

Preparation of star copolymers with three arms of poly(ethylene oxide-*co*-glycidol)-*graft*-polystyrene and investigation of their aggregation in water

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Abstract

The star graft copolymers with three arms composed of poly(ethylene oxide) (PEO) as main chain and polystyrene (PS) as side chains were prepared by sequential anionic ring-opening copolymerization of ethylene oxide and ethoxyethyl glycidyl ether (EEGE), and then atom transfer radical polymerization (ATRP) of styrene. The anionic ring-opening copolymerization of EO and EEGE was carried out using 2-ethyl-2-hydroxy-methyl-1,3-propanediol as trifunctional initiator and diphenylmethyl potassium (DPMK) as deprotonating agent. The resulting three-arm star copolymer [poly(EO-*co*-EEGE)]₃ could be easily hydrolyzed to unmask the pendant hydroxyl groups without affecting the PEO chains. The switch from the first to the second mechanism was completed by the reaction of the multi-pendant hydroxyl groups of three-arm PEO chain with 2-bromoisobutyryl bromide. The obtained poly(ethylene oxide-*co*-2-bromoisobutyryloxyglycidyl ether), [poly(EO-*co*-BiBGE)]₃, was used as macroinitiators to initiate the polymerization of styrene in bulk at 90 °C by ATRP. The final products and intermediates were characterized by NMR, SEC and IR in detail. The amphiphilic star graft copolymers synthesized can form micelles in water. The critical micelle concentration (cmc) determined by fluorescence spectra was about 5×10^{-7} g/mL. Sphere micelles were observed by transmission electron microscopy (TEM) at low copolymer concentration (6×10^{-5} g/mL), but the micelle shape became irregular when the copolymer concentration increased to 6×10^{-4} g/mL.

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1. Introduction

The self-assembly of amphiphilic copolymers has been the subject of research for several decades [1–3]. It is well known that block or graft copolymers can form the micelles in select solvents [4,5], and it showed the potential applications as catalysis, nanoreactors [6] and in drug delivery system [7,8], etc. Although the studies of association behaviors of block copolymers showed that the critical micelle concentration, micelle size as well as micelle shape are influenced by the pH value and ionic strength of the solution, preparation condition, copolymer concentration and the molecular weight and

composition of the copolymer [9–11], the researches concerning the micellization of amphiphilic graft copolymers in water have been seldom carried out [12,13]. Star graft copolymers have more complicated and confined structure compared with linear graft copolymers, so it is interesting to explore their association behaviors in water.

However, the investigation of the self-assembly behaviors of star graft copolymers has been limited because of the difficulty in the synthesis of well-defined amphiphilic star graft copolymers with controlled molecular weights and low polydispersities [14]. Recently, star graft copolymers with three and four arms comprising poly(2-hydroxyethyl methacrylate) as main chain and poly(*n*-butyl acrylate) as side chains were synthesized by ATRP [15]. The similar architectures with both backbone and graft chains composed of polybutadiene were prepared through macromonomer technique and anionic

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polymerization [16]. The first synthesis of well-defined star copolymers with arms composed of hydrophilic poly(acrylic acid) (PAA) backbone and hydrophobic poly(methyl methacrylate) (PMMA) side chains by ATRP has also been reported [14] lately. However, the synthesis of star graft copolymer with arms consisting of hydrophilic PEO backbone has never been reported in the literature. PEO segments are not only hydrophilic, but also nonionic and crystalline, and can form the complex with monovalent metallic cations [17]. Branched PEO has potential application in biomedical and pharmaceutical areas and a lot of work have been done, such as the synthesis and characterization of star-like and dendrimer-like PEOs [18], or star-like [19,20] and comb-like [21] block copolymers containing poly(ethylene oxide) segments. Polyglycidol is a functional analogue of PEO and the polymerization of glycidol has been the subject of several studies [22–26]. Using linear polyglycidol modified by 2-bromoisobutryl bromide as initiator, an efficient and universal method was suggested by us to synthesize the well-defined amphiphilic linear graft copolymers of PEO as backbone [27,28].

In this paper, the preparation of star-like amphiphilic graft copolymers with hydrophilic PEO as backbone and hydrophobic PS as side chains is described. The molecular weights of the backbone and the side chains of star-like copolymers were both controllable, and the polydispersity was very low. The cmc of the final copolymer was measured, and the sizes and morphologies of the obtained micelles at different copolymer concentrations were studied with dynamic light scattering (DLS) and transmission electron microscopy (TEM), respectively.

2. Experimental

2.1. Materials

2-Ethyl-2-hydroxymethyl-1,3-propanediol (Aldrich, 99%) was dried under vacuum at 50 °C. EO (98%, Sinopharm Chemical Reagent Co., Ltd. (SCR)) was dried over calcium hydride for 48 h and then distilled under N₂ before use. Tetrahydrofuran (THF, SCR, 99%) was refluxed over sodium wire and distilled. Dimethyl sulfoxide (DMSO, SCR, 98%) was distilled over CaH₂ under reduced pressure just before use. Pyridine (SCR, 99.5%) was refluxed over sodium wire and distilled. Styrene (St, SCR, >99.5%) was washed with 10% NaOH aqueous solution and water successively, then dried over anhydrous MgSO₄, further dried over CaH₂ and distilled under reduced pressure. CuBr (98%, Acros) was purified by stirring overnight in acetic acid and filtered, then washed with ethanol and diethyl ether successively, and finally dried under vacuum. 2-Bromo-isobutryl bromide (98%), 2,2'-bipyridine (bpy, >99%), and pyrene were purchased from Aldrich and used as received. Ethoxyethyl glycidyl ether (EEGE) was prepared as described elsewhere [27] and the product (b.p. 152–154 °C) was a colorless liquid. Element Anal. Calcd for C₇H₁₄O₃: C, 57.58%; H, 9.66%. Found: C, 57.77%; H, 9.55%. GC (99.6%), MS (70 eV) *m/z* (%): 131 (33) [M – CH₃]⁺, 101 (27) [M – C₂H₅O]⁺, 73 (88) [M – C₄H₉O]⁺. ¹H NMR (CDCl₃, δ ppm): 4.76

[–O–CH(CH₃)–O–], 3.35–3.90 (–O–CH₂CH₃ and –O–CH₂–C₂H₅O), 3.15 (methine CH of oxirane ring), 2.61–2.91 (methylene CH₂ of oxirane ring), 1.33 [–OCH(CH₃)–O–], 1.19 (–O–CH₃). FTIR (film, ν) 1350, 1254 cm^{–1}. Diphenylmethyl potassium (DPMK) was synthesized according to procedure described in literature [27] and titrated with 0.1 M HCl and its concentration was 0.57 M. Other reagents (Sinopharm Chemical Reagent) were purified by common procedures. The linear graft copolymer used for comparison was PEO₂₂₆-*g*-26PS₂₂ with *M_w/M_n* = 1.14 (the subscripts 226 and 22 are the degrees of polymerization of the backbone and the side chains, respectively while 26 is the number of the grafted PS side chains). The detailed description of synthesis and characterization can be found elsewhere [27].

