

Effect of Degree of Branching on the Thermoresponsive Phase Transition Behaviors of Hyperbranched Multiarm Copolymers: Comparison of Systems with LCST Transition Based on Coil-to-Globule Transition or Hydrophilic–Hydrophobic Balance

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Received: January 25, 2010; Revised Manuscript Received: April 8, 2010

This work reports for the first time the influence of degree of branching (DB) on the thermoresponsive phase transition behaviors of hyperbranched multiarm copolymers. Two series of PEHO-star-PEOs (series A) and PEHO-star-PDMAEMAs (series B) with the hydrophobic DB-variable PEHO core and different kinds of linear arms (PEO arms or PDMAEMA arms) were synthesized. It was found these two series demonstrate thermoresponsive phase transitions with the lower critical solution temperature (LCST). The studies on the LCST transition mechanism indicate that series A belongs to the thermoresponsive polymer system with LCST transition based on hydrophilic–hydrophobic balance, while series B belongs to the thermoresponsive polymer system with LCST transition based on coil-to-globule transition. Correspondingly, there is a big difference in the DB dependence of LCST transition between series A and series B. For series A, the LCST phase transition is highly dependent on the DB of the PEHO core in copolymers. For series B, the LCST phase transition is independent of the DB but dependent on solution pH. Such results may extend the knowledge on the structure–activity relationship of thermoresponsive highly branched polymers.

Introduction

Stimuli-responsive polymers, also called “smart polymers”, have attracted considerable attention in the past several years due to their biological and medical applications.^{1–8} Water-soluble thermoresponsive polymers are one of the most appealing stimuli-responsive species, which undergo fast and reversible phase transition from a soluble to an insoluble state at the lower critical solution temperature (LCST).^{9–11} Hudson² has summarized the category of thermoresponsive polymers. Apart from biopolymers and artificial polypeptides, there are two important thermoresponsive polymers: type I is polymers based on LCST, and type II is polymers based on amphiphilic balance. For type I, poly(*N*-isopropylacrylamide) (PNIPAM) is a representative thermoresponsive polymer.^{12–15} Below the LCST, the polymer chains exist in random coil conformation because of the hydrogen-bonding interactions between the polymer and water molecules, so they are totally soluble in water. However, when the temperature exceeds the LCST, these interactions are broken, and the polymer chains collapse into globule conformation and then precipitate from the solution. For this type of thermoresponsive polymers, an intramolecular coil-to-globule transition occurs before intermolecular aggregation through LCST and the collapse of individual polymer chains increases the scattering of light in solution. Besides PNIPAM, some thermoresponsive polymers such as poly(*N,N*-diethylacrylamide) (PDEAM) and poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) also belong to this type.^{16–21} Different from type I, thermoresponsive polymers in type II are generally amphiphilic block copolymers,

and their LCST transitions are not originated from intramolecular interactions but from intermolecular interactions such as hydrogen bonding and hydrophobic interactions.² During the LCST transition, no intramolecular conformation collapse like the coil-to-globule transition occurs; however, the hydrophilic–hydrophobic balance of the block copolymers is broken, resulting in intermolecular aggregation (micellization). It is the intermolecular aggregation that increases the scattering of light in solution. In fact, besides in the micellization process, such an amphiphilic balance mechanism is also worked in other thermoresponsive systems based on the intermicellar aggregation such as the formation of hydrogels.²² Up to now, the thermoresponsive block copolymers in type II often contain hydrophilic polyethylene oxide (PEO) segments, such as PEO-*b*-PPO,^{23–25} PEO-*b*-PBO,²⁶ and PLA-*b*-PEO^{27,28} (PPO, PBO, and PLA refer to polypropylene oxide, poly(1,2-butylene oxide), and polylactide, respectively). As elaborated above, the category of thermoresponsive polymers is based on the difference in ability of intramolecular interaction. The typical characteristic of type I is intramolecular coil-to-globule transition of individual polymer chains, while for type II it is amphiphilic balance and the thermoresponsive transition originates from intermolecular rather than intramolecular interaction.

