

pH-responsive amphiphilic H-shaped supramolecular copolymer via the inclusion complexation between β -cyclodextrin and adamantane

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Abstract pH-responsive amphiphilic H-shaped copolymer was prepared by the supramolecular self-assembly between β -cyclodextrin-*graft*-poly(2-(*N,N*-diethyl-amino)ethyl methacrylate) (β -CD-(PDEAEMA)₂) and bi-adamantyl terminated poly(ϵ -caprolactone) (Ad-PCL-Ad). β -CD-(PDEAEMA)₂ was synthesized by click reaction of *alkynyl*-modified β -CD with azide PDEAEMA (PDEAEMA-N₃). Ad-PCL-Ad was synthesized by the DCC reaction of bi-hydroxyl terminated PCL (HO-PCL-OH) with adamantaneacetic acid. The supramolecular copolymer can self-assemble into micelles in water at room temperature. The micellization and pH-responsivity of the amphiphilic copolymer solution were investigated by transmittance, dynamic light scattering spectrophotometer, and transmission electron microscopy in water. Investigation shows that the micelles' sizes can be adjusted through the alteration of the pH values of solutions and the supramolecular copolymer will have the potential applications in biomedical field.

Keywords Supramolecular assembly · pH-responsive · Amphiphilic copolymer · Biomaterials · Polymers

Introduction

Stimuli-responsive polymers exhibit reversible property changes in response to changes in environmental factors such as pH [1–4] or temperature [5–7], and have

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attracted considerable attention due to their wide applications in nanotechnology and biotechnology fields. Among these polymers, poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) was usually used as pH-responsive segments in the functional copolymers for biomedical applications [8, 9]. The pK of PDEAEMA is about 7.2. Above its pK , PDEAEMA is hydrophobic as its amine groups are deprotonated. Below its pK , PDEAEMA becomes a cationic hydrophilic polymer as its amine groups are protonated.

Cyclodextrins (CDs) are a series of natural cyclic oligosaccharides that consist of 6, 7, or 8 D-(+)-glucose units held together by 1,4-linkages, namely α -, β -, or γ -CD. The geometry of CDs is like a hollow truncated cone form with a hydrophobic inner cavity of depth around 7 Å, and internal diameter of 4.5, 7.0, and 8.5 Å with increasing sugar units, and various molecules can be fitted into the cavities to form supramolecular inclusion complexes, which have induced a lot of interesting development of supramolecular systems for biomedical applications [10–12]. Herein, β -CD-*graft*-PDEAEMA and bi-adamantyl terminated poly(ϵ -caprolactone) (Ad-PCL-Ad) can form supramolecular self-assembly through host–guest complexation.

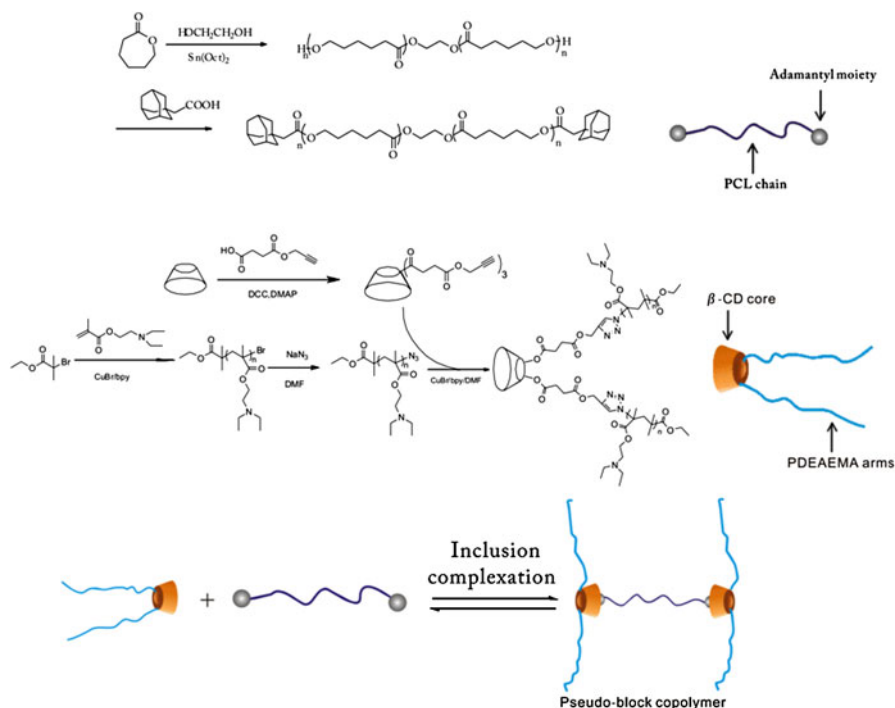
Click chemistry [13] has received considerable attention as a facile, highly efficient, and stereoselective reaction and was used to prepare polymeric materials with well-defined structure [14, 15]. Therefore, β -CD-*graft*-PDEAEMA can be synthesized by click chemistry of azide PDEAEMA (PDEAEMA- N_3) and *alkynyl*-modified β -CD (*alkynyl*- β -CD) easily.

