SCIENCE CHINA
Chemistry

• ARTICLES •
• SPECIAL TOPIC • Advances in Principles of Polymerization

August 2010 Vol.53 No.8: 1653–1662
doi: 10.1007/s11426-010-3181-2

A pH- and temperature-sensitive macrocyclic graft copolymer composed of PEO ring and multi-poly(2-(dimethylamino) ethyl methacrylate) lateral chains

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Received January 31, 2010; accepted March 19, 2010

A novel method for the synthesis of macrocyclic graft copolymers was developed through combination of anionic ring-opening polymerization (AROP) and atom transfer radical polymerization (ATRP). A linear α,ω-dihydroxyl poly(ethylene oxide) with pendant acetal protected hydroxyl groups (*l*-poly(EO-*co*-EEGE)) was prepared first by the anionic copolymerization of ethylene oxide (EO) and ethoxyethyl glycidyl ether (EEGE). Then *l*-poly(EO-*co*-EEGE) was cyclized. The crude cyclized product containing the linear byproduct was hydrolyzed and purified by being treated with α-CD. The pure cyclic copolymer [*c*-poly(EO-*co*-Gly)] was esterified by reaction with 2-bromoisobutyryl bromide, and then used as ATRP macroinitiators to initiate polymerization of 2-(dimethylamino) ethyl methacrylate (DMAEMA), and a series of pH- and temperature-sensitive macrocyclic graft copolymers composed of a hydrophilic PEO as the ring and PDMAEMA as side chains (*c*-PEO-*g*-PDMAEMA) were obtained. The behavior of pH- and temperature-sensitive macrocyclic copolymers was studied in aqueous solution by fluorescence and dynamic light scattering (DLS). The critical micellization pH values of macrocyclic graft copolymers and their corresponding linear graft copolymers (*l*-PEO-*g*-PDMAEMA) were measured. Under the same conditions, the cyclic graft copolymer with the shorter side chains gave the higher critical micellization pH value. The *c*-PEO-*g*-PDMAEMA showed the lower critical micellization pH value than the corresponding *l*-PEO-*g*-PDMAEMA. The average hydrodynamic diameters (D_h) of the micelles were measured by DLS with the variation of the aqueous solution pH value and temperature.

macrocyclic PEO, anionic ring-opening polymerization, ATRP, PDMAEMA, pH- and temperature-sensitive

1 Introduction

The properties of polymers are closely related to their molecular structure. Polymers with unique structures, such as block, star, gradient, and graft structures, have been widely used in tissue engineering, drug delivery, and micelle preparation [1–5]. Recent progress in controlled living polymerization has provided an effective tool for preparing polymers with well-defined structure [6–8] because of its versatility and simplicity.

The stimuli-responsive polymers, also designated as "smart polymers", which undergo phase transitions in response to environmental stimuli such as pH and temperature, have been widely investigated in various fields [9–11]. Poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA), which is a pH- and temperature-sensitive polymer, is one of the most studied stimuli-responsive polymers in various fields [12–14] (paints, membranes, and gene delivery systems).

The cyclic molecules with special properties could find applications in many fields, such as the formation of organic

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nanotubes, ion complexes, and ion transport across membranes [15, 16]. Cyclic polymers, also designated as ring-shaped polymers, have special properties caused by the lack of chain ends and circular architecture [17, 18]. Looking for an efficient rout for the preparation of macrocyclic polymers have been a goal for polymer chemists for a long time, owing to their unique topology and physical properties [19]. Previous studies about cyclic polymers exclusively focused on the preparation of single polymer ring including hydrophobic polymers such as cyclic PS, PE [20, 21], and hydrophilic macrocyclic polymers such as macrocyclic poly(ethylene oxide) [22, 23]. However, there are relatively limited reports on the preparation of macrocyclic graft copolymers, especially stimuli-responsive cyclic graft copolymers, due to the difficulty of synthesis. The macrorings of PEO with multi-PDMAEMA lateral chains (*c*-PEO-*g*-PDMAEMA) may offer interesting properties as their special properties. The lack of data related to *c*-PEO-*g*-PDMAEMA motivated us to investigate this system.

We report a novel route for preparing the macrocyclic graft copolymers (*c*-PEO-*g*-PDMAEMA) with pH- and temperature-sensitive properties by a combination of anionic ring-opening polymerization (AROP) and atom transfer radical polymerization (ATRP). Their pH- and temperature-sensitive properties were investigated.

2 Experimental

2.1 Materials

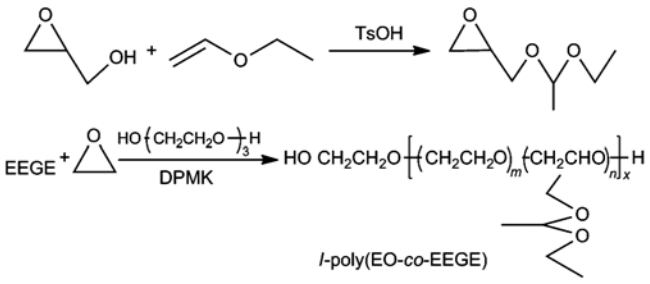
2-Bromoisobutyryl bromide (98%), 2,2′-bipyridyl (bpy, > 99%), and pyridine (99.5%) were purchased from Aldrich and Sinopharm Chemical Reagent Co., Ltd. (SCR), respectively, and used as received. CuBr (98%, Acros) was stirred overnight in acetic acid and filtrated, washed with ethanol and diethyl ether successively, and dried in vacuo. α-Cyclodextrin (α-CD, Aldrich) was used as received. *p*-Toluenesulfonyl chloride (TsCl, 98%, Aldrich) and potassium hydroxide (KOH, 96%, Aldrich) were dried under vacuum prior to use. 2-(Dimethylamino) ethyl methacrylate (DMAEMA, Acros organics 99%) was passed through the basic alumina column to remove the stabilizer and distilled over CaH_2 under reduced pressure, and stored at −20 °C prior to use. DMSO (SCR, 98%) was distilled over CaH_2 under reduced pressure just before use. Triethylene glycol was distilled from CaH_2 under reduced pressure and the fraction at 134 °C/90 Pa was collected. THF (99%) was refluxed over potassium wire and distilled from potassium naphthalenide solution. EO (SCR, 98%) was dried by CaH_2 for 48 h and then distilled under N_2 before use. Ethoxyethyl glycidyl ether (EEGE) and diphenylmethylpotassium (DPMK) were prepared according to previous reports [24, 25]. All the other reagents were purified by common purification procedures.