2.2. Measurements

Gas chromatographic–mass spectrometric (GC/MS) analysis of monomer EEGE was carried out using a Finnigan Voyager system with mass selective detection operating in electronic ionization (EI). A silica capillary column was 30 m × 0.25 mm (i.d.) with 0.25 μm film thickness (DB-5 Restek). The GC/MS parameters were as follows – ion source temperature: 200 °C; carrier gas: helium; column flow: 1 mL/min; temperature program: from 100 to 200 °C at 15 °C/min; splitless injection at 250 °C; and the ionization was achieved at 70 eV. ¹H NMR spectra were obtained on a DMX 500 MHz spectrometer with tetramethylsilane (TMS) as the internal standard and CDCl₃ as the solvent. Size exclusion chromatography (SEC) was performed on an Agilent 1100 equipped with a G1310A pump, a G1362A refractive index detector, and a G1314A variable wavelength detector using THF as eluent at 35 °C with an elution rate of 1.0 mL/min. One 5 μm LP gel column (500 Å, molecular weight range 500–2 × 10⁴ g/mol) and two 5 μm LP gel mixed bed column (molecular weight range 200–3 × 10⁶ g/mol) were calibrated by polystyrene standard samples. For [poly(EO-*co*-Gly)]₃, SEC was performed in 0.1 M NaNO₃ at 40 °C with an elution rate of 0.5 mL/min on the same instrument except a G1315A diode-array detector was used to substitute the G1314A variable wavelength detector. Three TSK-gel PW columns in series (bead size: 6, 13, 13 μm; pore size: 200 Å, greater than 1000 Å, less than 100–1000 Å; molecular weight range: 0–5 × 10⁴, 5 × 10⁴–8 × 10⁶, 5 × 10⁶–8 × 10⁶ g/mol, respectively) were calibrated by PEO standard samples. The injection volume was 20 μL and the concentration was 5 mg/mL. IR spectra were obtained on a Magna-550 Fourier transform infrared spectrometer. The Ultra Filtration Separator was purchased from Shanghai Institute of Nuclear Research Chinese Academy of Science and the cut-off molecular weight of used poly(ether sulfone) membrane was 10,000 g/mol (calibrated by globular protein). Steady-state fluorescence spectra of the samples were recorded on an Edinburgh Instruments 920 spectrometer operating at 25 °C. A commercial laser light scattering (LLS) spectrometer (Malvern Autosizer 4700) was used for dynamic light scattering measurement. TEM was performed on a Hitachi H-600 electron microscope operated at an accelerating voltage of 75 kV.

2.3. Synthesis of three-arm star copolymer [poly(EO-co-EEGE)]₃

A 150 mL stainless steel kettle was evacuated at 80 °C for 24 h, and then cooled to –20 °C. Anhydrous 2-ethyl-2-hydroxymethyl-1,3-propanediol (0.295 g, 2.2 mmol) was first dissolved in 50 mL mixed solvent of DMSO and THF (v/v: 3/2) and a solution of DPMK in THF (2.64 mL, 0.57 M solution) was introduced. The orange-red color of DPMK changed to yellow as the alkoxides were formed. The homogeneous solution obtained was introduced into the cooled kettle and EEGE (6.35 g, 43.5 mmol) and EO (20 g, 454.5 mmol) were added. After the solution was stirred at 60 °C for 48 h, the polymerization was terminated by adding of a few drops of acidified methanol. Then all the solvents were removed under reduced pressure. The crude product was dissolved in CH₂Cl₂, filtered, dried over anhydrous MgSO₄ overnight and then filtered again to remove MgSO₄. The yellowish viscous product [poly(EO-co-EEGE)]₃ was obtained in the yield of 89% after CH₂Cl₂ was removed.

2.4. Preparation of three-arm poly(ethylene oxide-co-glycidol), [poly(EO-co-Gly)]₃, by cleavage of the ethoxyethyl groups of [poly(EO-co-EEGE)]₃

[Poly(EO-co-EEGE)]₃ ($M_{n(\text{SEC})} = 9500$, 6 g) was first mixed with 30 mL of formic acid. The obtained solution was stirred at room temperature for 30 min and then formic acid was evaporated under reduced pressure at 50 °C. The crude product was dissolved in a mixture of dioxane (27 mL) and methanol (16 mL), hydrolyzed with KOH aqueous solution (1 mol/L, 5 mL) under refluxing for 24 h, and then neutralized with 5% HCl. After the solvents were removed under reduced pressure, the polymer was dissolved in water and purified by ultra filtration membrane. Then the filtered aqueous solution was concentrated to dryness. The resulting solid dissolved in CH₂Cl₂ was dried over anhydrous MgSO₄ overnight and then filtered again to remove MgSO₄. The transparent viscous product [poly(EO-co-Gly)]₃ was obtained in yield of 91% after removal of CH₂Cl₂ and dried in vacuo at 50 °C.

2.5. Preparation of macroinitiator [poly(EO-co-BiBGE)]₃

A 4 g sample of [poly(EO-co-Gly)]₃ (11.1 mmol hydroxyl groups) was dissolved in 100 mL anhydrous degassed pyridine and 3.4 mL (27.75 mmol) of 2-bromoisobutryl bromide was added dropwise at 0 °C in 60 min under vigorous stirring. The mixture was stirred for 3 h at 0 °C followed by stirring at room temperature for 24 h. After the insoluble salt was removed by filtration, the pyridine was removed by azeotropic distillation with dry toluene. The residue was dissolved in CH₂Cl₂, washed with KOH aqueous solution (1 mol/L), hydrochloric acid aqueous solution (1 mol/L) and distilled water, sequentially. After CH₂Cl₂ was removed by distillation under vacuum, the polymer dissolved in ethanol was purified using an ultra filtration membrane to remove low molecular weight

impurities. Ethanol was removed by distillation under reduced pressure and the product was dried in vacuo at 50 °C. The yellowish product with a yield of 83% was obtained.

2.6. Synthesis of three-arm star graft copolymers by ATRP

In a typical process, an ampoule charged with the initiator [poly(EO-co-BiBGE)]₃ (0.2573 g, 0.4210 mmol of bromoisobutryl groups), CuBr (60.4 mg, 0.4210 mmol), bpy (65.7 mg, 0.4210 mmol) and St (12 mL, 0.1053 mol) was degassed by three freeze–pump–thaw cycles, sealed, and immersed in an oil bath at 90 °C. After a certain time, the ampoule was dipped into liquid nitrogen to stop the polymerization. The solution was diluted with THF and passed through a neutral alumina column to remove the catalyst and then precipitated into cold MeOH. After filtration, the product was purified by dissolution/precipitation with THF/cold MeOH twice and dried at 40 °C in vacuo for 2 days.