Being regarded as a significant kind of functional polymer, hyperbranched polymers (HBPs) are highly branched macromolecules with three-dimensional dendritic globular architecture, which have the advantages of facile one-pot fabrication, a large population of terminal functional groups, lower viscosity, and better solubility.^{29–31} Up to now, the majority of research on temperature-responsive polymers has focused on the linear polymers. Recently, a number of studies on thermosensitive dendritic polymers (including dendrimers and HBPs) have been

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reported.^{32–46} Two different strategies have been employed to prepare thermoresponsive HBPs. The first strategy involves the incorporation of temperature-responsive groups or polymers which undergo coil-to-globule transition with temperature increase onto the surface of HBPs,^{37,38} and thus, these thermoresponsive HBPs belong to type I. The second strategy involves grafting PEO chains or ether-based units onto HBPs,^{44–46} and the obtained thermoresponsive HBPs belonging to type II are rendered temperature-responsive by adjusting the balance of hydrophilic and hydrophobic moieties.

For HBPs, the degree of branching (DB) is one of the most important intrinsic parameters, which leads to various polymer topological structures and corresponding different properties. Up to now, the effect of DB on the glass transition temperature (T_g), crystallinity, self-assembly behavior, and gene delivery of HBPs has already been reported.^{47–55} However, to our knowledge, the effect of DB on the polymer thermoresponsibility has not been disclosed hitherto. Recently, Kono⁵⁶ has reported the influence of polymer topology on thermosensitive properties by comparing NIPAM-end-capped poly(amidoamine) dendrimers with linear PNIPAM or PNIPAM-based copolymer. Therefore, we guess the DB will have a pronounced effect on the thermoresponsive phase transition of HBPs. Besides, such a DB-dependent thermoresponsibility might be different between the polymer systems with LCST transition based on the coil-to-globule transition and hydrophilic–hydrophobic balance. However, the difficulty to control the DB of HBPs blocks the progress.

Previously, the Hult^{57,58} and Penczek^{59,60} groups independently reported the synthesis of hyperbranched poly(3-ethyl-3-(hydroxymethyl)-oxetane) (PEHO). Subsequently, we found that the DB of PEHO can be well controlled through the variation of reaction temperature:⁶¹ it is as low as 5% at a lower temperature such as $-50\text{ }^{\circ}\text{C}$ while close to 50% at a higher temperature such as $30\text{ }^{\circ}\text{C}$. In addition, it was also found that the molecular weight of PEHO did not change much as the reaction temperature varied. Thus, we have a unique advantage to synthesize thermoresponsive copolymers based on such a DB-controllable PEHO core and study the DB-dependent thermoresponsibility therein. For this purpose, two series of PEHO-star-PEOs (series A) and PEHO-star-PDMAEMAs (series B) with DB-variable PEHO cores were prepared. Series A belongs to the thermoresponsive polymer system with the LCST transition based on hydrophilic–hydrophobic balance, while series B belongs to the polymer system with the LCST transition based on coil-to-globule conformation transition. Then, we carefully explored the relationship between the DB and thermoresponsive phase transition behaviors in both series. The results indicate that the DB dependence of thermoresponsive phase transition behaviors is different between series A and series B.

Experimental Section

Materials. 3-Ethyl-3-(hydroxymethyl)-oxetane was prepared according to a literature procedure.⁶² $\text{BF}_3\cdot\text{Et}_2\text{O}$ (A.R. grade, Shanghai Chemical Reagent Co.) was refluxed with CaH_2 and then distilled under reduced pressure prior to use. CH_2Cl_2 and THF (A.R. grade, Shanghai Chemical Reagent Co.) were refluxed with CaH_2 and then distilled prior to use. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, 97%, Aldrich) and 2-bromoisobutryl bromide (98%, Aldrich) were used as received. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 99%, Acros) was passed through a column of basic alumina to remove the stabilizing agents. Copper(I) bromide (CuBr , C.P.

grade, Shanghai Chemical Reagent Co.) was stirred over 24 h in acetic acid, filtered, washed with ethanol, and then dried in a vacuum. All of the other chemical reagents were purchased from Shanghai Chemical Reagent Co. and used as received. A deuterated phosphate buffer solution (*d*-PBS) was prepared by dissolving NaH_2PO_4 and NaHPO_4 in D_2O to give a pD 8.5 solution.⁶³

