl out-of-plane bending, 762, 812; and —SO_3^-: 1209, 1115, 1041, 630.

The UV–Vis absorption spectra of PAAO and PAAHO copolymers dissolved in deionized water were measured and shown in Figure 3. The inset is the UV spectrum of HMBA comonomer. In the UV region, besides the band at around 205 nm corresponding to the absorption of amide group in HMBA, two bands at 230 and 280 nm were also observed which can be attributed to the aromatic ring structure of HMBA. Due to the auxochromous groups (hydroxyl group and methoxyl group) attached to the aromatic group, the absorption bands of HMBA exhibit a red shift compared to those of benzene. For PAAO, only one band assigned to the absorption of amide group occurs at around 205 nm. In contrast, PAAHO exhibits two additional bands at 230 and 280 nm, which are consistent with those of HMBA. From the above description, we could safely draw the conclusion that HMBA comonomers have been incorporated into PAAHO copolymer molecules.

The thermal stability of PAAO and PAAHO copolymers was evaluated by TGA and differential thermal analysis (DTA), and the corresponding measuring curves are presented in Figure 4. The variation tendency of TGA diagrams for the two copolymers is consistent basically, both showing three steps for the weight loss. Corresponding to the degradation process, several marked endothermic or exothermic peaks can be found in the DTA curves. In the first heating step when temperature increased from 40 to 140°C, a small weight loss occurred for both copolymers due to the evaporation of intra- and intermolecular moisture.[32] The copolymers contain a large number of hydrophilic groups which induce the sample to combine with water molecules.[33] The weight loss occurred at around 215–350°C was attributed to the second stage due to the thermal rupture and decomposition of the copolymer side chain. In the second step, it was found that the weight loss was prolonged for PAAHO compared with that of PAAO. The onset degradation appeared at 328.6°C for PAAHO, which was higher than that of PAAO, i.e., at 299.2°C, indicating the thermal stability of PAAHO was enhanced due to the incorporation of HMBA. The reason for this is that HMBA's incorporation enhances the rigidity of the copolymer chains, which leads to the increase of the steric hindrance for the internal motion of molecular chains. As a result, the thermal motion of molecular chains is limited, and the thermal stability of the copolymer is improved. As shown in Figure 4, a significant weight loss was found at the third step corresponding to the temperature range in 350–590°C due to the thermal decomposition of copolymer backbones. When the temperature exceeded 590°C, the TGA curves went to mild and no longer changed. The residual of PAAHO was 10.95%, higher than that of PAAO, i.e., 7.89%. Moreover, from the DTA curves, one can also find that the maximum decomposition temperatures of both the second and third weight loss regions were increased after HMBA's incorporation, which were, respectively, 320°C and 566.1°C for PAAO and 345°C and 582.6°C for PAAHO, indicating that the weight loss of PAAHO was slower than that of PAAO. These thermal behaviors are expected and again indicate that the global thermal stability of PAAHO is enhanced by the incorporation of HMBA.

Solution Behavior

Effect of Polymer Concentration and Hydrophobe Content on Hydrophobic Association Properties of PAAHOs in Pure Water. Viscometry is a convenient and reliable method for determining associative properties of amphiphilic copolymers in

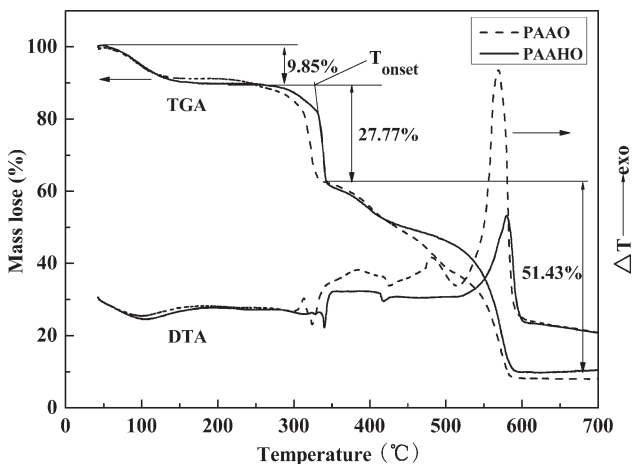

Figure 4. Thermal gravimetric curves of the PAAO and PAAHO copolymers.

