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Temperature- and pH-responsive star amphiphilic block copolymer prepared by a combining strategy of ring-opening polymerization and reversible addition–fragmentation transfer polymerization

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ABSTRACT

The star-shaped poly(ε-caprolactone)-b-poly(2-(dimethylamino)ethyl methacrylate) (HPs-Star-PCL-b-PDMAEMA) was synthesized by ring-opening polymerization and reversible addition–fragmentation chain transfer (RAFT) polymerization. Star-shaped polycaprolactones (HPs-Star-PCL) were synthesized by the bulk polymerization of ε-caprolactone (CL) with a hyperbranched polyester initiator and tin 2-ethylhexanoate as a catalyst. The number-average molecular weight of these polymers linearly increased with the increase of the molar ratio of CL to hyperbranched initiator. HPs-Star-PCL was converted into a HPs-star-PCL-RAFT by an esterification of HPs-Star-PCL and 4-cyanopentanoic acid dithiobenzoate. Star amphiphilic block copolymer HPs-Star-PCL-b-PDMAEMA was obtained via RAFT polymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA). The molecular weight distribution of HPs-Star-PCL-b-PDMAEMA was narrow. Furthermore, the micellar properties of HPs-Star-PCL-b-PDMAEMA in water were studied at various temperatures and pH values by means of dynamic light scattering (DLS). The results indicated that the star copolymers had the pH- and temperature-responsive properties. The release behaviors of model drug aspirin from the star polymer indicated that the rate of drug release could be effectively controlled by pH value and temperature.

1. Introduction

Amphiphilic block copolymers can form a micellar structure with a hydrophobic inner core and a hydrophilic outer shell in an aqueous media [1]. Micelles of this type can act as nanocontainers to deliver and release water insoluble drugs in a controlled way [2–4]. However, the formation and subsequent stability of such micelles are sensitive to temperature, dilution, salinity fluctuations and mechanical shear [5], which limits their use for in vivo applications. An alternate and facile approach is to prepare unimolecular micelles which contain only covalently linked branching points, and not liable to be affected by the external condition, such as temperature, concentration and pH, etc. Star-shaped block copolymers in which hydrophobic and hydrophilic arms are connected together demonstrate behavior of unimolecular micelles [6], and may potentially be useful to replace block copolymers in applications such as drug delivery systems, microencapsulation and catalysis [7].

The aliphatic polyester poly(ε-caprolactone) (PCL) and its copolymers have attracted much research interest due to their excellent biodegradability, biocompatibility and high drug permeability [8,9]. These desirable properties render PCL and its copolymers potential candidates for biomedical [10,11] and environmental friendly [12,13] applications. Star-shaped amphiphilic block copolymers containing the

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hydrophobic PCL block and different hydrophilic blocks, such as poly(ethyleneoxide) (PEO) [14–19], poly(acrylic acid) (PAA) [2], poly(*N*-vinyl-2-pyrrolidone) [20], poly (2-(dimethylamino)ethyl methacrylate) (PDMAEMA) [21] have been synthesized. Poly(ethylene oxide) is most commonly used as hydrophilic segment due to its biocompatibility. However, the temperature- and pH-responsive hydrophilic polymers, which have potential applications in controlled drug delivery [2], have been rarely associated with PCL segments to form star-shaped amphiphilic block copolymers. Ternat et al. described the synthesis of amphiphilic multi-arm star-block copolymers H40-$(PCL)_p$-$(PAA)_q$ by a combination of ROP and ATRP [2]. These copolymers were shown to effectively encapsulate and disperse small hydrophobic and volatile molecules. Liu et al. reported the synthesis of PCL-b-$(PDMAEMA)_2$ and $(PCL)_2$-b-(PDMAEMA) Y-shaped miktoarm star copolymer, and investigated the chain architectural effects on the micellization properties [21]. However, the micelles were formed by intermolecular force, so they were sensitive to external conditions.

Poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) is a hydrophilic polymer with temperature and pH sensitivities, excellent biocompatibility, and a well-studied environmentally responsive polymer [22]. Therefore, the potential applications of PDMAEMA are drug delivery, DNA transfer, etc. [23,24]. The amphiphilic copolymers consisting of poly(ε-caprolactone) and poly(2-(dimethylamino)ethyl methacrylate) segments arranged in diblock [25–27], triblock [28], graft [27,29,30], brush [31] and miktoarm star [21] architectures have been synthesized. To the best of our knowledge, there is no report on the preparation of multi-arm star-shaped poly(ε-caprolactone)-block-poly(2-(dimethylamino)ethyl methacrylate) copolymers, although it is remarkable that amphiphilic star copolymers are known to generate stable unimolecular micelles in water.

In this paper, we describe the synthesis and characterization of star amphiphilic block copolymers HPs-Star-PCL-b-PDMAEMA via ROP and RAFT polymerization. The micellar properties of HPs-Star-PCL-b-PDMAEMA were investigated by dynamic light scattering (DLS). Using aspirin (ASP) as a model drug, the drug release behaviors from the HPs-Star-PCL-b-PDMAEMA polymer were studied.

2. Experimental

2.1. Materials

2,2-Bis(hydroxymethyl)propionic acid (bis-MPA; Aldrich Organics), 2-(hydroxymethyl)-l,3-propanediol (TMP; Tianjing Chemical Reagent Research Institute), and 4,4′-azobis(4-cyanopentanoic acid) (ACVA; Fluka) were used as received. Dithiobenzoic acid (DTBA) was synthesized according to a literature procedure [32]. ε-Caprolactone (Aldrich) was dried over CaH_2 for 2 days and distilled under reduced pressure prior to use. 4-(Dimethylamino)pyridinium toluenesulfonate (DPTS) was synthesized according to the literature [33]. Tin 2-ethylhexanoate ($Sn(Oct)_2$; Shanghai First Chemical Reagent Co.) was distilled under reduced pressure and then dissolved in dry toluene prior to use. 2-(Dimethylamino)ethyl methacrylate (DMAEMA; Aldrich Organics) was dried over CaH_2 and distilled under reduced pressure. Tetrahydrofuran (THF) was refluxed for 24 h over sodium and distilled prior to use. Aspirin was purchased from Hefei Bomei Biotechnology Co., Ltd. and used as received. All other reagents were purchased from Shanghai First Chemical Reagent Co. and used as received.

