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ORIGINAL PAPER

Synthesis and self-assembly of pH-responsive amphiphilic poly(ε-caprolactone)-*block*-poly(acrylic acid) copolymer

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Abstract The pH-responsive amphiphilic poly(ε-caprolactone)-*block*-poly(acrylic acid) (PCL-*b*-PAA) copolymer was prepared by selective hydrolysis of one novel poly(ε-caprolactone)-*block*-poly(methoxymethyl acrylate) (PCL-*b*-PMOMA) block copolymer, which was synthesized by combining ring-opening polymerization (ROP) of ε-caprolactone (ε-CL) and atom transfer radical polymerization (ATRP) of methoxymethyl acrylate (MOMA). Selective hydrolysis of the hemiketal ester groups on the PMOMA block gave 100% deprotection without the cleavage of the PCL block. The self-assembly behavior of PCL-*b*-PAA was investigated by fluorescence spectroscopy, DLS and TEM. The spherical micelles were formed with the hydrophobic PCL block as the core and the hydrophilic PAA as the shell by a co-solvent evaporation method. Moreover, the size and size distribution of the micelles varied with pH value and ionic strength in aqueous solution. The cytotoxicity of the PCL-*b*-PAA was lower, which was confirmed by MTT assay.

Keywords Poly(ε-caprolactone) · Poly(acrylic acid) · PH-responsive · Self-assembly

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Introduction

In the past decades, the amphiphilic stimuli-responsive block copolymer has become the hotspot due to the special environmental responsiveness and the self-assembly behavior. In selective solvent, amphiphilic copolymers can self-assemble into the micelles with the hydrophobic segment as the inner core, and the hydrophilic segment as the outer shell to stabilize the micelles and respond to environmental change such as temperature [1–4], pH [5–7], and ionic strength [8, 9]. As reported [10, 11], there exist several pH gradients in human body, for example pH 1–2 in the stomach, pH 5–8 in intestine, and the extracellular pH value of the tumors is lower than that of blood and normal tissue. Therefore, pH-responsive amphiphilic copolymers have caused wide attention for their practical application and feasibility in drug delivery systems.

The pH-responsive polymers are usually composed of monomeric units containing acidic (e.g. carboxyl group) or basic (e.g. ammonium salt) groups, which can either accept or release protons in response to pH changes [12–14]. PAA is a typical pH-responsive polymer with pendent carboxyl group in each repeating unit, and its chain conformation evidently is changed in different pH environments [15–18]. Meanwhile, PAA is also a bioadhesive and FDA-approved polymer which has good and instantaneous mucoadhesive properties [19–21]. Hence, amphiphilic block copolymers with PAA as pH-responsive segment have gained great interest. Traditional strategy to synthesize this kind of block copolymer depended on anionic polymerization method, which demanded relatively rigorous conditions. Although the atom transfer radical polymerization (ATRP) has provided a facile method to synthesize various well-defined copolymers [22]. The direct synthesis of PAA via ATRP of acrylic acid has still faced the challenge due to the complexation of carboxyl group with the copper ion and the quaternization of the nitrogen ligands [22]. In most cases, the block copolymers containing PAA block have been prepared via the ATRP of (*tert*)-butyl acrylate [23–27] and the subsequent selective deprotection reaction by trifluoroacetic acid or trimethylsilyl iodide, however, these reagent could lead to the incomplete deprotection or the cleavage of other ester bonds [24, 25]. Jansen et al. [28–30] have reported that the ester bonds of hemiketal ester could be easily hydrolyzed to carboxyl group in a mild condition. Recently, Peng [31, 32] and Yang [33] have synthesized the hemiketal ester of acrylic acid by an easy method and prepared the well-defined graft copolymer with PAA as backbone, and also found that the hydrolysis of hemiketal ester bond did not affect other ester bonds.

Poly(ε-caprolactone) (PCL) is one of the most attractive and promising aliphatic polyesters for drug delivery system because of its good biocompatibility, biodegradability, drug permeability, and nontoxicity [34, 35]. As a result, various functional amphiphilic block copolymers with PCL as hydrophobic segment have gained substantial attention. In this article, we synthesized pH-responsive amphiphilic copolymer poly(ε-caprolactone)-*block*-poly(acrylic acid) (PCL-*b*-PAA) via ring-opening polymerization (ROP) of ε-CL and atom transfer radical polymerization (ATRP) of an easy-hydrolytic monomer methoxymethyl acrylate (MOMA), followed by a selective hydrolysis. Moreover, the self-assembly of the amphiphilic

copolymer and the stimuli-responsiveness of the micelles were investigated in aqueous solution.

