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Novel amphiphilic copolymer with pendant tris(trimethylsiloxy)silyl group: synthesis, characterization and employment in CE DNA separation

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Abstract

Hydrophobically modified water-soluble polymers have been prepared by micellar copolymerization of hydrophilic monomer acrylamide and the silicone-containing hydrophobic comonomer, tris(trimethylsiloxy)methacryloxypropylsilane in various molar ratios. Capillary viscometer, FT-IR, gel permeation chromatography-multi-angle laser light scattering, transmission electron microscopy, and ^{1}H NMR were used to characterize these polymers. The results showed that the resulting copolymers are amphiphilic in nature and associate into micelles in water. 9-chloromethyl-anthracene was used as the fluorescent probe to further confirm the copolymer self-aggregates in water by fluorescence spectroscopy. The fluorescence intensity increased as a function of the hydrophobicity of copolymers. And the critical concentration of the self-aggregate formation of copolymer (molar ratio of acrylamide to hydrophobic silicone monomer being 75:1) determined fluorometrically was 0.5 g l^{-1}. The amphiphilic copolymers (molar ratio of acrylamide to hydrophobic silicone monomer being 20:1 and 100:1, respectively) were used as separation media in capillary electrophoresis for the separation of DNA. The experimental results indicate that the copolymer with higher hydrophobe content showed no separation efficient while the lower one separated most DNA fragments clearly in ϕX174/Hae III digest at very low copolymer concentration of 0.1 wt%.

Keywords: Amphiphilic copolymer; DNA; Capillary electrophoresis

1. Introduction

Amphiphilic block copolymers consist of both hydrophobic and hydrophilic chain segments combined in a single macromolecule and are typically found to aggregate and adsorb at surfaces, similar to low molecular weight surfactants [1]. The phase behavior of such system can show a high degree of richness and complexity, such as the formation of large variety of lyotropic liquid crystalline phases at higher block copolymer concentrations and in the presence of a single selective solvent or two immiscible selective solvents [2], and the generation of a variety of supermolecular structures in aqueous solution such as spherical or rodlike micelles, vesicles, fibers, network structures, and lamellar or helical aggregates [3]. These copolymers have received a lot of attention because of their application as gel-formers, surface modifiers, foam and colloid stabilizers, thickeners, wetting agents, compatibilizers, microreactors and nanostructure materials [4]. Recently a special focus is placed on the use of self-assembled block copolymer in pharmaceutical and biomedical applications [5].

In most cases the hydrophobic groups are either alkyl chains, from octyl to octadecyl, or contain an aromatic ring (phenyl, naphthyl, pyrenyl, etc.). Extensive investigations have been conducted on amphiphilic cellulose [6], PEG [7], poly(acrylic acid) [8], and others [9], as well as on polyacrylamide-based copolymers [10]. The former polymers were mainly prepared by chemical modification of a preformed polymer, and the latter were obtained by copolymerization of the appropriate monomers. It is known that solution copolymerization leads to a statistical copolymer, while a copolymer with a somewhat blocky structure can be obtained by micellar polymerization due to the microheterogeneous nature of the polymerization medium [10]. The formation of a blocky structure in the latter process was first suggested in 1987 [11] and experimentally confirmed in 1989 [12].

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On the other hand, due to bubble formation, gel inhomogeneity, short column life and low reproducibility, the separation media for DNA capillary electrophoresis (CE) have been gradually changed from traditional gels to a variety of polymer solutions including many non cross-linked hydrophilic polymer solutions [13]. Solutions of random, block, or graft copolymers, interpenetrating network, polymer mixtures [14] are also used to get high sieving ability, low viscosity and dynamic coating ability, which are not available simultaneously for a homopolymer solution.

There are a few reports concerning amphiphilic polymers as separation media for DNA in CE. When the amphiphilic block copolymer $E_{99}P_{69}E_{99}$ (with E and P denoting oxyethylene and oxypropylene, respectively) was used as separation medium at the concentration higher than 20% (w/v), it aggregated to closely packed cubic structure and showed good sieving ability for small size DNA fragments or allowed the use of very short columns [15]. Menchen et al. [16] synthesized a block copolymer consisting of PEGs end-capped with fluorocarbon (C_nF_{2n+1}) tails. The self-assembled flower-like micellar structure in aqueous solution above the critical micelle concentration (CMC) could further aggregate to form a network structure. It was found that these terminal hydrophobic groups had subtle effects on DNA sequencing since they formed the micellar core as well as the cross-linking points that affect the mesh size. Another block copolymer, PEO end-capped with *n*-dodecane, was synthesized and used in the separation of oligonucleotide [17]. In aqueous solution such system could also form micelle structure. Magnusdottir et al. used oligonucleotide to explore the performance and achieved baseline resolution under a concentration between 5.1 and 10.1% (w/v) with UV detection, which was much better than that of PEO having the same molecular weight and under same conditions.

