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ORIGINAL CONTRIBUTION

^{1}H NMR study on microstructure of a novel acrylamide/methacrylic acid template copolymer in aqueous solution

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Abstract A novel acrylamide/methacrylic acid template copolymer was prepared using polyallylammonium chloride (PAAC) as a template. This copolymer contains acrylamide (PAM), phenoxy acrylate (POA), and acylic acid (PAA) blocks. The investigation by high resolution nuclear magnetic resonance (^{1}H NMR) shows that intramolecular hydrogen bonds between the PAM and PAA blocks lead to compact molecular arrangement at quite low pH values, and the motion of the phenoxy side chain of the POA blocks is somewhat restricted. With the increase in pH value of the solution, the carboxylic acid of the PAA block gradually dissociates, which weakens hydrogen bonds between the PAM and PAA blocks. The decrease in D_w, self-diffusion coefficient of water, indicates the growth in aggregate size of the template copolymer. The cross peaks between amide protons and backbone protons shown in 2D nuclear overhauser spectroscopy (NOESY) spectra imply the existence of the intermolecular hydrogen bonding interaction between PAM and PAA blocks. After the carboxylic acid of the PAA block is completely dissociated in alkaline solution, the electrostatic repulsion of the carboxylic ion makes the molecular chain of the copolymer exhibit more outstretched. Consequently, the phenoxy groups (the side chain of the POA block) have more space to move.

Keywords NMR · Template copolymer · Hydrogen bond · pH

Introduction

Template polymerization, also termed replica or matrix polymerization, has been first suggested by Szwarc [1]. As template copolymers have different polymerization processes from that of the ordinary copolymers, many efforts have been focused on the template effects in polymerization aiming to synthesize more stereoregular copolymers [2, 3]. The mechanism of propagation of the growing chain may affect not only the reaction rate but also the molecular weight and the microstructure of the polymer formed. In comparison with the similar copolymer prepared in the absence of the template, polymerization in the presence of template tends to produce copolymers with significantly longer block lengths of the repeated units. This is probably due to the development of a special attractive interaction between the growing chain and the template. Rainaldi et al. [4] found that stronger interaction between acrylic acid and the template occurred in the copolymerization of acrylic acid and 2-hydroxyethyl methacrylate systems in aqueous solution by means of high performance liquid chromatography (HPLC). The template copolymer *N*-isopropylacrylamide (NIPAM)/*N*, *N*-(dimethylaminopropyl) methacrylamide

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(MADAP) was investigated by viscometry, fluorescence, and turbidity study [5]. This copolymer contains longer NIPAM sequence and forms stronger, more compact and more hydrophobic hydrogen-bonding inter-polymer complexes with polyacrylic acid (PAA) at low pH values.

Nuclear magnetic resonance (NMR) is an effective method to investigate the microstructure and the arrangement of the molecules in aqueous solution. Frisch's research groups [6, 7] have studied the template copolymerization effects on the structure of the copolymer by using ^{1}H and ^{13}C NMR. The overlap of the peaks in the NMR spectrum of the template copolymer makes it difficult to assign and analyze the spectral lines. The study of template polymerization is still confronted with some difficulties. In this paper, we investigate a novel acrylamide/methacrylic acid template copolymer in aqueous solution by 1D and 2D NMR techniques. The obtained results show that hydrogen bonds are formed between the PAM and PAA blocks at acidic and neutral solutions. The motion of the phenoxy side chain of the POA blocks is somewhat restricted for inter- or intramolecular hydrogen-bonding interaction.

Materials and methods

Materials

Acrylamide (AM) was the product of Jiangxi Changjiu Biochemical Engineering and used as received. Methylacrylic acid (MAA) was the product of Tianjin Chemical Reagent Research Institute and distilled under vacuum over copper turnings. The template poly (allylammonium chloride) (PAAC, Mw: 8,000) was synthesized by the Technical Institute of Physics and Chemistry of the Chinese Academy of Sciences. 2,2′-Azo-bi- (2-amidinopropane) dichloride (AIBA) was used as an initiator.

Template copolymers of AM and MAA were synthesized in the presence of PAAC as a template and AIBA as an initiator. The molar ratio of PAAC (monomer units) to MAA was 1. Traditional copolymer of AM and MAA was synthesized in the absence of PAAC. The experimental parameters were kept constant: the overall concentration of monomers (8 wt%), the temperature (318 K), pH about 6.5, and the concentration of initiator (AIBA at 0.2 wt% related to the total monomers feed). The polymerization reactions were conducted to exceed 90% (about 15 h). The separation of the resulting copolymers from template was performed as described in a previous paper [8].