Experimental section

Materials

ε-Caprolactone (ε-CL, Aldrich) was dried over calcium hydride for 48 h at room temperature, and distilled under reduced pressure just before use. Benzyl alcohol was dried over magnesium sulfate and vacuum distilled prior to use. Tin (II) ethyl hexanoate ($Sn(Oct)_2$, Sigma), 2-bromoisobutyryl bromide (Aldrich), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylene triamine (PMDETA, Aldrich), acrylic acid (Shanghai Chemical Reagent Co., China), and chloromethyl methyl ether (Shanghai Bangcheng Chemical Co., China) were used as received without further purification. Methoxymethyl acrylate (MOMA) was synthesized as the literatures [31–33]. Copper (I) bromide (CuBr, Aldrich) was washed with glacial acetic acid for the removal of any soluble-oxidized species, filtered, flushed, and then dried in vacuo. Tetrahydrofuran (THF) was dried over $CaCl_2$ and distilled over sodium/benzophenone. Other solvents were purified by the conventional procedures. All other reagents were purchased from Shanghai Chemical Reagent Co. and used as received.

Characterization

^{1}H-NMR spectra were recorded on a Bruker Avance DMX500 spectrometer in $CDCl_3$, D_2O, or DMSO-d_6 at ambient temperature with tetramethylsilane as internal standard. The molecular weights and molecular weight distributions of polymers (except for PCL-*b*-PAA) were determined by gel permeation chromatography (GPC) measurements on a Waters GPC system, equipped with a Waters 1515 HPLC solvent pump, three ultrastyragel columns (2×10^5, 10^5, and 5×10^4 Å) and a Waters 2414 differential refractive detector, with THF as the eluent at a flow rate of 1.0 mL/min at 35 °C. The molecular weight and molecular weight distribution of PCL-*b*-PAA copolymer were analyzed on PL-GPC50 integrated GPC system (Polymer Laboratories), equipped with PL refractive index detector using poly(methyl methacrylate) (PMMA) calibration standards, PLgel 5 μm mixed-C column (300 × 7.5 mm), with DMF containing 20 mM LiBr as the eluent at a flow rate of 0.8 mL/min at 50 °C (PCL-*b*-PAA copolymer). FT-IR spectra were operated on a Nicolet 5700 spectrometer as KBr pellets with a resolution of 4 cm^{-1} in the range of 500–4000 cm^{-1}. Critical micelle concentration of the amphiphilic copolymer was recorded on a Fluorolog-3-P Fluorescence spectrophotometer (Jobin-Yvon Corp. France). TEM were performed on a JEM 1200EXII microscope at an accelerating voltage of 75 kV. Samples for TEM were prepared by dropping droplets of polymer solution directly onto carbon-coated copper grids and allowing the solution to evaporate under ambient conditions. The size and size distribution of the freshly prepared micelles in aqueous solutions were measured using Zeta

Potential/Particle sizer (NICOMP^{TM} 380 ZLS). The micelle solutions had the final concentration at 1 mg/mL, and the solutions were filtered through 0.45 μm pore size filter prior to measurement. Cell viability was measured by the MTT methods on Human dermal fibroblast cells at 37 °C under the moist atmosphere containing 5% CO_2.

Synthesis of monohydroxyl-terminated poly(ε-caprolactone) (PCL-OH)

The PCL-OH was synthesized by ring-opening polymerization of ε-CL in bulk with $Sn(Oct)_2$ as a catalyst and benzyl alcohol as an initiator at 130 °C. Typically, benzyl alcohol (0.108 g, 1 mmol), ε-caprolactone (5.7 g, 50 mmol) and $Sn(Oct)_2$ (11.4 mg, 0.5 mmol) were added into a previously flamed 25-mL Schlenk tube with a magnetic stirring bar under nitrogen. The Schlenk tube was connected to a standard Schlenk line, and the reactive mixture was degassed via three freeze–pump–thaw cycles, left in vacuo, and then immersed in a thermostated oil bath at 130 °C. After 24 h, the resultant polymer was dissolved in CH_2Cl_2, and the solution was added dropwise into an excess amount of cold methanol to precipitate. The obtained product was purified twice by dissolving and precipitation, and then dried in a vacuum oven until a constant weight. The yield was 95%. $M_{n,NMR} = 5200$, $M_{n,GPC(THF)} = 9400$, PDI = 1.65. ^{1}H-NMR ($CDCl_3$, δ): 7.35 (br, 5H, C_6H_5), 5.12 (s, 2H, $C_6H_5CH_2$), 4.05 (m, 2H, CH_2O in PCL), 3.65 (t, 1H, terminal CH_2OH in PCL), 2.30 (m, 2H, $COCH_2$ in PCL), 1.60 (m, 4H, CH_2 in PCL), 1.37 (m, CH_2 in PCL). FT-IR 3300-3600 (ν_{O-H}), 2944 (ν_{C-H}), 2864 (ν_{C-H}), 1730 ($\nu_{C=O}$), 1472 (ν_{C-H}), 1243 ($\nu_{C\ (=O)-O}$), 1184 (ν_{C-O-C}).