Acrylamide (AM) is a desirable first choice as one of the monomer because polyacrylamide (PAM) is well known for its high sieving ability as a separation medium for DNA sequencing analysis in CE, as well as its water-solubility and potential utility. When separating the DNA fragments shorter than 1000 bps [18], high PAM concentration is needed, but this results in the high viscosity of the sieving agent. In this paper, the synthesis and characterization of amphiphilic polyacrylamide with bulky pendant tris(trimethylsiloxy)silyl group in various molar ratios were reported, in which the bulky hydrophobic groups differs from traditional investigation of the long alkyl chain. The polymerization method used was micellar copolymerization with sodium dodecyl sulfate as surfactant. TEM, ^{1}H NMR, fluorescence spectroscopy and viscometric measurement indicated that the copolymers are of amphiphilic nature and self-assemblized to micelles in aqueous solution, and this trend was strengthened with the increased content of hydrophobic comonomer tris(trimethylsiloxy)methacryloxypropylsilane (TTMAPS). The amphiphilic polymers were used as media for the separation of DNA in CE. It was found that the copolymer with higher TTMAPS content showed no separation efficient while the lower one separated most DNA fragments clearly in ϕX174/Hae III digest at very low copolymer concentration of 0.1 wt%.

2. Experimental section

2.1. Materials

Chlorotrimethylsilane and 3-methacryloxypropyltrimethoxysilane were purchased from Wuhan University Chemical Plant (Wuhan, China) and purified by distillation under reduced pressure. Acrylamide (AM, >99.0%), sodium dodecyl sulfate (SDS) and potassium persulfate (KPS) were purchased from Shanghai Chemical Reagents Co. (Shanghai, China) and used as supplied. YO-PRO-1 (1 mM in DMSO) was purchased from Molecular Probes Inc. (Eugene, OR). Polyvinylpyrolidone (PVP, $M_w = 360,000$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). ϕX174/Hae for DNA standard containing 11 fragments was prepared by digesting ϕX174 plasmid (SBS Genetech Co. Ltd., Beijing, China) with Hae III at final concentration of 0.15 μg ml^{-1}. All solvents were of analytical grade and used as received.

2.2. Synthesis of tris(trimethylsiloxy)methacryloxypropylsilane

3-methacryloxypropyltrimethoxysilane (59.5 ml, 0.25 mol) and chlorotrimethylsilane (192 ml, 1.52 mol) were charged to a 500 ml three-necked, round-bottomed flask equipped with a magnetic stirrer, a dropping funnel and a reflux condenser connected to a sodium hydroxide trap for evolving hydrochloride vapors. A solution of methanol (60.5 ml, 1.5 mol) in water (40.5 ml) was added dropwise in 30 min under stirring. The temperature was maintained at 30 °C for 13 h. The organic phase was separated and washed with water (100 ml × 3), then dried on anhydrous sodium sulfate overnight, filtered, and distilled under normal pressure then reduced pressure to give tris(trimethylsiloxy)methacryloxypropylsilane (TTMAPS).

Yield: 79.1 g (73.9%). bp: 124 °C/0.53 kPa; ^{1}H NMR ($CDCl_3$, ppm): $\delta = 0.105$ (s, 27H, $SiCH_3$), 0.500 (t, 2H, CH_2Si), 1.692 (m, 2H, $CH_2CH_2CH_2$), 1.939 (s, 3H, CCH_3), 4.083 (t, 2H, OCH_2CH_2), 5.516 and 6.083 (2 × m, 1H each, CH_2=C); GC: 94.3%.