This paper presents the preparation of amphiphilic H-shaped supramolecular copolymer (PDEAEMA)₂- β -CD/Ad-PCL-Ad/ β -CD-(PDEAEMA)₂ via inclusion complexation of β -CD-*graft*-PDEAEMA (β -CD-(PDEAEMA)₂) and Ad-PCL-Ad (Scheme 1). The self-assembly and pH-responsive properties of the amphiphilic copolymer in water were investigated. The pH-responsive H-shaped supramolecular copolymer has potential applications in biomedical field.

Experimental

Materials

β -Cyclodextrin (β -CD, Aldrich) was dried 100 °C for 48 h under vacuum after recrystallization from water before use. CuBr and 2,2'-bipyridine (bpy) were recrystallized and dried under vacuum. ϵ -Caprolactone (CL) (Acros Organic, 99 %) was purified with CaH₂ by vacuum distillation. Ethylene glycol (Acros Organic) was dried over CaO and distilled under reduced pressure. Tin 2-ethylhexanoate [Sn(Oct)₂, Aldrich] was used without further purification. 2-(*N,N*-diethylamino)ethyl methacrylate (DEAEMA, Acros Organic) was dried over CaH₂ and distilled under reduced pressure. Adamantaneacetic acid (Aldrich), dicyclohexylcarbodiimide (DCC, Alfa Aesar), 4-dimethylaminopyridine (DMAP, Fluka), ethyl 2-bromoisobutyrate (Aldrich), pentamethyldiethylenetriamine (PMDETA, Acros Organic) and sodium azide (NaN₃, Alfa Aesar) were used as received. Propargyl 3-carboxylic-propanoate was prepared according to the literature [16].



Scheme 1 Synthesis of the amphiphilic H-shaped supramolecular copolymer

Synthesis of HO-PCL-OH

HO-PCL-OH was synthesized from the ring-opening polymerization (ROP) of CL with ethylene glycol as initiator. A typical procedure was as follows. CL (21.04 g, 184 mmol), ethylene glycol (0.143 g, 2.3 mmol), and $\text{Sn}(\text{Oct})_2$ (131 μL) were added into a 50-mL round-bottom flask. The flask was degassed with three freeze–evacuate–thaw cycles. The flask was put into an oil bath at 120 °C under argon atmosphere with stirring and cooled to room temperature after polymerization for 24 h. The resulting product was dissolved in chloroform and precipitated three times in methanol. The purified polymer was dried in vacuum at room temperature. Yield: 14.2 g, $\eta = 67\%$ ($M_n = 7945$, $M_w/M_n = 1.05$).

Synthesis of Ad-PCL-Ad

The dried HO-PCL-OH (5.327 g, 0.65 mmol), adamantaneacetic acid (0.785 g, 3.9 mmol), DCC (0.805 g, 3.9 mmol), DMAP (0.262 g, 1.95 mmol), and CH_2Cl_2 (10 mL) were added into a 25-mL round-bottom flask. The reaction was carried out at room temperature for 36 h under argon atmosphere with stirring. The reaction byproduct dicyclohexylcarbodiurea (DCU) was removed by filtration, and the solution was washed with aqueous NaHCO_3 solution and deionized water. The organic layer was dried over anhydrous MgSO_4 overnight. The solution was

concentrated and then precipitated in methanol. The sediments were collected and dried in vacuum at room temperature. Yield: 3.9 g, $\eta = 72\%$.