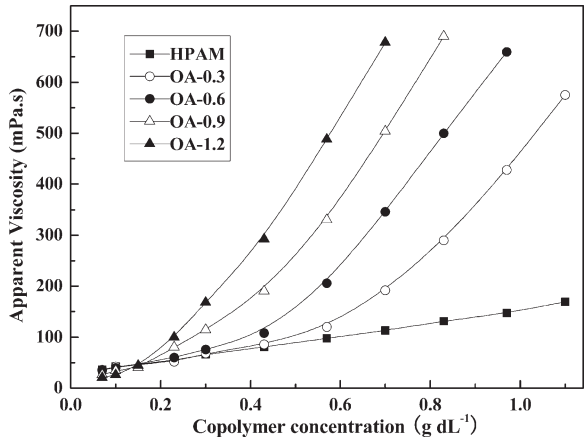

Figure 5. Dependence of viscosity on copolymer concentration for copolymers with different OA contents.

aqueous solutions.[34] Figure 5 presents the concentration dependence of apparent viscosity for hydrophobically modified PAAHOs and unmodified HPAM for comparison. As expected, the solution viscosity of HPAM increases regularly with its concentration, exhibiting common viscosity behavior of a polyelectrolyte. The slight enhancement of viscosity is usually ascribed to the strengthening of chain entanglement due to the increase of polymer concentration. In contrast, the thickening ability of HAPAMs stems mainly from the intermolecular associations which are strongly effected by the polymer concentration and hydrophobe content.[35] As shown in Figure 5, typical HAPAM behavior occurs for PAAHOs as polymer concentration increases. At low polymer concentration ($c \leq 0.15$ g dL^{-1}), the viscosities of OA-0.3 and OA-0.6 are nearly equal to those of HPAM, while for OA-0.9 and OA-1.2, the viscosities are even lower. This can be explained that at low concentration, hydrophobic microblocks have little chance of interacting intermolecularly with each other and presumably intramolecular associations in each of the individual suspended polymer coils prevail,[36] which lead to the collapse of coils and the decrease of viscosity. With the increase of polymer concentration, the formation of intermolecular aggregations gradually surpasses that of intramolecular ones. When the concentration exceeds a critical value (C^*), the viscosity increases sharply due to the formation of hydrophobic microdomains which are caused by strong intermolecular associations and act as physical network-like structures.

By plotting two tangents of the curves about the point at which an obvious increase of viscosity appears, C^* can be estimated, which are the corresponding concentrations of intersection points for two tangents.[26] The C^* values for OA-0.3, OA-0.6, OA-0.9, and OA-1.2 are, respectively, 0.56, 0.48, 0.36, and 0.18 g dL^{-1}, implying that the higher the hydrophobe content, the lower the C^*. It can be explained the higher OA content in the chains, the stronger the hydrophobic associations, and consequently, the physical network-like structure can be formed at lower concentration. Moreover, upon increasing the hydrophobe level in the copolymer, a strong increase of the viscosity at identical concentration can be observed, and the enhancement in viscosity with increasing polymer concentration becomes sharper. For instance, OA-1.2 shows the largest values of viscosity for all concentrations above 0.15 g dL^{-1}, and the viscosity can reach up to 678 mPa s at the concentration of 0.7 g dL^{-1}, exhibiting strong thickening capability.

In order to have a better insight into the association process, we have performed fluorescence spectrum using pyrene as a probe. The intensity ratio of the first to the third vibronic peak for pyrene, I_1/I_3, is sensitive to local polarity,[37,38] and the weaker the polarity of the microenvironment around pyrene, the smaller the value of I_1/I_3. The variation of the I_1/I_3 ratio as a function of copolymer concentrations is plotted and displayed in Figure 6. For HPAM solution, the I_1/I_3 ratio is around 1.8, which is typical value in water and only decreases slightly to 1.72 at the copolymer concentration of 2.5 g dL^{-1}. The result indicates there were no hydrophobic microdomains in the HPAM solutions. For PAAHOs, the I_1/I_3 values are smaller than those of HPAM and decrease with increasing polymer concentrations. This behavior reflects the formation of hydrophobic microdomains in which pyrene preferentially locates. With increasing polymer concentrations, the I_1/I_3 values of PAAHOs decrease slightly first and then abruptly at a certain concentration, which is an information of the association type transforming from intramolecular association into intermolecular association. Since intermolecular associations lead to form large hydrophobic microdomains, the polarity of the microenvironment around pyrene is weaken when pyrene molecules penetrate into these domains, and consequently a dramatical decrease of I_1/I_3 value is observed. By adopting the manner of plotting tangents as described above, the values of C^* also can be obtained from Figure 6, and the result agrees well with that obtained from solution viscosity measurement. As copolymer concentration continues increasing, the number as well as the volume of hydrophobic microdomains gets larger, so the I_1/I_3 values progressively decrease. When the concentration reaches a certain value, the I_1/I_3 ratio tends to stabilize at a fixed level (0.95–1.10), characteristic of low polarity media. Moreover, it is noted that the decrease of I_1/I_3 value is more pronounced for copolymers with higher hydrophobe content. For example, at the concentration of 2.2 g dL^{-1}, the I_1/I_3 values of OA-0.3 and OA-1.2 drop to 1.10 and 0.95, respectively. This reveals that higher