2.3. Micellar copolymerization and solution preparation

A 250 ml four-necked, round-bottomed flask was fitted with reflux condenser, overhead mechanical stirrer, thermometer, and nitrogen inlet/outlet. 3.00 g (0.042 mol) AM was dissolved in 100 ml water in the flask; TTMAPS and

3.00 g SDS were transferred into it with gently stirring to get an optically transparent solution, then purging with nitrogen under stirring for 1 h. After heating to 50 °C, initiator KPS (at 0.3 wt% relative to the monomer feed, dissolved in a little amount of water) was injected with a syringe. The reaction was run for 6 h. After cooling, the copolymer was precipitated by addition of a 4-fold excess of acetone dropwise with stirring. The copolymer was purified by repeated dissolution/precipitation cycles and extracted in Soxhlet extractor with acetone for 4 h, then dried 24 h at 40 °C under vacuum, and stored in a desiccator.

A series of copolymers were synthesized by micellar copolymerization in various AM/TTMAPS molar ratios: 10:1, 15:1, 20:1, 25:1, 40:1, 50:1, 75:1, and 100:1. For reference, homopolyacrylamide (PAM) was prepared under identical experimental conditions in the presence of surfactant.

The homopolyacrylamide (PAM) dissolves easily in water and gives a clear solution, but hydrophobically associating PAM dissolves not so easily. So copolymers were suspended and swelled in water or 0.2 M NaCl aqueous solutions for 24 h, then magnetically stirred for another 24 h at room temperature to give optically clear solutions.

2.4. Characterization and property studies

2.4.1. Gel permeation chromatography-muti-angle laser light scattering. Gel permeation chromatography-muti-angle laser light scattering (GPC-MALLS) is convenient for determination of the true molecular weight and distribution of polymer without standard samples. Molecular weights (M_w), radii (R_z), and M_w/M_n of the samples were determined by a DAWN®DSP multi-angle laser photometer with a pump P100 (Thermo Separation Products, San Jose, USA) equipped with TSK-GEL G6000 PWXL with a G4000 PWXL column (7.8 mm × 300 mm) for aqueous solutions, and differential refractive index detector (RI-150) at 25 °C. The mobile phase was 0.2 M NaCl at a flow rate of 1.00 ml min^{-1}. Scattering light intensity was measured in the angles of 42, 49, 63, 71, 81, 90, 109, 118, and 127° at 25 °C, where the He–Ne laser used at 632.8 nm (DAWN®DSP, Wyatt Technology Co., USA). Refractive index increments (d*n*/d*c*) were measured with a double-beam differential refractometer (DRM-1020, Otsuka Electronics Co.). Concentrations of polymers for measurement were all about 2.0 g l^{-1} in 0.2 M NaCl, which were filtered with sand filter and with 0.45 μm filter (CA, PuradiscTM 13 mm Syringe Filters, Whattman, England). Astra software was utilized for data acquisition and analysis.

2.4.2. ^1H NMR measurements. ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 spectrometer operating at a proton frequency of 300 MHz, and chemical shifts were referenced to solvents peaks. To 0.5 ml of D_2O was added 5.0 mg of polymer, keeping for several days with frequent vibration to get optically transparent solutions. After measurements a small amount of $CDCl_3$ was added as solubilized agent and the ^{1}H NMR spectra were recorded again for comparison.

2.4.3. FT-IR measurements. FT-IR (KBr pellet) spectra were recorded on a Nicolet 670 FT-IR Fourier transform infrared spectrometer (Nicolet Co., USA).

2.4.4. Viscometric measurements in dilute solution. The intrinsic viscosities [η] of the (co)polymers were determined in 0.2 M NaCl aqueous solutions at 25 ± 0.05 °C, using a capillary viscometer (Ubbelohde type) at polymer concentrations in the range 0.075–3.0 g l^{-1}. From molecular weight values ($< 2.5 \times 10^6$) and [η] values (< 550 ml g^{-1}), it was inferred that the shear rate imposed in the capillary did not affect the viscosity data [19]. During the experiments care must be taken to avoid foaming of the copolymer solutions, which would result in erroneous flow times.

2.4.5. Fluorometric measurements. A 0.1 ml $CHCl_3$ solution of 9-chloromethyl-anthracene (as probe) was added to a vial and the solvent was evaporated to form a thin film at its bottom. A polymer stock solution (5.0 ml) was added to the vial and the final probe concentration was 5.734×10^{-6} M in water. The solutions were allowed to equilibrate for 24 h prior to fluorescence runs. Steady-state fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrophotometer (Shimadzu, Japan). The slit setting was 3 nm for measurements, and emission spectra were monitored at 408 nm with an excitation wavelength of 363 nm. Polymer concentration was 3.0 g l^{-1} or diluted to 0.06–3.0 g l^{-1}.