Synthesis of alkynyl-modified β -CD

Alkynyl- β -CD was prepared by the DCC reaction between β -CD and propargyl 3-carboxylic-propanoate. The typical procure was as follows. 20 mL of anhydrous DMF, β -CD (0.91 g, 0.8 mmol), propargyl 3-carboxylic-propanoate (1 g, 6.4 mmol), DCC (1.32 g, 6.4 mmol) and DMAP (0.43 g, 3.2 mmol) were added into a 50-mL round-bottom flask. The reaction was carried out for 36 h at room temperature under stirring. The reaction byproduct DCU was removed by filtration. The solution was concentrated on a rotary evaporator and then precipitated in *n*-hexane. The sediments were collected and then dried in vacuum at room temperature. Yield: 0.82 g, $\eta = 80\%$.

Synthesis of PDEAEMA-Br

A dried 50-mL round-bottom flask with a magnetic stirrer was charged with CuBr (0.46 g, 3.24 mmol), bpy (1.01 g, 6.48 mmol), ethyl 2-bromoisobutyrate (0.63 g, 3.23 mmol), DEAEMA (15 g, 81 mmol), and methanol (10 mL). The flask was degassed with three freeze–evacuate–thaw cycles. Then, the polymerization was performed at 40 °C for 3 h. After being cooled to room temperature, the reaction flask was open to air, and the crude product was diluted with methanol and passed through a neutral alumina column to remove the copper catalysts. The eluent was concentrated and precipitated in *n*-hexane. The product was collected and then dried in vacuum at room temperature. Yield: 13.44 g, $\eta = 86\%$ ($M_n = 3862$, $M_w/M_n = 1.05$).

Synthesis of azide-modified PDEAEMA

Azide-modified PDEAEMA (PDEAEMA- N_3) was synthesized by the reaction of PDEAEMA-Br and excess NaN_3 . A typical procure was as follows. NaN_3 (5.20 g, 80 mmol), PDEAEMA-Br (7.77 g, 2 mmol), and 50 mL of DMF were charged in a 100-mL round-bottomed flask. The reaction mixture was allowed to stir at 45 °C for 24 h. After all solvents were removed on a rotary evaporator, the residues were dissolved in THF and passed through a neutral alumina column to remove residual sodium salts. The eluent was concentrated and then precipitated in *n*-hexane. The sediments were collected and dried in a vacuum at room temperature. Yield: 5.32 g, $\eta = 77\%$.

Synthesis of β -CD-(PDEAEMA)₂

β -CD-(PDEAEMA)₂ was obtained by the click chemistry between alkynyl- β -CD with an excess of PDEAEMA- N_3 . The typical procedure was as follows. Alkynyl- β -CD (0.040 g, 0.035 mmol), PDEAEMA-Br (0.54 g, 0.14 mmol), CuBr (0.015 g, 0.10 mmol), bpy (0.033 g, 0.21 mmol), and anhydrous DMF (10 mL) were added

into a 25-mL round-bottom flask. The reaction was carried out for 48 h at 70 °C under stirring. After being cooled to room temperature, the reaction flask was open to air, and the crude product was diluted with ethanol and passed through a neutral alumina column to remove the copper catalysts. Then, the filtered solution was purified by dialysis (molecular weight cut-off 5,000 Da) against water. Water was removed by azeotropic distillation with ethanol and the product was dried in vacuum at room temperature. Yield: 0.31 g, $\eta = 84\%$ ($M_n = 7198$, $M_w/M_n = 1.03$).

Supramolecular self-assembly between Ad-PCL-Ad and β -CD-(PDEAEMA)₂

Ad-PCL-Ad and β -CD-(PDEAEMA)₂ were dissolved in DMF and the solution was dialyzed against water with dialysis membrane with 8,000–14,000 molecular weight cut-off at 25 °C. Then, the resulting copolymer was obtained by freeze-dried method.