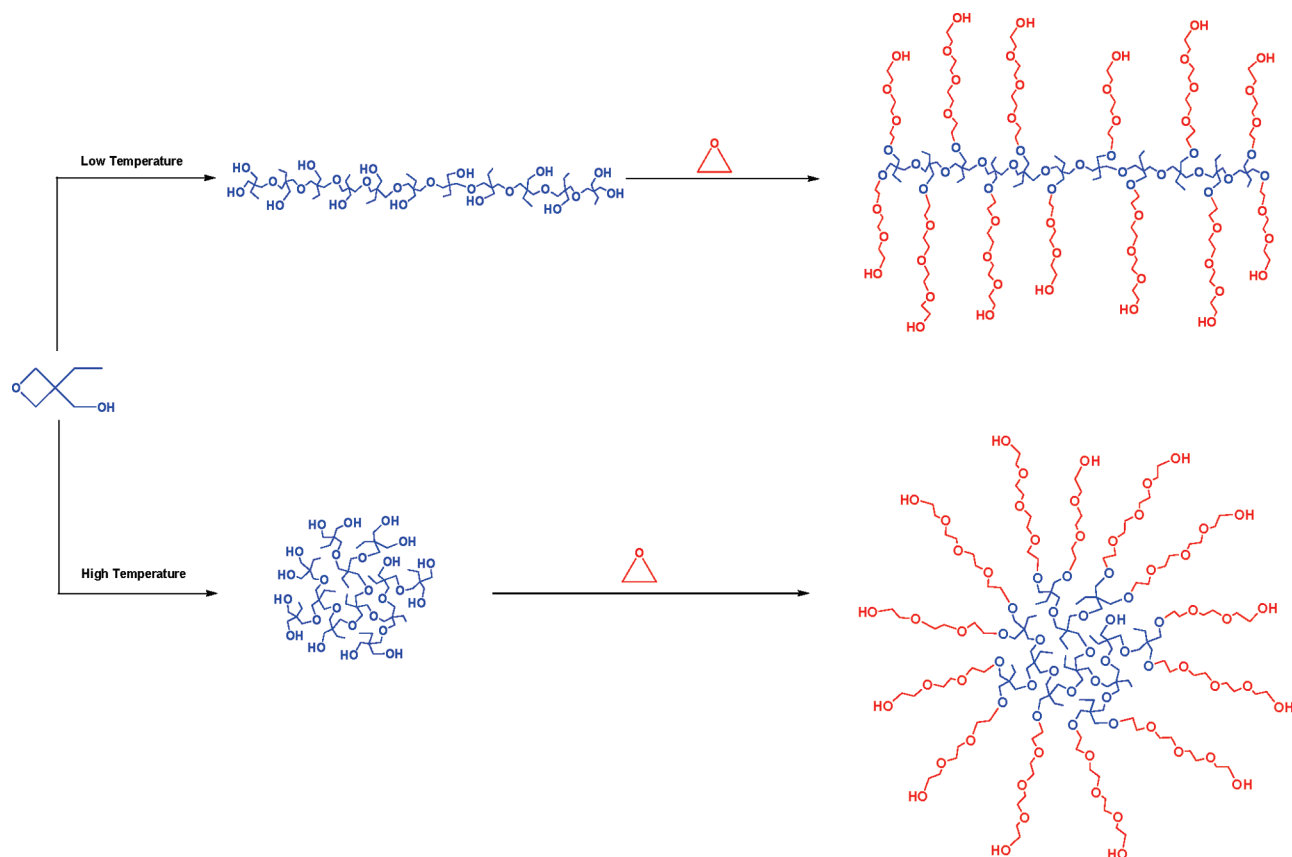
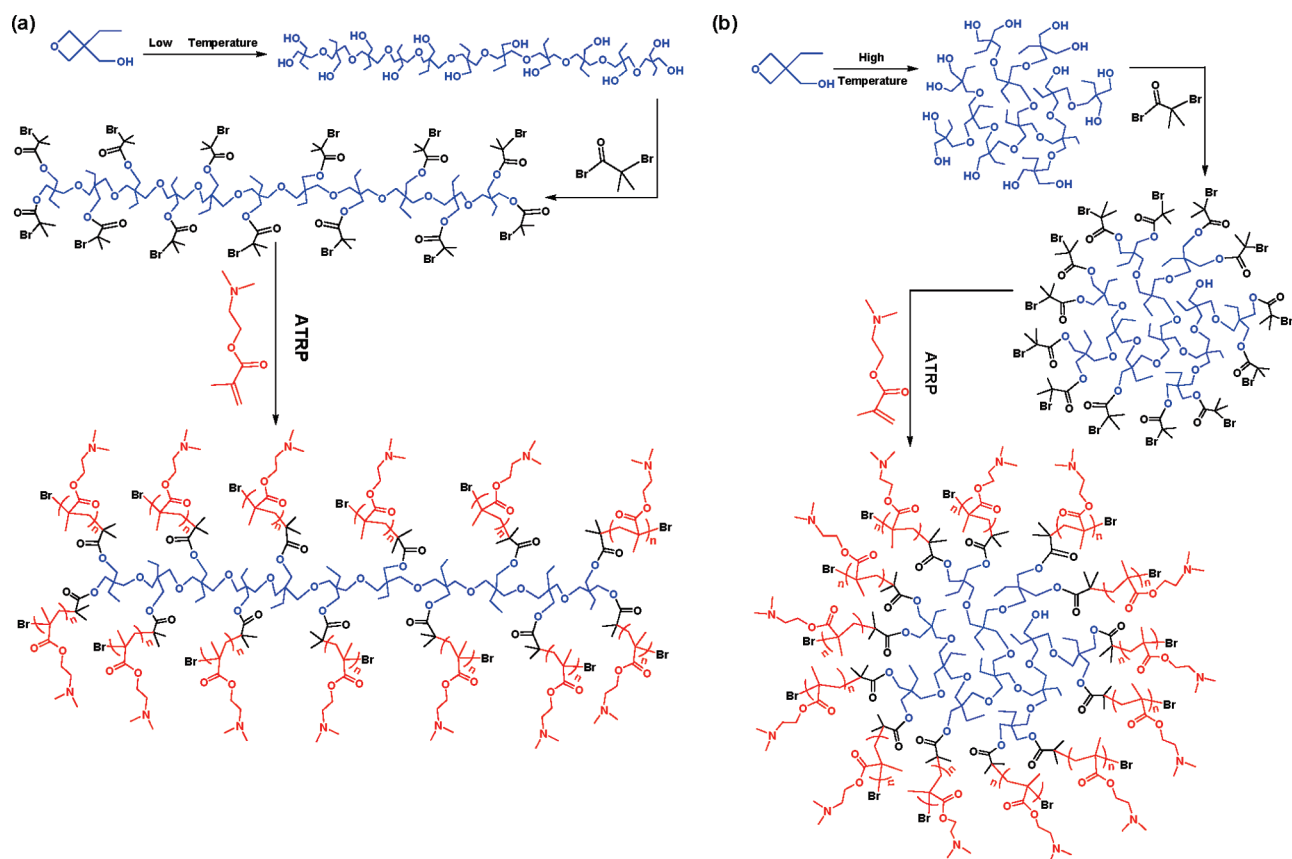
Preparation of PEHO-star-PEOs (Series A) with DB-Variable PEHO Cores. The synthesis of PEHO-star-PEOs (HP1–HP4) with DB-variable but molecular-weight-analogous PEHO cores, and similar PEO arms, was carried out in our preceding paper.⁵⁴ The synthesis processes and structure of copolymers with linear or highly branched PEHO cores are shown in Scheme 1.