2.4.6. Transmission electron microscopic observation. The size and morphology of polymer particles in aqueous solutions were determined by a JEM-100CXII Transmission electron microscope (Hitachi, Japan). Dilute (co)polymer solutions were sonicated at 40 W for 2 min, applied onto formvar-membrane-coated copper grids and evaporated under infrared light to form a thin film. Then it was negatively stained by phosphotungstic acid and observed.

2.5. DNA separation by capillary electrophoresis

The copolymers (20:1, and 100:1) and PAM were used as separation media, respectively, on an Agilent 3D capillary electrophoresis instrument (Palo Alto, CA, USA) equipped with a ZETALIF laser induced fluorescence detector (Picometrics, Ramonville, France). The excitation wavelength was chosen as 488 nm. Data collection was performed on a HP Chemstation (Palo Alto, CA, USA). In all experiments, 65 cm (effective length 50 cm) × 75 μm I.D. × 375 μm O.D., fused-silica capillaries (Yongnian Optic Fiber Inc., Hebei, China) were used without coating. The buffer used was 100 mM Tris, 100 mM boric acid, and

2 mM EDTA (TBE buffer), which naturally reaches a pH of 8.3. Conditions: 0.1 wt% P(AM-*b*-TTMAPS) or PAM; 0.5 wt% PVP; 2 μM YO-PRO-1; the total concentration of DNA fragments: 0.15 μg ml^{-1}; 15 kV applied voltage (231 V cm^{-1}), negative polarity; electrokinetic injection at 10 kV (154 V cm^{-1}) for 5 s.

3. Results and discussion

3.1. Synthesis and characterization of polymers

The copolymerization method used in the paper was 'micellar copolymerization'. Such process was initially reported in 1982 [20]. Peer and Hill et al. [10] suggested the mechanism and detail description of polymerization. In the present case, hydrophobic TTMAPS was solubilized within SDS surfactant micelles, whereas AM was dissolved together with the KPS initiator in the aqueous continuous medium. The propagating species walked randomly in the aqueous solution to form long hydrophilic sequences and encountered with the micelles to form more or less short hydrophobic blocks. A series of P(AM-*b*-TTMAPS) block copolymers were prepared by changing the hydrophobe level. The compositions and characterization of polymers are summarized in Table 1.

The chemical structure of (co)polymers was characterized by FT-IR and ^{1}H NMR. The IR spectra verified the existence of the stretching vibration of C=O bond of methacrylate and Si–O–Si bond with the bands at ca 1721 and 1060 cm^{-1}, respectively, and the intensity of the stretching vibration band of Si–O–Si bond weakening with decreasing TTMAPS content in the feed. The stretching vibration bands of N–H and C=O bonds of acylamide group were also found at ca 3420 and 1660 cm^{-1}, respectively. The ^{1}H NMR spectra showed protons on main chains (1.3–1.7 ppm, and 2.0–2.3 ppm), and protons of TTMAPS moieties $Si(CH_3)_3$ (−0.1–0.1 ppm). While the characteristic signal of unsaturated proton was not observed in the range of 4.8 to 6.5 ppm on ^{1}H NMR spectrum.

The molecular weights of the polymers were determined by GPC-MALLS without standards. Chromatograms of polymers in aqueous NaCl were obtained as well; however, they do not give an accurate estimate of polymer molecular weights, since in aqueous media copolymers tend to form aggregates, as described later.

Examination of the data of Table 1 leads to the comments that the polymers (to PAM) or their aggregates (to copolymers) are all rather monodisperse because the values of M_w/M_n are all in the range 1.14–1.29; the radii are also rather uniform (75 ± 3 nm), and the molar mass of copolymers prepared by micellar polymerization are similar to the PAM obtained under the same conditions. While for higher hydrophobe content, the molecular weights increase, which displays the opposite trend compared with that observed by Hill et al. [10]. This may be attributed to the stronger intermolecular aggregation of higher hydrophobe content copolymers because of the vigorous hydrophobicity of bulky tris(trimethylsiloxy)silyl groups. At the similar measurement condition they have larger aggregation numbers.

The dissolution of polymers in water becomes more difficult with the increasing of hydrophobe levels, qualitatively indicates the self-assemblized microstructure of the copolymers, and this is confirmed by other measurements below. High foaming ability is observed during the preparation of sample solutions and the measurement of viscosities, but PAM exhibits foaming properties to a less extent. This behavior also reflects the amphiphilic nature of the copolymers [10].