Preparation of poly(ε-caprolactone) macroinitiator (PCL-Br)

The synthesis of the macroinitiator was carried out according to the literatures [36, 37]. In a 100-mL two-necked round-bottom flask, the resulting PCL-OH (5.0 g, 0.96 mmol) was dissolved in 50 mL of dried CH_2Cl_2, and then the solution was cooled to 0 °C in an ice bath. To this solution was added triethylamine (1.4 mL, 10.0 mmol) under nitrogen. After 15 min of stirring, 2-bromopropionyl bromide (1.3 mL, 10.5 mmol) was added dropwise into the mixture solution over a period of 30 min by microsyringe. The reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 72 h. After the insoluble salts were removed by filtration, the filtrate was washed sequentially with saturated $NaHCO_3$ and NaCl aqueous solution. The organic layer was dried over anhydrous $MgSO_4$ overnight, and the solution was concentrated using a rotary evaporator. The remained solution was dropped into an excess of cold methanol. The crude product was purified from methanol three times and dried overnight in vacuo at 40 °C. The macrointiator was recovered in about 90% yield after purification. $M_{n,NMR} = 5400$, $M_{n,GPC(THF)} = 10200$, PDI = 1.56. ^{1}H-NMR ($CDCl_3$, δ): 7.35 (m, 5H, C_6H_5), 5.12 (s, 2H, $C_6H_5CH_2$), 4.05 (m, 2H, CH_2O in PCL), 2.30 (m, 2H, $COCH_2$ in PCL), 1.93 (s, 6H, $OCOC(CH_3)_2Br$), 1.60 (m, 4H, CH_2 in PCL), 1.37 (m, 2H, CH_2 in PCL). FT-IR (KBr): 2946 (ν_{C-H}), 2863 (ν_{C-H}), 1730 ($\nu_{C=O}$), 1472 (ν_{C-H}), 1243, 1184 ($\nu_{C\ (=O)-O}$), 1045 (ν_{O-C-C}).

Synthesis of poly(caprolactone)-*block*-poly(methoxymethyl acrylate) (PCL-*b*-PMOMA)

The synthesis of the PCL-*b*-PMOMA was carried out with PCL-Br as macroinitiator and CuBr/PMDETA as catalyst/ligand in DMF solution. PCL-Br (1.2 g, 0.22 mmol), CuBr (68.8 mg, 0.47 mmol), and degassed DMF (3 mL) were added into 25-mL Schlenk tube under argon, and then the mixture was cycled between vacuum and argon three times to remove oxygen. MOMA (5 mL, 42.8 mmol) and PMDETA (0.16 mL, 0.47 mmol) were introduced into the solution through a rubber septum with syringe equipped with a stainless steel capillary. After degassed again via three freeze–pump–thaw cycles, the Schlenk tube was then placed in a constant-temperature (110 °C) oil bath with magnetic stirring for 48 h. The polymerization was terminated by quenching the Schlenk tube in liquid nitrogen. The resultant solution was diluted by THF and passed through a basic alumina column to remove the copper catalyst. After being concentrated, the crude copolymer solution was precipitated in an excess of hexane to remove unreacted MOMA monomers, and the product was dried in vacuo at 40 °C. The yield was about 40%. $M_{n,NMR} = 13500$, $M_{n,GPC(THF)} = 18900$, PDI = 1.37. ^{1}H-NMR ($CDCl_3$, δ): 5.30 (s, 2H, $COOCH_2O$ in PMOMA), 4.05 (m, 2H, CH_2O in PCL), 3.50 (s, 3H, OCH_3 in PMOMA), 2.43 (s, 1H,–C*H* in PMOMA backbone), 2.30 (m, 2H, $COCH_2$ in PCL), 1.79-2.02 (br, 2H, $–CH_2$ in PMOMA backbone), 1.60 (m, 4H, CH_2 in PCL), 1.37 (m, CH_2 in PCL),1.16 (s, 6H, $OC(O)C(CH_3)_2Br$). IR (KBr): 2949 ($\nu_{C\text{-}H}$), 2867 ($\nu_{C\text{-}H}$), 1730 ($\nu_{C=O}$), 1370 ($\nu_{\text{-}CH3}$), 1243, 1181 ($\nu_{C\ (=O)\text{-}O}$), 1088, 1046 ($\nu_{C–O–C}$), 929.

Preparation of poly(ε-caprolactone)-*block*-poly(acrylic acid) (PCL-*b*-PAA)

