Carbohydrate Polymers 84 (2011) 195–202

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis, self-assembly and drug release behaviors of pH-responsive copolymers ethyl cellulose-*graft*-PDEAEMA through ATRP

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ARTICLE INFO

Article history:
Received 26 August 2010
Received in revised form 19 October 2010
Accepted 10 November 2010
Available online 18 November 2010

Keywords:
Ethyl cellulose
Poly(2-(diethylamino) ethyl methacrylate)
pH-responsive
Graft copolymer
ATRP
Drug loading

ABSTRACT

The pH-responsive ethyl cellulose graft poly(2-(diethylamino) ethyl methacrylate) (EC-*g*-PDEAEMA) copolymers were synthesized through atom transfer radical polymerization (ATRP). Kinetic analysis confirmed the reaction is living and controllable. The graft copolymers can form micelles in acid aqueous medium. The critical micelle concentration (CMC) of the graft copolymers decreases with the increase of the graft length and graft density. The resultant micelles show the pH-sensitivity. The decrease in the hydrodynamic radius of the micelles at pH 6–6.9 attributes to collapse of the side PDEAEMA chains in the shell of the micelles due to the deprotonation, which was further confirmed by TEM observation. The loading and controlled release of drugs in the micelles was investigated by using rifampicin (RIF) as the model drug. It was found that the cumulant release of RIF in the buffer solution at pH 6.6 is higher than that at pH 7.4.

1. Introduction

Cellulose and its derivatives have the biocompatible and biodegradable properties and have been investigated extensively for the application in industry and pharmacy (Bontempo et al., 2006; Carlmark & Malmstrom, 2003; Gupta & Khandekar, 2003; Hinterstoisser, Akerholm, & Salmen, 2003; Li, Mai, & Ye, 2000; Meng et al., 2009; Mertoglu, Garnier, Laschewsky, Skrabania, & Storsberg, 2005; Ohya et al., 1991; Sturcova, His, Apperley, Sugiyama, & Jarvis, 2004; Tang, Gao, Fan, & Zhou, 2007; Wada, Heux, & Sugiyama, 2004). However, as one of the most abundant renewable polymers in nature, the applications of cellulose materials are still limited for the efficient green producing process and specific functions. Researches on cellulose were mainly focused on the developing new solvents for cellulose to replace rayon process (Liu & Hu, 2006; Liu, Shen, Shao, Wu, & Hu, 2001a; Swatloski, Spear, Holbrey, & Rogers, 2002; Zhang, Wu, Zhang, & He, 2005) and offering new properties to cellulose materials by synthesizing new cellulose derivatives (Loscher, Ruckstuhl, Jaworek, Wegner, & Seeger, 1998; Sannino et al., 2004; Liebert & Heinze, 2005) and graft copolymers (Gupta & Khandekar, 2003; Gupta, Sahoo, & Khandekar, 2002; Kang, Liu, Liu, & Huang, 2008; Li, Liu, & Huang, 2008; Li et al., 2008; Lönnberg et al., 2006; Ma et al., 2010; Shen & Huang, 2004; Shen, Yu, & Huang, 2005; Sui et al., 2008; Tang et al., 2007; Teramoto & Nishio, 2003; Vlcek et al., 2006, 2008; Yan et al., 2009). Among the methods to prepare cellulose graft copolymers, atomic transfer radical polymerization (ATRP) is popular for synthesizing cellulosic graft copolymer with well-defined architecture without by-produced homopolymer that is unavoidable in traditional free radical graft copolymerization (Matyjaszewski & Xia, 2001). Some of the cellulosic graft copolymers can be potentially applied for controlled release drug delivery system (Gupta et al., 2002; Lönnberg et al., 2006; Shen & Huang, 2004; Shen et al., 2005; Teramoto & Nishio, 2003; Vlcek et al., 2006). Furthermore, the specific topological molecular structure of graft copolymers offers them special properties for the promising applications in biological system, such as the immobilizing enzymes via the interaction between the polyelectrolyte copolymers and oppositely charged colloidal particles (Turner, Spear, Holbrey, & Rogers, 2004) or nonspecific association of DNA with basic proteins.(Kötz, Kosmella, & Beitz, 2001)

Stimuli-responsive polymers with environmental sensitivities, such as pH, temperature, ionic strength, or photochemical reaction, have attracted increasing interests in recent years. These stimuli-responsive polymers can be used in biological and medical fields,

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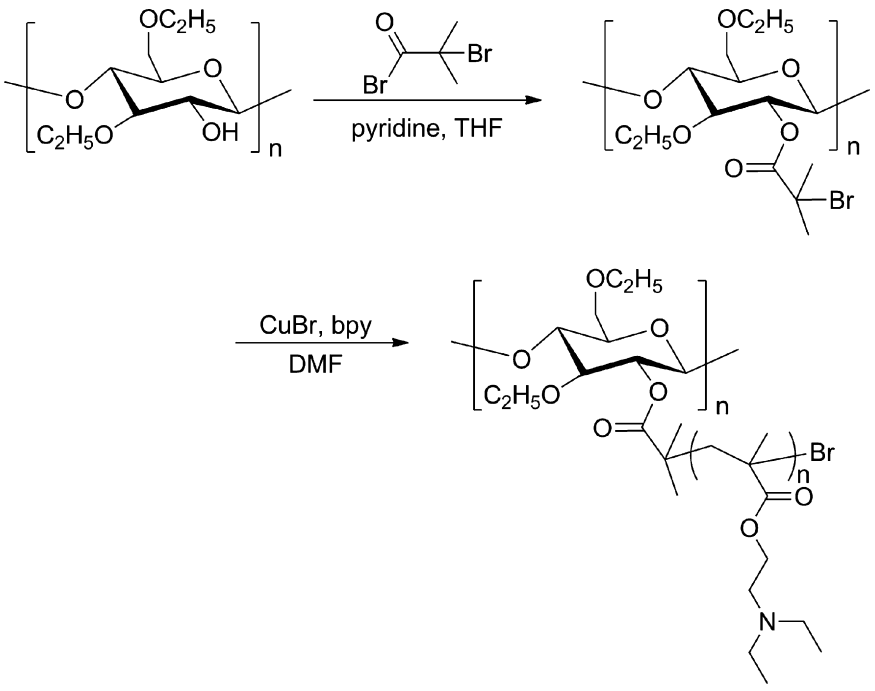
doi:10.1016/j.carbpol.2010.11.023