Self-assembly behavior of the amphiphilic supramolecular copolymer

(PDEAEMA)₂- β -CD/Ad-PCL-Ad/ β -CD-(PDEAEMA)₂ copolymer (1 mg) was dissolved in DMF (10 mL) and the solution was dialyzed against water with dialysis membrane with 8,000–14,000 molecular weight cut-off at 25 °C.

Measurements

¹H NMR data were obtained by a Bruker DMX-500 NMR spectrometer with CDCl₃ or DMSO-*d*₆ as solvent. ATR FT-IR spectra of the samples were recorded on an AVATAR 360 ESP FT-IR spectrometer equipped with a single reflection ATR system. The transmittances of copolymer aqueous solutions at various pH were measured at a wavelength of 500 nm on a UV–visible spectrophotometer. The hydrodynamic diameters (*D*_h) of the copolymer micelles were determined using a dynamic light scattering spectrophotometer (DLS). Samples for transmission electron microscopy (TEM) images were taken on an H-600 transmission electron microscope (Hitachi, Japan) operating at 120 kV.

Results and discussion

As shown in Fig. 1a, the peak at 3.66 ppm (g) should be assigned to the methylene protons next to the terminal hydroxyl groups of OH–PCL–OH. After DCC reaction with adamantaneacetic acid, the peak disappeared, and three new peaks at 1.97, 2.06, and 2.18 ppm assigned to adamantyl group appeared (Fig. 1b). This indicated the successful preparation of Ad-PCL-Ad. Figure 2 shows the ¹H NMR spectra of the alkynyl- β -CD and pure β -CD. By comparing the integration of the signals for the 1-positioned protons (–C(1)H, around 4.65 ppm) to those remaining hydroxyl protons of β -CD (O(2)H, O(3)H), around 5.81 ppm and O(6)H around 4.30 ppm), the average number of alkynyl groups grafted to β -CD was estimated to be about 3.