2.2 Measurements

GPC was performed on an Agilent1100 with a G1310A pump, a G1362A refractive detector, and a G1314A variable wavelength detector. THF was used as the eluent at 35 °C at 1.0 mL/min. One 5 μm LP gel column (500 Å, molecular range 500–2×10^4 g/mol) and two 5 μm LP gel mixed bed column (molecular range 200–3×10^6 g/mol) were calibrated with polystyrene standard samples. For *l*-poly($EO_{43.5}$-*co*-EEGE) and *c*-poly($EO_{10.6}$-*co*-Gly), GPC analyses were performed in 0.1 M aqueous $NaNO_3$ at 40 °C with an elution rate of 0.5 mL/min on the same instrument except that 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 range: 0–5×10^4, 5×10^4–8×10^6, (5–8) × 10^6 g/mol, respectively) were calibrated with PEO standard samples. The injection volume was 20 μL, and the concentration was 5 mg/mL. MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight) analysis was carried out on a Voyager DE-STR from Applied Biosystems. The matrix α-cyano-4-hydroxycinnamic acid was dissolved in THF at a concentration of 40 mg/mL. Potassium trifluoracetate was used as a cationization agent at typical concentrations of 5 mg/mL. The sample was dissolved in THF at approximately 1 mg/mL. Finally, the matrix, salt, and polymer solutions were premixed in a molar ratio of 5:1:5. The premixed solutions were hand-spotted on the target well and left to dry. All mass spectra were recorded in the reflector mode and about 1000 laser shots were collected per spectrum. ^{1}H NMR spectra were obtained at a DMX 500 MHz spectrometer. All the samples were dissolved in $CDCl_3$. The ultrafiltration separator was purchased from the Shanghai Institute of Nuclear Research, Chinese Academy of Sciences, and the cutoff molecular weight of the poly(ether sulfone) membrane was 20000 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.

2.3 Anionic copolymerization of EEGE with EO

The copolymerization of EEGE with EO is shown in Scheme 1. The typical procedure was shown as follows: A 150 mL kettle was vacuumed at 80 °C for 2 h, and cooled to room temperature and then to 0 °C. Given volumes of the initiator solution [triethylene glycol (0.29 mL, 2.19 mmol) with DPMK (3.5 mL, 2.0 mmol) in a mixture of THF and DMSO (10/40 *v*/*v*, 50 mL)], EEGE (11.1 g, 75.8 mmol), and EO (30.0 g, 681.8mmol) were introduced successively into the kettle under magnetic stirring. It was subsequently



Scheme 1 Synthesis of linear copolymer *l*-poly(EO-*co*-EEGE).

heated to 60 °C under stirring for 48 h. The reaction was terminated by addition of a few drops of acidified methanol. After all the solvents were removed by reduced distillation, the crude product was dissolved in CH_2Cl_2, dried over anhydrous $MgSO_4$ and filtered. The yellowy viscous wax-like product, a linear α,ω-dihydroxyl poly(ethylene oxide) with pendant protected hydroxylmethyls [*l*-poly($EO_{10.6}$-*co*-EEGE)], was obtained in a yield of 94% after CH_2Cl_2 was removed. The copolymer *l*-poly($EO_{43.5}$-*co*-EEGE) was synthesized by the same method.

^{1}H NMR ($CDCl_3$, δ, ppm): 4.70–4.73(m, –OC**H**(CH_3)O–), 1.30 (d, –OCH(C**H$_3$**)O–), 1.19 (t, C**H$_3$**CH_2O–), 3.53–3.80 (m, –C**H$_2$**C**H$_2$**O– and –C**H$_2$**C**H** ($C_8H_{12}O_2$)O– for PEO main chain).

2.4 Cyclization of *l*-poly(EO-*co*-EEGE)

In a flask, finely-ground KOH (0.44 g) was dispersed in a mixture of THF and heptane (70/30, *v*/*v*, 100 mL) and stirred under nitrogen at 40 °C. *l*-Poly($EO_{43.5}$-*co*-EEGE) (5.0 g) and TsCl (223 mg) were dissolved in 100 mL of THF in a separate flask. This solution was added dropwise to the KOH dispersion via a syringe pump over 48 h. After a further 72 h under reflux (40 °C) the mixture was filtered and the filtrate was evaporated under reduced pressure. The crude cyclized product was obtained.

2.5 Purification of the crude cyclized copolymer

The crude product (4.0 g) was dissolved in 40 mL of distilled water (100 g/L), to which an aqueous solution of α-CD (80 mL, 100 g/L) was added at room temperature. The ratio of α-CD to the linear byproducts (about 20 %), which was estimated from the GPC, was 10/1 (*w*/*w*). The resulting clear solution was ultrasonically stirred for 30 min and then turbid solution appeared. It was allowed to stand overnight at room temperature and a white precipitate formed. The mixture was centrifuged and filtered to obtain a clear aqueous solution. The solvent was distilled and a solid mixture of the cyclic product, unthreaded α-CD or a small quantity of linear byproducts remained. This solid mixture was dissolved in dichloromethane and filtered to remove the unthreaded α-CD. The filtrate was precipitated in diethyl ether to obtain the product. The procedure was repeated twice to obtain the pure cyclized product [*c*-poly($EO_{43.5}$-*co*-EEGE)].