Scheme 1. Synthesis route for EC-*g*-PDEAEMA graft copolymers.

e.g. drug carriers and sensors (Butun, Billingham, & Armes, 1998; Butun et al., 2001; Weaver, Armes, & Butun, 2002). Compared to the popular stimuli-responsive block copolymers, the investigations on the synthesis and applications of graft copolymers with stimuli-responsive properties are still limited. Specifically, for the purpose of using cellulose graft copolymers in drug loading and controlled release system, stimuli-responsive cellulose graft copolymers are preferred. Among many candidates of the stimuli responsive side chains for cellulose graft copolymers, poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA) has the properties of pH-stimuli responsibility. In aqueous media with pH < 6.5, the tertiary amine groups on PDEAEMA chain are in protonated state and PDEAEMA is soluble. If pH > 6.5, PDEAEMA will coagulate due to the deprotonation of the tertiary amine groups (Liu, Billingham, & Armes, 2001b). Stimuli-responsive block copolymers with PDEAEMA block have been reported to self-assemble into reversible pH-responsive micelles at low-pH and high-pH solutions (Bronstein et al., 2005; Butun, Top, & Ufuklar, 2006; Cai & Armes, 2004; Dai, Ravi, Tam, Mao, & Gan, 2003; Ge et al., 2007; Gohy et al., 2000; He, Ravi, & Tam, 2007; Jiang, Luo, Armes, Shi, & Liu, 2006; Tan, Ravi, Too, Hatton, & Tam, 2005; Tan, Too, Hatton, & Tam, 2006; Xu et al., 2006; Zhang, Ni, He, & Liu, 2008). In this work, ethyl cellulose graft poly(2-(diethylamino)ethyl methacrylate) (EC-*g*-PDEAEMA) was synthesized via atom transfer radical polymerization (ATRP). The resultant EC-*g*-PDEAEMA graft copolymers have hydrophobic EC backbones and pH-responsive PDEAEMA side chains. Micelles were prepared from EC-*g*-PDEAEMA copolymers. The pH-sensitivity of the micelles was investigated by dynamic light scattering (DLS) and transmission electron microscopy. The drug loading and release properties were investigated by using rifampicin (RIF) as the model drug.

2. Experimental

2.1. Materials

Ethyl cellulose (EC) (Fluka, η = 9–11 mPa s) with the degree of substitution of ethyl groups (DS_{Et}) of 2.5 (determined by ^{1}H NMR) was dried at 35 °C under vacuum for three days before use. The monomer 2-(diethylamino) ethyl methacrylate (DEAEMA) (99%, Aldrich) was distilled under reduced pressure before use. The other chemicals, e.g. 2,2′-bipyridine (bpy, Aldrich), 2-bromoisobutylryl bromide (BrB*i*B, 98%, Aldrich) and rifampicin (RIF, 97%, Aladdin) were used as received. Copper(I) bromide (CuBr, Alfa) was stirred in the mixture of glacial acetic acid with a little amount of water to remove any soluble oxidized species, filtered, washed with acetone, and dried under vacuum at 25 °C. Dimethylformamide (DMF), tetrahydrofuran (THF) and pyridine were distilled prior to use. All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Synthesis and characterizations of EC-*g*-PDEAEMA graft copolymers

The synthesis route of EC-*g*-PDEAEMA is shown in Scheme 1. The macroinitiator for ATRP was synthesized according to the literature (Shen et al., 2005). The degree of substitution of 2-bromoisobutyryl groups (DS_{Br}) of the macroinitiator (EC-Br) was controlled by varying the molar ratios of 2-bromoisobutyryl bromide to hydroxyl groups on EC backbone. The macroinitiators with different DS_{Br} as listed in Table 1 were used for graft copolymerization.

The ATRP graft copolymerization for DEAEMA was carried out according to the following procedure. EC-Br with DS_{Br} of 0.1 (50 mg, 0.02 mmol, 1 equiv.) was dissolved in a Schlenk flask with 2 mL anhydrous DMF with 1.10 g DEAEMA (6 mmol, 300 equiv.). The flask was sealed, immersed in an ice-water bath, and purging via three freeze-thump-thaw cycles with nitrogen. Then CuBr (4.3 mg, 0.03 mmol, 1.5 equiv.) and 2,2′-bipyridine (bpy) (7.8 mg, 0.05 mmol, 2.5 equiv.) were added under nitrogen, and the reaction mixture was transferred to a water bath with a controlled temperature at 20 °C after one freeze-thump-thaw cycles with nitrogen for ATRP reaction. The reaction kinetics was investigated by determining the DEAEMA conversion at different reaction time intervals. The reaction mixture that taken out at a predetermined time was exposed to air to stop the reaction, diluted with THF, and passed through an alumina column. The solution was then condensed and

Table 1
The experimental details and information for the EC-*g*-PDEAEMA graft copolymers.