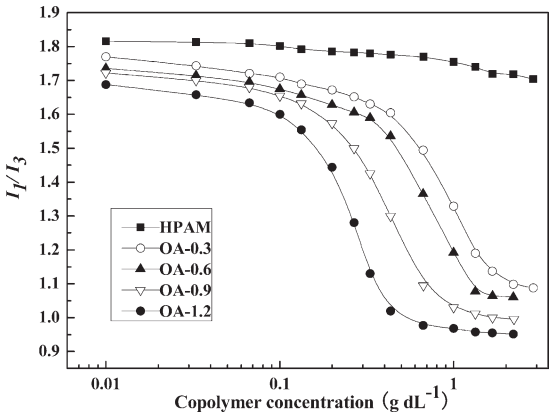

Figure 6. Fluorescence intensity ratio I_1/I_3 of pyrene as a function of copolymer concentration.

Applied Polymer SCIENCE

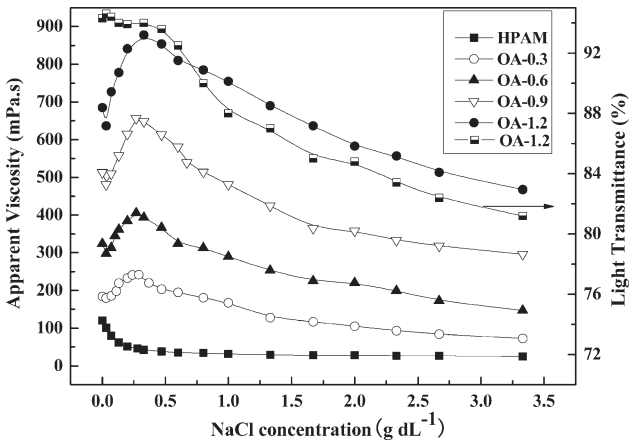

Figure 7. Effect of NaCl concentration on apparent viscosity and light transmittance of copolymers with different OA contents (copolymer concentration: 0.7 g dL^{-1}).

hydrophobe content increases the extent of intermolecular hydrophobic association.

Effect of NaCl Addition on Rheological Properties. The effect of NaCl addition on polymer viscosity provides additional evidence for hydrophobic association. Figure 7 shows the variation of apparent viscosity with NaCl concentration for the copolymers at a concentration of 0.7 g dL^{-1} that is in the semi-dilute regime. For unmodified HPAM, the solution viscosity decreases rapidly as the salinity increases at a low solution ionic strength. This behavior is attributed to a shielding effect of the electrolyte ions, which reduces the repulsion between anions in macromolecular chains and causes chain contraction. For PAAHOs containing both ionic groups and hydrophobic groups, however, things are quite different. The effect of salt on hydrophobic association of HAPAMs has been investigated for several decades and extensively documented.[39,40] On one hand, addition of salt shields the repulsive ionic interactions and makes the molecule contract. On the other hand, the addition of salt facilitates the macromolecular interpenetration and thus promotes the extent and strength of intermolecular associations.[41] As a consequence, depending on whether which of the effects is dominant, a great variety of behaviors occur as a function of ionic strength.

From Figure 7, one can see that the solution viscosities of series PAAHOs assume a regular variation against increasing NaCl concentration. The viscosities first decrease below 0.05 g dL^{-1} NaCl concentration due to the chain contraction. In the range of 0.05–0.33 g dL^{-1} NaCl, the viscosities increase dramatically and far surpass the original values, exhibiting a good salt-thickening effect. This behavior indicates that the enhanced degree of intermolecular associations prevails on the chain contraction effect in the solution. When the salt concentration further increases, the very strong hydrophobic associations lead to coil collapse and phase separation, resulting in the decrease of viscosity and a decreased rate of light transmission in the polymer solution.[42] Moreover, one can find out that the viscosity retention ratio of test samples at brine solution exhibited increases with an increase in their hydrophobe content. This may be explained that since the ratio of anionic monomer AMPS in PAAHOs is constant, the decreased degree of viscosity caused by charge screening is basically the same. As the hydrophobe content increases, the increased viscosity by strengthened hydrophobic associations becomes larger, and the combined effect results in a higher retention ratio at brine solution. For OA-0.3 copolymer, the viscosity and viscosity retention ratio at 3.3 g dL^{-1} NaCl solution are 73 mPa s and 39.7%, higher than those of HPAM which are 25 mPa s and 20.8%, respectively. Compared with OA-0.3, those of OA-1.2 are much higher, respectively, 468 mPa s and 68.3%, exhibiting excellent salt-resistance performance.