2.7. Determination of critical micelle concentration

Pyrene was used as fluorescence probe [29]. An acetone solution of pyrene 9.5 μL (5.4×10^{-2} mg/mL, 2.67×10^{-4} mol/L) was added to 5 mL water and the concentration of pyrene was 5.0×10^{-7} mol/L. Next, different amounts of copolymer solutions in *N,N*-dimethylformamide (DMF) (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} g/mL) were added to water containing pyrene to obtain the solution with the copolymer concentration as 10^{-4} – 10^{-9} g/mL. All fluorescence spectra were recorded at 25 °C.

2.8. DLS and TEM studies

The samples used for fluorescence measurement were then used for DLS and TEM studies.

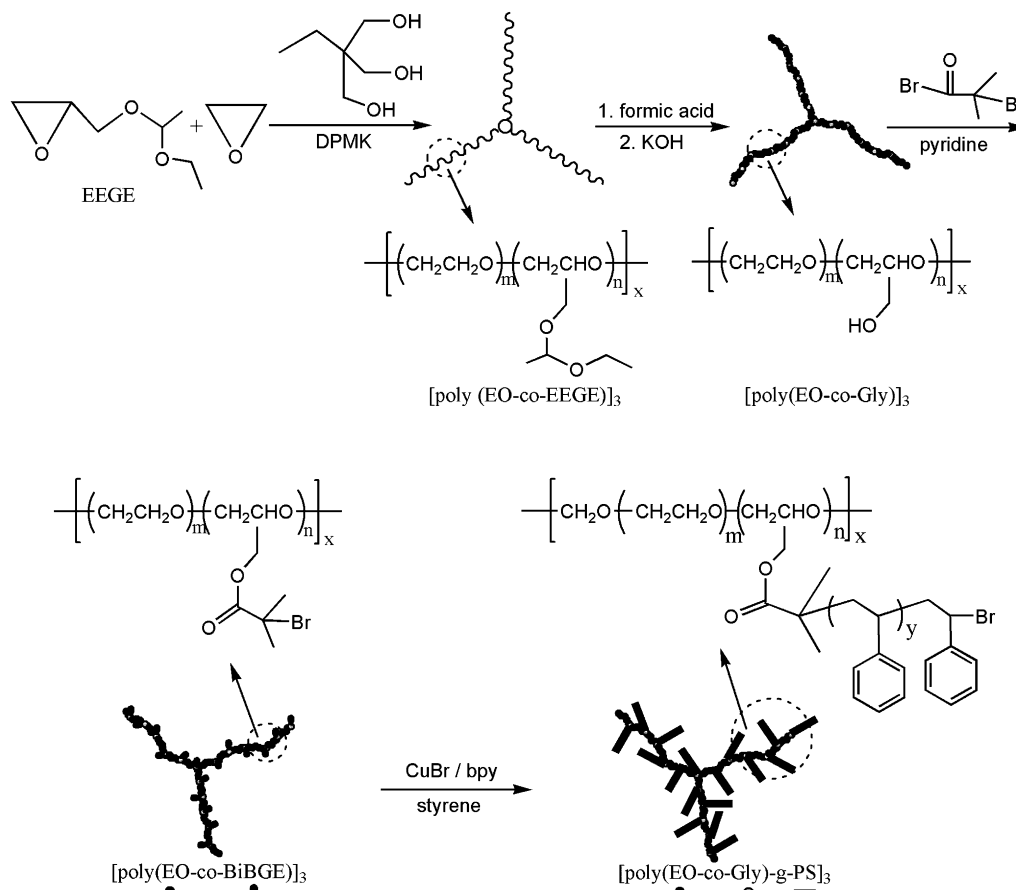
For DLS studies, the samples were measured after they were filtered through a membrane filter with a nominal pore size of 0.8 μm. For TEM studies, a drop of micelle solution was deposited on an electron microscopy copper grid coated with carbon film and the water evaporated at room temperature overnight before TEM studies.

3. Results and discussion

3.1. Synthesis of star macroinitiator with three arms of [poly(EO-co-BiBGE)]₃

The anionic ring-opening copolymerization of EO and EEGE was performed using 2-ethyl-2-hydroxymethyl-1,3-propanediol and DPMK as co-initiator shown in Scheme 1.

A mixed solvent of DMSO and THF (v/v: 3/2) was used for polymerization to prevent the aggregation of propagating alkoxides which is unavoidable in pure THF [18]. For a controlled polymerization, the amount of DPMK introduced was adjusted so as to deprotonate only 20% of the hydroxyl groups of the



Scheme 1. Schematic synthesis route of star graft copolymers.

2-ethyl-2-hydroxymethyl-1,3-propanediol [19]. Because of the rapid exchange of protons between dormant hydroxyls and propagating alkoxides, all the three hydroxyl groups of 2-ethyl-2-hydroxymethyl-1,3-propanediol could efficiently initiate the copolymerization of EO and EEGE, and all the three arms grew at the same rate [18,20]. The [poly(EO-co-EEGE)]₃ with very narrow molecular weight distribution was obtained as shown in Fig. 1.

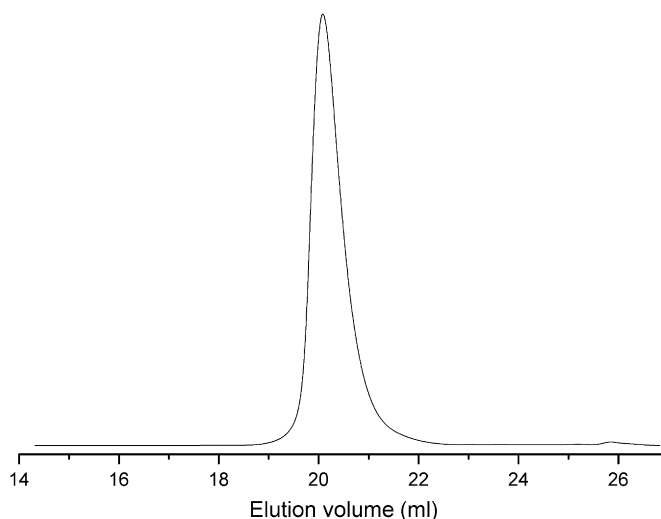
The ¹H NMR spectrum of [poly(EO-co-EEGE)]₃ is shown in Fig. 2(A). The quartet at δ = 4.68–4.72 ppm is the methine proton of the ethoxyethyl groups in EEGE units. The protons of the two different methyl groups in the EEGE units appeared at δ = 1.29, 1.30 ppm as a doublet and at δ = 1.18–1.21 ppm as a triplet, respectively. The signal of the protons in main chain and methylene protons in lateral chains is in the range of δ = 3.46–3.75 ppm. The methylene protons linked to the core were detected at 3.41 ppm and the methyl protons of the core are clearly detected at 0.82 ppm. The ratio (*R*_T) of EEGE to EO in the copolymer can be calculated from the following Eq. (1) on the basis of Fig. 2(A):