Run	DS_{Br}[a]	$n_{Br}:n_{DEAEMA}:n_{CuBr}:n_{bpy}$	Time (h)	Conv. (%)[b]	$DP_{PDEAEMA}$[b]	$DP_{PDEAEMA}$[c]	$M_{n,PDEAEMA}$[b]
1	0.1	1:50:1.5:2.5	1	5.02	15	11.9	0.28×10^4
2	0.1	1:150:1.5:2.5	1	7.03	21	13.8	0.39×10^4
3	0.1	1:300:1.5:2.5	1	11.7	35	21.6	0.65×10^4
4	0.1	1:300:1.5:2.5	2	14.7	43	25.5	0.80×10^4
5	0.1	1:300:1.5:2.5	3	20.7	58	38.7	1.07×10^4
6	0.1	1:300:1.5:2.5	4	25	75	66.3	1.39×10^4
7	0.1	1:300:1.5:2.5	5	28	85	92.4	1.57×10^4
8	0.06	1:450:1.5:2.5	4	24.1	90	81.5	1.66×10^4
9	0.2	1:200:1.5:2.5	6.5	41.7	87	73	1.59×10^4

[a] The macroinitiator EC-Br, for example, 0.1 stands for the number of initiating sites per repeating unit of cellulose. That is, there is one among ten glucose rings.
[b] Conversion yield, degree of polymerization (DP) and number average molecular weight for each PDEAEMA side chain calculated from ^{1}H NMR (see Section 2).
[c] Degree of polymerization of each PDEAEMA side chain is determined by elemental analysis.

precipitated in twice from water and freeze-dried to obtain the graft copolymers. The experimental details and the resultant EC-*g*-PDEAEMA copolymers are summarized in Table 1.

The ^{1}H NMR measurements were carried out on Bruker 400 MHz Avance NMR instrument using $CDCl_3$ as the solvent. Elemental analysis of the graft copolymer was performed on a Flash EA 1112 Elemental Analyzer. The FTIR spectra (thin casting films on KBr pallets) were recorded on a Bruker-Equinox 55 FT-TR spectrometer.

2.3. Preparation and characterizations of EC-*g*-PDEAEMA graft copolymer in aqueous solutions

EC-*g*-PDEAEMA micelles in aqueous solutions were prepared as follows. EC-*g*-PDEAEMA solutions in THF (2 mg/mL, 10 mL) were added into 20 mL aqueous solution (pH = 3.2) dropwise with continuous stirring. The resultant micelle solutions were dialyzed against water at pH 3.2 for 3 days using dialysis bag with cutoff molecular weight 3500 to remove the organic solvent THF. The out water was replaced every 12 h (Wu, Li, & Gao, 2009). This technique allowed the continuous and slow exchange of solvents, avoiding the formation of large aggregations.(Riess, 2003) The resultant micelle aqueous solutions at pH 3.2 were about 40 mL after dialysis, corresponding to micelle solutions with copolymer concentration of 0.5 mg/mL.

Fluorescence emission spectra were recorded on Perkin Elmer LS 55 fluorescence spectrometer equipped with a 20 kW Xenon discharge lamp. The excitation wavelength was 333 nm and the fluorescence emission spectra were recorded from 350 to 600 nm. The slit width was 10 nm. The concentration and loading conditions of pyrene into copolymer micelle solutions were referred to the literature (Bromberg & Barr, 1999). The intensity ratios of the first to the third vibronic peak (I_1/I_3) in the emission spectra of the monomer pyrene were used to estimate the polarity of the pyrene microenvironment (Tang, Liu, Armes, & Billingham, 2003). The steady-state fluorescence spectra of a series of copolymer micelle aqueous solutions (pH 3.2) with the copolymer concentration in the range of 5×10^{-8} to 5×10^{-2} mg/mL were measured. The pyrene concentration in all the micelle solutions is kept at 1.0×10^{-6} M. By the profile of I_1/I_3 as a function of copolymer concentration, the critical micelle concentration (CMC) was determined.

For the investigation of pH responsive behavior of the micelles, the pH value of the micelle solution was adjusted by NaOH aqueous solutions. Dynamic light scattering (DLS) experiments were carried out on the ALV/SP-150 spectrometer equipped with an ALV-5000 multi-τ digital time correlator and a solid-state laser (ADLS DPY 425II, output power ca. 400 MW at λ = 632.8 nm) as the light source. All the copolymer solutions were filtered through the Millipore Millex-FH nylon filter (0.45 μm) before DLS experiments. All measurements were carried out at the scattering angle of 90° at 25 °C. The hydrodynamic radius (R_h) was obtained by fitting the correlation function with the CONTIN program.

TEM observations were carried out on a Hitachi H-800 transmission electron microscope at an acceleration voltage of 100 kV. TEM samples were prepared by dropping a small drop of the micelle solutions on carbon-coated copper grids and air dried.