Moreover, it was found that the solution behavior of PAAHO with addition of NaCl varied greatly depending on the copolymer concentration. Taking OA-0.6, for example, the solution behavior of OA-0.6 at high concentration of 0.7 g dL^{-1} has been illustrated above. At a low concentration of 0.3 g dL^{-1} that is in the dilute regime, the viscosity initially decreased dramatically with increasing NaCl concentration, and addition of only 0.40 wt % NaCl reduced the viscosity about a half. Then the viscosity decreased slowly and tended to be constant without any salt-thickening process. In addition, the viscosity retention ratio at 3.3 g dL^{-1} NaCl solution was 29.8%, much lower than that of 0.7 g dL^{-1} OA-0.6 solution, i.e., 45.4%. In the dilute solution, intramolecular associations are dominant. Upon adding salt, the chains shrink due to electrostatic screening, and then the distances between the chains are even longer, and the intermolecular association is further weakened. Moreover, the increased solution polarity enhances the intramolecular associations which produce a decrease in the hydrodynamic radius of copolymer coils, leading to the decrease of viscosity.

Effect of Temperature on Rheological Properties. The effect of temperature on apparent viscosity of the copolymers is shown in Figure 8. The solution viscosity of HPAM decreases monotonously with increasing temperature, showing a general trend following Arrhenius' law,[43] due to the destruction of hydrogen bonds and the entanglement of polymer chains. For OA-0.3 copolymer, the viscosity versus temperature behavior is similar

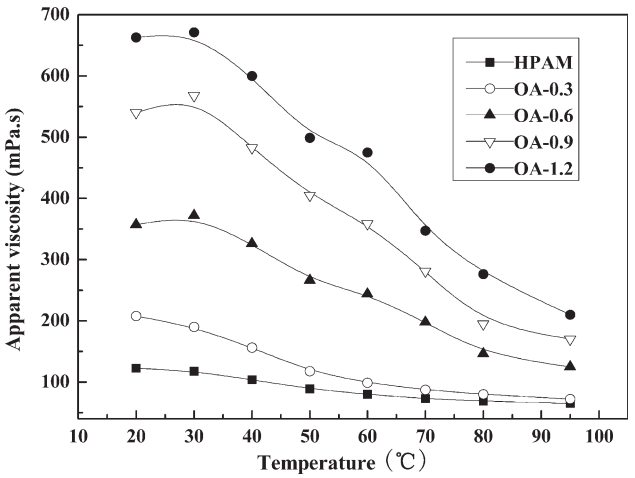

Figure 8. Effect of temperature on apparent viscosity of copolymers with different OA contents (copolymer concentration: 0.7 g dL^{-1}).

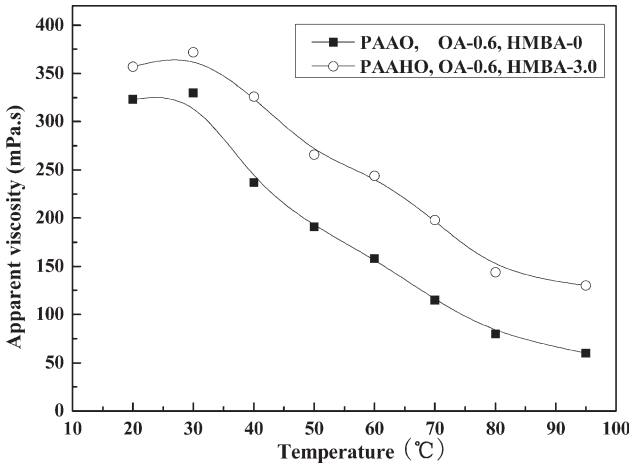

Figure 9. Effect of temperature on apparent viscosity of PAAO and PAAHO copolymers (copolymer concentration: 0.7 g dL^{-1}).

to that of HPAM. However, for copolymers with higher OA content, with increasing temperature, there is a slight viscosity increase followed by a decrease, and a maximum viscosity appears at about 30°C. The heat-thickening effect of the copolymers within 20–30°C should be owing to the intermolecular association, which is an endothermic process of entropy increase in a certain temperature range. This association includes two entropy increasing physical processes: the endothermic destruction of the hydration between hydrophobic groups and their surrounding water molecules in order, and the exothermic interaction between hydrophobic groups.[12,44] The viscosity increase observed upon heating implies that during the temperature range of 20–30°C, the endothermic energy of the former is higher than the exothermic energy of the latter. However, when the temperature is further increased, water molecules and polymer chains move faster, and the hydration spheres of hydrophobic groups change a great deal, which are unfavorable for interaction associations. Moreover, the exothermic energy by the aggregation of hydrophobic groups surpasses the endothermic energy by the destruction of the hydration of hydrophobic groups. As a result, the intermolecular association is weakened, and consequently, the solution viscosity decreases. As shown in Figure 8, the influence of temperature is more pronounced for copolymer solutions containing higher OA content where the intermolecular association is more common compared to the copolymer containing low OA content.