$$R_T = \frac{A_f}{\frac{A_{\text{sum}} - 7A_f}{4}} \quad (1)$$

where *R*_T is the molar ratio of EEGE to EO in the copolymer. *A*_f and *A*_{sum} are the peak area of methine protons (f) of the

ethoxyethyl groups in EEGE units, and the sum of the protons in main chain and methylene protons in lateral chains (*A*_T, d, and g), respectively. The number average molecular weight (*M*_n) of the polymer was determined by end group analysis using the following Eq. (2)

$$M_n = \frac{3A_f}{A_a}(146 + 44/R_T) + 134 \quad (2)$$

Fig. 1. SEC traces of three-arm star copolymer [poly(EO-co-EEGE)]₃.

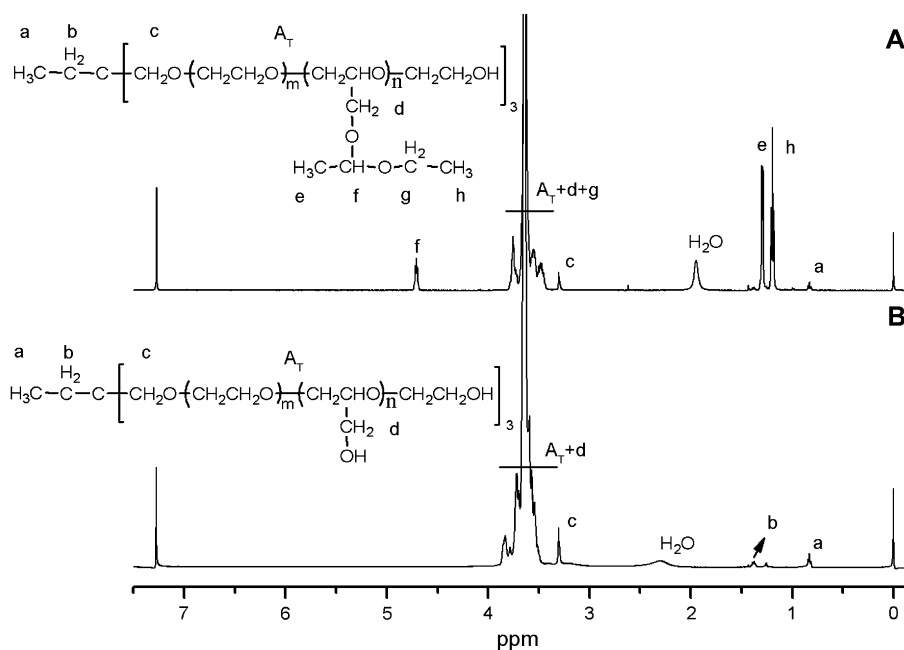


Fig. 2. ^1H NMR spectra of three-arm star copolymer and its hydrolyzed product in CDCl_3 : (A) $[\text{poly}(\text{EO-co-EEGE})]_3$ (entry 1 in Table 1); (B) $[\text{poly}(\text{EO-co-Gly})]_3$ (entry 2 in Table 1).

where A_a represents the peak areas of methyl protons (H_a) for the 2-ethyl-2-hydroxymethyl-1,3-propanediol core. A_f and R_T have the same meaning as in Eq. (1). The mole mass of 2-ethyl-2-hydroxymethyl-1,3-propanediol is 134, and that of the monomer units EEGE and EO are 146 and 44, respectively.

Table 1 lists the copolymerization data of EO and EEGE. As shown in Table 1, the composition of the copolymer ($\text{EO}/\text{EEGE} = 9.2$) is very close to the feed ratio of monomers ($\text{EO}/\text{EEGE} = 10$), indicating that the two monomers have the similar reactivity [25]. Thus, the content of EEGE units in the main chain can be controlled by changing the feed ratio of EO and EEGE.

In order to obtain the reactive hydroxyl groups on the $[\text{poly}(\text{EO-co-EEGE})]_3$, the polymers were treated with formic acid first, then the formed formate was saponified in a KOH

dioxane/methanol solution [25] and $[\text{poly}(\text{EO-co-Gly})]_3$ with multi-pendant hydroxyl groups was produced.

The complete removal of the ethoxyethyl groups was confirmed by ^1H NMR analysis. The peaks at $\delta = 4.68\text{--}4.72$ ppm $[-\text{O}-\text{CH}(\text{CH}_3)-\text{O}-]$ (f), at $\delta = 1.30, 1.29$ ppm $[-\text{OCH}(\text{CH}_3)-\text{O}-]$ (e) and at $\delta = 1.18\text{--}1.21$ ppm $(-\text{O}-\text{CH}_2\text{CH}_3)$ (h) assigned to the ethoxyethyl group of $[\text{poly}(\text{EO-co-EEGE})]_3$ in Fig. 2(A) disappeared completely after hydrolysis (Fig. 2(B)). The small peak at $\delta = 1.25$ ppm in Fig. 2(B) and following Fig. 5(A) may be attributed to hydrocarbon impurities introduced during the measurement.

Fig. 3 shows the SEC curve of $[\text{poly}(\text{EO-co-Gly})]_3$, in which water was used as eluent. The unimodal distribution indicated that there was no chain degradation during hydrolysis. The M_n value of $[\text{poly}(\text{EO-co-Gly})]_3$ determined by SEC (6500 g/mol), however, was lower than that of $[\text{poly}(\text{EO-co-EEGE})]_3$ measured by SEC (9500 g/mol), even the mass variation due to the removal of the ethoxyethyl groups was considered (Table 1). The $[\text{poly}(\text{EO-co-EEGE})]_3$ is soluble in THF and insoluble in water, so its molecular weight was derived using THF as eluent and PS as standard. However, $[\text{poly}(\text{EO-co-Gly})]_3$ is soluble in water and insoluble in THF, so the molecular weight was derived in water phase using PEO as standard. Thus the sharp difference in the molecular weight between them may be attributed to the different SEC measurement conditions.