2.4. Loading of RIF into the graft copolymer micelles and its release behavior

The incorporation of RIF into graft copolymer micelles was carried out as follows. In 5 mL $EC_{0.1}$-*g*-$PDEAEMA_{60}$ THF solution with 80 mg copolymers, 5 mL RIF THF solution (4 mg/mL) was added and stirred for half an hour. The mixed solution was then added dropwise into 20 mL Milli-Q water, and stirred for another 2 h. The resultant micelle solution was then dialyzed (cutoff molecular weight, 14,000) against Milli-Q aqueous solution (replaced every 12 h) for 24 h to remove the organic solvent THF. The resultant RIF loaded micelle was adjusted to 40 mL after dialysis to result micelle solutions with copolymer concentration of 2 mg/mL. The hydrodynamic radius (R_h) distribution of RIF loaded micelles was determined by dynamic light scattering (DLS).

The loading efficiency (E_L) and RIF content (c_p) in the micelles were estimated by using following equations (Riley et al., 1999):

$$E_L\,(\text{wt.\%}) = \frac{w_p}{w_t} \times 100 \quad (1)$$

$$c_p\,(\text{wt.\%}) = \frac{w_p}{w_m} \times 100 \quad (2)$$

where w_p, w_t, and w_m are the mass of RIF in micelles, total RIF that used, and micelles, respectively. The loading efficiency and RIF content were determined as follows. A certain volume RIF loaded micelle solution was diluted to a certain volume with THF, which makes RIF and graft copolymer dissolved completely. The mass concentration of RIF was estimated by comparing the absorption intensity at λ = 474 nm on a Shimadzu UV-1601PC spectrophotometer, and then the mass of RIF in micelles (w_p) was calculated by multiplying the mass concentration by volume. A calibration curve of the absorption intensity at λ = 474 nm as a function of RIF content in THF in the linear range was used as the multipoint working curve. The $EC_{0.1}$-*g*-$PDEAEMA_{60}$ graft copolymer and the solvent THF had no obviously contribution to the UV–vis spectra and the background was subtracted.

The release behavior of the loading RIF from the micelles was carried out as follows. A dialysis bag (molecular weight cutoff, 14,000) containing 4 mL of the RIF loaded micelle aqueous solution was immersed in 200 mL phosphate buffer solution (PBS) (pH = 6.6 and 7.4) at 37 °C in a beaker with constant stirring, by which the RIF could diffuse from the solution in the dialysis bag to the buffer solution. The copolymer concentration is fixed at 2 mg/mL. 2.5 mL phosphate buffer solution containing RIF was taken from the beaker at a certain time intervals. The concentration of RIF phosphate