2.2. Synthesis of RAFT agent 4-cyanopentanoic acid dithiobenzoate (CPDB) [34]

The DTBA (4.62 g, 30 mmol) and a catalytic amount of I_2 (50 mg) were dissolved in 15 mL of ethyl acetate in a 100 mL two-neck flask. Into this solution, a solution of dimethyl sulfoxide (DMSO) (1.2 g, 15 mmol) in 5 mL of ethyl acetate was added slowly while stirring vigorously. The reaction mixture was stirred in the dark for 10 h. Without further purification, the crude disulfide was used directly in the next step. 4,4′-Azobis-(4-cyanopentanoic acid) (6.3 g, 23 mmol) and 15 mL of ethyl acetate was added into the flask. The reaction solution was heated at reflux for 18 h. The ethyl acetate was removed in vacuo. The crude product was isolate by silica gel column chromatograph using ethyl acetate/hexane (1:2, v/v) as eluent. The pink red fractions were combined. The solvent was removed in vacuo to give red oil (4.8 g). The target compound was recrystallized from benzene. ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 7.91(d, 2H, *o*-ArH), 7.58 (t, 1H, *p*-ArH), 7.40 (t, 2H, *m*-ArH), 2.40–2.80 (m, 4H, $-CH_2CH_2-$), 1.94 (s, 3H, $-CH_3$).

2.3. Synthesis of hyperbranched polyester (HPs) [35]

Bis-MPA (6.71 g, 50.0 mmol), TMP (0.745 g, 5.55 mmol), and *p*-toluenesulfonic acid (*p*-TSA) (33.6 mg, 0.195 mmol) were uniformly mixed in a three-necked flask equipped with an argon inlet, a drying tube, and a stirrer. The flask was placed in an oil bath previously heated to 140 °C. The mixture was left to react under a stream of argon, removing the water formed during the reaction. After 2 h, the argon stream was turned off and the flask was sealed and connected to a vacuum line for 1 h. After the pressure was increased to atmospheric, bis-MPA corresponding to the third generation (8.94 g, 66.7 mmol) and *p*-TSA (44.8 mg, 0.260 mmol) were added and the argon flow was started. After 2 h of reaction at normal pressure, vacuum was applied for 1 h before the reaction mixture was removed from the flask. The product was dissolved in acetone, and precipitated into cold hexane, then dried in vacuum.

2.4. Synthesis of star-shaped PCL (HPs-Star- PCL) [36,37]

The hyperbranched polyester (0.2960 g, 0.15 mmol,) was added to a two-neck round-bottomed flask. The polyester was dried at 100 °C for at least six successive cycles of Ar(g)-vacuum followed by a final fill of the flask with Ar(g). 0.3% $Sn(Oct)_2$ in toluene solution (7 mL) was then added and temperature raised to 110 °C. The reaction mixture was refluxed at 110 °C for 30 min. The toluene was removed under reduced pressure. ε-Caprolactone (7.8253 g, 68.6 mmol) was added and stirred for 24 h at 120 °C. The

product was dissolved in CH_2Cl_2 and precipitated into hexane to give 7.4659 g of a white crystalline powder.

2.5. Synthesis of star-shaped RAFT agent (HPs-Star-PCL-RAFT)

The HPs-Star-PCL (0.9501 g, 2×10^{-2} mmol) was charged into a two-necked flask equipped with a magnetic stirrer and dried by heating at 110 °C under vacuum for 1 h. The contents were placed under an argon atmosphere and cooled to room temperature. Under stirring, the contents were dissolved in 50 mL of dried dichloromethane after which CPDB (0.3353 g, 1.2 mmol) was added to the HPs-Star-PCL solution. Stirring continued for 30 min, before a dichloromethane solution containing DCC (0.4951 g, 2.4 mmol), DPTS (0.3532 g, 1.2 mmol) and dichloromethane (30 mL) was added drop-wise to the vigorously stirred reaction solution. The esterification reaction was continued at room temperature for 48 h. After completion of the reaction, the dicyclohexylurea (DCU) that had formed as a white solid was removed by filtration and washed with small amounts of CH_2Cl_2, and evaporated to dryness under reduced pressure. The product was dissolved in ethyl acetate, filtered to remove DPTS, and precipitated in ethyl ether twice to remove remaining CPDB and DCC. Ethyl ether was decanted and the precipitate was exposed to reduced pressure.

2.6. Synthesis of star-shaped amphiphilic block copolymer HPs-Star-PCL-b-PDMAEMA

HPs-Star-PCL-RAFT (0.2812 g, 5.36×10^{-3} mmol), 2-(dimethylamino)ethyl methacrylate (12.78 mL, 76.01 mmol) and THF (5.4 mL) were added into a glass tube. After the polymerization mixture was degassed by three freeze-evacuate-thaw circles, the tube was sealed under a vacuum. The polymerization was performed in oil bath at 105 °C for 5 h. The reaction was stopped by plunging the tube into liquid nitrogen. The reaction product was dialyzed in a dialysis bag (molecular weight cut off: 8000–14,000) against distilled water for 72 h. It was refreshed at an interval of at least 4 h. Then HPs-Star-PCL-b-PDMAEMA product was precipitated into *n*-hexane. The product was collected by filtration and then dried in a vacuum oven at room temperature for 24 h.

2.7. Preparation of micellar solutions

HPs-Star-PCL-b-PDMAEMA (10 mg) was dissolved in DMF (2 mL). Deionized water (5 mL) was added slowly (1 drop/10 s) under vigorous stirring, the mixture was left stirring for an additional 12 h. DMF was then removed by dialysis against deionized water for 2 days.

2.8. Controllable drug release of HPs-Star-PCL-b-PDMAEMA [23,38]

Drug-loaded films of aspirin and HPs-Star-PCL-b-PDMAEMA copolymer (5/100, wt/wt) were cast from THF solution. The solvent was volatilized at room temperature. Residual solvent was removed in vacuo at room temperature for 2 days until a constant weight was obtained. The film thickness was found to be about 0.2 mm.

Films loading aspirin were sealed separately using dialysis bags. The dialysis bags were immersed into 40 mL of buffer solutions (pH 2.0, 7.0 or 10.0) at 37 °C or buffer solution with pH 10.0 at 25 °C. In a certain time interval, 4.0 mL of buffer solution was withdrawn and replaced by 4.0 mL of fresh buffer solution. The aspirin release was measured by UV–Vis spectrophoto meter using 228 nm (pH 2.0), 294 nm (pH 7.0), 296 nm (pH 10.0) as characteristic bands. The cumulative release was calculated by using the following equation

$$\text{Cumulative release } (\%) = \frac{100 \times (40C_{\mathrm{ASP}(n)} + 4\sum C_{\mathrm{ASP}(n-1)})}{W_0}$$

where W_0 (mg) is the weight of drug in the polymer, $C_{\mathrm{ASP}(n)}$ (mg/mL) is the concentration of aspirin in buffer solution which was withdrawn for n times, $C_{\mathrm{ASP}(n-1)}$ (mg/mL) is the concentration of aspirin in buffer solution which was withdrawn for $n-1$ times.