2.6 Preparation of *c*-poly(EO-*co*-Gly) by cleavage of the ethoxyethyl groups of *c*-poly(EO-*co*-EEGE)

The hydrolysis of EEGE segments of the copolymer [25] required two steps: (1) the cyclized product (3.0 g) was mixed with 40 mL of formic acid, and the solution was stirred at 20 °C during 30 min and then poured into methanol. The precipitate was separated and dried in vacuo at 50 °C; (2) the dried product dissolved in a mixture of dioxane (25 mL), methanol (15 mL) and KOH methanol solution (1 N, 10 mL) was refluxed for 24 h, and neutralized with 5% HCl. After the solvents were removed under reduced pressure, the polymer was dissolved in water and purified by ultra filtration. The filtrated aqueous solution was concentrated to dryness, dissolved in CH_2Cl_2, dried over anhydrous $MgSO_4$ and filtered. The filtrate was precipitated in diethyl ether and dried in vacuo at 50 °C. The hydrolyzed cyclic product *c*-poly($EO_{43.5}$-*co*-Gly) was obtained in a yield of 90 %. It was a white powder.

2.7 Preparation of macroinitiator *c*-poly($EO_{43.5}$-*co*-Gly)$_{(ATRP)}$

The esterification of hydroxymethyl groups on glycidol segments of *c*-poly($EO_{43.5}$-*co*-Gly) with 2-bromoisobutyryl bromide [26] was performed to prepare the cyclic macroinitiator. A typical example was described as follows: 2.0 g of *c*-poly($EO_{43.5}$-*co*-Gly) was dissolved in 50 mL anhydrous pyridine under dry nitrogen, and 0.4 mL of 2-bromoisobutyryl bromide was added dropwise at 0 °C over 20 min under vigorous stirring. The initial yellow color disappeared immediately, and pyridinium bromide with red-brown color was precipitated. It was continuously stirred for another 15 min. After that, 5 g of K_2CO_3 was added to the system at room temperature. The pyridine was removed by azeotropic distillation with dry toluene (3 × 40 mL). The residue was dissolved in water and separated by ultra filtration. The aqueous solution was concentrated to dryness and dissolved in CH_2Cl_2, dried over anhydrous $MgSO_4$ and then filtered. The CH_2Cl_2 of the filtrate was precipitated in diethyl ether. The precipitate was dried in vacuo at 50 °C and the yellowy product with a yield of 95% was obtained. ^{1}H NMR ($CDCl_3$, δ, ppm): 4.13–4.42 (m, –C**H$_2$**OOCC(CH_3)$_2$Br)), 1.86(s, –OOCC(C**H$_3$**)$_2$Br), 3.35–3.90 (m, –C**H$_2$**C**H$_2$**O– and –C**H$_2$**C**H**O– for PEO main chain).

2.8 Synthesis of macrocyclic graft copolymer *c*-PEO-*g*-PDMAEMA by ATRP

The graft copolymerization of DMAEMA was carried out using *c*-poly($EO_{43.5}$-*co*-Gly) with pending bromoisobutyryl

groups [*c*-poly($EO_{43.5}$-*co*-Gly)$_{(ATRP)}$] as macroinitiators. In a typical process, an ampoule charged with CuBr (31.9 mg, 0.22 mmol), bpy (34.7 mg, 0.22 mmol), *c*-poly($EO_{43.5}$-*co*-Gly)$_{(ATRP)}$ (M_n = 5400, 0.4 g, 0.074 mmol), and 6 mL of distilled water was stirred for 20 min and vacuumed by three freeze-thaw-cycles at the temperature of liquid nitrogen. At the same time, another ampoule charged with DMAEMA (4 mL, 23.6 mmol) was vacuumed by three freeze-thaw-cycles at the temperature of liquid nitrogen. The DMAEMA was then added rapidly to the first ampoule via a syringe needle and stirred strongly at 10 °C. The reaction solution turned dark brown and became progressively more viscous. The released polymerization heat raised the solution temperature. After about 2 h, the ampoule was dipped in liquid nitrogen to stop the polymerization. The products were dissolved in anhydrous methanol, the solution was passed through the neutral alumina column to remove the catalyst and then precipitated in cold heptane. The copolymers were purified twice by dissolution/precipitation with methanol/heptanes.

2.9 Synthesis of linear graft copolymers *l*-PEO-*g*-PDMAEMA

The *l*-PEO-*g*-PDMAEMA was synthesized to be compared with *c*-PEO-*g*-PDMAEMA for investigating their pH- and temperature-sensitive behaviors. A linear α,ω-dihydroxyl poly(ethylene oxide) with pendant protected hydroxylmethyls *l*-poly(EO-*co*-EEGE) was used. The *l*-poly(EO-co-Gly) with pending bromoisobutyryl groups [*l*-poly(EO-*co*-Gly)$_{(ATRP)}$] was prepared, and a series of linear graft copolymers *l*-PEO-*g*-PDMAEMA were obtained (the same as the synthesis route of *c*-PEO-*g*-PDMAEMA).

2.10 Determination of critical micellization pH

Pyrene was used as the fluorescence probe [27]. An acetone solution of 190 μL pyrene (2.67×10^{-4} mol/L) was added to 100 mL water with different pH values and the concentration of pyrene was 5.0×10^{-7} mol/L. Next, 50 μL of the copolymer solution in *N,N*-dimethylformamide (DMF) (0.1 g/mL) was added to water containing pyrene to obtain the solution with the copolymer concentration as 1 mg/mL. Each copolymer solution was left for 30 min at room temperature to ensure equilibration between the pyrene in the micelles and that in the aqueous solution. All fluorescence spectra were recorded at 25 °C.

2.11 Dynamic light scattering studies

The samples used for fluorescence measurement were used for dynamic light scattering (DLS) studies. The samples were measured after they were filtered through a membrane filter with a nominal pore size of 0.8 μm.

3 Results and discussion

3.1 Synthesis of parent copolymers *l*-poly(EO-*co*-EEGE)

The anionic ring-opening copolymerization of EO and EEGE was performed using DPMK as the deprotonating agent which could readily react with hydroxyl groups to form alkoxides. In order to control the polymerization reasonably, it is significant that only 20%–40% of the hydroxyl groups of the triethylene glycol were activated, otherwise alkoxides would be precipitated [28]. A mixture of DMSO and THF (*v/v*: 4/1) was used as the solvent for polymerization instead of THF because propagating alkoxides would be aggregated in pure THF [26]. Under such conditions, all of the hydroxyl groups of triethylene glycol could efficiently initiate the copolymerization of EO and EEGE because of the rapid exchange of protons between dormant hydroxyls and propagating alkoxides, and all the chains grew at the same rate [29]. The parent copolymers with different contents of EO and different molecular weights were synthesized through variation of the monomer feed ratio and initiator volume as shown in Table 1.