D_2O (99.8%) is the product of Aldrich Chemical. The concentration of storage solution is 1 g/l by weighing the desired amounts of copolymer and dissolved in D_2O. Its pH value is about 6.5. The samples of different pH values (2.0, 4.0, 9.0, 11.0) were prepared by extracting definite quantities of this storage solution, and the pH value of which were adjusted with HCl and NaOH solutions, respectively.

NMR experiments

NMR experiments were performed on a Varian INOVA-500 spectrometer with a proton frequency of 500.13 MHz at 298 K. D_2O was used as solvent instead of water to weaken the water signal. Meanwhile, the presaturation method was used to further suppress the proton signal of solvent. Self-diffusion coefficients were measured by using the bipolar pulse-longitudinal eddy current delay sequence [9]. Two-dimensional nuclear overhauser enhancement (2D NOESY) experiments were performed with the standard three-pulse sequence [10].

Results and discussion

Copolymer composition

Acrylamide/methacrylic acid template copolymer contains nonionic acrylamide (PAM) block, phenoxy acrylate (POA) block and 20 wt% negatively charged acylic acid (PAA) block. Figure 1 shows its formula and proton numberings. ^{1}H NMR spectrum of the template copolymer solution is shown in Fig. 2. The resonance peaks of the backbone protons of the template copolymer are located in the lower frequency region [11]. Chemical shifts of the side chain protons are at a higher frequency region due to the effect of electron attraction by the phenyl and acyl groups [12]. The peaks at about δ 7.1 and δ 7.4 are typical resonances of phenyl protons. The appearance of cross-peaks between proton d and c, e in the 2D COSY spectrum (Fig. 3) indicates that these protons belong to the same phenyl group. The ratio of integral areas of these two peaks is 0.40: 0.62, approximately 2:3. It is evidenced that the peaks at about δ 7.4 and δ 7.1 are the meta-, otho-, and para-phenyl groups, respectively. Meanwhile, they do not form cross-peaks with the proton chemical shifts of which are at about

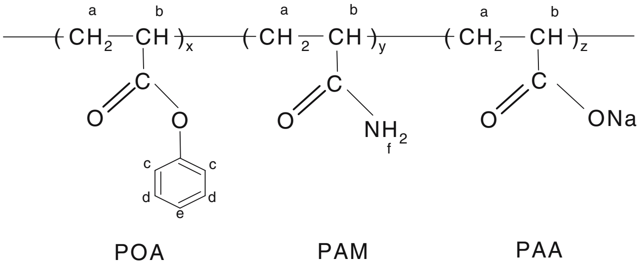

Fig. 1 The formula and proton numberings of acrylamide/methacrylic acid template copolymer