The hydroxyl groups of $[\text{poly}(\text{EO-co-Gly})]_3$ were then esterified with 2-bromoisobutryl bromide, and the macroinitiator $[\text{poly}(\text{EO-co-BiBGE})]_3$ with narrow molecular weight distribution was obtained. In addition, the molecular weight of the macroinitiator derived by SEC using THF as eluent was comparable to that of $[\text{poly}(\text{EO-co-EEGE})]_3$ (see Table 1),

Table 1
Data of three-arm star macroinitiator and its precursor

Sample	R_f^a	R_T^b	M_n^c	M_w/M_n^c	M_n^d	N_{EEGE}^e
$[\text{Poly}(\text{EO-co-EEGE})]_3$	1/10	1/9.2	9500	1.10	8396	15
$[\text{Poly}(\text{EO-co-Gly})]_3$			6500	1.14	7316	
$[\text{Poly}(\text{EO-co-BiBGE})]_3$			10,100	1.11	9866	

^a The feed ratio of EEGE to EO in the copolymerization.

^b The molar ratio of EEGE to EO in the copolymer $[\text{poly}(\text{EO-co-EEGE})]_3$ measured by ^1H NMR.

^c Number average molecular weight (M_n) and molecular weight distribution (M_w/M_n) determined by SEC, calibrated against PS standard using THF as elution except in the case of $[\text{poly}(\text{EO-co-Gly})]_3$ the elution is 0.1 M NaNO_3 and the standard for calibration is PEO.

^d Determined by ^1H NMR.

^e Number of the EEGE units in $[\text{poly}(\text{EO-co-EEGE})]_3$, calculated by the formula $N_{\text{EEGE}} = 3A_f/A_a$. A_f , A_a is the integration area of protons (H_f , H_a) in Fig. 2(A), respectively.

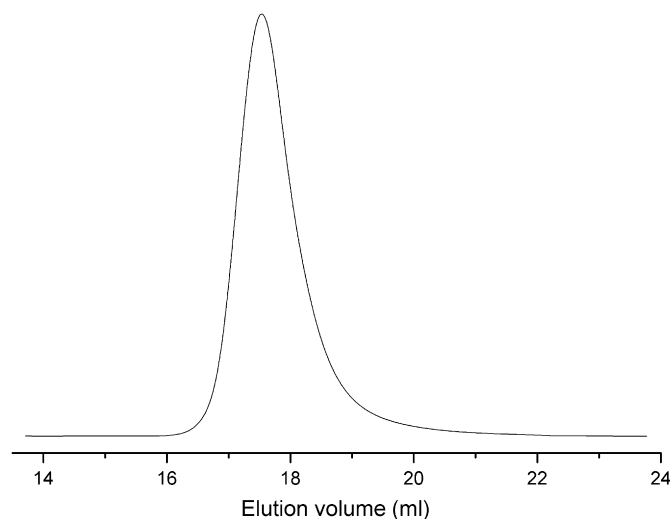


Fig. 3. SEC traces of the hydrolyzed product [poly(EO-co-Gly)]₃.

indicating no chain degradation during the hydrolysis and esterification procedure.

The degree of esterification of the hydroxyl groups of [poly(EO-co-Gly)]₃ could be calculated from ¹H NMR (Fig. 5(A)) by the following Eq. (3):

$$E_C = \frac{\frac{A_A}{2}}{\frac{A_a}{3} \times (N_{EEGE} + 3)} \times 100\% \quad (3)$$

where E_C is the conversion efficiency of hydroxyl groups of [poly(EO-co-Gly)]₃. A_a and A_A represent the integral area of the methyl protons (H_a) of the core (the peaks at $\delta = 0.82$ ppm) and the integral area sum of the methylene protons (H_h , H_j) linked to ester group (the peaks at $\delta = 4.18$ – 4.35 ppm), respectively. N_{EEGE} is the number of EEGE units in [poly(EO-co-EEGE)]₃.

The calculated E_C value was nearly 100%, which suggested that hydroxyl groups were converted into bromoisobutryl groups completely. The FTIR spectra before and after esterification of [poly(EO-co-Gly)]₃ provided another evidence for complete esterification, the peaks at 3473 cm^{-1} attributed to hydroxymethyl of glycidol units of [poly(EO-co-Gly)]₃ disappeared after esterification, and new peaks at 1736 cm^{-1} attributed to ester-band of [poly(EO-co-BiBGE)]₃ appeared.

3.2. Synthesis of three-arm star graft copolymers

Three-arm star graft copolymers with PS as side chains and PEO as backbone were synthesized by ATRP of St using [poly(EO-co-BiBGE)]₃ as macroinitiator as shown in Scheme 1. The results are compiled in Table 2. The M_n data measured by SEC was lower than the value calculated from ¹H NMR, and it may be attributed to the sharp difference of hydrodynamic volume between the star graft copolymers and linear polystyrene standard used in the SEC measurement.

The ATRP of styrene using bpy as ligand was terminated at low monomer conversion to avoid the coupling of propagating PS radicals; prolonged polymerization time may result in

Table 2
Data of the three-arm star graft copolymers [poly(EO-co-Gly)-g-PS]₃

Sample ^a	Time (h)	Conversion ^b (%)	M_n^c	M_w/M_n^c	M_n^d	DP _{PS} ^e
A	1.5	5.9	27,400	1.17	36,100	13.4
B	2.5	9.1	33,900	1.15	42,824	17

^a The molar ratio of CuBr, bpy and monomer to initiating bromine groups in ATRP was 1:1:250:1.

^b Monomer conversion determined by gravimetric method.

^c Number average molecular weight (M_n) and molecular weight distribution (M_w/M_n) determined by SEC, calibrated against PS standards using THF as elution.

^d Number average molecular weight of [poly(EO-co-Gly)-g-PS]₃, obtained from formula $M_n = M_{n,\text{NMR}([\text{poly}(\text{EO-co-BiBGE})]_3)} + (N_{\text{EEGE}} + 3) \times \text{DP}_{\text{PS}} \times 104.16$.

^e Degree of polymerization of St on each PS side chain (DP_{PS}) of [poly(EO-co-Gly)-g-PS]₃, calculated from the ¹H NMR data in Eq. (5) in text.

broader molecular weight distributions due to irreversible couplings of the growing radicals between different graft copolymer chains. The obtained three-arm star graft copolymers [poly(EO-co-Gly)-g-PS]₃ show the monomodal SEC curves (Fig. 4), which means in our system radical coupling reactions and homopolymerization of styrene did not occur. The narrow molecular weight distributions in both cases ($M_w/M_n < 1.2$) demonstrated that the star graft copolymers had uniform molecular weights.