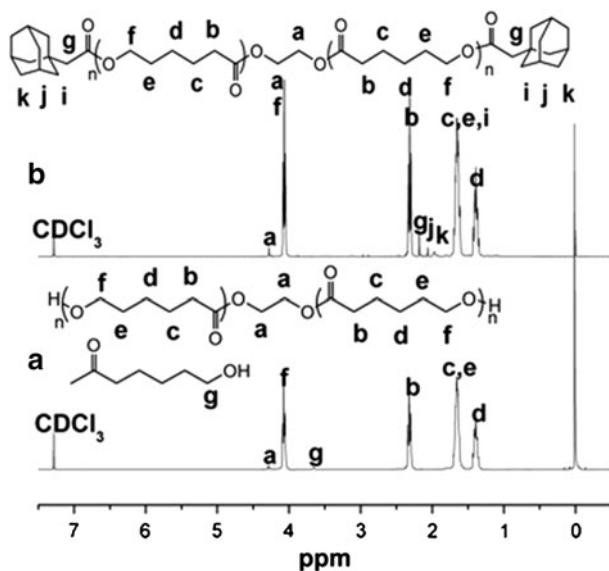


Fig. 1 ^1H NMR spectra of **a** HO-PCL-OH and **b** Ad-PCL-Ad in CDCl_3

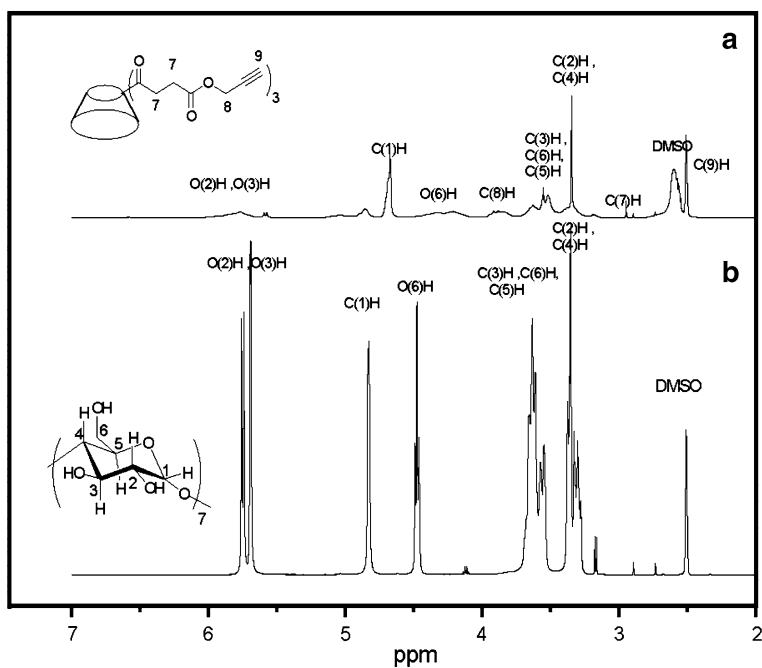


Fig. 2 ^1H NMR spectra of **a** β -CD and **b** β -CD-(PDEAEMA) $_2$ in $\text{DMSO}-d_6$

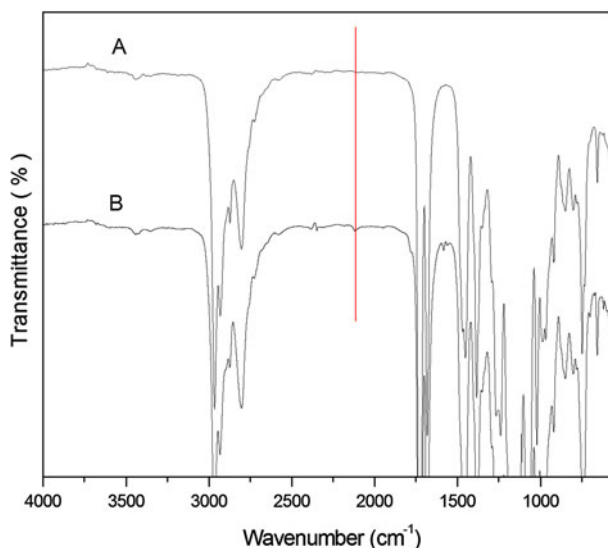


Fig. 3 FTIR spectrum of **a** PDEAEMA and **b** azide-modified PDEAEMA

From the ATR FT-IR spectra (Fig. 3), a peak at 2,120/cm can be observed. It is the characteristic peak of azide group absorbance in PDEAEMA- N_3 . Due to the weak signal intensity of protons of β -CD in graft polymer, it is difficult to confirm the obtaining of β -CD-*graft*-PDEAEMA after click reaction by 1H NMR spectrum. However, the molecular weights measured by GPC obviously increased from 3,800 to 7,500 g/mol, indicating the successful preparation of β -CD-*graft*-PDEAEMA (Fig. 4). And average two PDEAEMA chains were grafted to β -CD (β -CD-(PDEAEMA) $_2$). The ROESY NMR measurement was used to investigate the inclusion complex of β -CD-(PDEAEMA) $_2$ with the Ad-PCL-Ad (Fig. 5). It was found that the three signals of the methylene (Ha, Hc) and methine (Hb) protons of the adamantyl group were correlating well with the inner protons C(3)H or C(5)H of β -CD, indicating that the supramolecular copolymer was obtained successfully.

(PDEAEMA) $_2$ - β -CD/Ad-PCL-Ad/ β -CD-(PDEAEMA) $_2$ amphiphilic H-shaped copolymer can self-assemble to micelles in water at low pH. In this condition, PDEAEMA is hydrophobic as its amine groups are deprotonated. The hydrophilic PDEAEMA arms are mainly in the corona of the micelles, while the hydrophobic PCL is mainly in the core of the micelles. However, when the solution pH rises, PDEAEMA becomes a cationic hydrophilic polymer as its amine groups are protonated. This will lead the micelles to shrink (Scheme 2).

Figure 6 shows the transmittance curves of the micelles solutions. It can be seen that the transmittance decreased with the increase of pH values, because the deprotonation of amino groups of PDEAEMA occurred when pH values increased, and the PDEAEMA conformation changed from an expanding shape to a compact coil. The micelles shrunk and tended to aggregate, which led to the decrease of transmittance.