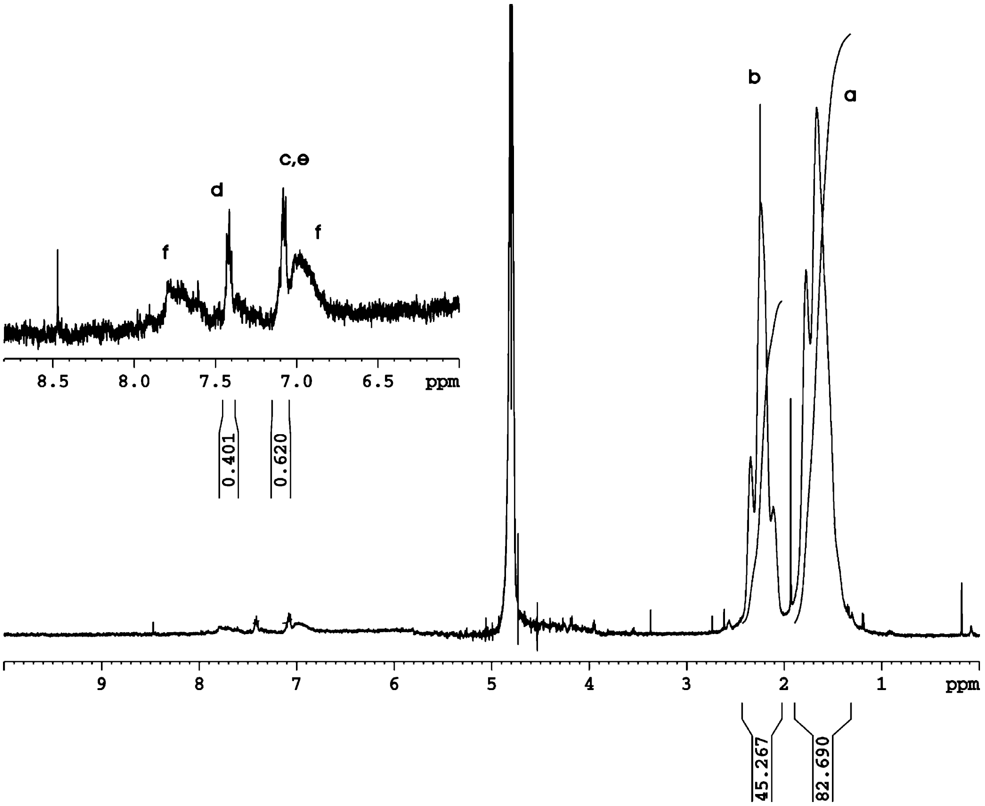

Fig. 2 ^{1}H NMR spectrum of the template copolymer at pH=6.5

7.7 and 7.0 ppm, so we can conclude that protons at 7.7 and 7.0 ppm belong to another functional group. Sun et al. [13] investigated the microstructure of PAM in aqueous solution by ^{1}H NMR. The chemical shifts of amide protons are located at 7.7 and 7.0 ppm, respectively. That is similar to our spectrum (Fig. 2). Accordingly, that the signals at 7.0

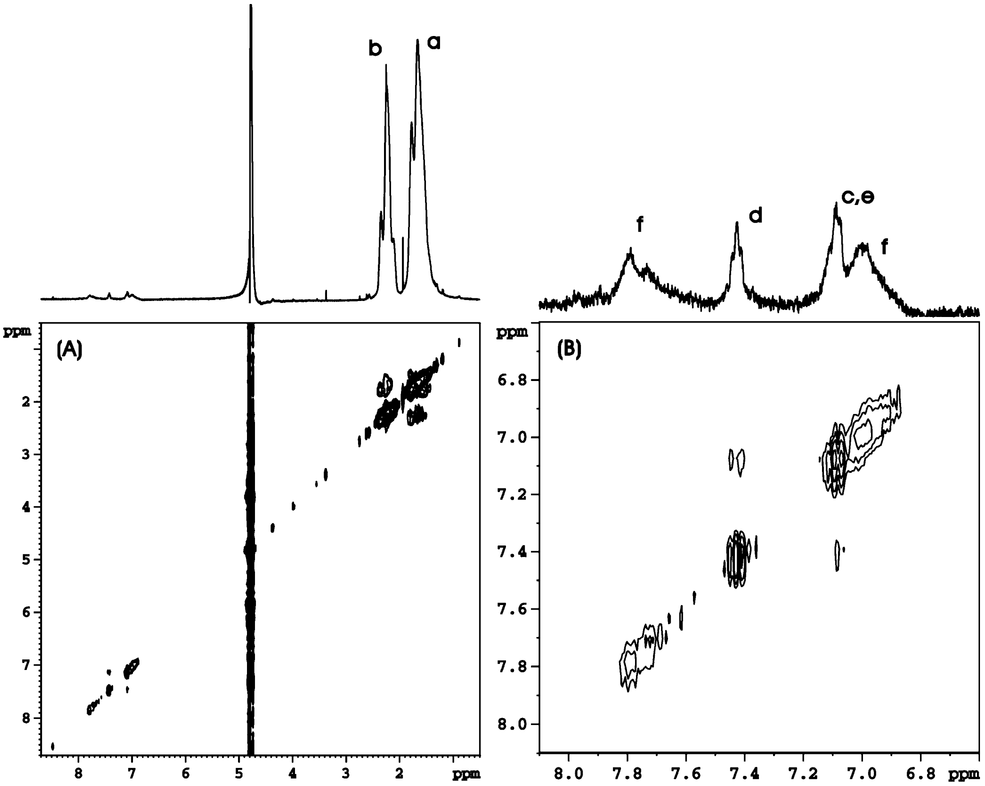

Fig. 3 **a**, **b** Contour plots of the 2D COSY spectrum of template copolymer solutions at pH=6.5. **a** Whole spectrum, **b** enlarged spectrum of the region 6.6–8.1 ppm