Fig. 5 shows the ¹H NMR spectrum of the resultant star graft copolymer A (see Table 2) and its precursor. The resonances at $\delta = 6.33$ – 7.31 ppm are due to the aromatic protons of polystyrene chains, and the resonances at $\delta = 3.41$ – 3.75 ppm are due to the protons of the PEO main chain. The resonances near TMS at 0.07 ppm are due to silicone grease that was used to airproof ampoule in the ATRP. After ATRP of styrene initiated by [poly(EO-co-BiBGE)]₃, the peaks at $\delta = 4.18$ – 4.35 ppm assigned to the methylene protons (H_h , H_j) linked to ester group and the peak at $\delta = 1.90$ – 1.93 ppm

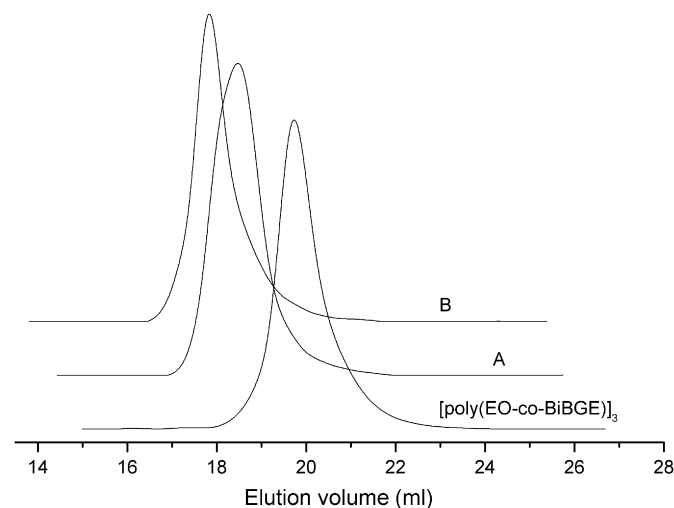


Fig. 4. SEC traces of three-arm star graft copolymers (samples A, B were prepared as shown in Table 2) and its precursor [poly(EO-co-BiBGE)]₃ ($M_n = 10,100$).

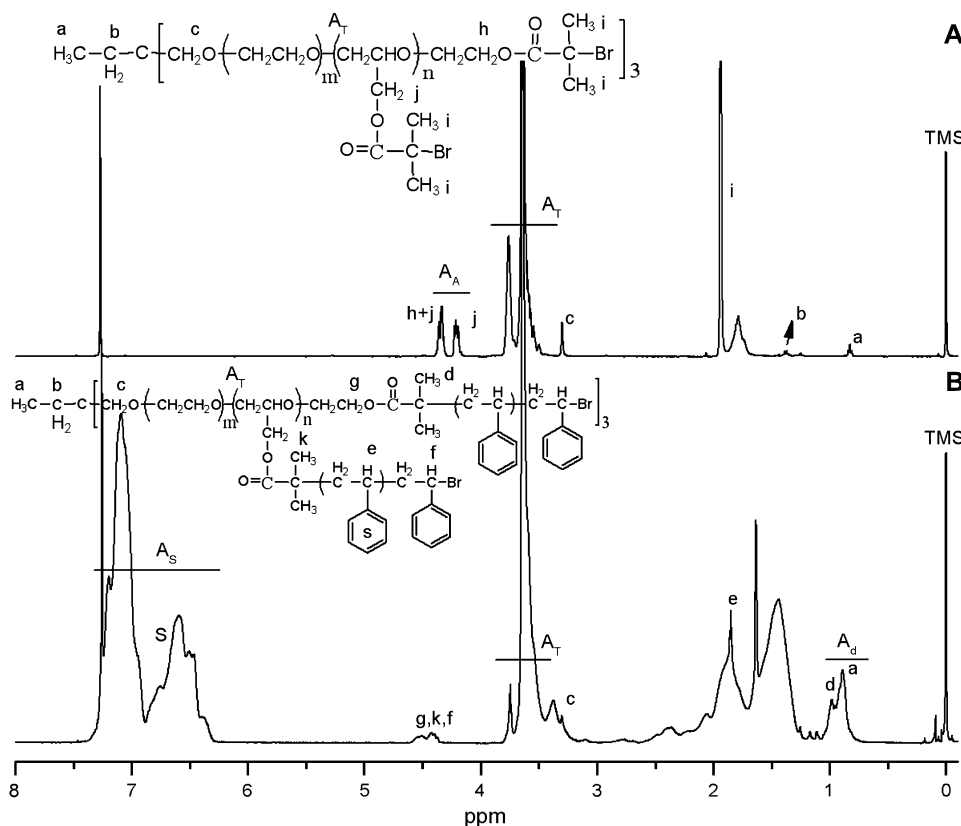


Fig. 5. ^1H NMR spectra of three-arm star graft copolymer (sample A in Table 2) and its precursor in CDCl_3 : (A) $[\text{poly}(\text{EO-co-BiBGE})]_3$; (B) $[\text{poly}(\text{EO-co-Gly})\text{-g-PS}]_3$.

for the methyl protons at 2-bromoisobutyryl groups (H_i) in Fig. 5(A) disappeared while new peaks at 4.28–4.65 ppm representing end group protons ($-\text{CH}(\text{Ph})\text{Br}$, H_f) and peak at 0.89–0.97 ppm representing methyl protons (H_d) at the α -end of PS chains appeared as shown in Fig. 5(B), indicating that the graft copolymer with PS side chains has been synthesized successfully.

The initiation efficiency of macroinitiator can be obtained by Eq. (4):

$$E_R = \frac{A_d - 3Q}{6Q \times (N_{\text{EEGE}} + 3)} \times 100\% \quad (4)$$

where E_R is the reaction efficiency of bromoisobutyryl for ATRP, $Q = A_T / [(4/R_T) + 3] \times N_{\text{EEGE}}$, A_d represents the integral area sum of the methyl groups at the α -end of PS chains and the methyl groups due to the core. A_T is the integral area of all protons of the PEO main chain, and N_{EEGE} is the number of EEGE units in $[\text{poly}(\text{EO-co-EEGE})]_3$ (see Table 1). R_T is the molar ratio of EEGE to EO in copolymer $[\text{poly}(\text{EO-co-EEGE})]_3$ measured by ^1H NMR. The calculated E_R values were nearly 100%, which means nearly all the bromoisobutyryl groups took part in the radical polymerization. Thus the average number of St in each PS side chain can be obtained by Eq. (5)

$$\text{DP}_{\text{PS}} = \frac{A_S}{5Q \times (N_{\text{EEGE}} + 3)} \quad (5)$$

where DP_{PS} is the average number of St on each PS side chain (see Table 2), A_S represents the integral area of phenyl protons on the grafted PS chains, Q and N_{EEGE} have the same meaning as in Eq. (4). Thus the molecular weight of PS side chains could be derived from DP_{PS} and mass of St.