To reveal the effect of HMBA on the thermal stability of the copolymers, the apparent viscosities versus temperature for PAAO and PAAHO copolymers were measured and shown in Figure 9. It is obvious that the initial viscosity of PAAHO at room temperature is larger than that of PAAO. This is attributed to the rigidity enhancement of PAAHO chains owing to HMBA's incorporation, which results in the formation of expanded copolymer chains and favors the formation of associating structures.[12] For both copolymers, a slight increase in viscosity is observed for temperature ranging from 20 to 30°C. As the temperature further increases, the viscosity of PAAO decreases sharply from 330 mPa s (30°C) to 61 mPa s (95°C) with a retention ratio of 18.8%. But for PAAHO, the decline is gentle from 372 mPa s (30°C) to 128 mPa s (95°C), and the retention ratio is 35.9%. The fact shows that PAAHO has better thermal stability than PAAO, corresponding to results of TGA. This can be confirmed further by comparing the viscous activation energy (E_a) of the copolymers which can be used to characterize the ability of the copolymer to retain viscosity under high temperature.[42] The relationship between E_a and viscosity can be expressed by the Arrhenius formula:

$$\eta = \mathrm{A}\times\exp\left[E_a/(RT)\right] \tag{2}$$

It can also be written as

$$\ln(\eta) = (E_a/R)(1/T) + \ln A \tag{3}$$

The $\ln(\eta) - 1/\mathrm{T}$ curves of the two copolymers were plotted from viscosities under various temperatures, and then the equations were obtained and are shown in Figure 10. By combining the equations with the Arrhenius formula, E_a of the two copolymers were calculated, which are 16.1 kJ mol^{-1} for PAAHO and 23.8 kJ mol^{-1} for PAAO. For an individual polymer, a higher E_a means higher sensitivity to temperature; therefore, to some degree, the lower E_a of PAAHO indicates again that PAAHO has better heat resisting property than PAAO owing to the covalent attachment of HMBA. The rigid aryl groups on HMBA are stable and could effectively interfere with the hydrolysis of amido groups on the copolymer.[7] Also, HMBA's incorporation enhances the stiffness of the copolymer chains. The resistance of molecular thermal motion increases due to the steric hindrance effect, resulting in the weakness of the molecular chain movement. Therefore, the destruction of interaction associations between copolymer chains is weakened to some extent, and the copolymer solution could maintain a relatively high viscosity at high temperature.

Antibacterial Properties

The antibacterial activity of the copolymers against Gram-negative *E. coli* and Gram-positive *S. aureus* was initially investigated using disk diffusion method. The diameters of inhibition zone against the two bacteria for the copolymers are displayed in Table III. For PAAO copolymer, it is evident that the inhibited areas against *E. coli* and *S. aureus* are similar to those of solvent

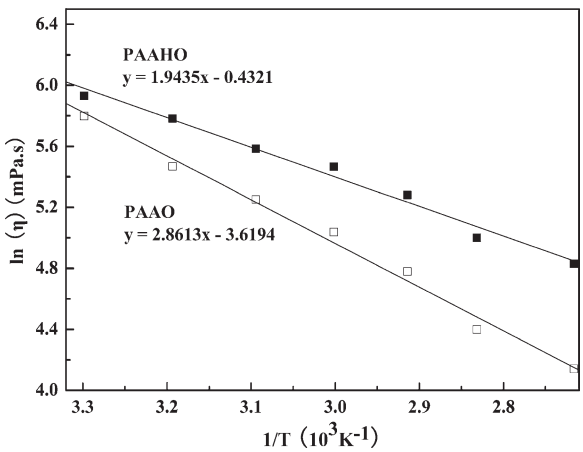

Figure 10. Plots of $\ln(\eta)$ versus $1/T$ for PAAO and PAAHO copolymers.

Table III. Inhibited Area (mm) of PAAO and PAAHO Copolymers[a] against *E. coli* and *S. aureus*

	Diameter of inhibition zone (mm)		
Test organisms	Control	PAAO	PAAHO
E. coli	7.47	7.94	11.52
S. aureus	7.79	8.49	13.57

[a] Polymer concentration: 0.7 g dL^{-1}.

control (sterile deionized water), indicating that PAAO is inactive against the two bacteria. On the contrary, the inhibition zone diameters of PAAHO against both bacteria are much larger compared to those of control, implying PAAHO exhibits considerable inhibitory effects on the two bacteria due to the incorporation of HMBA. The result is consistent with the findings reported by Gao's group[45,46] that the modification of ultrafiltration membrane with capsaicin-like function structure could improve its antibacterial performance significantly. It is well known that capsaicin, an extract from pepper fruits, could paralyze bacteria cells and interfere with normal nerve transmission. Also, sharing similar structure with flavonoids, capsaicin can disrupt the integrity of the cell wall and membrane, causing the release of intracellular components and then destroying the action of electron transfer, nutrient absorption, and ribonucleotide synthesis, as a result, leading to the death of bacteria cells.[47,48] Being a capsaicin derivative, HMBA possesses the characteristics as above and exhibits remarkable antibacterial activity. Moreover, the existence of the lipophilicity methoxyl group in HMBA could make it more active to interact with cytoplasmic membranes increasing the panniculus permeability and consequently facilitate the crack of panniculus followed by the death of cells.[49] Therefore, as HMBA participates in the polymerization, the functionalities that bear remarkable antimicrobial activity are attached to the copolymer backbone, and the copolymer is endowed with high antimicrobial activity.