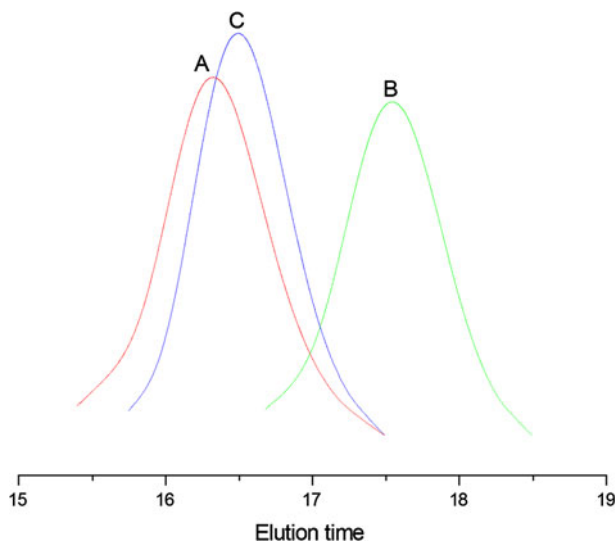


Fig. 4 GPC trace of **a** PCL, **b** PDEAEMA and **c** β -CD-(PDEAEMA)

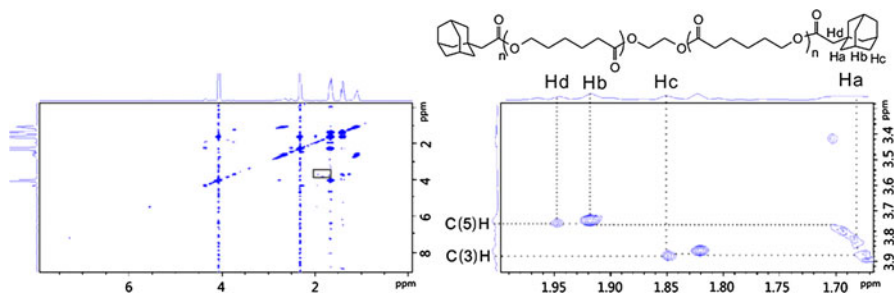
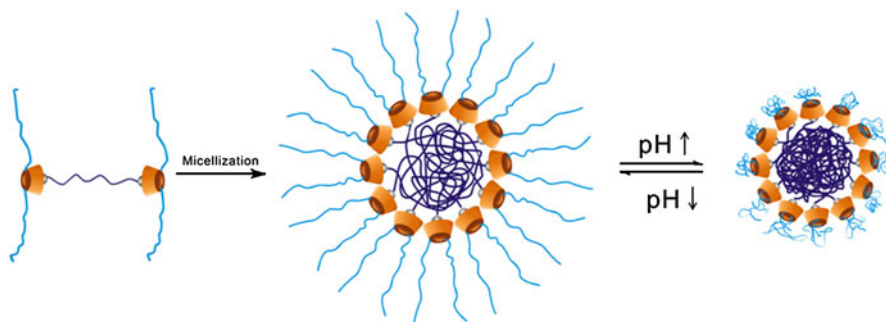


Fig. 5 ROESY NMR spectrum of the supramolecular copolymer $(\text{PDEAEMA})_2\text{-}\beta\text{-CD/Ad-PCL-Ad/}\beta\text{-CD-(PDEAEMA)}_2$ in CDCl_3



Scheme 2 The micellization behavior and the shrink process of micelles with the increase of pH values

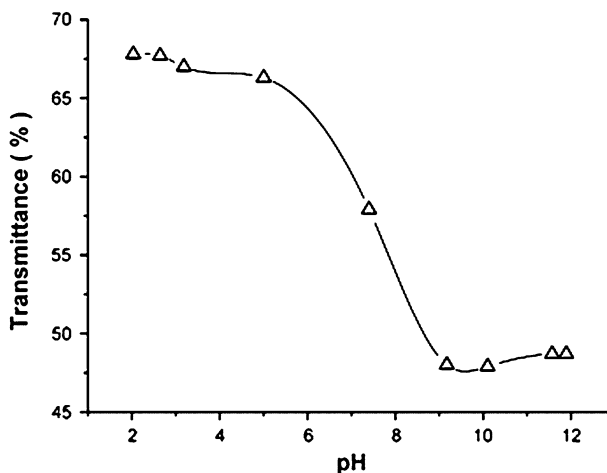


Fig. 6 pH dependence of transmittance for the supramolecular copolymer micelles solutions (0.1 mg/mL)