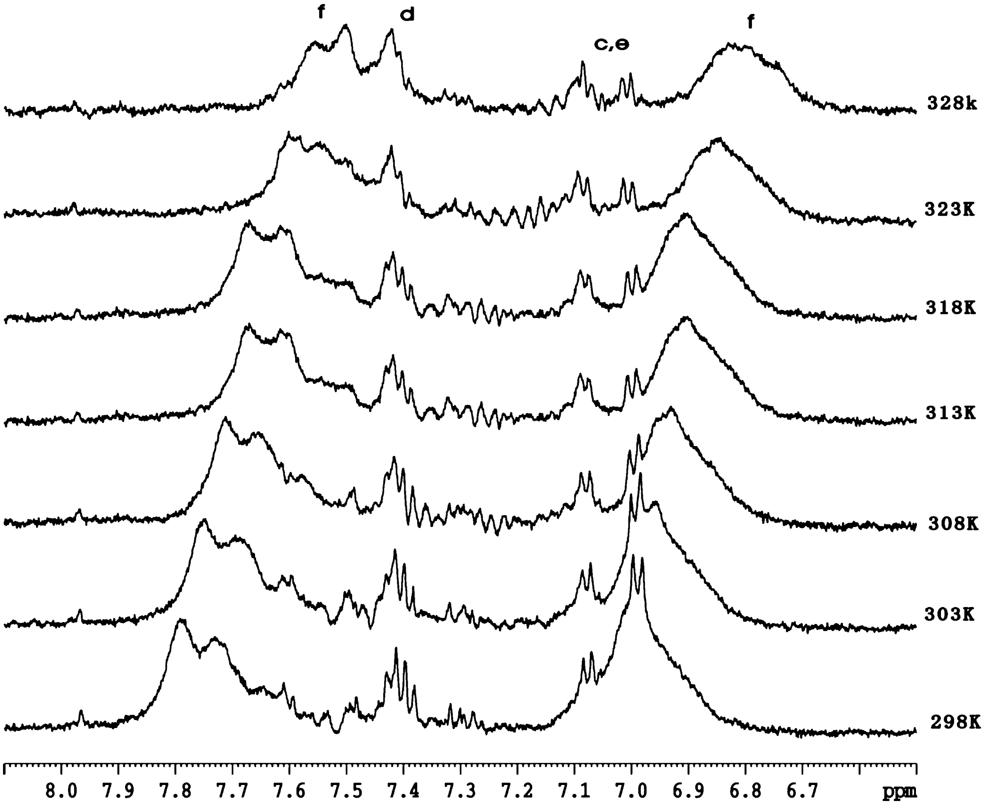

Fig. 4 ^{1}H NMR spectra of the template copolymer at different temperatures at pH=4.0

and 7.7 ppm belong to PAM segments is reasonable. The POA hydrophobic contents can be determined by using Eqs. 1, 2.

$$S_d/(S_a + S_b) = 2x/3(x + y + z) \tag{1}$$

$$x + y + z = 1 \tag{2}$$

where S_d is the integrating areas of meta-phenoxy protons in the side chain of the POA blocks, S_a and S_b are the integrating areas of the methylene protons and of the methine protons in the copolymer backbone, respectively. Standing for POA, PAM, and PAA contents, respectively, are x, y, and z, in mol%. The data of S_d=0.40, S_a=82.69, and S_b=45.27 are indicated in Fig. 2. The content of the POA blocks was estimated at about 0.47 mol%. By the same means, the content of the POA blocks at various pH value of the solution can be easily estimated at 0.50 mol%.

Hydrogen-bonding interaction

Ordinarily, the proton peak of amide group is hardly observed in D_2O solution, due to the exchange between the proton of the amide group and the deuteron of the heavy water. The signals at 7.0 and 7.7 ppm in Fig. 2 indicate that the exchange of H-D between the protons of amide group with the solvent D_2O is somewhat restricted. Figure 4 shows a series of ^{1}H NMR spectra of the copolymer solution at different temperatures (298, 303, 308, 313, 318, 323, and 328 K). This chemical shift dependence of the template copolymer on temperature indicates the existence of hydrogen bond in the copolymer solution. Chemical shifts depend on the inverse third power of the distance between amide proton and hydrogen bond acceptor [14]. The thermal motion is enhanced with the increase in temperature, and it results in lengthening of average hydrogen bond lengths [15]. This, in turn, leads to a decrease in the deshielding effect induced by an acceptor, and in consequence, at higher temperature amide proton peaks shift to lower frequency side [16, 17]. In contrast, the chemical shifts of the phenoxy proton peaks remain unchanged. As is well-known, the basicity of the amide protons is too weak for protonation to occur to any extent in water solution. Even at low pH values, the amide groups of the PAM unit remain neutral [18]. The carbonyl oxygen can readily be an electron donor; consequently, the lower frequency shift of the amide protons by increasing the