3.2. ^{1}H NMR studies. The ^{1}H NMR spectra of PAM, PAM + TTMAPS (PAM with the addition of a little of TTMAPS), and P(AM-*b*-TTMAPS) in D_2O are shown in Fig. 1. The proton signals of the CH_2–Si and Si–CH_3 certainly did not appear in PAM (B), but such signals appeared in P(AM-*b*-TTMAPS) (A) and PAM + TTMAPS (C) with apparent difference. In the former they appeared at the chemical shifts $\delta = 0.006$ and -0.096 ppm,

Table 1
Results of copolymerization and characterization of polymers

Polymer	AM/TTMAPS[a]	AM/TTMAPS[b]	M_w (10^6 g mol^{-1})[c]	R_z (nm)[c]	M_w/M_n[c]
1	10:1	9.6:1			
2	15:1	15.2:1			
3	20:1	19.0:1	1.886	79.9	1.285
4	25:1	24.2:1	2.259	79.4	1.172
5	40:1	41.9:1	2.025	78.7	1.141
6	50:1		2.228	76.4	1.135
7	75:1		1.626	74.9	1.165
8	100:1		1.727	78.5	1.144
9	100:0		1.519	78.0	1.161

Typical reaction conditions: AM: 3.00 g (0.042 mol), SDS: 3.00 g, KPS: 0.3 wt% of monomers, water: 100 ml, 50 °C, 6 h.
[a] Molar ratio of AM to TTMAPS in the feed.
[b] Molar ratio of AM to TTMAPS in polymer calculated from elemental analysis.
[c] From GPC-MALLS analysis.

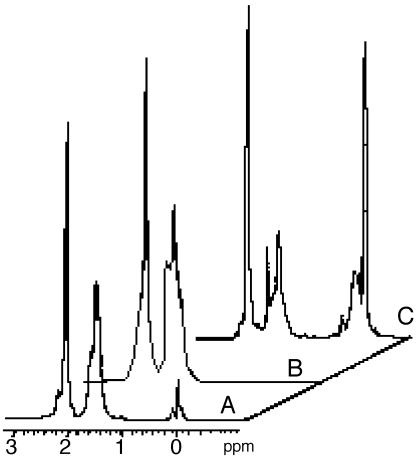

Fig. 1. ^{1}H NMR spectra of P(AM-*b*-TTMAPS) (A), PAM (B), and PAM + TTMAPS (PAM with the addition of a small amount of TTMAPS) (C) in D_2O.

respectively. While in the latter each sharp peak appeared accompanied by a broad one at lager chemical shifts: $\delta =$ 0.303(0.500) and -0.102(0.091) ppm. This indicates that the protons in copolymer do not come from the monomer but the TTMAPS segment attached to the copolymer.

The ^{1}H NMR spectra of polymers (AM/TTMAPS ratios: 25:1, 40:1, 50:1, and 100:0) in D_2O are shown in Fig. 2. The proton signals of the Si–CH_3 ($\delta = -0.105 - 0.05$ ppm) strengthened with the increasing of TTMAPS content in the feed, but still not so strong as that of monomer. This implied the self-aggregations in amphiphilic copolymers. The protons signals of the Si–CH_3 of lower hydrophobe level copolymers were almost not recorded such as in copolymer 50:1, 70:1, and 100:1.

The spectra of P(AM-*b*-TTMAPS) in D_2O and the mixed solvent of D_2O and $CDCl_3$ are shown in Fig. 3. Here a little $CDCl_3$ was added into D_2O as the solubilizer of TTMAPS segment to make copolymers more swelling. The relative

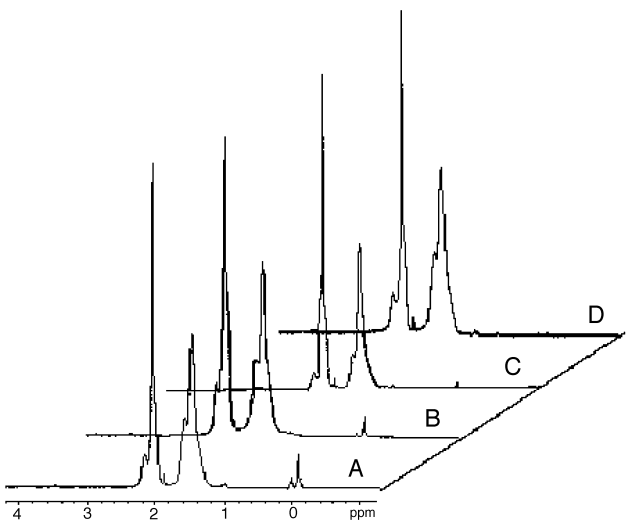

Fig. 2. ^{1}H NMR spectra of polymers: (A) 25:1, (B) 40:1, (C) 50:1, and (D) 100:0 in D_2O.