The PCL-*b*-PAA was obtained by the hydrolysis of the precursor PCL-*b*-PMOMA as described by the literatures [31–33]. Briefly, PCL-*b*-PMOMA (1.0 g) and 10 mL of dried THF were added to a previously flamed round-bottom flask. After the copolymer being dissolved, the solution was cooled to 0 °C. To the copolymer solution was added dropwise HCl solution (1.0 mL). The mixture was kept stirring for 2 h at 0 °C, and then washed with saturated NaCl solution until the aqueous phase became neutral. After dried over anhydrous $MgSO_4$, the copolymer solution was added into an excess of hexane, and the precipitate was collected by filtration and dried in vacuo at 40 °C. $M_{n,NMR} = 10400$, $M_{n,GPC(DMF)} = 7400$, PDI = 1.31. ^{1}H-NMR (DMSO-d_6 δ): 12.3 (s, 1H, –COO*H* in PAA), 4.01 (m, 2H, CH_2O in PCL), 2.28 (m, 2H, $COCH_2$ in PCL), 1.55 (m, 4H, CH_2 in PCL), 1.29 (m, CH_2 in PCL), 2.17 (s, 1H, C*H* in PAA), 1.76 (br, CH_2 in PAA backbone). IR (KBr): 3442 (ν_{COOH}), 2952 ($\nu_{C–H}$), 2868 ($\nu_{C–H}$), 1731 ($\nu_{C=O}$), 1241 ($\nu_{C(=O)–O}$), 1164 ($\nu_{C–O–C}$).

Preparation of self-aggregated nanoparticles

PAA-*b*-PCL block copolymer nanoparticles were prepared by the co-solvent evaporation method. Briefly, PCL-*b*-PAA (20 mg) was dissolved in 2 mL of dried THF, and the solution was then dropped in ultrapure-deionized water (20 mL) with the rate at 0.1 mL/min under moderate stirring. Then, the solution was kept stirring

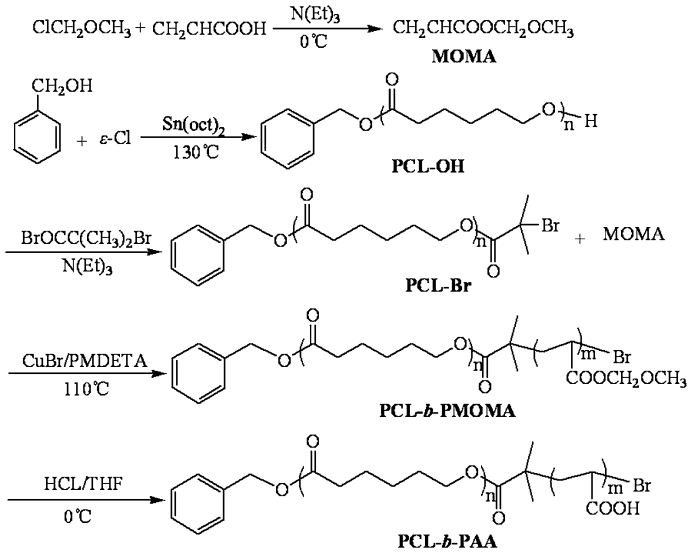
until THF was thoroughly evaporated at ambient temperature. To assure the final concentration of the copolymer to be 1 mg/mL, a little of ultrapure-deionized water was added into the remained solution.

Results and discussion

Synthesis of PCL macroinitiator

To obtain the macroinitiator PCL-Br, the monohydroxyl-terminated PCL was firstly synthesized by ROP of ε-CL with benzyl alcohol as an initiator and $Sn(Oct)_2$ as a catalyst as illustrated in Scheme 1. Figure 1a showed the ^{1}H-NMR spectrum of the PCL-OH, apart from the dominant PCL signals (b–f), the characteristic proton signals of the benzyl (t, a) ($C_6H_5CH_2$–) and terminal methylene group (w) (–CH_2OH) were observed clearly at 7.35, 5.12, and 3.65 ppm, respectively, and this clarified the benzyl alcohol successfully initiated the ROP of ε-CL. The $M_{n,NMR}$ value of the PCL-OH was calculated by the integral ratio of the aliphatic methylene protons (f) to the aromatic protons (t) to be 5200, which was lower than that of measurement by GPC (9400), and the discrepancy was most likely ascribed to the GPC analysis, in which polystyrene was used for calibration.

The monohydroxyl-terminated PCL was transformed into macroinitiator PCL-Br for ATRP by esterification reaction with 2-bromoisobutyryl bromide in the presence of triethylamine. The obtained macroinitiator was characterized by ^{1}H-NMR (Fig. 1b). After esterification, the signals of the terminal methylene protons (w) at 3.65 ppm completely disappeared, whereas a new sharp single peak (g) appeared at 1.93 ppm corresponding to the two methyl protons adjacent to the bromine atom,