The copolymer composition could be obtained by using the following formula based on the ^{1}H NMR data (see Anionic Copolymerization of EEGE with EO in Experimental Section2.3):

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

where R_T is the molar ratio of EEGE to EO in the copolymer, A_{sum} and A_f represent the peak area sum of the protons of the main chain and protons of lateral chains methylene and the peak areas of the methine protons of the EEGE moiety, respectively. The R_T values of copolymers A and B are 1/10.6 and 1/43.5, respectively, which are nearly equivalent to the monomer feed ratio of EEGE to EO (1/9 and 1/39). Therefore, the linear α,ω-dihydroxyl PEO with pendant acetal protected hydroxyls (*l*-poly(EO-*co*-EEGE)) could be described as *l*-poly($EO_{10.6}$-*co*-EEGE) (A) and *l*-poly($EO_{43.5}$-*co*-EEGE) (B). The number of the protected hydroxyls on the PEO chain could be evaluated by the

Table 1 The data of parent copolymers *l*-poly(EO-*co*-EPEE)

Sample	R_f [a]	R_T [b]	M_n [c]	M_w/M_n [d]	N_{EPEE} [e]
A	1/9	1/10.6	12,500	1.06	20
B	1/39	1/43.5	6,400	1.16	3

a) The feed ratio of EEGE to EO. b) The molar ratio of EEGE to EO in copolymer poly(EO-*co*-EEGE) measured by ^{1}H NMR. c) Number-average molecular weight determined by GPC, sample A: calibrated against PS standards using THF as the eluent; sample B: calibrated against PEO standards using 0.1 M $NaNO_3$ as the eluent. d) The polydispersity determined by GPC. e) The number of EEGE units in poly(EO-*co*-EEGE) calculated by the integration of protons from ^{1}H NMR.

combination of the molecular weight determined by GPC and ^{1}H NMR data using eq. (2):

$$N_{\mathrm{EEGE}} = \frac{M_{\mathrm{n}}}{\left(146 + 44/R_{\mathrm{T}}\right)} \quad (2)$$

where M_{n} is the molecular weight of *l*-poly(EO-*co*-EEGE), 146 and 44 are the molar masses of EEGE and EO, and R_{T} is the molar ratio of EEGE units to EO units in *l*-poly(EO-*co*-EEGE). The calculated N_{EEGE} values are listed in Table 1.

3.2 Cyclization of *l*-poly(EO-*co*-EEGE) and purification

The essential work is the closure of linear copolymers *l*-poly(EO-*co*-EEGE) and the purification of the closed product for the synthesis of macrocyclic copolymers**.** The ring closure of *l*-poly(EO-*co*-EEGE) was achieved via ether linkage by reaction with tosyl chloride (TsCl) in the presence of solid KOH. End-to-end intramolecular coupling was promoted over intermolecular chain extension by conducting the reaction at high dilution [$C^* < 10^{-5}$ mol/L]. This synthesis was based on a method reported by Booth *et al*. for the preparation of cyclic PEO [30, 31]. They also reported a second method for ring closure via an acetal linkage (reaction of the α,ω-dialkoxide with CH_2Cl_2) [32]. We have followed the first method because of the relative chemical stability of the ether linkage compared with the acetal linkage, which is subject to scission under acidic conditions.

Figure 1(a) shows the GPC curve of the crude cyclized product using the *l*-poly($EO_{43.5}$-*co*-EEGE) as a linear precursor. It is apparent that the chain-extended copolymer, which is the intermolecular reaction product, coexisted with the cyclized product. Compared with the original linear copolymer (Figure 1(b)), the major peak of the cyclized product moved to the higher elution time due to its smaller hydrodynamic volume and the peak molecular weight of M_{pl} = 8000 was changed to M_{pc} = 6,500 ($M_{\mathrm{pc}}/M_{\mathrm{pl}}$ = 0.81), indicating formation of the cyclic polymer [33], and a broad shoulder assigned to chain extended polymer was found at lower elution time.

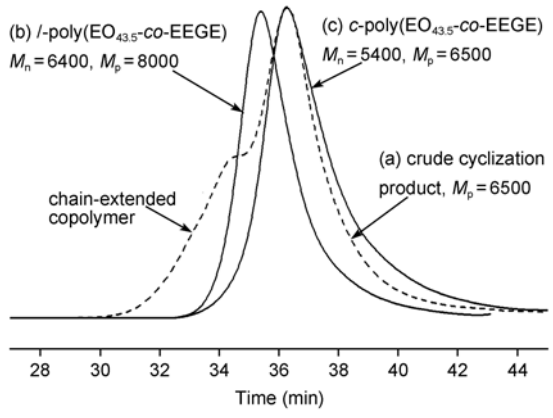


Figure 1 Typical GPC traces. (a) Crude cyclized product of *l*-poly ($EO_{43.5}$-*co*-EEGE). (b) Linear *l*-poly($EO_{43.5}$-*co*-EEGE). (c) Purified cyclized product by α-CD (0.1 M $NaNO_3$ as the eluent).

The separation and purification of the cyclized product is a significant step for the next syntheses of macrocyclic graft copolymers. It was reported that the cyclodextrins (α, β, and γ) could form inclusion complexes with a wide variety of low molecular weight compounds as well as linear polymers, both organic and inorganic [34–36]. After dealing with by the α-CD, as shown in Figure 1(c), the peak of linear chain extended polymer at lower elution time disappeared .