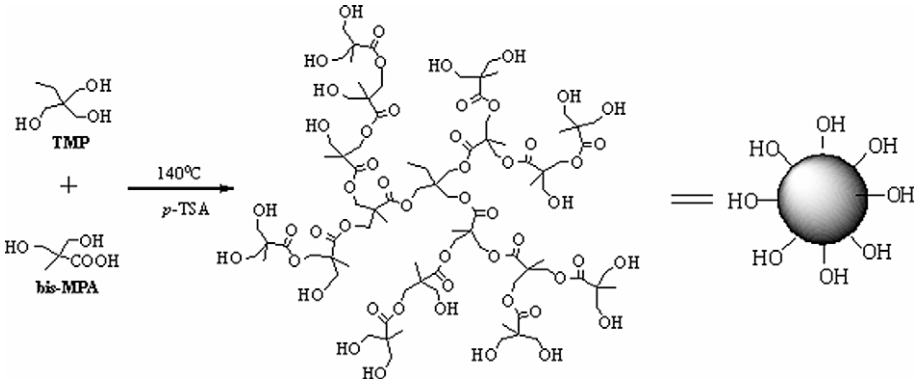
2.9. Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz AVANCE NMR spectrometer (Model DMX400) using $CDCl_3$ or acetone-d_6 as solvent. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 5700 infrared spectrometer. Molecular weights and molecular weight distributions were determined on a Waters 201 gel permeation chromatograph (GPC) equipped with Ultra Styragel columns with pore sizes of 10^3–10^5 Å and a refractive index detector. The eluent was dimethylformamide (DMF) at a flow rate of 1.0 mL/min. The column system was calibrated by monodispersed linear poly (methyl methacrylate) standards. Dynamic laser light scattering (DLS) measurements on polymer micelles were carried out using Zetasizer 3000 (Malvern Instruments Co.) equipped with the Multi Angle Option and with a 30 mW solid state laser operated at a wavelength of 633 nm. The sizing measurements were performed at 25 °C at an angle of 90°. All solutions were filtered through Millipore membranes with pore sizes of 0.45 μm prior to measurements. Transmission electron microscopy (TEM) measurement was performed on a Philips CM200 instrument at a voltage of 160 kV. A drop of the filtered (0.45 μm filter) sample solution was cast on a carbon-coated copper grid, followed by drying at room temperature and negative staining with 2% phosphotungstic acid. UV spectra were performed on a Varian CARY 100 Bio UV–Vis spectrophoto meter.

3. Results and discussion

3.1. Synthesis and characterization of hyperbranched polyester (HPs)

The hyperbranched polyester was prepared using 2,2-bis(hydroxymethyl) propionic acid (bis-MPA) as an AB_2 monomer, 2-ethyl-2-(hydroxymethyl)-l,3-propanediol (TMP) as a core moiety, and *p*-toluenesulfonic acid as catalyst, as shown in Scheme 1. The ^{1}H NMR spectrum of polyester dissolved in acetone-d_6 is illustrated in Fig. 1. The ^{1}H NMR spectrum of polyester shows peaks with the following



Scheme 1. Synthesis procedure of hyperbranched polyester.

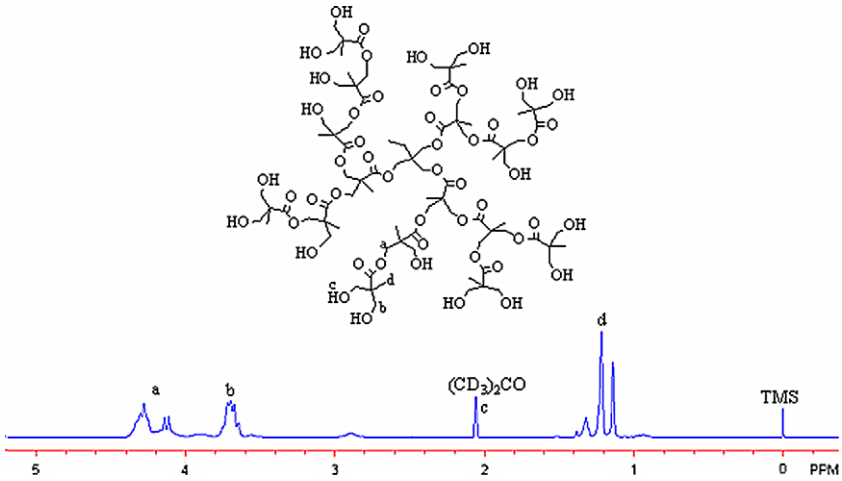


Fig. 1. ^{1}H NMR spectrum of hyperbranched polyester in acetone-d_6.

shifts: 4.10–4.30 (–COOC$\underline{H}_2$), 3.6–3.7 (–C$\underline{H}_2$OH), 2.05 (–CH$_2$O$\underline{H}$), 1.09–1.33 (–C$\underline{H}_3$). The ^{1}H NMR result is thus consistent with the structure of the polyester. The FT-IR spectrum of the hyperbranched polyester is shown in Fig. 2. The characteristic absorption band for ester carbonyl groups occurs at 1733.6 cm^{-1}. The broad signal at 3422.8 cm^{-1} result from hydroxyl groups of primary alcohol. Furthermore, the characteristic absorption band for carboxyl C=O (1680–1720 cm^{-1}) disappears, indicating no remaining carboxylic acid in the reaction system. The result reveals the successful preparation of hydroxyl-terminated hyperbranched polyester [35].

Gel permeation chromatography (GPC) analysis of hyperbranched polyester was carried out in dimethylformamide (DMF). The number-average molar mass determined by GPC is 1980 g/mol and the polydispersity, M_w/M_n, is 1.60. The degree of polymerization, DP$_n$, was estimated to be 16 as follows:

$$\mathrm{DP}_n = \frac{M_n^{\mathrm{exp}} - M_{\mathrm{core}}}{M_{\mathrm{BMPA}}}$$

where M_n^{exp} is the experimental number-average molar mass, M_{core} = 131.17 g/mol is the molar mass of the TMP core and M_{BMPA} = 115.06 g/mol is the molar mass of the bis-MPA branch units. The corresponding average number of hydroxyl end-groups per molecule, N_{OH} is 19 (N_{OH} = DP$_n$ + 3) [2].

3.2. Synthesis and characterization of HPs-Star-PCL

Star-shaped poly(ε-caprolactone) (HPs-Star-PCL) was synthesized using hydroxyl-terminated hyperbranched polyester as a macroinitiator for the ring-opening polymerization of ε-caprolactone (CL) at 120 °C in the bulk under an inert atmosphere, as shown in Scheme 2.