Scheme 1 Synthesis of block copolymer PCL-*b*-PAA

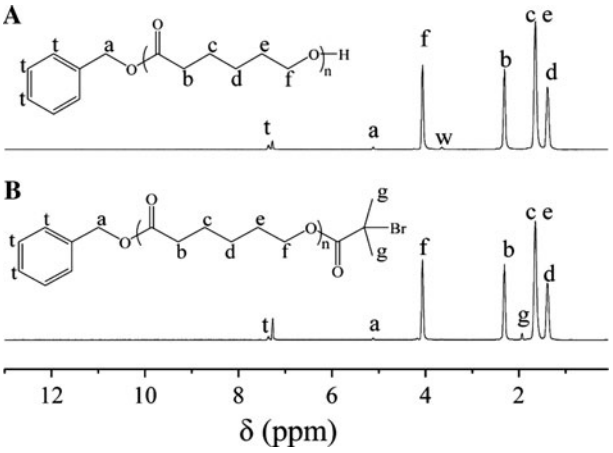


Fig. 1 ^{1}H-NMR spectra of PCL-OH (**a**) and PCL-Br (**b**) in $CDCl_3$

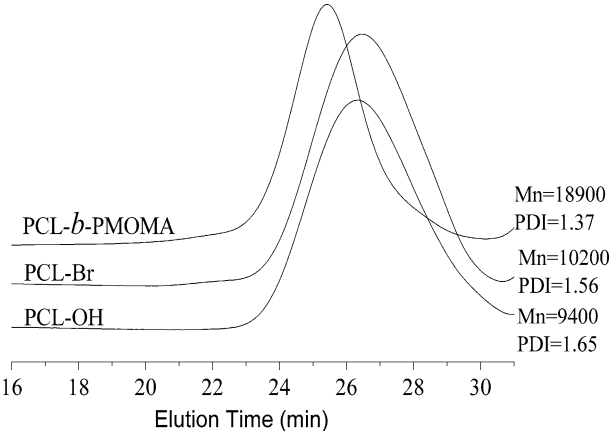


Fig. 2 GPC traces of PCL-OH, PCL-Br, and PCL-*b*-PMOMA

which indicated that the terminal hydroxyl groups had been quantitatively functionalized by the 2-bromoisobutyryl bromide. The GPC traces of PCL-OH and PCL-Br polymers were shown in Fig. 2, the two polymers displayed the symmetrical and unimodal traces, which further confirmed that monohydroxyl-terminated PCL-OH completely turned into a corresponding PCL-Br macroinitiator.

Preparation of PCL-*b*-PMOMA by ATRP of MOMA

The block copolymer of PCL-*b*-PMOMA was synthesized by ATPR of MOMA with PCL-Br as a macroinitiator and CuBr/PMDETA as a catalyst/ligand in DMF solution at 110 °C as shown in Scheme 1. Figure 3a showed ^{1}H-NMR spectrum of PCL-*b*-PMOMA. Besides the characteristic signals of PCL (a–f), the new signals appeared at 1.7–2.3, 5.21, and 3.47 ppm ascribed to the proton signals of the

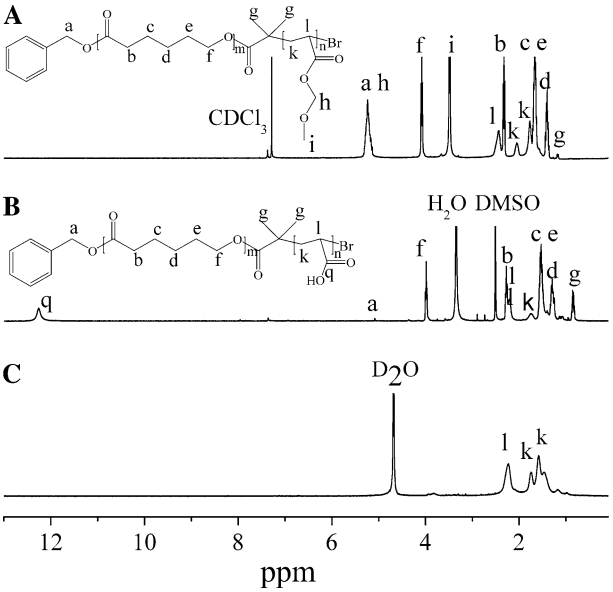


Fig. 3 ^{1}H-NMR spectra of PCL-*b*-PMOMA in $CDCl_3$ (**a**) and PCL-*b*-PAA in DMSO-d_6 (**b**) and D_2O (**c**)

methylene (k), methyne (l) (–C*H*$_2$–C*H*–), methylene (h), and methyl groups (i) (–COOC*H*$_2$OC*H*$_3$) in PMOMA block, and the signals of methyl (g) in isobutyryl group (–CO(C*H*$_3$)$_2$Br) transferred to 1.16 ppm. These results indicated that PMOMA segments were connected to PCL blocks by ATRP of MOMA. Furthermore, the GPC analysis (in Fig. 2) of PCL-*b*-PMOMA showed a symmetrical and unimodal trace and shifted to the higher molecular weight region compared to the macroinitiator, which demonstrated the high initiation efficiency of PCL-Br macroinitiator and successful introduction of PMOMA block to PCL segment. The degree of polymerization (DP) and the length of PMOMA block were estimated to be 70 and 8100 by comparing the integrated peak areas of the methylene protons (i) of PMOMA block with those of the methylene protons (f) of PCL block in ^{1}H-NMR spectrum (Fig. 3).