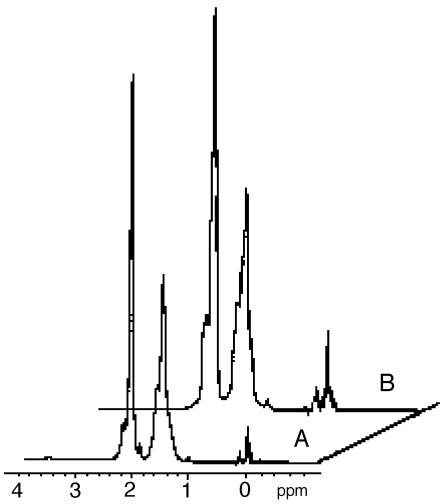

Fig. 3. ^{1}H NMR spectra of P(AM + TTMAPS) (25:1) in D_2O (A) and $D_2O + CDCl_3$ (B).

intensity of the proton signals of the Si–CH_3 ($\delta =$ $-0.105 - 0.05$ ppm) appeared in D_2O solvent [Fig. 3(A)] increased in the mixed solvent [Fig. 3(B)]. This means that the copolymer self-aggregated and form polymer micelles, consisting of a rigid core of TTMAPS segments and a mobile shell of PAM segments [9,21]. And the mobility of the hydrophobe segments was held or ascribed until the swelling of the selective solvent ($CDCl_3$) of hydrphobe (TTMAPS groups).

3.3. Viscosity in dilute solutions. The intrinsic viscosity $[\eta]$ and the Huggins coefficient κ_H of some copolymer samples are reported in Table 2 and that of PAM is given for comparison. The intrinsic viscosity $[\eta]$ of the polymers decreases and their Huggins coefficient κ_H increases with the increasing of TTMAPS content. The lowering in intrinsic viscosity reflects the contraction of the polymer coil because of the intramolecular interactions, and as expected, this effect is stronger upon increasing the hydrophobe level. While the increasing in Huggins coefficient reflects the fluid dynamic interactions between the polymer coils in dilute solution, and this effect is also stronger upon increasing the TTMAPS content.

Table 2
Intrinsic viscosity $[\eta]$ (ml g^{-1}) and the Huggins coefficient κ_H of copolymers and PAM in deionized water

Polymer	AM/TTMAPS[a]	$[\eta]$ (ml g^{-1})	κ_H[b]
1	10:1	216	1.08
2	15:1	281	0.99
4	25:1	320	1.21
6	50:1	337	0.54
8	100:1	338	0.49
9	100:0	345	0.42

[a] Molar ratio of AM to TTMAPS in the feed.
[b] Calculated from Huggins equation: $\eta_{SP}/C = [\eta] + \kappa_H[\eta]^2 C$.

3.4. TEM studies. Fig. 4 gives out the TEM photos of three copolymers and PAM (AM/TTMAPS ratios: 10:1, 20:1, 75:1, and 100:0). Spherical particles were observed in copolymers with the size decreasing upon increasing the hydrophobe levels, ca 100 nm in copolymer 100:1 and 20:1, but ca 40 nm in 10:1. But parent PAM without hydrophobically modified shows the morphology of random coil. So the hydrophobic association and its increment with the hydrophobe were confirmed also by stained electron microscopic observation.

3.5. Fluorometric measurements. The existence of hydrophobic microdomain in aqueous solutions of the copolymers was also tested by fluorescence spectroscopy [22], and as an extrinsic fluorescent probe, 9-chloromethyl-anthracene (5.734×10^{-6} M) was added.

The fluorescent emission spectra of different copolymer solutions at the same polymer concentration and the same probe concentration are reported in Fig. 5. The intensity of fluorescent emission bands increases with increasing hydrophobe level. This indicates the formation of hydrophobic microenvironments and increments of the solubilization of copolymers, and this trend was strengthened with the increasing of the TTMAPS content because of the increasing of hydrophobic interactions.