The chemical structure of the star-shaped poly(ε-caprolactone) was characterized by ^{1}H NMR and ^{13}C NMR spectroscopy. A representative ^{1}H NMR and ^{13}C NMR spectra of HPs-Star-PCL are shown in Figs. 3 and 4, respectively. In the ^{1}H NMR spectrum of HPs-Star-PCL (shown in Fig. 3), four major peaks are observed: the triplet peak at 3.99 ppm is assigned as (–C$\underline{H}_2$–O–) protons; the triplet peak at 2.24 ppm is assigned as (–C$\underline{H}_2$–CO–) protons; the multiple peak at 1.50–1.65 ppm are the protons of –CO–CH$_2$–C$\underline{H}_2$–CH$_2$–C$\underline{H}_2$–CH$_2$–O–; the multiple peak at 1.25–1.37 ppm is the protons –CO–CH$_2$–CH$_2$–C$\underline{H}_2$–CH$_2$–CH$_2$–O–. The small triplet peak at 3.65 ppm is assigned

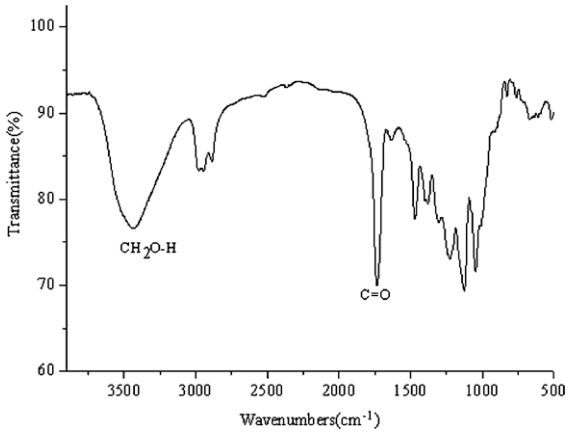
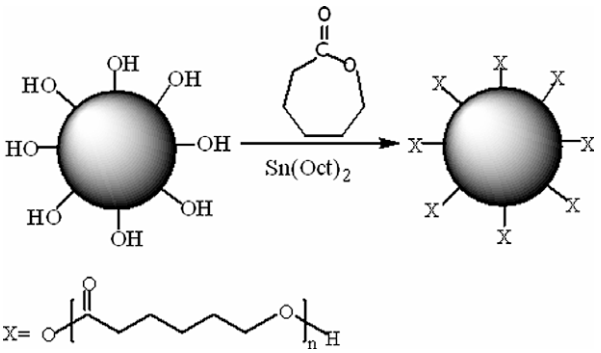


Fig. 2. FT-IR spectrum of hyperbranched polyester.



Scheme 2. Synthesis procedure of HPs-Star-PCL.

as the chain end terminal methylene protons (–C<u>H</u>$_2$–OH). The characteristic peaks for hyperbranched polyester appear at 4.20 ppm (–COOC<u>H</u>$_2$) and 1.22 ppm (–C<u>H</u>$_3$). Therefore, a star-shaped PCL with hydroxyl groups was successfully synthesized. The ^{13}C NMR spectrum of HPs-Star-PCL (Fig. 4) shows the typical signals of PCL at 173.55, 64.16, 34.13, 28.37, 25.55, and 24.59 ppm. The results indicate that multi-arm star-shaped PCL was obtained.

To obtain polymers with different molecular weights, [CL]/[OH] ratios were adjusted and varied. Fig. 5 depicts gel permeation chromatograms of a series of star-shaped PCL with different molecular weights revealing symmetrical and unimodal molecular weight distributions, which indicates that the star-shaped PCL homopolymer was synthesized. As shown in Fig. 6, the number-average molecular weight of the star-shaped PCL, determined by NMR spectroscopy and GPC, is close to the theory value ($M_{n,\mathrm{th}}$), and linearly increases with increasing [CL]/[OH] ratio, which indicate that the hydroxyl-terminated hyperbranched polyester could be used as effective propagation centers and all of the hydroxyl groups of the hyperbranched polyester molecule could initiate the ROP of CL. In addition, the number-average molecular weight distributions of these polymers are narrow ($1.16 \leqslant M_w/M_n \leqslant 1.37$). Therefore, the molecular weight of the star-shaped PCL could be well controlled in terms of the feed ratios.

3.3. Synthesis and characterization of star-shaped RAFT agent

RAFT polymerization is arguably the most versatile and effective means of living free radical polymerization currently available due to its compatibility with a very wide range of monomers and reaction conditions. There are now a number of literatures on the synthesis of star polymers using the RAFT process [39]. There are two types of multi-functional RAFT agents used in the synthesis of star polymers: 'Z-connected' RAFT agents and 'R-connected' RAFT agents [39]. For Z-groups RAFT approach, the growth occurs at the nexus of the core and the arm. As the molecular weight of the arm builds up, a remarkable "shield effect" is found, which prevents formation of a well-defined star polymer. For R-groups RAFT approach, chain growth occurs on the surface of core. When conversion is high, there is no shield effect. Therefore, we selected

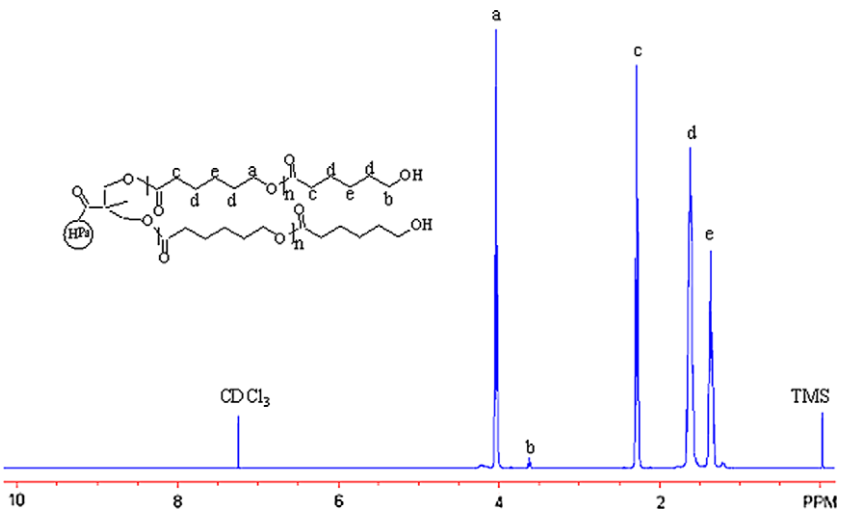


Fig. 3. ^{1}H NMR spectrum of HPs-Star-PCL.

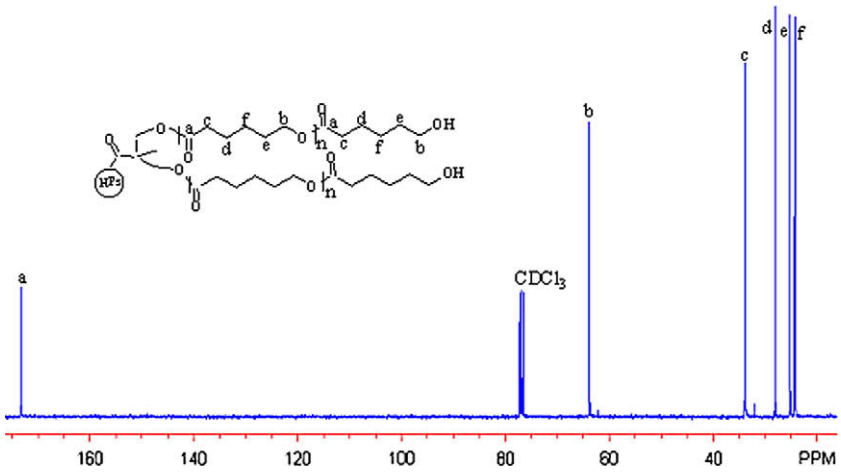


Fig. 4. ^{13}C NMR spectrum of HPs-Star-PCL.