Preparation of PEHO-star-PDMAEMAs (Series B) with DB-Variable PEHO Cores. The preparation procedure was made up of three steps, including synthesis of PEHO, PEHO–Br, and PEHO-star-PDMAEMA. The synthesis processes and structure of copolymers with linear or highly branched PEHO cores are shown in Scheme 2. The synthesis of PEHO was carried out through a cationic ring-opening polymerization (CROP) method by using $\text{BF}_3\cdot\text{Et}_2\text{O}$ as the catalyst, EHO as the monomer, and CH_2Cl_2 as the solvent. The molar ratio of the monomer to catalyst is 4:1 (Table 1). The reactions were performed at different temperatures from -50 to $30\text{ }^{\circ}\text{C}$, and four PEHO samples with different DBs were acquired. The detailed synthesis process should be referred to in the literature.⁶¹ The synthesis of PEHO–Br involved the reaction of the terminal hydroxyl groups of the PEHO precursors with a 26% molar excess of 2-bromoisobutryl bromides. Then, a series of PEHO-star-PDMAEMAs were synthesized through an atom transfer radical polymerization (ATRP) path. For the polymerization, PEHO–Br, CuBr , DMAEMA, and HMTETA are used as the macroinitiator, the catalyst, the monomer, and the ligand, respectively. The detailed synthesis processes should be referred to in the literature.⁶⁴