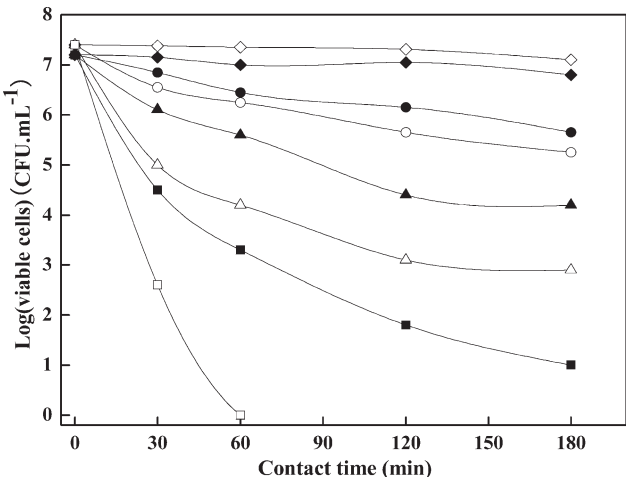

Figure 11. Plots of log(viable cells) versus contact time for PAAHO copolymers with different concentrations against *E. coli* and *S. aureus*: ◆, ●, ▲, and ■—blank, 0.6, 1.2, and 1.8 g dL^{-1}, respectively, of PAAHO against *E. coli*; ¤, ○, Δ, and □—blank, 0.6, 1.2, and 1.8 g dL^{-1}, respectively, of PAAHO against *S. aureus*.

To further study the antibacterial activities of PAAHO copolymers, the effect of contact time on the antibacterial activities against *E. coli* and *S. aureus* was investigated. Figure 11 shows plots of log (viable cells) versus contact time for PAAHOs with different concentrations against the two bacteria. It can be found that the viable cell number decreases gradually with the increase of copolymer concentration and the prolongation of contact time. At the concentration of 0.6 g dL^{-1}, 82.21% of *E. coli* and 92.05% of *S. aureus* were killed within 60 min of contact. And at 1.2 g dL^{-1}, 95.93% of *E. coli* and 99.87% of *S. aureus* were killed within 60 min when exposed to the copolymer. As the concentration increased to 1.8 g dL^{-1}, the copolymer was capable of killing 99.68% of *E. coli* and 99.99% of *S. aureus* within 30 min of contact, and 99.98% of *E. coli* and all of the *S. aureus* cells within 60 min, exhibiting high antibacterial activity. The results demonstrate that the higher the copolymer concentration, i.e., higher content of antimicrobial agent HMBA, the better the antibacterial activity, consistent with the general findings for normal antimicrobial polymers.[50,51] Moreover, it is noted that under identical copolymer concentration and contact time, PAAHO shows larger bacteriostasis rate to *S. aureus* than to *E. coli*, implying that PAAHO exhibits higher antibacterial activity against *S. aureus*. This may be attributed to the difference in the structure of cell walls between Gram-positive and Gram-negative bacteria. *S. aureus*, a genus of Gram-positive bacteria, has a simple cell-wall structure, in which outside the cytoplasmic membrane there is only a rigid peptidoglycan layer (20–80 nm). The peptidoglycan layer, though relatively thick, is composed of networks with plenty of pores which allow foreign molecules to come into the cell without difficulty.[52] However, the cell-wall structure of Gram-negative bacteria *E. coli* is more complicated. Besides the peptidoglycan layer (2 nm) and cytoplasmic membrane, there is a lipid polysaccharide layer (20–100 nm) outside which is a potential barrier against foreign molecules with high molecular weight. Moreover, the O-polysaccharide side chains in this layer could endow *E. coli* with drug-resistance in a certain.[49] Therefore, the inhibitory effect against *E. coli* is weaker than *S. aureus*. On the whole, PAAHO copolymer shows good antimicrobial activity, particularly against Gram-positive bacteria, owing to the incorporation of HMBA. These results indicate that the incorporated HMBA comonomers not only increase the rigidity of polymer backbones improving the thermal stability but also behave as antimicrobial functional groups. Desired as a new kind of HAPAM, PAAHOs may have wide application foreground in a variety of fields, such as EOR or water purification treatment.