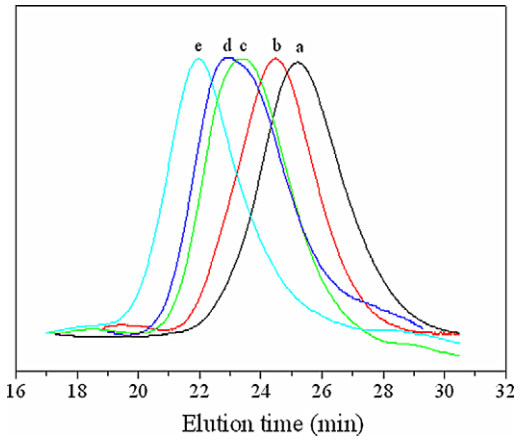


Fig. 5. GPC traces of star-shaped PCL: (a) [CL]/[OH] = 10.59, (b) [CL]/[OH] = 15.85, (c) [CL]/[OH] = 26.29, (d) [CL]/[OH] = 34.93, (e) [CL]/[OH] = 52.89.

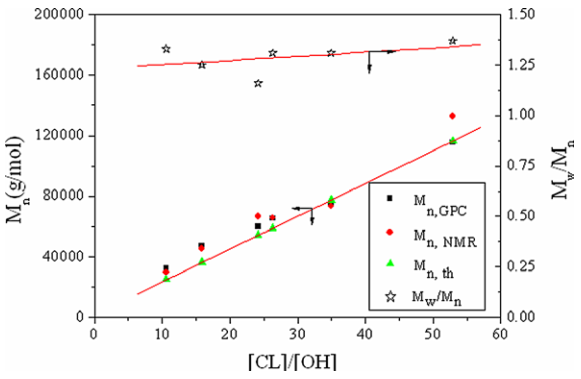


Fig. 6. Dependence of the molecular weight (M_n) on the molar ratio of [CL]/[OH] with hyperbranched polyester initiator, $M_{n,th} = [CL]/[OH] \times 19 \times M_{CL} + M_{initiator}$, $M_{n,NMR}$ was determined by ^1H NMR spectroscopy of HPs-Star-PCL from the integration ratio between the methylene protons in the repeat units (a) and those in the terminal unit (b).

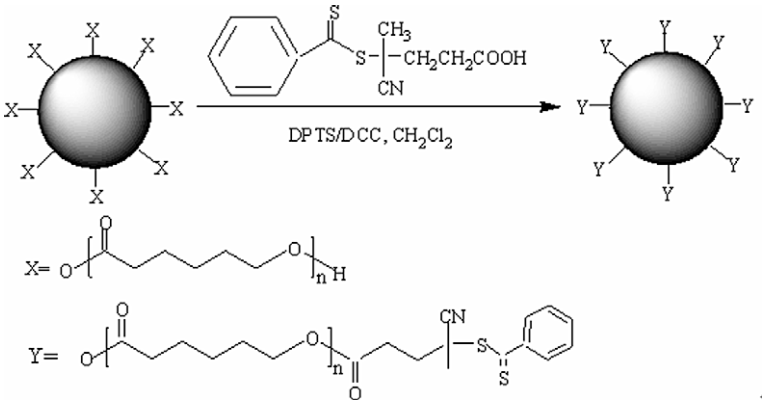
'R-connected' RAFT agents to synthesize star amphiphilic block copolymer.

The star-shaped RAFT agent (HPs-Star-PCL-RAFT) was obtained by the esterification of star-shaped PCL with 4-cyanopentanoic acid dithiobenzoate (CPDB) in dichloromethane at room temperature by using *N*,*N*-dicyclohexylcarbodiimide (DCC) as the coupling agent and 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) as the catalyst, as shown in Scheme 3.

In the experiment, the first attempt is to utilize (dimethylamino)pyridine (DMAP) as catalyst. From Table 1, we can see DMAP result in low yield. As previously reported, the formation of unreactive *N*-acylureas caused the low yield. To overcome this side reaction, the *p*-toluenesulfonic acid salt of the (dimethylamino)pyridinium (DPTS) reported by Moore and Stupp was substituted to DMAP [33]. DPTS can suppress the *N*-acylurea formation in the carbodiimide esterification process.

DPTS proved to be very effective for catalyzing the esterification reaction even in amounts as low as 0.2 equiv. with respect to the carboxyl acid [33]. As shown in Table 1, When the molar ratio of DPTS to CPDB is 0.2, the reactive yield is 54.82%, indicating the steric crowding at the periphery of the star-shaped PCL interfere in the esterification of PCL and CPDB. From Table 1, we see the yield of esterification is dependent upon the amount of DPTS, the increment of the amount of DPTS may obviously improve the esterification yield. When the molar ratio of DPTS to CPDB is 1, all the hydroxyl groups in the star-shaped PCL are esterified with CPDB.

A representative ^1H NMR spectrum of star-shaped RAFT agent is shown in Fig. 7. The signals at $\delta = 7.91$ (g), 7.58 (h), and 7.40 (i) ppm are assigned to the ortho, para, and meta aromatic protons relative to the dithiobenzoate group, respectively. The resonance labeled h ($\delta = 1.94$ ppm) represents the hydrogen of the methyl group on the cyanopentanoic acid fragment. Compared with ^1H NMR spectrum of star-shaped PCL, the shift associated with the methylene group adjacent to the hydroxyl (b) from 3.65 ppm to 4.11 ppm and the disappearance of peak at 3.65 ppm indicate the reaction to be complete. All of the evidence con-



Scheme 3. Synthesis procedure of HPs-Star-PCL-RAFT.

Table 1
Effect of catalyst on the esterification yield of star-shaped RAFT agent.[a]

Sample	Catalyst	Molar ratio of catalyst to CPDB	Yield[b] (%)
1	DMAP	0.2	9.96
2	DPTS	0.2	54.82
3	DPTS	0.5	74.63
4	DPTS	1	100

[a] Conditions: in 30 mL dichloromethane, room temperature, [HPs-Star-PCL] = 1.38×10^{-4} mol/L, [CPDB] = 5.23×10^{-3} mol/L, [DPTS] = 5.23×10^{-3} mol/L, [DCC] = 1.05×10^{-2} mol/L.
[b] The yield of star-shaped RAFT agent is calculated from ^{1}H NMR.

firmed that star-shaped RAFT agent was successfully achieved.

3.4. Synthesis and characterization of star amphiphilic block copolymers HPs-Star- PCL-b-PDMAEMA

The star amphiphilic block copolymer HPs-Star-PCL-b-PDMAEMA was synthesized by RAFT polymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA) in the presence of star-shaped PCL RAFT agent, as shown in Scheme 4. It was reported that, at the higher temperature and without a radical initiator, the molecular weight of star polymer was as expected [40]. DMAEMA can display thermal polymerization [41]. We therefore selected thermal polymerization to prepare HPs-Star-PCL-b-PDMAEMA.