3.3. Measurement of critical micelle concentration

The critical micelle concentration of star graft copolymers $[\text{poly}(\text{EO-co-Gly})\text{-g-PS}]_3$ (sample B in Table 2) and linear graft copolymer $\text{PEO}_{226}\text{-g-26PS}_{22}$ in water was examined by fluorescence technique using pyrene as probe. This method is based on the sensitivity of the probe to the hydrophobicity and polarity of its micro-environment [29,30]. In the presence of micelles, pyrene is solubilized within the interior of the hydrophobic part. As a result, the values of I_1/I_3 in the emission spectrum changed sharply. We plotted fluorescence peak intensity ratios (I_1/I_3) against the logarithm of copolymer concentrations to determine cmc as the onset of micellization (Fig. 6).

Both curves in Fig. 6 show a characteristic shape. At low copolymer concentrations, the I_1/I_3 ratio is approximately 1.7, a little below its typical value in water (1.8). When the copolymer concentration is increased above the value of cmc, the pronounced decrease in the ratio reflects the formation of hydrophobic microdomains in which pyrene is preferentially located.

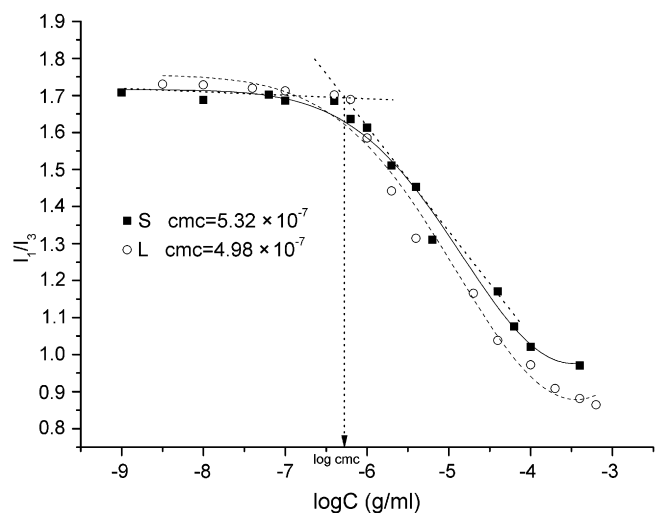


Fig. 6. Dependence of fluorescence intensity ratios of pyrene emission bands on the concentration of star graft copolymer S (sample B in Table 2) and linear graft copolymer L (PEO₂₂₆-g-26PS₂₂).

As obtained from Fig. 6, the values of cmc of the star and the linear graft copolymer are about 5.32 and 4.98×10^{-7} g/mL, respectively, which are very low compared with traditional surfactant or block copolymers [31]. These low cmc values are related to the branched structure of graft copolymers and the high percentage of the hydrophobic side chains. It can also be found that the cmc value of the star graft copolymer is larger than that of the linear graft copolymer. This difference may be mainly caused by the different ratio of hydrophilic PEO chain to the hydrophobic PS chains although the star graft copolymer has smaller molecular weight and different structure compared with the linear graft copolymer. Both the copolymers have about one hydrophobic PS chain in every 10 hydrophilic EO units, but the average length of hydrophobic PS chain of the star graft copolymer (17 St units) is shorter than that of the linear graft copolymer (22 units). And it has been proven that the shorter hydrophobic side chain results in higher cmc in the case of graft copolymer [13] while the star graft structure does not have distinct advantage in reducing cmc value than the linear graft structure [14].

3.4. The effect of copolymer concentration on the micelle sizes and morphologies

Usually, two different methods are used to prepare polymer micelles in water. One is direct solubilization of copolymers in

water and the other is dialysis of copolymer solutions in common solvents against water. The method we used was similar to the latter one. Firstly, the copolymers were dissolved in pure DMF to obtain stock solutions of various copolymer concentrations and then small amounts of the copolymer solutions were directly added to deionized water under vigorous stirring to obtain the polymer micelle solutions with the desired concentrations.

The DLS and TEM studies using the same samples for fluorescence spectra measurement were performed in order to obtain the real sizes and morphologies of the micelles formed in water at different copolymer concentrations. The DLS results are listed in Table 3.

As shown in Table 3, when the concentrations of the star graft copolymer were below 10^{-5} g/mL, the micelle sizes are relatively small compared with the values measured at higher copolymer concentration. When the concentrations of the star graft copolymer are near to 10^{-4} g/mL, the sizes of the micelle formed are about 200 nm and the micelle size distributions are narrow. In the case of the linear graft copolymer, however, the results are similar except the broader distributions of micelle size. Two factors may be attributed to this difference. One is the more confined structure and stronger repulsion among side chains of the star graft copolymers than linear graft copolymers [14] and another is the smaller molecular weight and the shorter hydrophobic side chain length of the star graft copolymers than linear graft copolymers.

TEM is used to observe the morphologies of the micelles formed in water at different copolymer concentrations. As shown in Fig. 7, when the concentration of star graft copolymer was 6×10^{-4} g/mL, the micelles were inhomogeneous in size and shape (Fig. 7(A)), and when the copolymer concentration was decreased to 1×10^{-4} g/mL, the shape of the micelles became more regular (Fig. 7(B)). When the copolymer concentration was decreased further to 6×10^{-5} g/mL, the regular sphere shape became the dominant morphologies (Fig. 7(D)). This phenomenon was also observed in the case of the linear graft copolymer PEO₂₂₆-g-26PS₂₂, but the latter was more difficult to form stable micelles than the star graft copolymer at the same copolymer concentration. When the concentration of PEO₂₂₆-g-26PS₂₂ was 6×10^{-4} g/mL, the micelle aggregated after the stirring was stopped for one day. After the concentration of PEO₂₂₆-g-26PS₂₂ was decreased to 1×10^{-4} g/mL, regular micelles could be observed as shown in Fig. 7(C), but it was only a small part of the TEM grid. These results were different from the reported micelle morphology changes for block copolymer. In the case of the

Table 3
Data of the micelle size and size distribution at different copolymer concentrations

Sample	6×10^{-4} (g/mL)		1×10^{-4} (g/mL)		6×10^{-5} (g/mL)		8×10^{-6} (g/mL)		6×10^{-6} (g/mL)		4×10^{-6} (g/mL)	
	D_h^b	PI ^b	D_h	PI	D_h	PI	D_h	PI	D_h	PI	D_h	PI
S ^a	241	0.317	215	0.213	211	0.187	132.7	0.368	159.8	0.488	117.2	0.571
L ^a	205.0	0.449	226.6	0.472	263.1	0.782	116.9	0.493				

^a S represents the star graft copolymer [poly(EO-co-Gly)-g-PS]₃ (sample B in Table 2) while L represents the linear graft copolymer PEO₂₂₆-g-26PS₂₂ (synthesized as described in Ref. [27]).

^b The hydrodynamic diameter (D_h) and size distribution of the micelle (PI) were measured by DLS.