In order to obtain direct evidence for the formation of the macro-ring, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) was used. Figure 2 shows the MALDI-TOF-MS spectra of linear precursor *l*-Poly($EO_{43.5}$-*co*-EEGE) (a) and cyclized product *c*-poly($EO_{43.5}$-*co*-EEGE) (b). The spacing 44.3 and 43.8 amu between the peaks are ascribed to the molar mass of the EO and the spacing 146.1 and 146.2 amu for the molar mass of the EEGE unit. The spacing 14.7, 29.6, 15.2 and 29.9 between the peaks are ascribed to the difference of the molar mass of different combinations of EEGE and EO unit, and the molecular-weight decrease of 18 amu after cyclization reaction supports the formation of the ether linkage which is consistent with loss of a water molecule upon ring closure. Thus the MALDI-TOF provides direct evidence for the closure of *l*-poly(EO-*co*-EEGE).

3.3 Synthesis of cyclic ATRP macroinitiator

In order to obtain the reactive hydroxyl groups on the *c*-poly (EO-*co*-EEGE), the polymers were treated with formic acid first [37], and the formed polyformate was further saponified in the KOH dioxane/methanol mixture solution, and

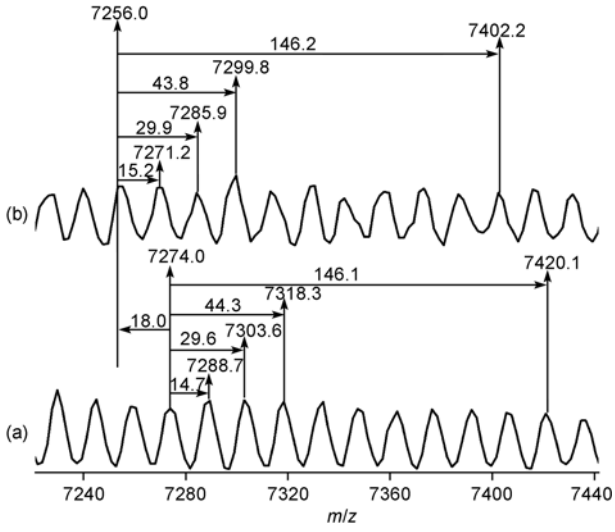


Figure 2 MALDI-TOF mass spectra of the model copolymer for (a) linear precursor *l*-poly($EO_{43.5}$-*co*-EEGE) and (b) cyclization product *c*-poly($EO_{43.5}$-*co*-EEGE).

hydroxyl groups were recovered. Thus the *c*-poly (EO-*co*-EEGE) was transformed to *c*-poly(EO-*co*-Gly) with multi-pending hydroxyl groups.

The hydroxyl groups of the cyclized product *c*-poly-(EO-*co*-Gly) were esterified with 2-bromoisobutyryl bromide to obtain cyclic macroinitiators with narrow molecular weight distribution as shown in Table 2, and the complete esterification of the hydroxyl groups of *c*-poly(EO-*co*-Gly) was confirmed by ^{1}H NMR. The hydroxyl group conversion was calculated by eq. (3)

$$E_T = \frac{\left(3+\frac{4}{R_T}\right)\times A_e}{2A_{sum}}\times 100\% \quad (3)$$

where E_T is the conversion efficiency of hydroxyl groups of *c*-poly(EO-*co*-Gly), A_{sum} and A_e represent the integral area of the protons of PEO main ring (the peaks at δ = 3.35–3.90) and the integral area of the protons linked to the ester (the peaks at δ = 4.13–4.42), and R_T is the molar ratio of EEGE to EO in the original linear copolymer *l*-poly(EO-*co*-EEGE) measured by ^{1}H NMR. The E_T value is nearly 100%, suggesting that hydroxyl groups are completely converted to bromoisobutyryl units.

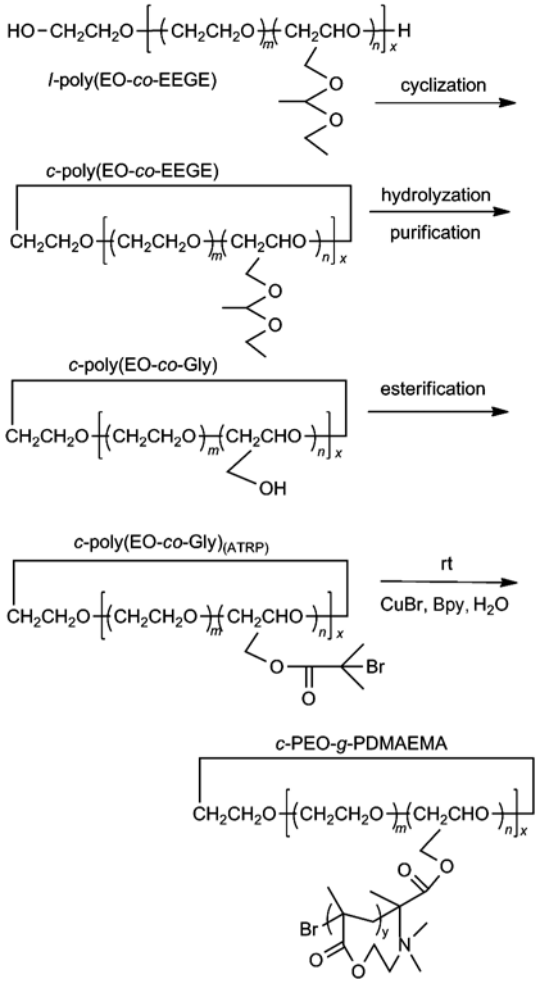
3.4 Synthesis of macrocyclic graft copolymer *c*-PEO-*g*-PDMAEMA by ATRP

The procedure for the synthesis of *c*-PEO-*g*-PDMAEMA using cyclic macroinitiators is shown in Scheme 2. The polymerization of 2-(dimethylamino) ethyl methacrylate (DMAEMA) has been intensively investigated because poly(DMAEMA) is a useful water-soluble, pH- and temperature-sensitive polymer. In this work, the ATRP of DMAEMA was carried out in water at 10 °C using the Bpy/CuBr catalyst system. The results of ATRP by using two kinds of *c*-poly(EO-*co*-Gly)$_{ATRP}$ with different bromoisobutyryl groups density as cyclic macroinitiators are shown in Table 3.