The intensity (I_f) of the band at 408 nm in the emission spectra was monitored as a function of polymer concentration (Fig. 6). A clear break point was observed in the intensity at the concentration around 0.05 wt% (0.5 g l^{-1}). This point observed should correspond to the critical concentration, where intermolecular aggregation of copolymer occurs. It is reasonable to consider that the copolymer undergoes a certain intramolecular aggregation even below the critical concentration because the binding of the probe accompanied by changes in the intensity is observed below the concentration. While after reaching a peak the intensity increases not very obviously, which is different from the somewhat amphiphilic systems [20,23]. This case may be corresponding to the rigid and compact micelles at higher

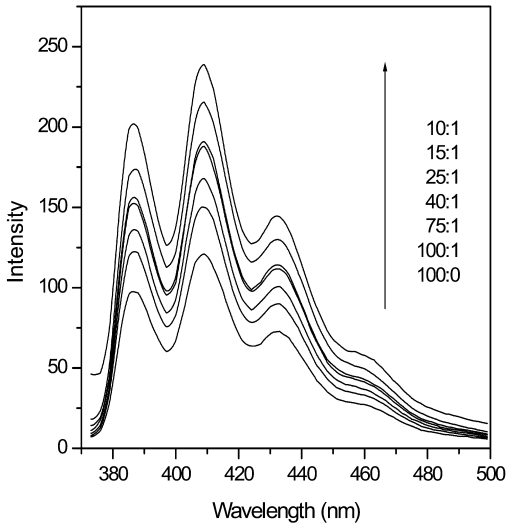

Fig. 5. Fluorescent emission spectra of probe in aqueous solutions of polymers with different molar ratios of AM/TTMAPS. [polymer] = 0.3 wt%, [probe] = 5.734×10^{-6} M.

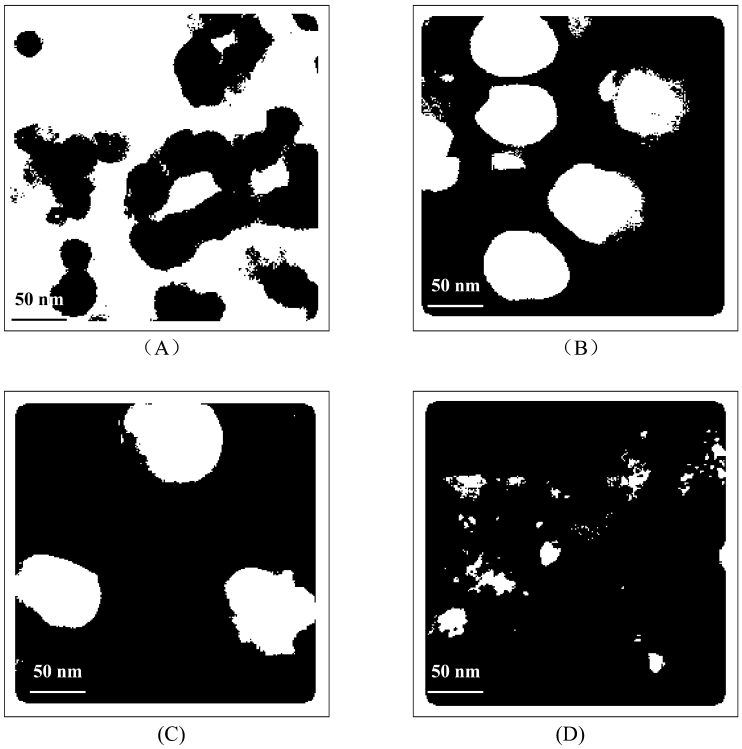

Fig. 4. TEM photos of copolymers with the AM/TTMAPS ratios: (A) 10:1 (B) 20:1 (C) 75:1, and PAM (D).