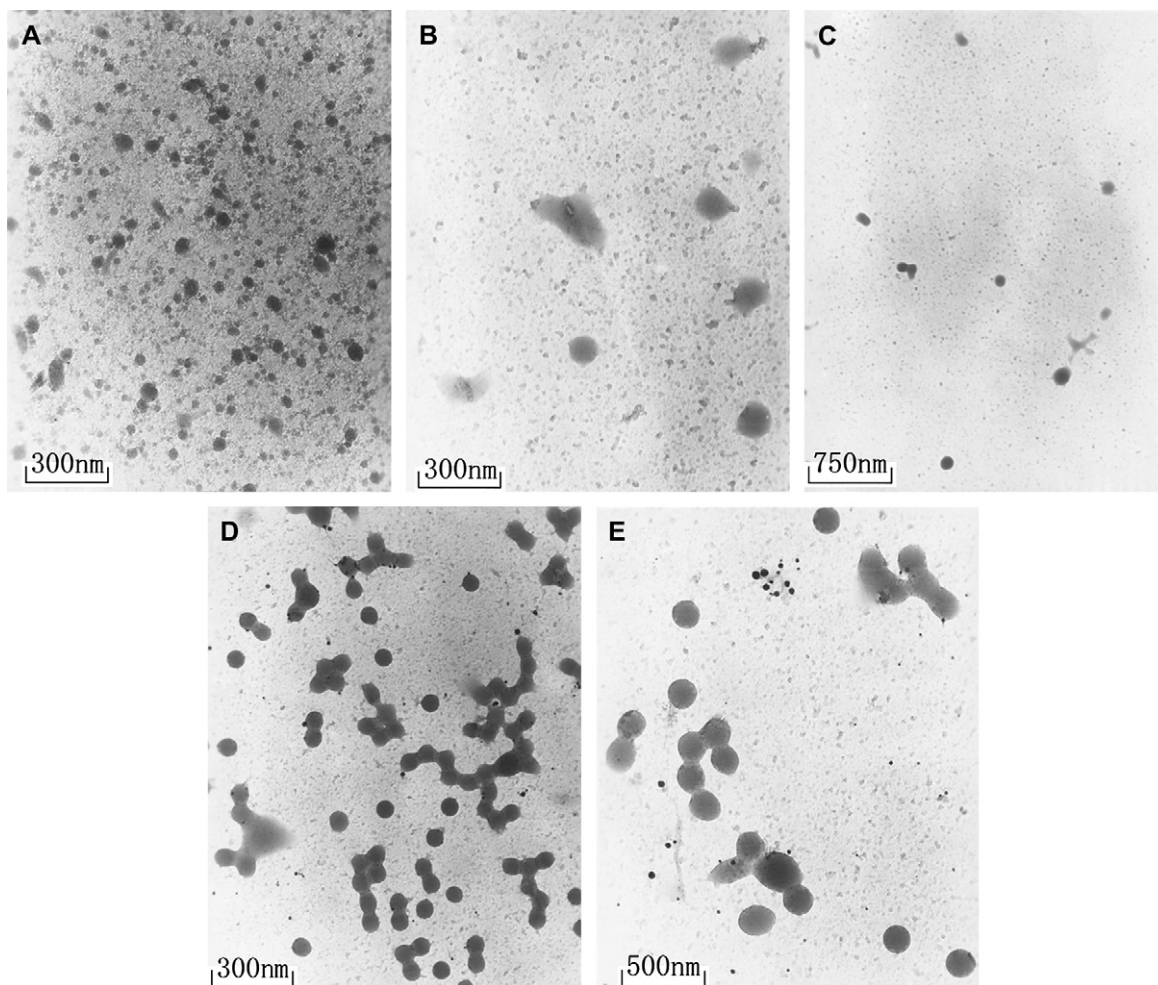


Fig. 7. TEM images of the micelles prepared by directly adding copolymer solution in DMF to water: (A) star graft copolymer (sample B in Table 2), 6×10^{-4} g/mL; (B) star graft copolymer (sample B in Table 2), 1×10^{-4} g/mL; (C) linear graft copolymer (PEO₂₂₆-g-26PS₂₂), 1×10^{-4} g/mL; (D) and (E) star graft copolymer (sample B in Table 2), 6×10^{-5} g/mL.

block copolymers, the copolymer concentration studied was always above 1×10^{-3} g/mL [32,33]. When copolymer concentration was increased, the aggregate morphology tended to change from spheres to wormlike cylinders and then to vesicles. In our study, both the star graft copolymer and linear graft copolymer can form regular micelles at copolymer concentration as low as 6×10^{-5} g/mL, but irregular micelles were found when the copolymer concentration was increased to 6×10^{-4} g/mL.

The micelles formed by directly adding star graft copolymer solution in DMF to water were spheres (Fig. 7(D)). During the sample preparation, the polarity of the environment of polystyrene increased quickly in a short time, which caused the abrupt decrease of entropy and increase of free energy, so the hydrophobic polystyrene side chains tended to aggregate to reduce the free energy and hydrophilic PEO backbone formed shell to prevent the micelle from precipitating. Small black dots can also be found in the TEM images (Fig. 7(A, B, D and E)), which may be due to the formation of single molecular micelles or micelles with small numbers of aggregated molecules. In a select solvent one graft copolymer chain can

aggregate together forming single molecular micelle [4,34], in which the PS side chains form the core and the PEO backbone form the shell around the core. A loop structure formed by PEO in the shell is also possible. The low polymer concentration favors the formation of single molecular micelle with small size. However, at a higher polymer concentration graft copolymer chains aggregate to form the multi-chain micelles. This is the reason why micelle sizes of the graft copolymers increase with copolymer concentration as shown in Table 3.

The micelle sizes obtained by TEM (about 120 nm) were smaller than that measured by DLS (above 200 nm). This phenomenon may be attributed to that both the PS cores and the PEO shells of micelle-like particles could be measured by DLS but only the inner cores of the micelle-like particles can be observed by TEM and the outer PEO shells with low contrast are difficult to be observed by TEM [35]. Another reason is that the light scattering is weighted in favor of larger objects (i.e. it is a *z*-average) and the dried micelles may undergo a change in volume because the shell structure of the PEO chains is likely to collapse when no longer solvated [36].

4. Conclusion

A novel star graft copolymer of PEO as main chain and PS as side chains was prepared by combination of the anionic ring-opening copolymerization with ATRP using 2-ethyl-2-hydroxymethyl-1,3-propanediol as core molecule. The copolymers have relatively narrow molecular weight distributions and well-defined structure, which was confirmed by SEC, NMR and IR.

The obtained amphiphilic star graft copolymer has very low critical micelle concentration at about 5×10^{-7} g/mL and can form sphere micelles with uniform diameters at low copolymer concentration, but the micelle shape became irregular when the copolymer concentration was increased.

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