Table 2 GPC data of the cyclic macroinitiators

Sample [a]	*c*-Poly(EO-*co*-Gly)		*c*-Poly(EO-*co*-Gly)$_{(ATRP)}$	
	M_n [b]	M_w/M_n [b]	M_n [c]	M_w/M_n [c]
A	6800	1.29	11,800	1.10
B	5200	1.23	5,400	1.12

a) Samples A and B are coincident with the sample number in Table 1. b) Number average molecular weight (M_n) and molecular weight distribution (M_w/M_n) determined by GPC, calibrated against PEO standards using 0.1 M $NaNO_3$ as the eluent. c) Number average molecular weight (M_n) and molecular weight distribution (M_w/M_n) determined by GPC, calibrated against PS standards using THF as the eluent.



Scheme 2 Synthesis of macrocyclic graft copolymer *c*-PEO-*g*-PDMAEMA.

Table 3 Data of macrocyclic graft copolymers *c*-PEO-*g*-PDMAEMA

Initiator	Entry	M_n [a] ($\times 10^4$)	M_w/M_n [b]	$M_{n,NMR}$ [c] ($\times 10^4$)	N_{DMAEMA} [d]	R_f [e]
A [f]	A_1	2.24	1.48	7.49	20.1	22
	A_2	2.59	1.36	8.28	22.6	30
	A_3	1.23	1.51	1.80	22.3	25
B [g]	A_4	2.22	1.46	2.54	38.1	40

a) Number-average molecular weight determined by GPC, calibrated against the PS standard. b) The polydispersity determined by GPC. c) M_{nNMR} is calculated by the formula: $M_{n,NMR} = M_{n\ c\text{-poly(EO-}co\text{-Gly)(ATRP)}} + N_{OH} \times N_{DMAEMA} \times M_{DMAEMA}$, $M_{n\ c\text{-poly(EO-}co\text{-Gly)(ATRP)}}$ is the molecular weight of $M_{n\ c\text{-poly(EO-}co\text{-Gly)(ATRP)}}$ derived by GPC, N_{OH} is the hydroxyl number on *c*-poly(EO-*co*-Gly), and M_{DMAEMA} is the molecular weight of DMAEMA. d) The average number of DMAEMA on the grafting chain of the final copolymers is calculated from the ^{1}H NMR data. e) The feed ratio of DMAEMA to Br-atom (N_{Br}). f) *c*-Poly($EO_{10.6}$-*co*-EEGE) as a precursor. g) *c*-Poly($EO_{43.5}$-*co*-EEGE) as a precursor.

The final products were characterized by GPC and ^{1}H NMR shown in Figures 3 and 4. According to ^{1}H NMR spectrum, the degree of polymerization of PDMAEMA side chains was obtained by eq. (4):

$$N_{\mathrm{PDMAEMA}} = \left(A_{\mathrm{j}}/2\right)\Big/\left(A_{\mathrm{f}}/6\right) \tag{4}$$

where N_{PDMAEMA} is the average number of DMAEMA on each side chain (Table 3), A_{j} and A_{f} represent the integral area of the methylene protons (δ = 2.55–2.80 ppm (m, $-CH_2C\boldsymbol{H}_2N-$)) of the grafted PDMAEMA chains and the integral area of methyl protons at the α-end of the PDMAEMA side chains, respectively.

The initiation efficiency in the synthesis of some copolymers by ATRP has been widely studied. Reports [38, 39] showed that not every initiating site in the backbone generated a side chain and incomplete initiation was always attributed to steric hindrance due to the high density of initiating centers. In our case, however, the density of initiating centers in the macrocyclic PEO is lower than that reported in previous reports, so higher initiation efficiency is anticipated. The initiation efficiency of bromoisobutyryl for ATRP could be estimated from the ^{1}H NMR spectrum (Figure 4) in eq. (5):

$$E_{\mathrm{T}} = \frac{\left(\dfrac{4}{R_{\mathrm{T}}}+3\right)\times A_{\mathrm{f}}}{6A_{\mathrm{sum}}}\times 100\% \tag{5}$$

where E_{T} is the reaction efficiency of bromoisobutyryl for ATRP, A_{sum} represents the integral area of all protons of the PEO main chain, and R_{T} is the molar ratio of EEGE to EO in copolymer *c*-poly(EO-*co*-EEGE). The calculated E_{T} value is as high as 97.7%, which indicated that nearly all the bromoisobutyryl groups took part in the polymerization.

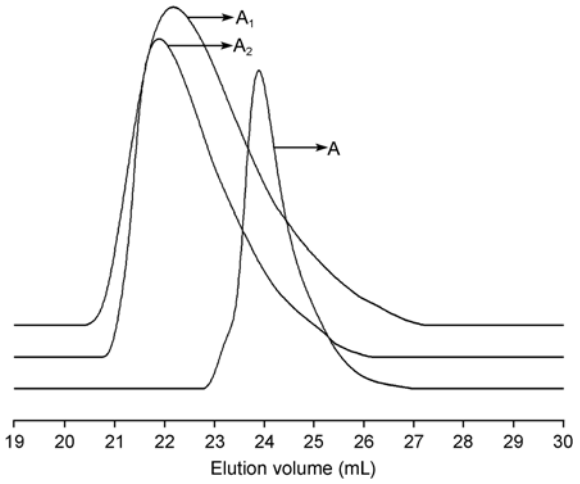


Figure 3 GPC traces of the graft copolymer *c*-PEO-*g*-PDMAEMA. Sample A *c*-poly($EO_{10.6}$-*co*-Gly)$_{(ATRP)}$ as the macroinitiator, entry A_1 and entry A_2 in Table 3.