Preparation of block copolymer PCL-*b*-PAA

As reported [31–33], the hydrolysis of the hemiketal esters are mild and do not destroy other ester bonds. In this study, PCL-*b*-PMOMA copolymer was hydrolyzed with 1 mol/L HCl in THF solution at 0 °C. The successful hydrolysis of the block copolymer was confirmed by ^{1}H-NMR spectrum, FT-IR spectrum, and GPC. In ^{1}H-NMR spectrum (Fig. 3), the signals of methyl (i) and methylene (h) of the pendent group (–OC*H*$_2$OC*H*$_3$) in MOMA repeating units disappeared, while a new proton signal (q, –COO*H*) appeared at 12.3 ppm, and the signals assigned to PCL were intact after the hydrolysis. Compared with the FT-IR spectra of PCL-*b*-PMOMA (Fig. 4), a new broad peak of carboxyl group appeared at 3442 cm^{-1} in the spectrum of

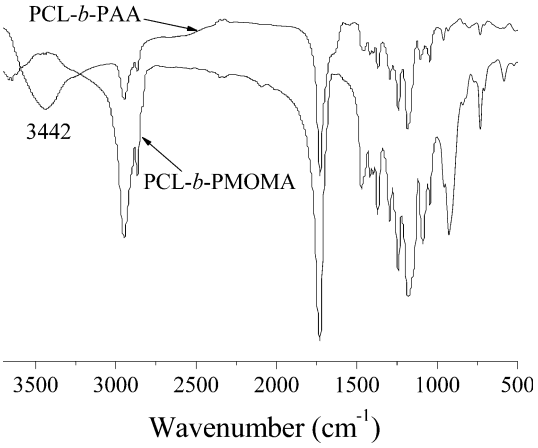


Fig. 4 FTIR Spectra of PCL-*b*-PMOMA and PCL-*b*-PAA

Table 1 Molecular weights and distributions of the precursor and hydrolyzed PCL

RUN	M_n^a	M_n^b	PDI[a]	PDI[b]	Peak
1	6000	6500	1.25	1.25	Unimodal
2	9400	8900	1.65	1.60	Unimodal

[a] PCL precursor, [b] hydrolyzed PCL

PCL-*b*-PAA copolymer. As for the GPC measurement of PCL-*b*-PAA copolymer, the value of $M_{n,GPC(DMF)}$ (7400) was lower than that of $M_{n,NMR}$ (10400), which was possibly owed to the stronger interaction with PAA block with PMMA calibration standards.

To investigate the influence of hydrolysis on PCL segment, two PCL-OH homopolymers were hydrolyzed at the same condition as the hydrolysis of PCL-*b*-PMOMA copolymer. It was noted (in Table 1) that the molecular weight and molecular weight distribution of the hydrolyzed PCL were similar to the corresponding precursors. The present minor discrepancy could be ascribed to the polydispersity of the polymers or the measurement error, which indicated that the hydrolysis had no effect on the PCL block of PCL-*b*-PMOMA.

Self-assembly behavior of amphiphilic block copolymer PCL-*b*-PAA

The self-assembly behavior of amphiphilic polymer could be judged preliminarily by the ^{1}H-NMR in deuterated common solvent and in selective solvent. Compared the proton signals of PCL-*b*-PAA in D_2O with that of DMSO-d_6 (in Fig. 3), signals assigned to PCL segment dramatically weakened, whereas the signals attributed to PAA segment strengthened, which indicated PCL-*b*-PAA self-associated into core–shell micelles with hydrophobic PCL chain as the core and hydrophilic PAA chain as the shell.

The fluorescence probe technique with pyrene as a hydrophobic fluorescence probe was used to further investigate the self-assembly of PCL-*b*-PAA. Pyrene is a sensitive fluorochrome to the polarity of environment and preferably enters into the hydrophobic inner core rather than into hydrophilic outer shell or aqueous phase.

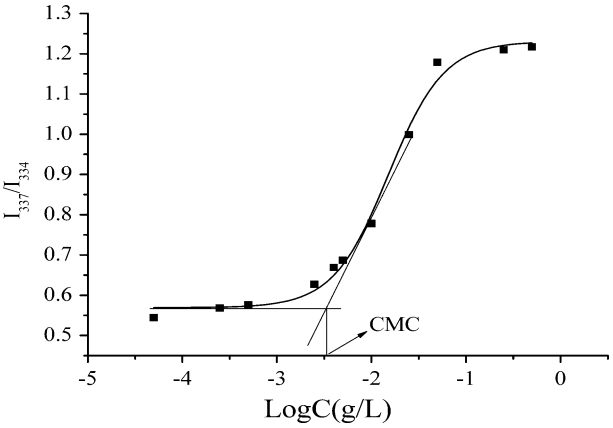


Fig. 5 CMC of PCL-*b*-PAA

The transfer from the polar region to the nonpolar region results in the increase of the fluorescence intensity and the shift of (0, 0) band in the emission spectra from 334 to 337 nm [38]. As a rule, the relationship between of the intensity ratio of the two peaks and the logarithm of the micelle concentrations is used to determine the CMC of micelle. Figure 5 showed the change of the intensity ratio (I_{337}/I_{334}) versus the logarithm of the copolymer concentration for PCL-*b*-PAA, it was obvious that the ratio was almost constant at low concentration and started to enhance with the increasing concentration after a certain value, which indicated that the surroundings of pyrene changed from aqueous phase to the hydrophobic area and the micelles were formed. Based on the CMC definition, the CMC of PCL-*b*-PAA was estimated as 3.55 mg/L, which was lower than the CMC of the common surfactants or similar polymer [9, 38].