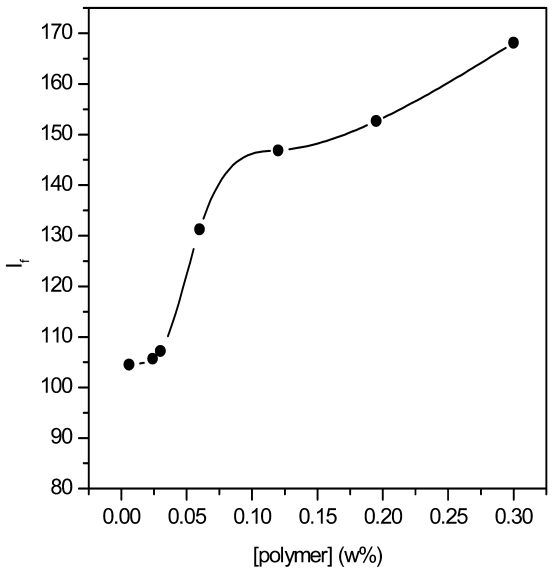

Fig. 6. Fluorescence intensity (I_f) of probe 9-chloromethyl-anthracene as a function of the polymer (75:1 was used in such study) concentration at 20 °C. [probe] = 5.734×10^{-6} M.

hydrophobe content, which cannot be normally incorporated by probe molecules.

In summary, the fluorescence probe experiments give unambiguous evidence for the formation, in aqueous solutions of copolymers, of hydrophobic microdomains that is able to solubilize preferentially the hydrophobic probe.

3.6. DNA separation by CE. Fig. 7 is the electropherogram of DNA sample by using 0.1 wt% P(AM-*b*-TTMAPS) (100:1). It shows that the 10 fragments in ϕX174 marker with YO-PRO-1 fragments have been clearly separated within 27.5 min in naked capillary column. While for the medium of copolymer 20:1 there was no sample peak appearing till a long time of 70 min. This indicates that as a separation medium, higher hydrophobic content copolymer (20:1) showed no separation efficient while lower one (100:1) clearly separated most DNA fragments in ϕX174/Hae III digest. This may be explained that although in the presence of PVP which is a good coating material for capillary walls and can substantially suppress electroosmotic flow (EOF) [24], higher hydrophobic content copolymers adsorbed strongly to the inner wall of the capillary due to the vigorous hydrophobicity of bulky tris(trimethylsiloxy)silyl groups. For reference, PAM was also used as separation medium under identical experimental conditions and results showed worse sensitivity, longer peak appearing time (32.5 min), and higher noise than copolymer 100:1.

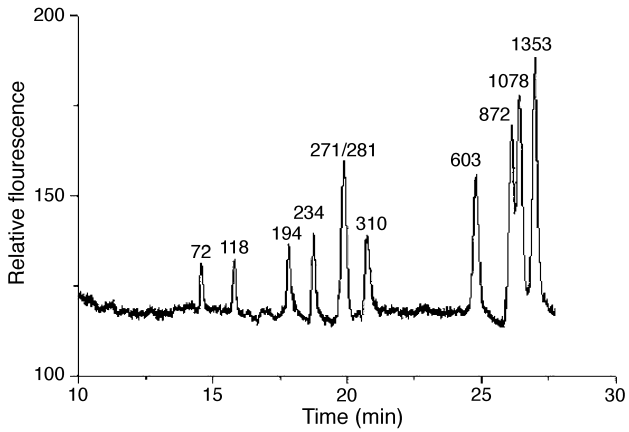

Fig. 7. Electropherogram of DNA sample by using 0.1 wt% P(AM-*b*-TTMAPS) (100:1). Conditions: 100 mM Tris-100 mM boric acid-2 mM EDTA, pH 8.3; 0.5% PVP; 2 μM YO-PRO-1; the total concentration of DNA fragments: 0.15 μg ml^{-1}; Voltage: − 15 kV; the effective length of column: 50 cm; the total length of column: 65 cm; electrokinetic injection time: 5 s at − 10 kV.

4. Conclusion

A series novel silicone-containing amphiphilic PAM were prepared and many means were used to testify the formation of self-assemblized microenvironments of copolymers in aqueous solution. The results of TEM, ^{1}H NMR spectra, capillary viscometer, and fluorescent emission spectra etc showed that the resulting copolymers are amphiphilic in nature and self-assemble to form structurally rigid micelles in water, and this trend is strengthened with the increasing of hydrophobic comonomer tris(trimethylsiloxy)methacryloxypropylsilane (TTMAPS) in the feed. The critical concentration of the self-aggregate formation of copolymer (molar ratio of acrylamide to hydrophobic silicone monomer being 75:1) determined fluorometrically was 0.5 g l^{-1}. Two amphiphilic copolymers were used as separation media for DNA separation in CE; results indicate that the copolymer solution of lower hydrophobic monomer content showed good sieving ability toward DNA and markedly separated most DNA fragments in ϕX174/Hae III digest. And such separation medium runs at a very low polymer concentration of 0.1 wt%.

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