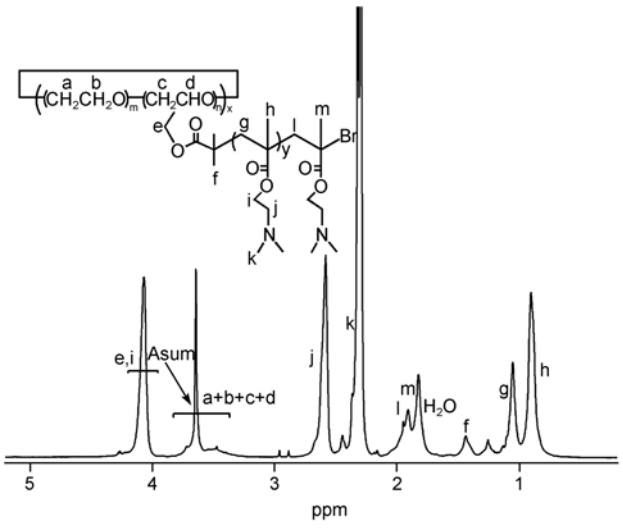


Figure 4 ^{1}H NMR spectrum of *c*-PEO-*g*-PDMAEMA (entry A_1 in Table 3; solvent $CDCl_3$).

3.5 Measurement of critical micellization pH

The pH-induced micellization behaviors of copolymers have been investigated [40, 41]. However, no report was published on the macrocyclic graft copolymers. Therefore, we investigated the pH-induced micellization behaviors of cyclic graft copolymers in water solution by determining their critical micellization pH values.

The critical micellization pH values were examined by fluorescence technique using pyrene as the probe. Pyrene fluorescence is very sensitive technique for detecting the formation of copolymer micelles. Pyrene is highly hydrophobic and has very low solubility in water so it migrates preferentially into the hydrophobic micelle cores. A red shift was observed in the pyrene fluorescence spectra, and there were changes in relative peak intensities [42–44]. In our work, the protocol based on the analysis of pyrene emission spectra reported by Gast *et al*. was employed [45]. Figure 5 shows the variation in the intensity ratio (I_1/I_3) vs solution pH for macrocyclic graft copolymers *c*-PEO-*g*-PDMAEMA. As the solution pH increases, the PDMAEMA becomes progressively deprotonated. The critical pH values for micellization were estimated from the reduced I_1/I_3 ratio, which indicates a more hydrophobic environment for the pyrene probe. The critical micellization pH value estimated for c-PEO-g-PDMAEMA (sample A_2 in Table 3) is pH 6.05. As a comparison, the critical micellization pH value for the corresponding linear graft copolymer *l*-PEO-*g*-PDMAEMA is pH 6.68 (Figure 5). The critical micellization pH values of other macrocyclic graft copolymers (Table 3) and their corresponding linear graft copolymers were determined under the same conditions, and all the results are listed in Table 4. These results indicate that cyclic graft copolymer *c*-PEO-*g*-PDMAEMA with the same PEO macrocycle and

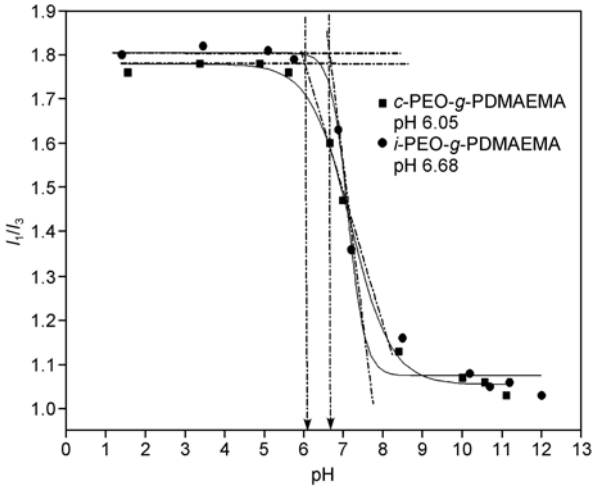


Figure 5 Determination of the critical pH for micellization of the macrocyclic graft copolymer *c*-PEO-*g*-PDMAEMA (sample A_2 in Table 3) and the corresponding linear copolymer *l*-PEO-*g*-PDMAEMA at 25 °C from pyrene emission spectra (excitation wavelength λ_{ex} = 333 nm; the copolymer concentration was 1 mg/mL in each case).

longer side chains shows lower critical micellization pH value. This difference may be mainly caused by the different percentages of PDMAEMA side chains. With the solution pH increasing, PDMAEMA side chains become progressively deprotonated and hence hydrophobic, so the cyclic graft copolymer with longer PDMAEMA side chains is more hydrophobic, leading to the formation of micelles with PDMAEMA cores in lower pH water solution.

The data listed in Table 4 shows that the critical micellization pH value of cyclic graft copolymer is lower than that of the corresponding linear graft copolymer. These lower critical micellization pH values are related to the cyclic structure of graft copolymers. Although the graft chains PDMAEMA of either cyclic copolymers or linear copolymers are progressively hydrophobic with water solution pH increasing, the cases are different for them. For *c*-PEO-*g*-PDMAEMA, the whole macrocyclic molecule is considered as a cage and its compact structure makes cyclic molecules readily assemble into micelles in lower pH water solution, compared with the corresponding ring-opening graft copolymer *l*-PEO-*g*-PDMAEMA.

Table 4 The critical micellization pH values of the macrocyclic graft copolymer and the corresponding linear copolymer

Sample	A_1/a_1	A_2/a_2	A_3/a_3	A_4/a_4
Critical pH	6.18/6.85	6.05/6.68	6.40/6.97	6.23/6.83

A_1/a_1 and A_2/a_2: *l*-poly($EO_{10.6}$-*co*-EEGE) as the precursor (sample A_1 and A_2 in Table 3, their corresponding comb-like copolymer a_1 and a_2 (a_1: $N_{PDMAEMA}$ = 19.7; a_2: $N_{PDMAEMA}$=23.1)). A_3/a_3 and A_4/a_4: *l*-poly($EO_{43.5}$-*co*-EEGE) as the precursor (sample A_3 and A_4 in Table 3, their corresponding linear copolymer a_3 and a_4 (a_3: $N_{PDMAEMA}$ = 22.9; a_4: $N_{PDMAEMA}$ = 40.1)).