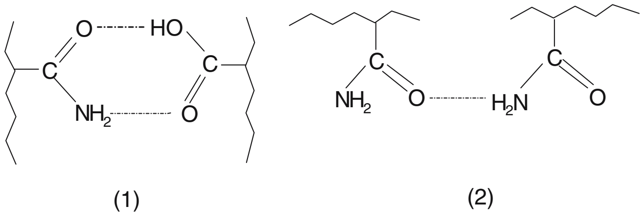

Fig. 5 Schematic diagram of hydrogen bonding in template copolymer solution

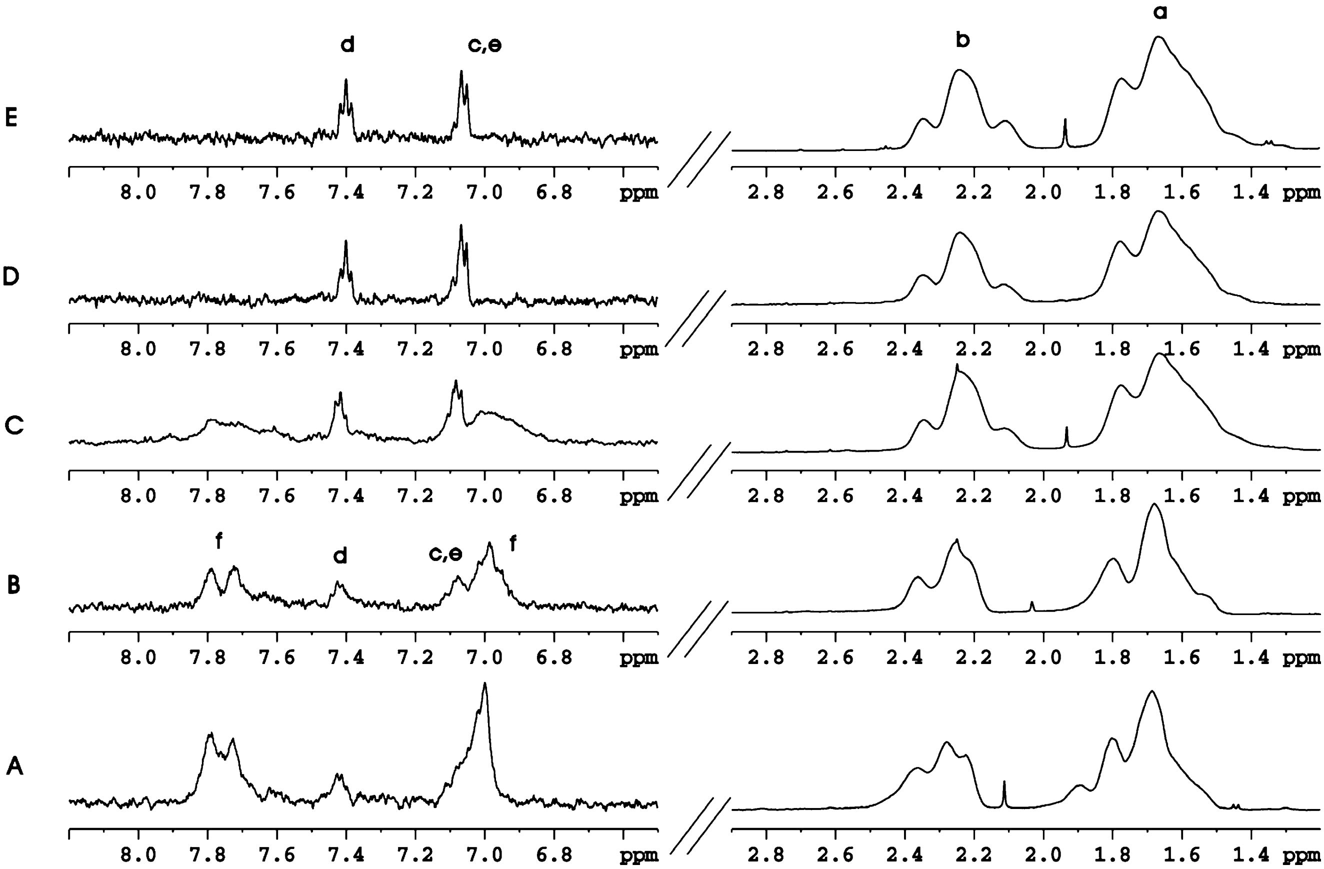

Fig. 6 **a**–**e** ^{1}H NMR spectra of the template copolymer at different pH values: **a** 2.0, **b** 4.0, **c** 6.5, **d** 9.0, and **e** 11.0; *asterisk* (*): Spectra of the high frequency region are enlarged

temperature suggests that the amide protons of the PAM unit form hydrogen bonds with carbonyl oxygen in the template copolymer solution. It has been reported that hydrogen bonding between the non-dissociated acidic proton of PAA unit and the carbonyl oxygen of PAM unit is the main factor stabilizing the PAM/PAA complex [19, 20]. Considering that the content of the POA unit was about 0.50 mol%, we can conclude that the hydrogen bonds mostly form between PAA and PAM unit as shown in Fig. 5.