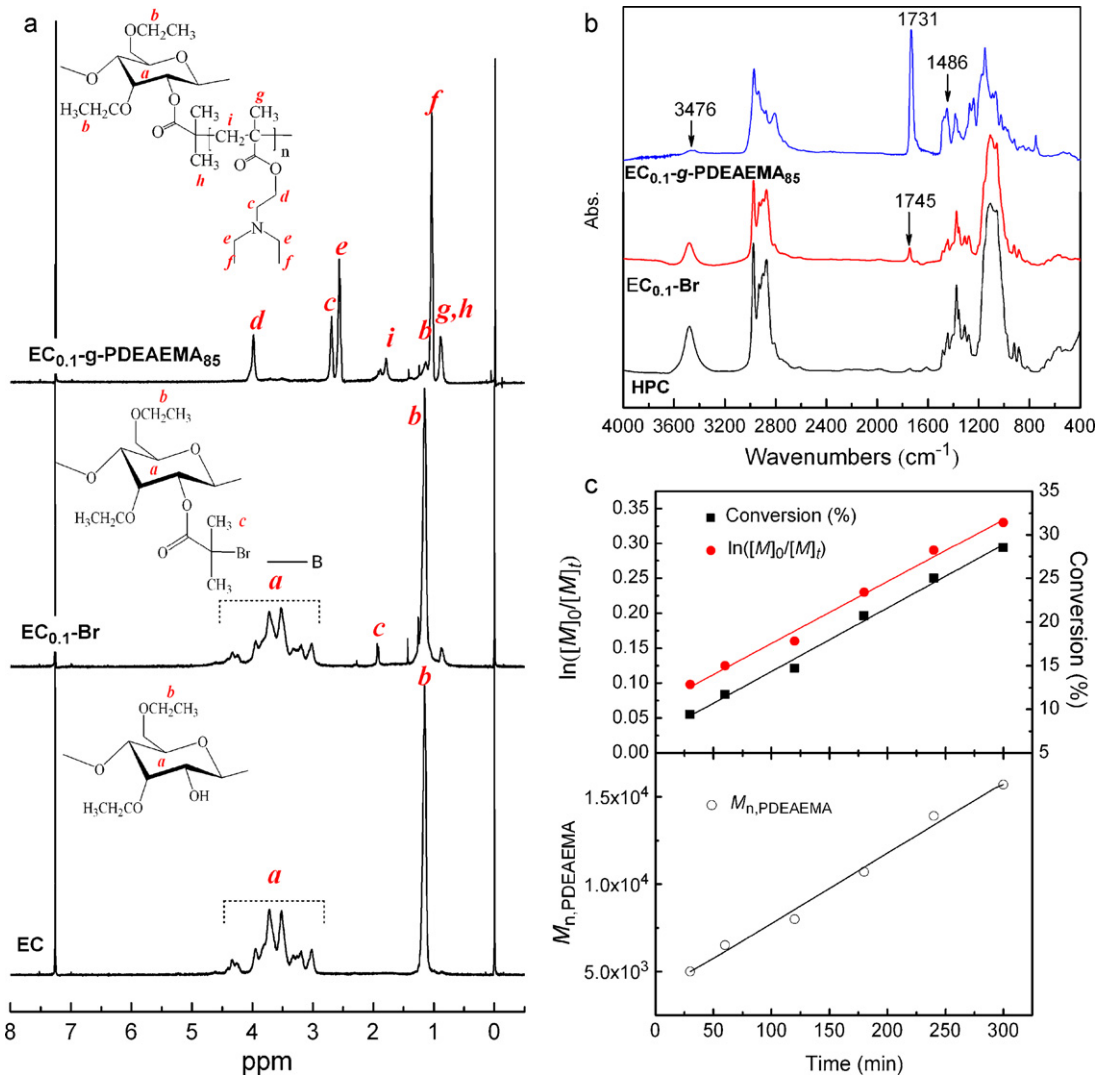


Fig. 1. ^{1}H NMR (a) and FTIR (b) spectra of EC, $EC_{0.1}$-Br, and $EC_{0.1}$-*g*-$PDEAEMA_{85}$ in $CDCl_3$ at 400 MHz. (c) Conversion of monomer, $\ln([M]_0/[M]_t)$, and M_n of PDEAEMA side chain versus reaction time for the ATRP of DEAEMA initiated by EC-Br (DS_{Br} = 0.1). The feeding ratio n_{Br}:n_{DEAEMA}:n_{CuBr}:n_{bpy} = 1:300:1.5:2.5.

buffer solution at different time intervals was determined by the UV–vis spectra.

3. Results and discussion

3.1. Synthesis of EC-g-PDEAEMA copolymers

The synthesis route of EC-*g*-PDEAEMA copolymers is shown in Scheme 1. The first step was to synthesize the macroinitiator EC-Br with desirable substitution degree of bromoisobutyryl groups according to the previous work (Shen et al., 2005). The successive synthesis of EC-Br macroinitiator was confirmed by ^{1}H NMR and FTIR spectra. A new peak appears at chemical shift of δ = 1.8–2.1 ppm on ^{1}H NMR spectrum of EC-Br besides the typical peaks from protons of EC backbones at δ = 1.05–1.3 and 2.6–5.0 ppm (Fig. 1a). This peak comes from the methyl protons of bromoisobutyryl groups. Moreover, on the FTIR spectrum of EC-Br, the new absorption peak appears at 1745 cm^{-1}, corresponding to the C=O stretching vibration band (ν(C=O)) of the graft bromoisobutyryl groups. Meanwhile, the absorption peak attributes to the –OH groups around 3400 cm^{-1} becomes relatively weaker, which indicates that part of the –OH groups have reacted with 2-bromoisobutyryl bromide. Both ^{1}H NMR and FTIR spectra confirmed the success of the initiating groups for ATRP. The degree of substitution of bromoisobutyryl groups (DS_{Br}) of the macroinitiator can be estimated by $DS_{Br} = (12.5A_1/A_2)/(6 + A_1/A_2)$, where 12.5 is all the hydrogen numbers of per anhydroglucopyranose unit, 6 is the methyl hydrogen numbers of 2-bromoisobutyryl groups, A_1 and A_2 are the integrated areas of the methyl hydrogen of 2-bromoisobutyryl at around δ = 1.8–2.1 ppm and all the hydrogen of EC backbones at δ = 2.6–5.0 ppm on ^{1}H NMR spectra, respectively. The DS_{Br} of the macroinitiators that used for the ATRP graft is listed in Table 1.

In the synthesis of EC-*g*-PDEAEMA graft copolymers, the feeding molar ratios of the macroinitiator, catalyst CuBr, and the ligand bpy are listed in Table 1. The graft copolymerization was taken in anhydrous DMF at the reaction temperature of 20 °C. The results show that the side chain length can be tailored by varying the feeding ratio and the reaction time. The success of the synthesis of EC-*g*-PDEAEMA graft copolymers was confirmed by ^{1}H NMR and FTIR spectra. On the ^{1}H NMR spectra of resultant EC-*g*-PDEAEMA copolymer (Fig. 1a), the new peaks at δ = 3.99 (s, 2H), 2.7 (s, 2H), 2.57 (s, 4H), 1.8-2.1 (m, 2H), 1.15 (s, 6H), 0.9 (s, 2H) come from the protons of the PDEAEMA side chains. The characteristic C–N stretching vibration band (ν(C–N)) at 1486 cm^{-1} appears on the FTIR spectrum of the EC-*g*-PDEAEMA copolymer (Fig. 1b). Moreover, the

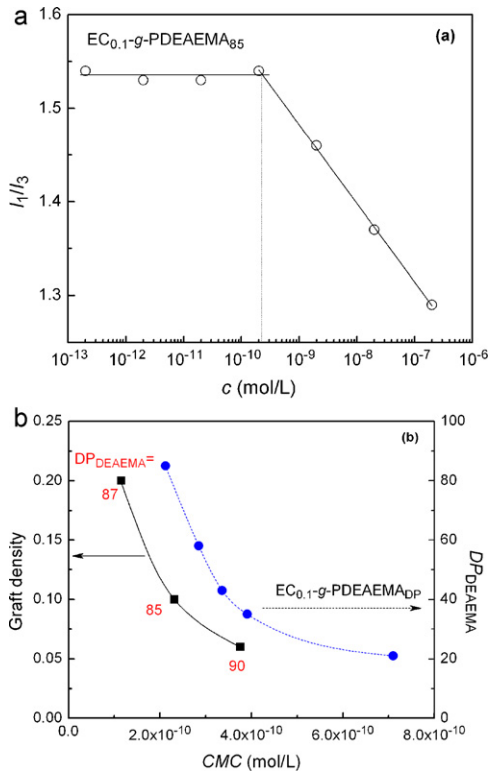


Fig. 2. Typical profile of I_1/I_3 as a function of the copolymer concentration for the estimation of CMC (a) and the dependence of CMC on the length of the side PDEAEMA chains and graft density (b). The pH of the solution is pH 3.2 and λ_{ex} = 333 nm.