The molecular weight and its distribution of HPs-Star-PCL-b-PDMAEMA were characterized by GPC in DMF using poly(methyl methacrylate) calibration. The narrowly distributed HPs-Star-PCL-b-PDMAEMA ($M_n = 6.26 \times 10^4$, $M_w/M_n = 1.56$) was obtained (Table 2). The GPC curve of HPs-Star-PCL-b-PDMAEMA in Fig. 8 demonstrates monomodal peaks, and exhibits a decrease in retention time compared to that of HPs-Star PCL. However, the GPC curve of HPs-Star-PCL-b-PDMAEMA shows a tailing toward the low molecular weigh, which is attributed to the absorption of PDMAEMA onto the GPC column [23]. Due to the fact that hydrodynamic volume of the star polymer probably is lower than that of linear PMMA having the same molecular weight, GPC tends to underestimate the molecular weight, and the actual molecular weight of HPs-Star-PCL-b-PDMAEMA could be easily determined by means of ^{1}H NMR spectrum by comparing the intensity of the methylene protons (H_h) of PDMAEMA at 2.6 ppm to that of the intensity of the methylene protons of PCL at 1.65 ppm (H_b). The actual molecular weight estimated by ^{1}H NMR spectrum ($M_{n,NMR}$) was 3.14×10^5, which was 5 times higher than the molecular weight obtained by GPC($M_{n,GPC} = 6.26 \times 10^4$).

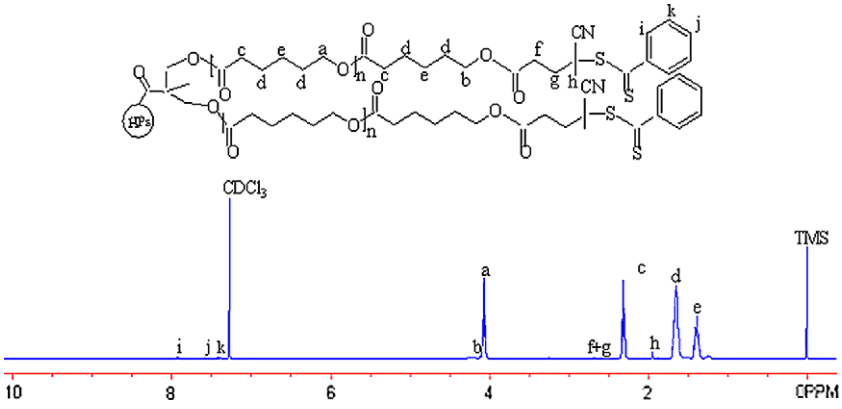
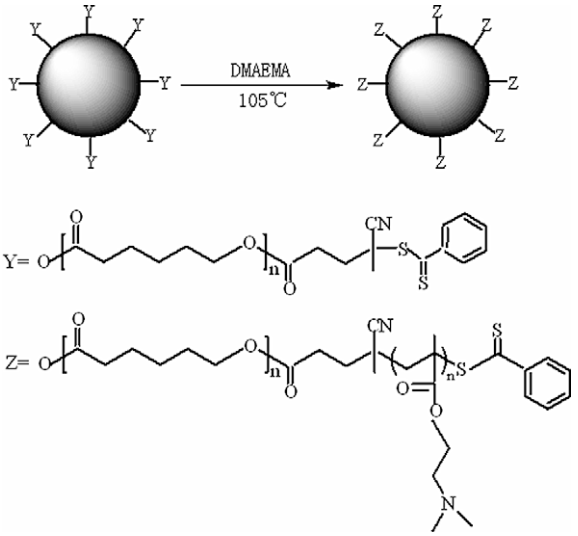


Fig. 7. ^{1}H NMR spectrum of star-shaped RAFT agent.



Scheme 4. Synthesis of the HPs-Star-PCL-b-PDMAEMA.

Table 2
Molecular weight and molecular weight distribution for HPs-Star-PCL and HPs-Star-PCL-b-PDMAEMA.[a]

Sample	M_n[b]	M_w/M_n[b]
HPs-$(PCL_{20})_{19}$[c]	4.75×10^4	1.25
HPs-$(PCL_{20}$-b-$PDMAEMA_{90})_{19}$[d]	6.26×10^4	1.56

[a] Conditions: in tetrahydrofuran (THF), temperature = 105 °C, reaction time = 5 h, [HPs-Star-PCL-RAFT] = 9.93×10^{-4} mol/L, [DMAEMA] = 14.08 mol/L.

[b] Determined by GPC in DMF, 1.0 mL min^{-1}, calibrated using linear poly(methyl methacrylate) standards.

[c] Degree of polymerization of the polycaprolactone block measured by ^1H NMR spectroscopy.

[d] Degree of polymerization of the PDMAEMA block estimated by comparing the intensity of the methylene protons (H_h) of PDMAEMA at 2.6 ppm to that of the intensity of the methylene protons of PCL at 1.65 ppm (H_b).

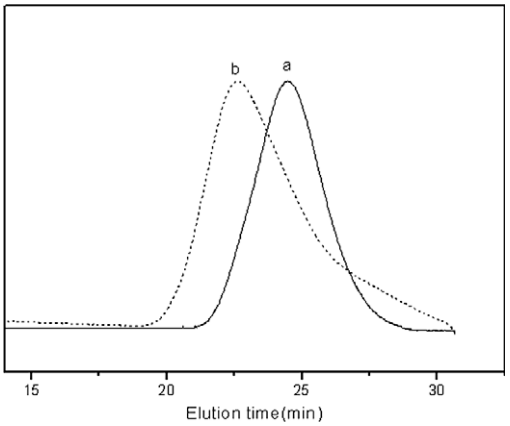


Fig. 8. GPC chromatograms of HPs-$(PCL_{20})_{19}$ (a) and HPs-$(PCL_{20}$-b-$PDMAEMA_{90})_{19}$ (b).

^1H NMR spectrum of HPs-$(PCL_{20}$-b-$PDMAEMA_{90})_{19}$ is shown in Fig. 9. The characteristic chemical shifts of PDMAEMA segments are clearly assigned: the signals at 2.3, 2.6, and 4.1 ppm are attributed to the methyl and methylene protons on the tertiary amine, the ester methylene protons, respectively. ^1H NMR results confirm the successful polymerization of DMAEMA on the surface of polycaprolactone (PCL).

3.5. Micellar properties of HPs-Star-PCL-b-PDMAEMA

The HPs-Star-PCL-b-PDMAEMA copolymer does not dissolve directly in water due to the hydrophobicity of the polycaprolactone. For this reason, a dialysis method was employed to prepare polymeric micelles. The polymer was first dissolved in DMF, which is a good solvent for both PCL and PDMAEMA segments, and micellization was induced by the drop-wise addition of water followed by dialysis.