3.6 Dynamic light scattering studies on stimuli-sensitive properties of macrocyclic graft copolymer *c*-PEO-*g*-PDMAEMA in aqueous media

The hydrophilicity of PDMAEMA may vary with the solution pH: when the pH is lower than the critical micellization, PDMAEMA chains are hydrophilic and soluble in water. However, when the solution pH increases, PDMAEMA becomes progressively deprotonated and turns to hydrophobic. In our case, at low pH values, the macrocyclic graft copolymer *c*-PEO-*g*-PDMAEMA was dissolved in the aqueous solution as mono-molecules. As the solution pH was raised over the critical micellization pH, the PDMAEMA graft chains turned to hydrophobic which caused the formation of micelles composed of the PDMAEMA core. Figure 6 shows the dynamic light scattering (DLS) results for *c*-PEO-*g*-PDMAEMA at 1 mg/mL concentration. When the pH was lower than the critical micellization pH, cyclic copolymers were molecularly dissolved, with an intensity-average hydrodynamic diameter (D_h) of approximately 10 nm and very low scattering intensity. As the solution pH was raised, the cyclic copolymer A_2 with longer graft chain PDMAEMA started to form the micelles, and the micellization occurred above pH 6.05. Between pH 6.05 and 8.50, micellization was not complete: dynamic light scattering revealed two populations at 10 and 80 nm, corresponding to dissolved chains (unimers) and micelles, respectively. At pH 8.50 or higher, DLS revealed solely one population corresponding to micelles. The D_h of the micelles decreased from 80 to 68 nm as the solution pH was further increased from 8.50 to 10.40. It was caused by the fact that the micelles became more compact due to further deprotonation of the PDMAEMA chains. Above pH 10.40, the micelle size does not change significantly.

In addition to the pH-sensitive property, the side chain PDMAEMA is temperature-sensitive. It exhibits lower critical

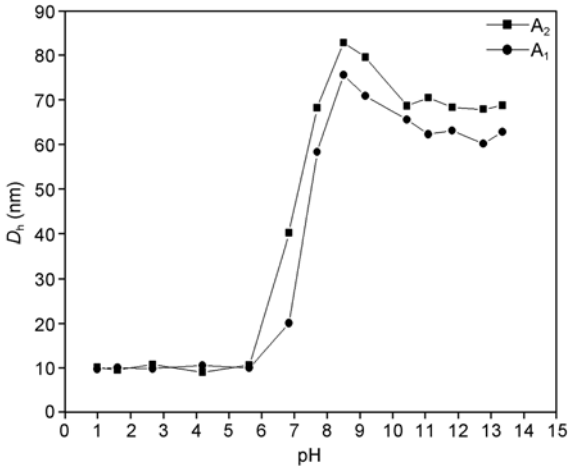


Figure 6 Variation of intensity-average hydrodynamic diameter (D_h) with pH for the macrocyclic graft copolymers (sample A_1 and A_2 in Table 3) at 1 mg/mL in water at 25 °C.

solution temperature (LCST) phase behavior and precipitates from neutral or basic aqueous solution between 32 and 50 °C, depending on its molecular weight and solution pH [46–49]. When the temperature is lower than LCST, PDMAEMA chains are hydrophilic and soluble in water, and when the temperature is higher than LCST, PDMAEMA turns to hydrophobic. Figure 7 shows the variation of D_h as a function of temperature for the *c*-PEO-*g*-PDMAEMA (sample A_2 in Table 3) at 1 mg/mL at different solution pH. At pH 2.68, the copolymer is soluble in water solution with D_h of approximately 10 nm (the whole temperature range: 14–53 °C). At pH 8.50, below 47 °C the micelle size shows little change with D_h being about 90 nm. Above 47 °C, the scattering intensity increased, accompanied by an increase in the aggregate size from 90 to about 200–300 nm in diameter. Concerning pH 12.76, above 35 °C the scattering intensity increased abruptly, accompanied by a dramatic increase in the aggregate size from about 100 to about 3000–4000 nm in diameter. Because of progressive increase of the solution temperature, the PDMAEMA graft chains approach its cloud point and become hydrophobic. Presumably, the changing hydrophilic-hydrophobic balance leads to micellar fusion.

4 Conclusions

A novel method for the synthesis of macrocyclic graft copolymers was developed through combination of anionic ring-opening polymerization (AROP) and atom transfer radical polymerization (ATRP). A series of pH- and temperature-sensitive macrocyclic graft copolymers composed of a hydrophilic PEO as the ring and PDMAEMA as side chains (*c*-PEO-*g*-PDMAEMA) were obtained. The behavior of pH- and temperature-sensitive macrocyclic copolymers was studied in aqueous solution using fluorescence and dynamic light scattering (DLS). The critical micellization pH values of macrocyclic graft copolymers and their corresponding linear graft copolymers (*l*-PEO-*g*-PDMAEMA) were measured. Under the same conditions, the cyclic graft copolymers with the shorter side chains gave the higher critical micellization pH values. The *c*-PEO-*g*-PDMAEMA shows the lower critical micellization pH values than the corresponding *l*-PEO-*g*-PDMAEMA. The average hydrodynamic diameters (D_h) of the micelles were measured by DLS with the pH value and temperature's variation.

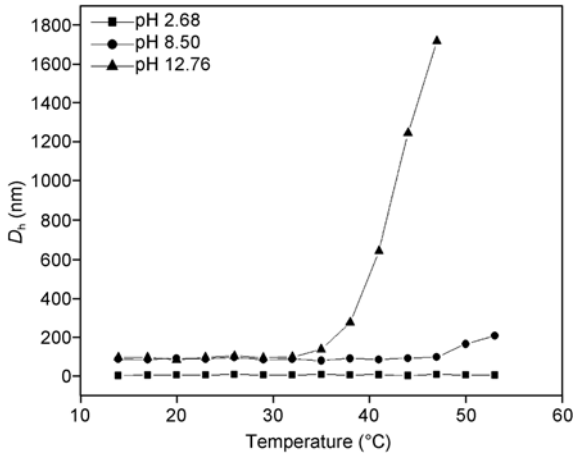


Figure 7 Variation of the intensity-average hydrodynamic diameter (D_h) with temperature for the macrocyclic graft copolymer (sample A_2 in Table 3) at 1 mg/mL and different pH values in water.

We appreciate the financial support from the National Natural Science Foundation of China (20874013).

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