C=O stretching vibration band (ν(C=O)) is stronger, and shifted from 1745 cm^{-1} to 1731 cm^{-1}, which mainly comes from the C=O groups of the PDEAEMA side chains. The even weak of the hydroxyl groups on EC backbones at around 3476 cm^{-1} also indicates the lower hydroxyl group content in the graft copolymers (Fig. 1b). The kinetic results of the graft copolymerization show the linear relationship of $\ln([M_0]/[M_t])$, conversion and $M_{n,PDEAEMA}$ versus the reaction time t, which indicate that the graft copolymerization is a first-order kinetics reaction and the graft copolymerization is living and controllable (Fig. 1c). GPC analysis was tried and it was found to be unreliable in the characterization of the copolymers due to their too high molecular weights. We have attempted to hydrolyze the EC-*g*-PDMAEMA to cleave PDMAEMA from cellulose backbone. However, it was unsuccessful due to that the ester bonds of the linkage between the PDMAEMA side chains and the EC backbones and those on the PDMAEMA side chains can be hydrolyzed.

The degree of polymerization (DP) of side PDEAEMA chains was estimated by ^{1}H NMR spectra by $DP = (A_4 DS_{Et})/(2A_3 DS_{Br})$, where 2 is the methylene hydrogen at δ = 3.99 numbers connect with –O– per monomer, A_3 and A_4 are the integrated areas of the methyl protons on EC backbone at δ = 1.15 and methylene protons connect with –O– of the PDEAEMA side chains at δ = 3.99 on the ^{1}H NMR spectra, respectively. The details of the polymerization of the side chains are listed in Table 1.

3.2. Micellization and pH-responsive behaviors of the EC-g-PDEAEMA copolymers in aqueous solution

Amphiphilic copolymers with a suitable hydrophobic/hydrophilic components can self-assemble into micelle structure in selective solvents (Wu et al., 2009). In present work, EC-*g*-PDEAEMA copolymers have the hydrophobic backbones and the hydrophilic side PDEAEMA chains. Moreover, the hydrophilic PDEAEMA side chain is a weak polyelectrolyte, and becomes hydrophobic at pH > 7.0. The micellization behavior of EC-*g*-PDEAEMA copolymers was investigated by using pyrene as fluorescent probe. Pyrene is the most frequently used dye in fluorescence studies of labeled polymers (Winnik, 1993) and has been widely used in the studies on amphiphilic polymers, polymer chain dynamics, polymer/particle interfaces, polymer films, polymer gels, and biological samples (Duhamel, 2005). The critical micelle concentration (CMC) of block copolymers in aqueous phase could be determined by pyrene excitation spectra (Kim et al., 2000; Wilhelm et al., 1991). The state fluorescence spectrum of pyrene has five characteristic peaks, the variation in the intensity ratio of the first I_1 (373 nm) to the third vibrational peak I_3 (383 nm) is quite sensitive to the polarity of microenvironment where pyrene is located. Fig. 2a shows the typical profile of I_1/I_3 as a function of copolymer concentration of the EC-*g*-PDEAEMA copolymers in aqueous solutions at pH 3.2. The abrupt decrease of I_1/I_3 corresponds to the critical micelle concentration (CMC). The results indicate that the CMC depends on both the length of the side PDEAEMA chain and the graft density. At the fixed graft density of 0.1, that is, one graft chain per ten glucose rings, the CMC decreases with the increasing of side chain length (Fig. 2b). For the graft copolymers with the similar length of the side PDEAEMA chains, the CMC of the copolymer decreases with the increasing of graft density. The decrease in CMC with the increasing of graft density and side chain length attributes to the higher content of