The micelle size distribution of the star polymer solution was measured by DLS at 25 °C. As an example, a DLS graph of size distribution of HPs-Star-PCL-b-PDMAEMA micelles in a polymer solution with a concentration of 1 mg/mL and pH 6.58 is shown in Fig. 10. It is a bimodal distribution with a smaller micelle diameter in the range of 28–32 nm (13%) and a larger micelle diameter in the range of 79–98 nm (87%). The average hydrodynamic diameter is 89.5 nm with a polydispersity index of 0.183. Assuming a fully extended conformation of star polymer and considering length per monomeric unit for the PCL block is 0.73 nm [42] and for the PDMAEMA block is 0.25 nm [43], the contour length for the HPs-$(PCL)_{20}$-b-$(PDMAEMA)_{90}$ would be 74.2 nm. However, the DLS experiment was performed in water and the PCL segment was contracted, and the PDMAEAM chains were not fully extended at neutral aqueous solution due to the pK_a of star PDMAEMA being about 6.8 [44]. Therefore, the nonaggregated star polymer in water should have much smaller diameter than 74.2 nm. It appears thus that in all likelihood the small species in DLS with 28–32 nm diameters can be identified as the expected "unimolecular" micelle, and the large species with 79–98 nm diameters can be assigned to micelles formed from aggregated star polymers. It is possible that such aggregates form as a result of the hydrophobic-hydrophobic interactions or van der Waals interactions between the exposed PCL cores of the individual star polymer molecules.

The morphology of the star polymer micelles was further examined by transmission electron microscopy (TEM) using phosphotungstic acid as a staining agent. Fig. 11 shows a TEM image of the star polymer micelles. The micelles are close to spherical morphology. Analysis of TEM image revealed the broad size distribution with the smaller size micelles of about 6 nm and the larger size micelles of about 25 nm. The diameters of the particles determined from the TEM data were smaller than that determined by DLS. This is not surprising, since the DLS determines the hydrodynamic diameter in solution, reflecting the conformation of the aggregates in the wet state, while TEM image is obtained in the absence of the solvent. Nevertheless, both methods exhibited the same trend in the particle sizes of the micelles.

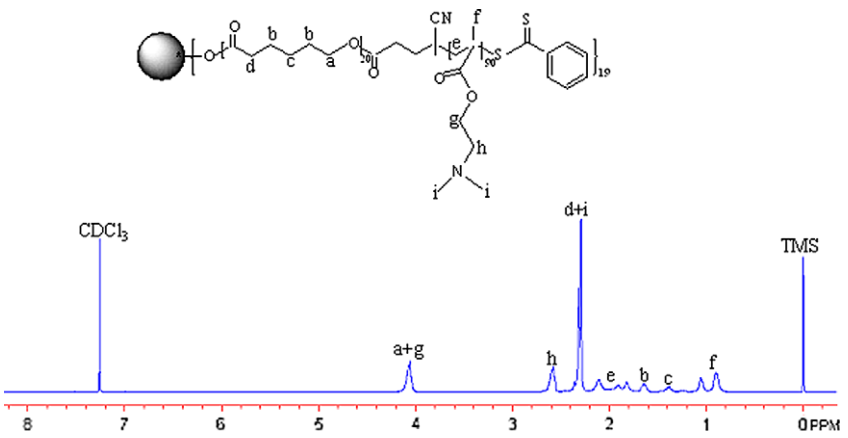


Fig. 9. ^{1}H NMR spectrum of HPs-Star-PCL-b-PDMAEMA.

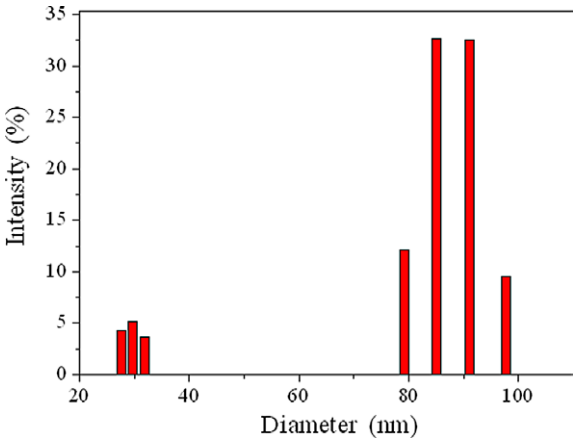


Fig. 10. DLS graph of the micelle size distribution of HPs-Star-PCL-b-PDMAEMA with a concentration of 1 mg/mL at 25 °C and pH 6.58.

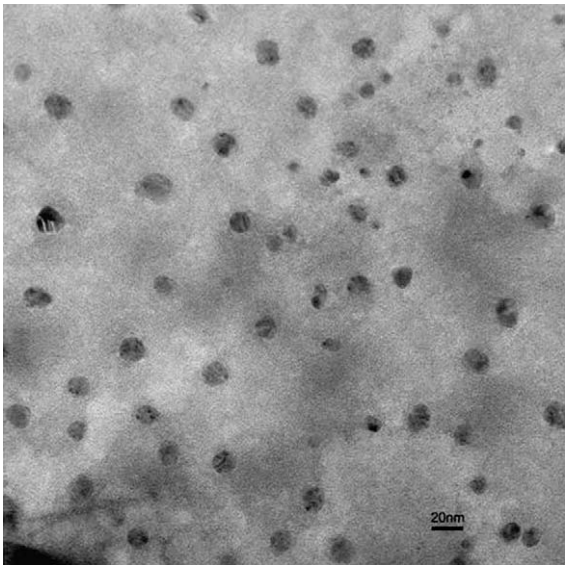


Fig. 11. TEM micrograph of HPs-$(PCL)_{20}$-b-$(PDMAEMA)_{90}$ micelles obtained using phosphotungstic acid staining.

3.6. The pH and temperature sensitivities of star amphiphilic block copolymer HPs-Star-PCL-b-PDMAEMA

PDMAEMA is a weak polybase. It is a water-soluble polymer at room temperature over a wide range of pH. However, it exhibits inverse solubility behavior and precipitates from neutral or basic solutions between 32 °C and 58 °C [30]. The pH and temperature sensitivities of star amphiphilic block copolymers HPs-Star-PCL-b-PDMAEMA were investigated by measuring the sizes of star polymer micelles. The effect of pH on the hydrodynamic diameter of micelles of HPs-Star-PCL-b-PDMAEMA copolymers with a concentration of 1 mg/mL is plotted in Fig. 12. At low pH, the tertiary amino groups of the star polymer are completely charged [29]. Hence, the PDMAEMA segments are stretched fully due to the electrostatic repulsion among PDMAEMA segments, and the star polymer shows a maximum in hydrodynamic diameter (D_H) of 143 nm at pH 2. When pH increases to basic values, the apparent hydrodynamic diameter of the micelles decreases as a result of the deprotonation of the ammonium groups of the corona. In