Morphology and size distribution of micelles

The morphology was observed by TEM as shown in Fig. 6a, it was apparent that the micelles from PCL-*b*-PAA were well dispersed as individual nanoparticle with regular spherical shape and a diameter about 70 nm, which further testified the self-assembly behavior of PCL-*b*-PAA in water. The size and size distribution of micelles in aqueous solution were analyzed by DLS. As shown in Fig. 6b, the micelles of PCL-*b*-PAA showed a narrow distribution (PDI = 0.104) with the mean size of 143.1 nm which was larger than the measurement by TEM. The difference was ascribed to the fact that the former was the size in aqueous solution, whereas the latter was the size in dry state. Similar results were already reported in literatures [2, 9].

Stimuli-responsiveness of PCL-*b*-PAA copolymer

PAA block shows different hydrophobicity or hydrophilicity owing to the deprotonation or protonation of carboxyl groups of repeating unit in different pH solutions, which could influence the size and size distribution of the PCL-*b*-PAA

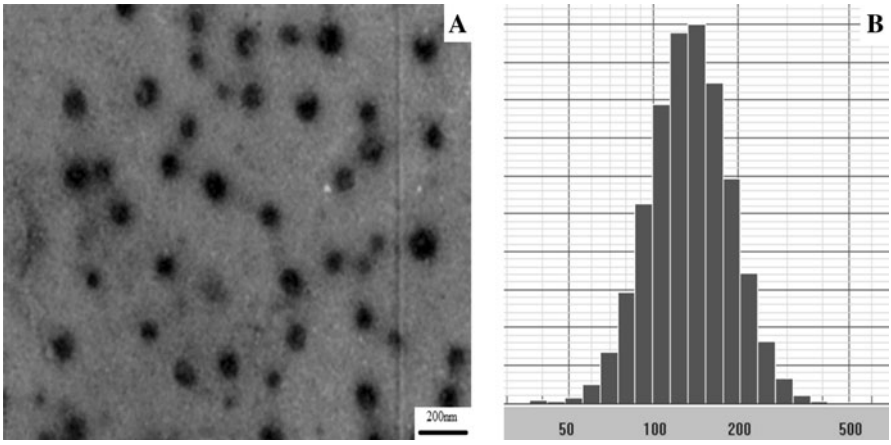


Fig. 6 TEM image of PCL-*b*-PAA micelles (**a**) and size distribution of micelle (**b**) in aqueous solution

micelles and reflect the stimuli-responsiveness of the copolymer in different pH environment.

The pH values of the micelle solutions were adjusted by the addition of 1 N HCl or NaOH. As shown in Fig. 7a, the mean size of micelles decreased from initial 143.1 to 123.5 nm when pH decreased from initial 3.60 to 2.20, which was probably due to the hydrogen bonding within the protonated PAA outer shell [39]. Whereas pH below 2.20, the mean size and size distribution of micelle increased, which were probably ascribed to the hydrogen bond among the protonated PAA outer shell [9, 40]. As far as the effects of NaOH on the size and distribution of micelles are concerned, when pH increased from initial 3.60 to 5.55, the mean size of micelles escalated from 143.1 to 212.5 nm due to the intramicellar increasing electrostatic repulsion between PAA outer shell, caused by the deprotonation of carboxyl groups. While pH further increased to 8.00, the size of micelles gradually decreased from 212.5 to 147.1 nm with the increased size distribution from 0.10 to 0.22. The rational explanation was that the micelles became unstable and partly disintegrated to free polymer chains due to the overwhelming intramicellar repulsive force, and then the free polymer chain re-self-assembled into micelles by the strong hydrophobic interaction of PCL segment [8, 9, 23, 33]. The schematic diagram of the pH-responsiveness of micelle was drawn in Scheme 2.

The effect of ionic strength on the mean size of micelles was investigated by adding NaCl into the micelle solution. As shown in Fig. 7b, the mean size of micelles with almost constant size distribution slightly decreased below 0.27 mol/L ionic strength. While ionic strength was above 0.27 mol/L, the size and distribution of the micelles drastically increased, and the obvious aggregation occurred. At lower ionic strength, the intramicellar repulsion within the PAA outer shell was weaken owing to charge neutrality of positive charge (Na^+) with the negative charges (COO^-), and then the micelle shrank. However, as more negative charge neutralized by an excess of Na^+, the electric repulsion among the outer shell of micelles decreased and the micelles tended to associate [9].