CONCLUSIONS

A series of hydrophobically associating AM-based quadripolymers (PAAHO) with NaAMPS, HMBA, and OA were prepared via micellar copolymerization, and the solution properties were studied. The initiator amount used in polymerization affected not only the molecular weight of the produced copolymers but also the degree of conversion and the rheological properties. The existence of critical association concentration in aqueous solution was observed, over which PAAHO exhibits much higher viscosity and much lower I_1/I_3 value than HPAM, and it

gives us evidence of the formation of physical networks due to intermolecular hydrophobic associations. As hydrophobe content increases from 0.3 mol % to 1.2 mol %, the hydrophobic association is enhanced and C^* decreases from 0.56 g dL^{-1} to 0.18 g dL^{-1}. The addition of NaCl can lead to the enhancement of intermolecular associations over certain polymer concentration, which is reflected by the increase in viscosity. The copolymer exhibits excellent salt-resistance performance, and the higher the hydrophobe content, the higher the viscosity retention in brine solution.

The capsaicin-like HMBA comonomer incorporated into PAAHO backbones displays multifunctional effects. On one hand, the rigid aryl groups of HMBA interfere with the hydrolysis of amido groups, increase the copolymer chain rigidity, and consequently improve the thermal stability of PAAHO. The solution viscosity exhibits a heat-thickening behavior at the low temperature from 20 to 30°C due to the enhanced intermolecular association. Even at 95°C, the solution viscosity of PAAHO can retain 35.9%, much higher than that of PAAO; moreover, the E_a of PAAHO is lower than that of PAAO, indicating that the temperature-tolerance property of PAAHO is greatly improved, consistent with the results of TGA. On the other hand, the implementation of HMBA comonomer into PAAHO could impart its remarkable antimicrobial activity to the copolymer. Therefore, the PAAHO copolymer exhibits considerable antibacterial activity, particularly against Gram-positive bacteria.

ACKNOWLEDGMENTS

This research was sponsored by the National Natural Science Foundation of China (contact grant number: 50673085), the National High Technology Research and Development Program of China (863 Program; contact grant number: 2010AA09Z203 and 2010AA065104), and the Ocean Public Welfare Scientific Research Projects, State Oceanic Administration (contact grant number: 201005028-2).