Microstructure at different pH values

Figure 6a–e gives the ^{1}H spectra of the template copolymer solutions at pH values of 2.0, 4.0, 6.5, 9.0, and 11.0, respectively. Variation of the amide proton resonance peaks with the pH value is remarkable. The two relatively sharp peaks at pH=2.0, 4.0 become less pronounced at pH=6.5 and finally vanished when the pH value exceeded 9.0. This variation could be understood if we consider that the undissociated carboxylic acid will gradually dissociate as

Table 1 ^{1}H spin-lattice relaxation time (T_1), spin–spin relaxation time (T_2), and T_R (T_2/T_1) for template polymers in aqueous solution at different pH values at 298 K

pH	Ph			CH (b)			CH$_2$ (a)		
	T_1 (ms)	T_2 (ms)	T_R	T_1 (ms)	T_2 (ms)	T_R	T_1 (ms)	T_2 (ms)	T_R
2.0	*	*	*	1,009	72	0.07	594	51	0.09
4.0	*	*	*	650	34	0.05	508	20	0.04
6.5	1,730	170	0.10	544	13	0.02	508	10	0.02
9	2,950	1,260	0.42	766	22	0.03	620	14	0.02
11.0	3,070	1,570	0.50	615	54	0.09	413	33	0.08

*At pH=2.0 and 4.0, the relaxation time of the phenoxy protons is difficult to be obtained because they are *broad and weak* and partly overlapped with the amide proton peaks.

Experimental error <10%

the pH value of the solution increases. The negatively charged carboxylic groups weakened the hydrogen bond between the PAM and PAA units. When the carboxylic groups are completely dissociated, the ion repulsion makes the backbone chain more rigid and the hydrogen bonds collapse. Thus, because of the D–H exchange between amide protons and the deuterium of the solvent D_2O, the amide proton peaks disappear at pH=9.0 and 11.0 as shown in Fig. 6d,e.

The variation of the resonance peaks of the phenoxy protons of the POA block exhibits an inverse trend to that of the amide protons of the PAM block with the increases in pH value of the solution. Their broad and poorly resolved peaks at pH=2.0 and 4.0 become narrower and better resolved with the increase in pH value of the solution. It seems difficult to imagine that the resonance peaks of protons of a nonionic group would be pH dependent.

The spin-lattice relaxation times (T_1), spin–spin relaxation times (T_2), and the ratio, T_R (T_2/T_1) of the main protons of copolymer in aqueous solutions at various pH values are shown in Table 1. From the ratio, T_2/T_1 (T_R) one can identify the different motional regimes. A deviation of T_2/T_1 from the unit shows that the motion is restricted. It is evident that T_2 values of all the methylene and methine protons of the backbone are several milliseconds at various pH values, and the corresponding T_R values are less than 0.1. This suggests that motions of main chain protons are seriously restricted, especially for those at pH=4.0, 6.5 or 9.0.

It is worth noting that T_R values of the phenoxy protons, the side-chain protons of PAO blocks, is 0.1 at pH=6.5, markedly shorter than those in alkaline solution. It indicates that the motion of the phenoxy protons is more restricted in neutral solution, whereas, more freely in alkaline solution. We have discussed that the hydrogen bonds between the PAM and PAA blocks are weakened with the increase in pH value. The hydrogen bonding interaction leads to compact molecular arrangement; thus, it would exert steric effect on the motion of the side chain of the POA block. In alkaline solution, the PAA block gradually leaves the PAM block by the dissociation of the carboxylic acid of the PAA block, leading to the increase in space for the side chain of POA to move. So these phenoxy proton peaks have more space to move and became narrower at high pH values as shown in Fig. 6d,e.