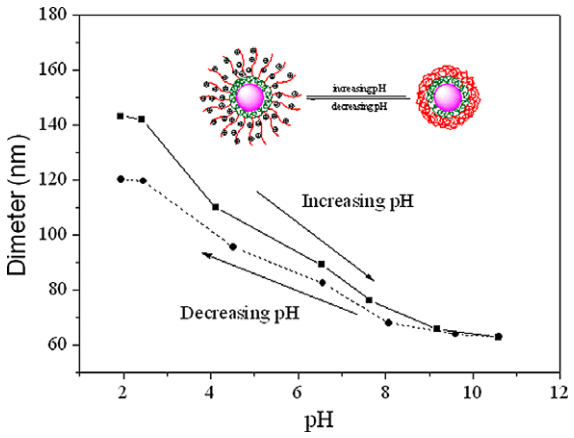


Fig. 12. Hydrodynamic diameters of HPs-Star-PCL-b-PDMAEMA at varying pH and 25 °C, and schematic representations of possible star configurations. Increasing pH from acidic conditions (solid squares) and decreasing pH from alkaline conditions (solid dots).

this case, the PDMAEMA blocks are in a coiled conformation and hence a smaller hydrodynamic diameter (62.7 nm) is observed. When the pH was decreased again, an increase in the diameters was observed, showing the conformational changes are largely reversible. Under these conditions, the maximum D_H observed was 120 nm at pH 2. This value is slightly lower than the initial size of the star polymer and can be explained by the increase in salt concentration of the solution as the pH is changed [45]. This additional salt would cause screening of the charges on the star polymer, leading to a smaller size.

Fig. 13 shows the relative dependence of the hydrodynamic diameter of micelles of HPs-Star-PCL-b-PDMAEMA copolymers as a function of temperature with a solution concentration of 1 mg/L and pH 6.58. The hydrodynamic diameter of star amphiphilic polymer was about 89.5 nm at 25 °C. The increase of temperature resulted in a noticeable decrease in hydrodynamic diameter. These observations indicate that the compact structure of star amphiphilic block copolymers facilitate intramolecular collapse at high temperature when the average distance between micelles is much larger than their hydrodynamic dimensions. At low temperatures, the PDMAEMA segments exist in random coil conformation owing to the predominantly intermolecular hydrogen-bonding interactions between $-CH_2N(CH_3)_2$ groups in PDMAEMA block and the water molecules. With the increase of temperature, the PDMAEMA segments dehydrate, intramolecular hydrophobic force between DMAEMA units become predominant, inducing the collapse of the PDMAEMA segments onto the surface of micelles.

3.7. Controllable drug release of HPs-Star-PCL-b-PDMAEMA

Considering the pH and temperature sensitivities of HPs-Star-PCL-b-PDMAEMA, the star copolymer could be expected to become an intelligent carrier in controllable drug release system. Aspirin is a kind of antipyretic analgesic drug and was used as a model drug to investigate the controllable release properties of the star polymer.

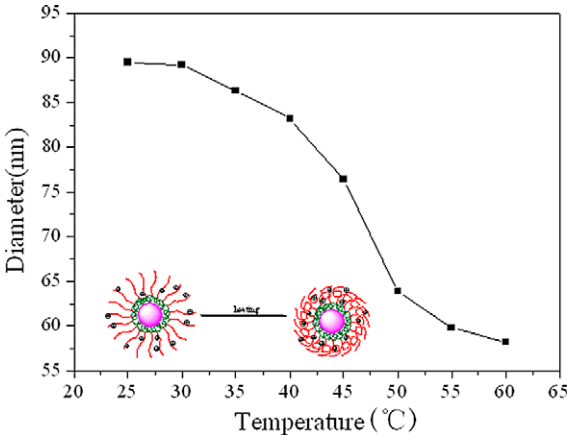


Fig. 13. Apparent hydrodynamic average diameters as a function of temperature at pH 6.58 for HPs-Star-PCL-b-PDMAEMA and schematic representation of possible star configurations.

Fig. 14 shows the cumulative release of aspirin from loading HPs-Star-PCL-b-PDMAEMA. The release rate for aspirin from the star polymer is increasing with the decrease of the pH value of the solution. The results should be ascribed to the conformational transition of PDMAEMA block from an extended chain (at lower pH) to a compact coil in accordance with the variation of the surrounding pH values [38]. In acidic solutions, the PDMAEMA segments are stretched fully due to the coulombic repulsive forces, and drugs can diffuse out from the star polymer easily. In basic solutions, PDMAEMA block shows compact conformation as a result of the deprotonation of the ammonium groups of PDMAEMA, and aspirin is prevented releasing from the star polymer. In addition, the results show that the release rate at 25 °C is faster than that at 37 °C in the solution with pH 10. With the increase of temperature, the dehydration of the PDMAEMA segments induces the conformational transition from the extended chains to compact coil structure, which slower the diffuse of loaded drug form the polymer. All these results demonstrate that the surrounding pH value and temperature could effectively control the release rate of the drug from HPs-Star-PCL-b-PDMAEMA.

4. Conclusions

Star-shaped polycaprolactones with narrow weight distribution were synthesized by living ROP of CL with hydroxyl-terminated hyperbranched polyester initiator. Then, star-shaped PCL was converted into a macroinitiator for the preparation of star amphiphilic block copolymer HPs-Star-PCL-b-PDMAEMA via RAFT polymerization. The novel star amphiphilic block copolymer HPs-Star-PCL-b-PDMAEMA was successfully synthesized. The polymerization displayed living characters, and the molecular weight distribution HPs-Star-PCL-b-PDMAEMA was narrow. DLS and TEM investigations showed unimolecular micelles and aggregated multimolecular micelles coexisted in the star polymer water solution, and that the micelles of HPs-Star-PCL-b-PDMAEMA in water exhibited well-defined pH and temperature-responsive behavior. The drug release experiments showed that the rate of drug release from HPs-Star-PCL-b-PDMAEMA could be effectively controlled by pH value and temperature.

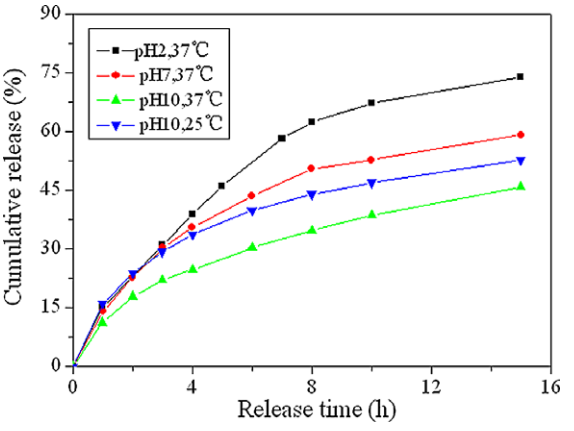


Fig. 14. Drug release profiles of aspirin from HPs-Star-PCL-b-PDMAEMA.

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