REFERENCES

1. Glass, J. E. Advances in Chemistry Series 213; American Chemical Society: Washington, DC, **1986**.
2. Gouveia, L. M.; Grassl, B.; Muller, A. *J. Colloid Interface Sci.* **2009**, *333*, 152.
3. Zhang, X. F.; Zhou, L.; Zhang, X.; Dai, H. *J. Appl. Polym. Sci.* **2010**, *116*, 1099.
4. Volpert, E.; Selb, J.; Candau, F. *Polymer* **1998**, *39*, 1025.
5. Xue, W.; Hamley, I. W.; Castelletto, V. *Eur. Polym. J.* **2004**, *40*, 47.
6. Ma, J. T.; Cui, P.; Zhao, L.; Huang, R. H. *Eur. Polym. J.* **2002**, *38*, 1627.
7. Zhong, C. R.; Huang, R. H.; Zhang, X.; Dai, H. *J. Appl. Polym. Sci.* **2007**, *103*, 4027.
8. Zhu, Z.; Jian, O.; Paillet, S.; Desbrieres, J.; Grassl, B. *Eur. Polym. J.* **2007**, *43*, 824.
9. Jiang, G. Q.; Liu, C.; Liu, X. L.; Chen, Q. R. *Polymer* **2010**, *5*, 1507.
10. Yahaya, G. O.; Ahdab, A. A.; Ali, S. A.; Abu-Sharkh, B. F. *Polymer* **2000**, *42*, 3364.
11. Feng, Y. J.; Billon, L.; Grassl, B.; Bastiat, G. *Polymer* **2005**, *46*, 9283.
12. Zhong, C. R.; Jiang, L. F.; Peng, X. H. *J. Polym. Sci. Part A: Polym. Chem.* **2010**, *48*, 1241.
13. Ye, L.; Mao, L. J.; Huang, R. H. *J. Appl. Polym. Sci.* **2001**, *82*, 3552.
14. Audibert, A.; Argillier, J. F. Presented at SPE International Symposium on Oilfield Chemistry, SPE 39037, San Antonio, TX, Feb 14–17, **1995**; p 81.
15. Yu, L. M.; An, Z. G.; Dong, L. *Paint Coat. Ind.* **2007**, *37*, 70.
16. Kenneth, J.; Fischer. *U.S. Pat. 5*,226,380, **1993**.
17. Watts, J. *U.S. Pat. 5*,397,385, **1995**.
18. Zhang, X.; Yu, L. M. *Appl. Chem. Ind.* **2010**, *39*, 793.
19. Cheng, W. H.; Xu, H. Z.; Yu, L. M. *Period. Ocean Univ. China* **2006**, *36*, 170.
20. Yu, L. M.; Xu, H. Z. CN Pat. ZL03111823.2, **2005**.
21. Huang, F.; Lu, X. Z. *Pet. Process. Petrochem.* **2002**, *33*, 5.
22. Shi, H. Q.; Li, M. Z.; Liu, C. D. *J. Daqing Pet. Inst.* **1998**, *22*, 21.
23. Taylor, K. C.; Nasr-El-Din, H. A. *J. Pet. Sci. Eng.* **1998**, *19*, 266.
24. Turner, S. R.; Siano, D. B.; Bock, J. *U.S. Pat. 4*,520,182, **1985**.
25. Umar, Y.; Al-Muallem, H.; Abu-Sharkh, B. *Polymer* **2004**, *40*, 47.
26. Gao, B. J.; Jiang, L. D.; Liu, K. K. *Eur. Polym. J.* **2007**, *43*, 4530.
27. Branham, K. D.; Davis, D. L.; Middleton, J. C.; McCormick, C. L. *Polymer* **1994**, *35*, 4429.
28. Zhang, Y. H.; Li, X. H.; Dong, Q. Z.; He, P. X. *J. Appl. Polym. Sci.* **2011**, *120*, 1767.
29. Candau, F.; Selb, J. *Adv. Colloid Interface Sci.* **1999**, *79*, 149.
30. Branham, K. D.; Shafer, G. S.; Hoyle, C. E. *Macromolecules* **1995**, *28*, 6175.
31. Piotr, K.; Annie, A. H.; Selb, J.; Candau, F. *J. Polym. Sci. Part A: Polym. Chem.* **2003**, *41*, 3261.
32. Wan, X.; Li, X.; Wang, X. *Eur. Polym. J.* **2007**, *43*, 3655.
33. Yang, Z. L.; Gao, B. Y.; Li, C. X. *Chem. Eng. J.* **2010**, *161*, 27.
34. Kramer, M. C.; Weich, C. G.; McCormick, C. L. *Macromolecules* **1995**, *28*, 5248.
35. Jiang, G. Q.; Huang, L.; Li, B.; Lv, C. S.; Li, R. *J. Appl. Polym. Sci.* **2012**, *123*, 66.
36. Zhou, H.; Song, G. Q.; Zhang, Y. X.; Chen, J. Y. *Macromol. Chem. Phys.* **2001**, *202*, 3057.
37. Dong, D. C.; Winnik, M. A. *Can. J. Chem.* **1984**, *62*, 2560.
38. Zhong, C. R.; Luo, P. Y.; Meng, X. H. *J. Appl. Polym. Sci.* **2010**, *116*, 404.
39. Effing, J. J.; Mclennan, I. J.; Kwak, J. C. T. *J. Phys. Chem.* **1994**, *98*, 2499.
40. Zhong, C. R.; Huang, R. H.; Xu, J. Y. *J. Solut. Chem.* **2008**, *37*, 1227.

41. McCormick, C. L.; Nonaka, T. *Polymer* **1988**, *29*, 731.

42. Li, Q.; Ye, L.; Cai, Y.; Huang, R. H. *J. Appl. Polym. Sci.* **2006**, *100*, 3346.

43. Wang, Y.; Feng, Y. J.; Wang, B. Q.; Lu, Z. Y. *J. Appl. Polym. Sci.* **2010**, *116*, 3516.

44. Zhong, C. R.; Luo, P. Y. *J. Polym. Sci. Part B: Polym. Phys.* **2007**, *45*, 826.

45. Wang, X.; Xu, J.; Jiang, Y. Y.; Gao X. L.; Gao, C. J. *Chem. J. Chin. Univ.* **2012**, *33*, 2129.

46. Xu, J.; Gao, C. J.; Gao, X. L.; Yu, L. M. CN Pat. 102580587 A, **2012**.

47. Li, Y.; Tang, H. G.; Liu, J. X. *Acad. Period. Farm Prod. Process.* **2008**, *12*, 53.

48. Xing, F. B. Doctoral dissertation, Study on the Microcapsules and Nanocapsules of Gelatin-Acacia-Tannin Containing Capsaicin, Tianjin University, **2003**.

49. Cong, W. W.; Yu, L. M. *Chem. Res. Chin. Univ.* **2011**, *27*, 803.

50. Jiang, S.; Wang, L.; Yu, H. J. *J. Appl. Polym. Sci.* **2006**, *99*, 2389.

51. Huang, J.; Huang, Z. M.; Bao, Y. Z. *J. Appl. Polym. Sci.* **2006**, *100*, 1594.

52. Costerton, J. W.; Cheng, K. J. *J. Antimicrob. Chemother.* **1975**, *1*, 363.