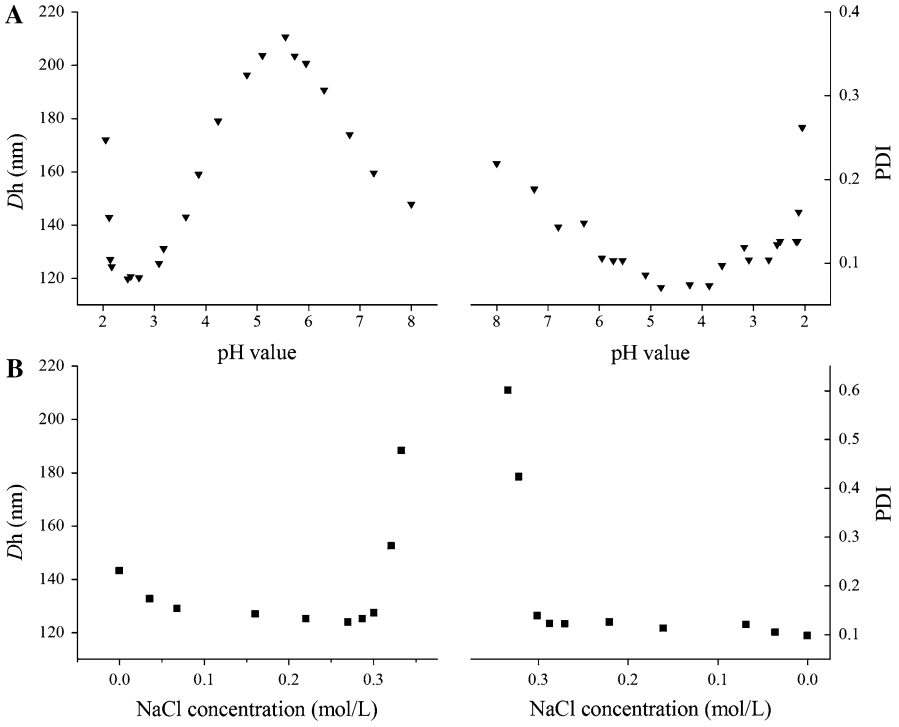
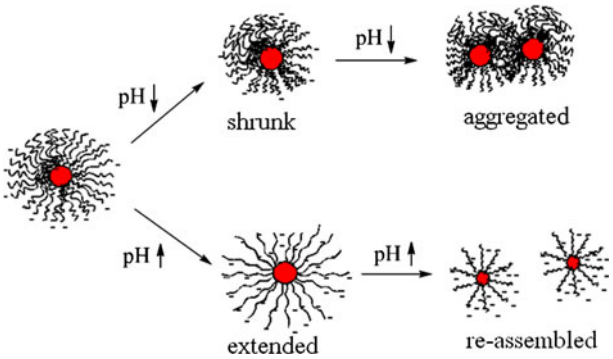


Fig. 7 The effect of pH and ionic strength on the mean size and size distribution



Scheme 2 Schematic diagram of the micelles in different pH aqueous solutions

Cytotoxicity of the PCL-*b*-PAA

The cytotoxicity of amphiphilic pH-responsive PCL-*b*-PAA was simply assayed by MTT assay. As shown in Fig. 8, the relative cell viability decreased slightly with increasing polymer concentration, but the cell viability was still 70% at high concentration (0.1 mg/mL). The result showed that the block copolymer

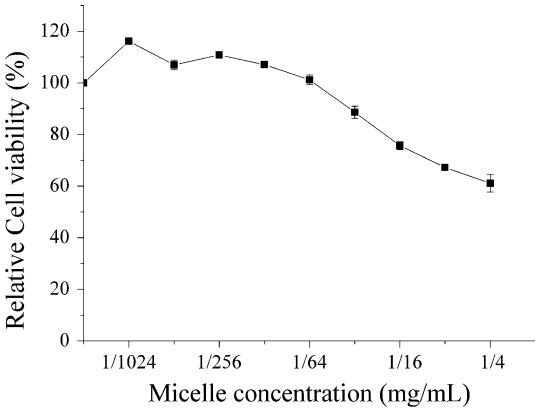


Fig. 8 In vitro cytotoxicity of PAA-*b*-PCL copolymer in fibroblast cells

PCL-*b*-PAA had no obvious toxicity to the fibroblast cells and could be expected to be used in biomedical fields.

Conclusion

One novel block polymer of PCL-*b*-PMOMA was synthesized by the combination of ATRP of MOMA and ROP of ε-CL. After the following selective hydrolysis in HCl/THF solution, amphiphilic pH-responsive polymer PCL-*b*-PAA was prepared. Moreover, the hydrolytic process did not cause the cleavage of the ester bond in PCL backbone. The formed PCL-*b*-PAA amphiphilic polymer could self-assemble into core–shell spherical micelles with PCL as inner core and PAA as outer shell in aqueous solution. The size and size distribution of the micelles were influenced by the pH value and ionic strength of the aqueous solution. The MTT assay showed that PCL-*b*-PAA block copolymers had good biocompatibility and could be used in biomedical fields.

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