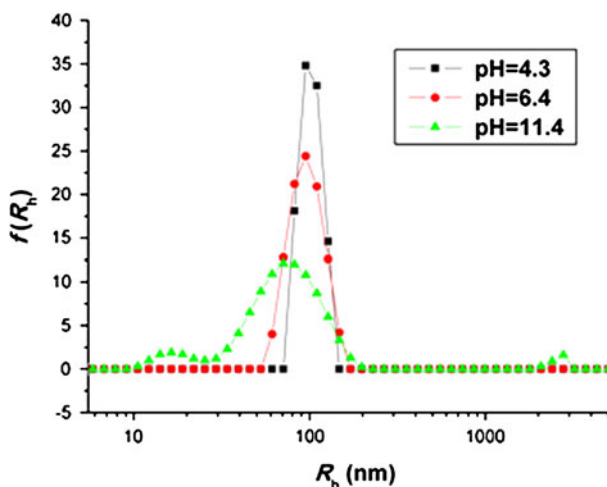


Fig. 7 Plots of hydrodynamic radius distribution, $f(R_h)$, obtained for 0.1 mg/mL aqueous solution of the copolymer

Figure 7 shows three typical plots of hydrodynamic radius distribution, $f(R_h)$, obtained for the micelles solution at pH 4.3, 6.4, and 11.4, and the values of R_h are 148.5, 102.7, and 61.3 nm, respectively. The result should be attributed to the protonation/deprotonation change of PDEAEMA segments from acidic to basic solutions. And the micelles shrunk with the pH increase, which led to the decrease of the size of micelles.

Figure 8 shows the typical TEM images of the micelles at different pH values. The spherical micelles can be observed at pH 2.4 (Fig. 8a), and the radius is about

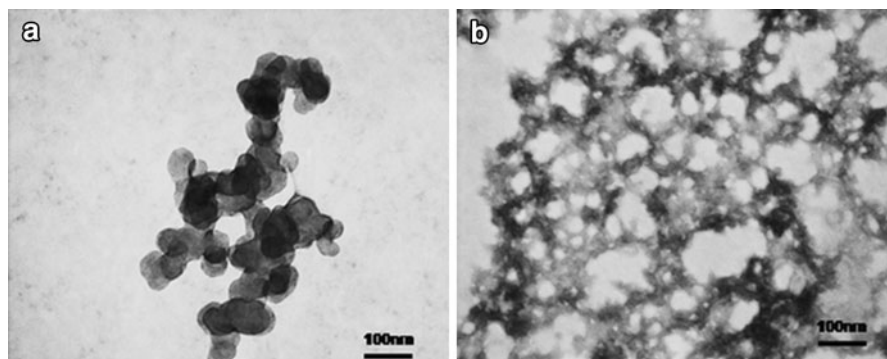


Fig. 8 TEM images of the supramolecular copolymer micelles (0.1 mg/mL)

30 nm. When pH value increased to 11.4, the size of the micelles decreased and its morphology became irregular (Fig. 8b). The results further indicated that the spherical micelles can be formed by self-assembly of the supramolecular copolymer in water, and the sizes of the micelles can be adjusted by the alteration of pH values.

Conclusion

Amphiphilic H-shaped supramolecular copolymer was synthesized by the combination of ATRP, click chemistry, and supramolecular assembly. The copolymer was characterized by ROESY NMR spectrum. The supramolecular copolymer can self-assemble to micelles in water. The self-assembly and pH-responsive properties of the copolymer were investigated by transmittance, TEM, and DLS. The micelles sizes and morphology can be adjusted through the alteration of the pH values of the solution. This supramolecular H-shaped copolymer may have the potential applications in biomedical filed.

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