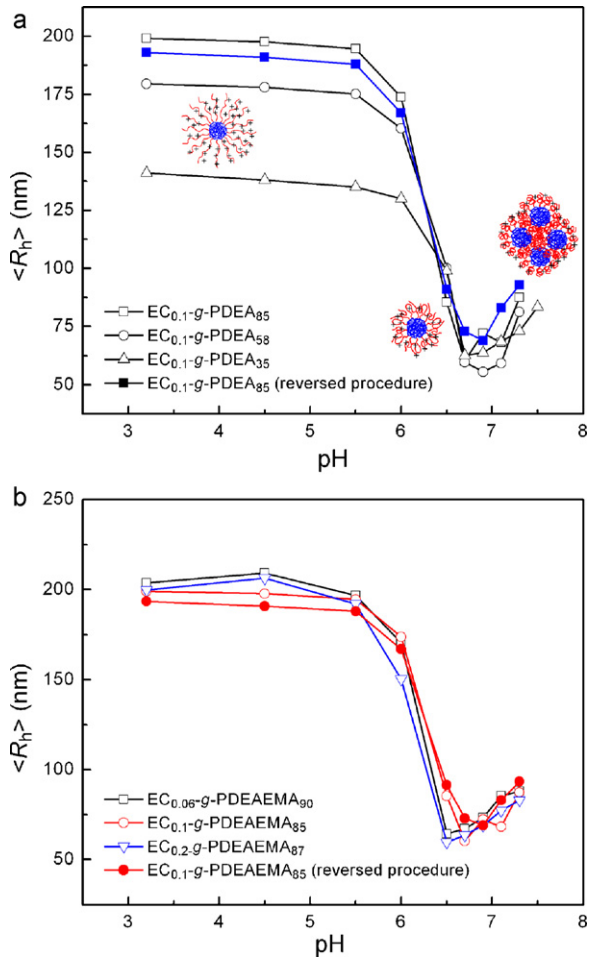


Fig. 3. The dependence of hydrodynamic radius ($\langle R_h \rangle$) as a function of pH values of graft copolymer with different side chain length (a) and graft density (b). All the DLS measurements were carried out at the scattering angle of 90° at the temperature of 25 °C. The copolymer concentration in all the solutions was kept at c = 0.5 mg/mL.

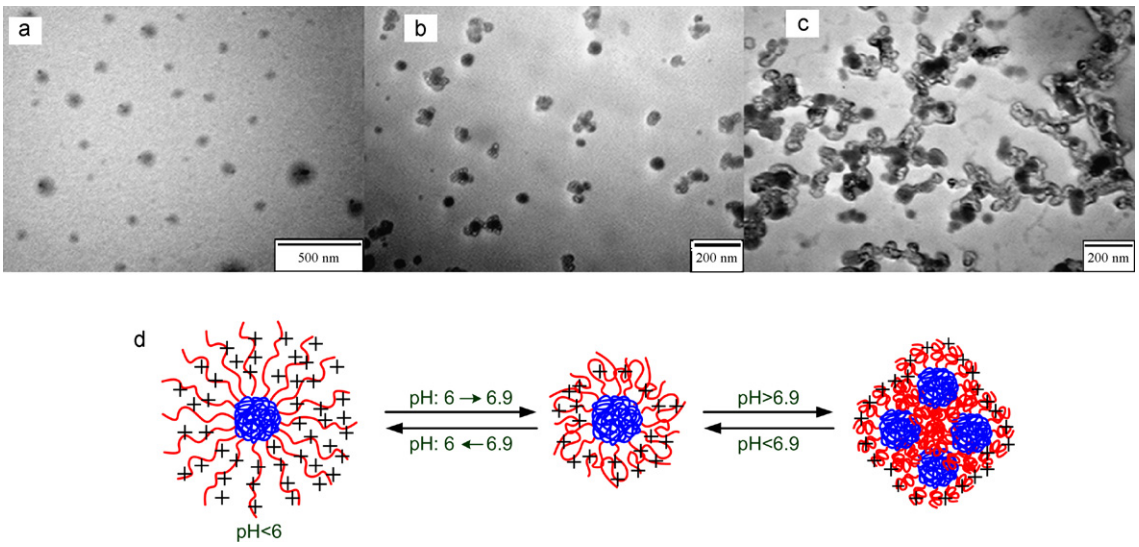


Fig. 4. TEM images of graft copolymer $EC_{0.1}$-*g*-$PDEAEMA_{85}$ micelle nanoparticles at pH of (a) 3.2, (b) 6.5, and (c) pH 7.3 at $c = 0.1$ mg/mL. (d) The schematically mechanism of the pH-responsive micelles prepared from EC-*g*-PDEAEMA copolymers.

hydrophilic component in the copolymers resulted in the fewer molecules to form stable micelles. Because the EC backbones are hydrophobic and the side PDEAEMA chains are hydrophilic, the EC backbones aggregate to form the core of the micelles that stabilized with PDEAEMA in the shell.

Fig. 3 shows the $\langle R_h \rangle$ of the EC-*g*-PDEAEMA micelles prepared from the copolymers as a function of pH values. The results show that at the pH value below 6.0, the $\langle R_h \rangle$ of the micelles keeps at a constant value for the micelles prepared from EC-*g*-PDEAEMA graft copolymers with different graft length and graft density. The micelles begin to shrink abruptly at around pH 6.5, which is due to the collapse of the deprotonated PDEAEMA side chains in the shell of the micelles. Further increase in pH (pH > 6.9) of the micelle solutions leads to the increase in $\langle R_h \rangle$, which attributes to the aggregation of the collapsed micelles (Dan & Tirrell, 1993; Wittmer & Joanny, 1993). Moreover, the pH influence on the $\langle R_h \rangle$ is a reversible procedure, and the micelles could be recovered when the pH of the micelle solution was adjusted back to the pH below 6.5 as shown by the solid symbols in Fig. 3.

Fig. 4 shows the TEM images of the micelles prepared from $EC_{0.1}$-*g*-$PDEAEMA_{85}$ at different pH 3.2, 6.5 and 7.3. The results indicate that the as prepared micelles have a spherical morphology with core-shell structure (Fig. 4a). The diameter of the micelles is smaller than that estimated by DLS, which is due to that the TEM samples were observed in their dried state and the micelles should be shrunk during the drying procedure. The diameter of the micelles prepared at pH 6.5 is obviously smaller than that of the as prepared micelles (Fig. 4b), and aggregation of micelles can be obviously observed at pH 7.3 (Fig. 4c).