NMR diffusion coefficient measurements can provide further evidence for the conformational changes by determining the long-range mobility of the components [21]. But the proton peaks of copolymer molecules can hardly give rise to decays in this diffusion experiment. It is probably due to the large aggregate size and high viscosity of the aqueous solution. The water self-diffusion can provide indirect evidence for the changes of the mobility of the copolymer, for it is influenced by obstruction effect due to the aggregates and by hydration [22]. Any growth in aggregate size and in hydration is concomitant to a decrease in water self-diffusion. In these diffusion experiments, the intensities of proton peak of water follow an exponential decay. The observed water diffusion coefficient, D_w, shown in Fig. 7 were obtained by nonlinear least-squares fitting procedures. It is obvious that the relatively lowest value of

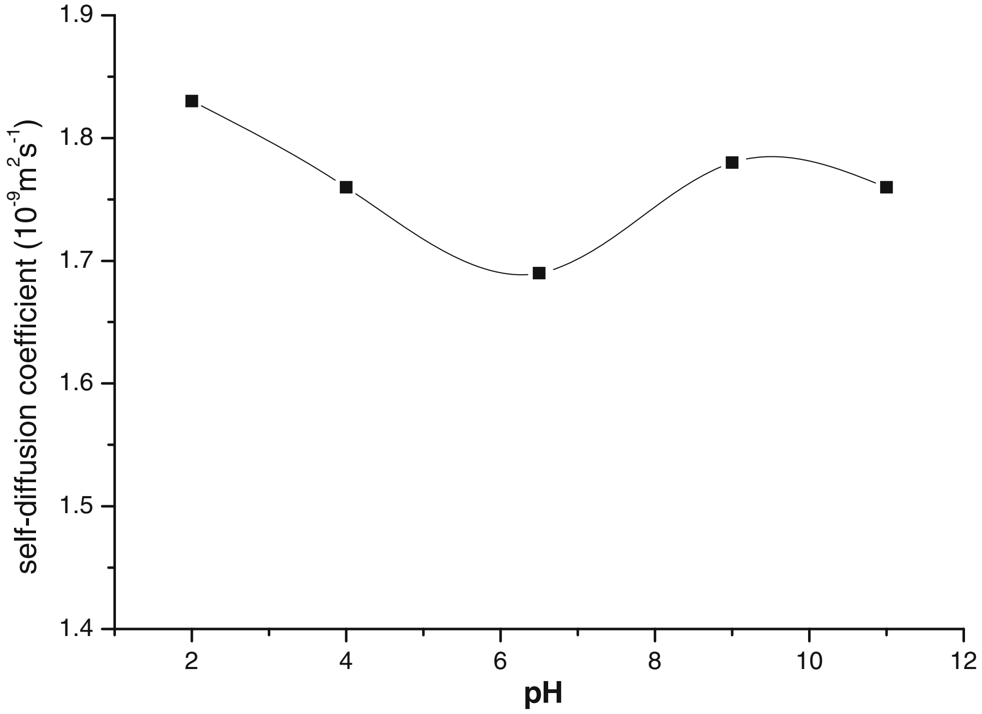

Fig. 7 pH dependence of water self-diffusion coefficients, D_w (10^{-9} m^2 s^{-1}), of the template copolymer solution

D_w occurs at pH=6.5. The results indicate that the aggregate size is relatively smaller in alkaline solution. This is easily understood if the collapse of hydrogen bonds is considered. However, the increase in D_w is inexplicable as the pH value decreases from 6.5 to 2.0, for it has been concluded above that the hydrogen bonds form in this range of pH values. Does it imply that there is a variation of the microstructure of copolymer aggregates with the pH increasing from 2.0 to 6.5?

Direct evidence about the space relationship among the protons of a molecule will help to confirm the microstructure of macromolecules, such as proteins, bio-molecules, and synthetic polymers. This can be achieved by the 2D NOESY experiment from which qualitative information about the distance in space between the proton pairs of interest can be obtained [23, 24]. Cross-peak between proton pairs separated by a distance in space shorter than 0.5 nm will appear in the 2D NOESY spectrum. 2D NOESY experiments of the template copolymer solution at pH values of 2.0, 4.0, and 6.5 have been done with a mixing time of 200 ms. Contour plots of these experiments are shown in Fig. 8a–c. Cross-peaks between proton pairs of the backbone are obvious in all of the plots (a–c). Cross-peaks between the amide protons of the PAM block and the protons of the backbone appeared when pH=4.0 or 6.5 as shown in Fig. 8b,c. It implies that the amide proton is much closer to the backbone. This could only be attributed to the hydrogen bonds among different copolymer chains. It is noticeable that the cross peaks between amide proton of PAM unit and the backbone disappeared at pH=2.0 in Fig. 8a. It can be concluded that the average distance between amide protons and backbone protons is not less than 0.5 nm when pH=2.0. In other words, the hydrogen bonds are more likely formed among different blocks within the copolymer chain when pH=2.0. The intra-copolymer hydrogen bonds generally lead to more compact aggregates, whereas, inter-copolymer hydrogen bonds induce bulky ones. This is consistent with the results of the self-diffusion coefficients of water.

Conclusions

Combination of the 1D and 2D NMR study indicates that the microstructure of the template copolymer in aqueous solution varies with the pH value of the solution. At pH=2.0, the intra-copolymer hydrogen bonds between PAM and PAA blocks lead to the compact molecular arrangement in aqueous

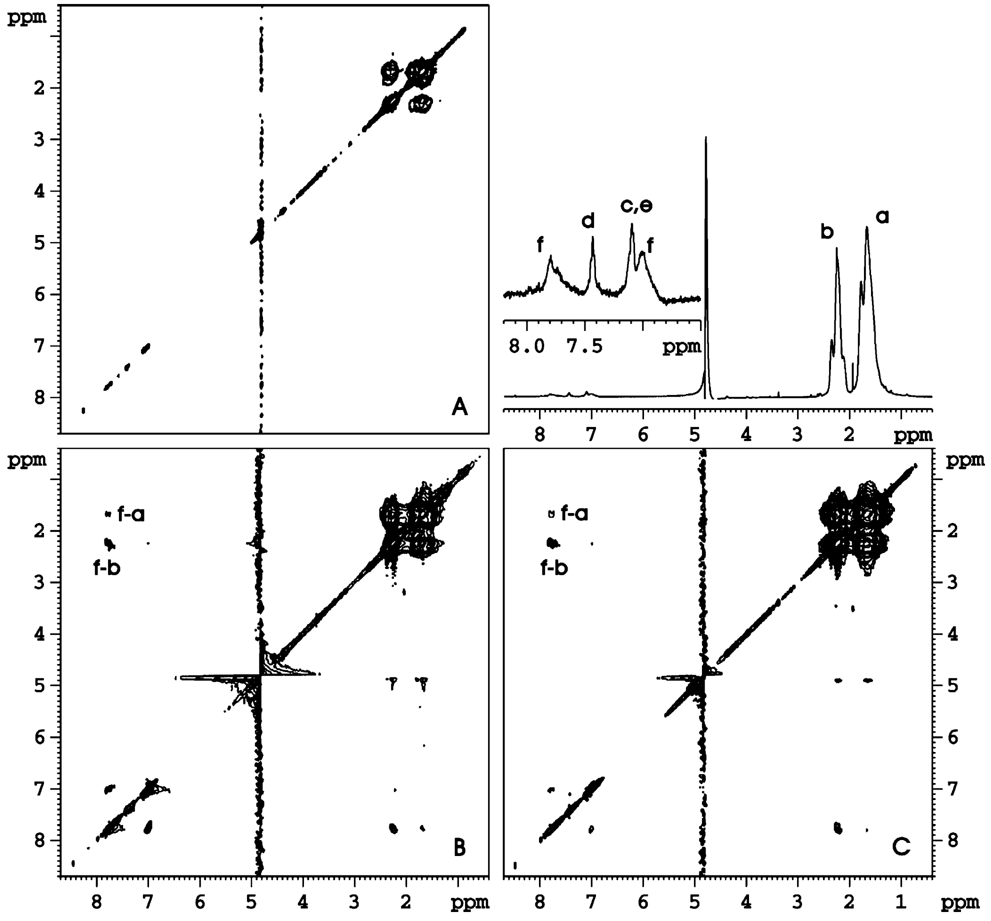

Fig. 8 **a–c** Contour plots of the 2D NOESY spectrum of template copolymer solutions with mixing times of 200 ms at various pH values: **a** 2.0, **b** 4.0, and **c** 6.5

solution, where the motion of the phenoxy side chain of the POA block is somewhat restricted. With the increase in pH value of the solution, the carboxylic acid of the PAA block gradually dissociates, which weakens hydrogen bonds between the PAM and PAA blocks. The two forces, the electrostatic repulsion of the carboxylic ions and the intercopolymer hydrogen bonding interactions, result in the growth in aggregate size. After the carboxylic acid of the PAA block is completely dissociated at alkaline solution, the hydrogen bonds collapse completely. Thus, the bulky mass PAA blocks leave the PAM blocks and the molecules of copolymer exhibit more outstretched. Consequently, the phenoxy groups of the POA block have more space to move.

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