The pH-responsive behavior of the micelles prepared from the EC-*g*-PDEAEMA copolymers can be illustrated in Fig. 4d. At the pH < 6, the micelles keep unchanged with different pH values due to the protonation of the PDEAEMA chains in the shell of the micelles. The PDEAEMA chains on the shell of the micelles are collapsed in the pH range of 6.0–6.9, and start to aggregate at pH > 6.9.

Table 2
Effect of added RIF total mass on the loading efficiency and RIF content of $EC_{0.1}$-*g*-$PDEAEMA_{60}$ graft copolymer micelles.

m(RIF):*m*($EC_{0.1}$-*g*-$PDEAEMA_{60}$)	Loading efficiency (E_L) (%)	RIF content (c_p) (%)
0.1:1	31.2	3.1
0.2:1	36.9	7.4
0.25:1	54.1	13.4
0.4:1	58.4	23.4

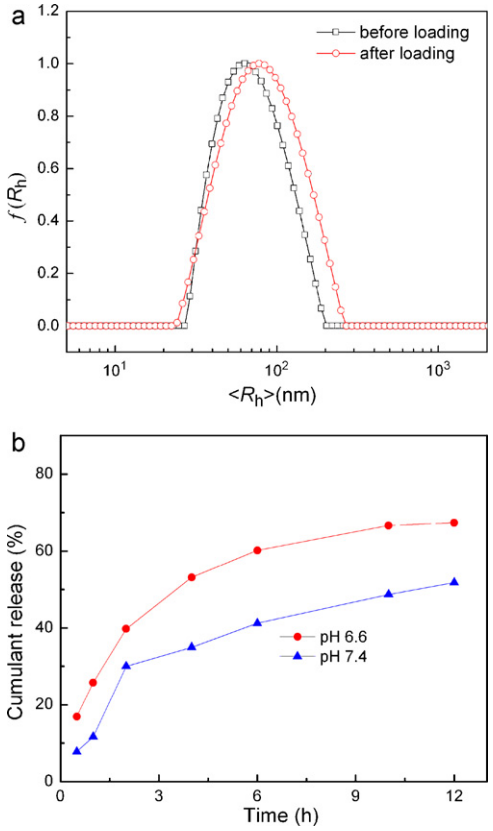


Fig. 5. Distribution of hydrodynamic radius of the micelles prepared from EC-*g*-PDEAEMA graft copolymers before and after loading RIF (a), and comparison of RIF release profile from graft copolymer micelle in phosphate buffer pH (6.6 and 7.4) (b).

3.3. Loading and pH-responsive release behavior of rif in graft copolymer micelles

In previous work, it has been reported to use graft copolymer for drug loading and controlled release (Opanasopit, Ngawhirunpat, Rojanarata, Choochottiros, & Chirachanchai, 2007; Tan et al., 2010). In present work, $EC_{0.1}$-*g*-$PPEGMA_{60}$ was selected to investigate the loading and controlled release of drugs in the prepared micelles using RIF as the model drug. The release behaviors were investigated in the buffer solutions at pH 6.6 and 7.4. RIF was selected due to that it is a drug with a poor water-solubility and has a powerful anti-tubercular action. The relationships of the loading efficiency and RIF content in the micelles with different feeding ratios of the RIF and micelles are listed in Table 2. The results indicate that the loading efficiency and RIF content increase with the increase of m(RIF):m($EC_{0.1}$-*g*-$PDEAEMA_{60}$). This is due to that there are the interaction of the N atoms in side PDEAEMA chains and the –OH groups in RIF, which enhanced the increase of the loading efficiency and content of RIF in the graft copolymer micelles (Manca, Loy, Zaru, Fadda, & Antimisiaris, 2008). The $\langle R_h \rangle$ of the RIF loaded micelles was determined by DLS and compared with unloaded micelles as shown in Fig. 5a. The results show that the $\langle R_h \rangle$ of the RIF loaded micelles is slightly increased, which also confirmed the success of the drug loading in the micelles. The release of the RIF from the micelles to the buffer solutions at pH 6.6 and 7.4, and the results are shown in Fig. 5b. The results show that resultant micelles from the graft copolymer have a controlled release behavior within 12 h. Moreover, the cumulant release of RIF in the buffer solution at pH 6.6 is higher than that at pH 7.4. This is due to the micelles in the weak acidic media is more stable, whereas the micelles aggregate at pH 7.4 as discussed above, which somewhat block the release of the RIF from the micelles.

4. Conclusion

EC-*g*-PDEAEMA graft copolymers with different graft length and graft density were synthesized by atomic transfer radical polymerization (ATRP). The graft copolymerization is living and controllable. The EC-*g*-PDEAEMA graft copolymers can self-assemble into micelles in acidic aqueous solution. The critical micelle concentration (CMC) decreases with the increase of graft density at the similar side chain length, and also with the increase of the side chain length at the same graft density. The hydrodynamic radius ($\langle R_h \rangle$) of the resultant micelles increases with the increasing of side chain length and is independent on the graft density at the similar side chain length that investigated. The micelles prepared from the EC-*g*-PDEAEMA graft copolymers have the reversible pH sensitivity. The micelles start to shrink at pH 6.0 and aggregate at pH > 6.9. The loaded RIF graft copolymer micelles show a controlled and pH responsive release in drug delivery systems.

Acknowledgements

The financial support of the National Natural Science Foundation of China (Grant Nos. 20974114, 20774105, and 50821062) and the Knowledge Innovation Program of the Chinese Academy of Sciences (Grant No. KJCX2-YW-H19) is greatly appreciated.

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