Instruments and Measurements. Nuclear Magnetic Resonance (NMR). ^1H NMR and ^{13}C NMR of PEHOs and copolymers in series A and series B were performed on a AVANCE III-400 M spectrometer (Bruker BioSpin Group, Switz.) using $\text{DMSO}-d_6$ or CDCl_3 as solvents at $20\text{ }^{\circ}\text{C}$. TMS was used as the internal reference. Variable temperature ^1H NMR of series A with external reference was conducted in D_2O by using a cylindrical coaxial tube. Potassium hydrogen phthalate solution was placed in the inner tube as an external standard, and copolymer solution was put in the outer tube. Each sample was kept stable at setting temperature for 10 min before recording. Variable temperature ^1H NMR of series B was conducted in *d*-PBS of pD 8.5; other conditions are the same as those for series A.

Size Exclusion Chromatography (SEC). The molecular weights of the products were measured by SEC on a Perkin-Elmer series 200 system at $70\text{ }^{\circ}\text{C}$ (100 μL injection column, PL gel (10 μm) 300 mm \times 7.5 mm mixed-B columns, polystyrene calibration). DMF was used as the solvent, and the flow rate was 1.0 mL/min.

UV–vis Spectrophotometry. The temperature-responsive phase transition behaviors of the PEHO-star-PEO copolymers in water (2 mg/mL) and PEHO-star-PDMAEMA copolymers in pH 7.5, 8.0, and 8.5 PBS (with the same concentration of 10 mg/mL) were measured with a GBC Cintra 10e UV–vis

SCHEME 1: Preparation of PEHO-star-PEOs with Linear (a) or Highly Branched (b) PEHO Cores**SCHEME 2: Preparation of PEHO-star-PDMAEMAs with Linear (a) or Highly Branched (b) PEHO Cores**

spectrophotometer equipped with a thermocell by monitoring the transmittance of a 500 nm light beam. The samples were

heated at a rate of 1 °C/min. The slit width and integration time were set as 1.5 nm and 10.0 s, respectively.

TABLE 1: Polymerization and Characterizations of PEHOs

sample	reaction temp (°C)	R_{feed}^a	DB (%)	M_n	polydispersity
1	30	4	48	5400	1.81
2	0	4	35	5600	1.82
3	-25	4	23	6500	1.90
4	-50	4	7	6800	1.87

^a The molar ratio of the monomer to the catalyst.

TABLE 2: Characterizations of PEHO-star-PEOs

sample	DB _{core} (%)	$R_{\text{A/C}}^a$	M_n^b	polydispersity ^b
HP1	44	3.4	6600	1.91
HP2	33	3.6	7100	1.83
HP3	20	3.4	7500	2.40
HP4	5	3.7	6100	1.45

^a Determined by ¹H NMR.⁵⁴ ^b Determined by SEC.

Results and Discussion

Preparation and Characterizations of PEHO-star-PEOs with DB-Variable PEHO Cores. As described in our recent paper,⁵⁴ PEHO-star-PEOs (series A) with DB-variable PEHO cores were obtained by the sequential CROP of monomers EHO and EO in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, and the DB of PEHO cores was controlled by changing the reaction temperature on the step of CROP of EHO. The detailed characterizations of PEHO-star-PEOs are shown in Table 2. In Table 2, $R_{\text{A/C}}$ (in the subscript, “A” is the abbreviation of arm and “C” is the abbreviation of core) means the molar ratio of the EO units (in PEO arms) to the EHO units (in PEHO core). Since almost all of the hydroxyl groups in PEHO cores have participated in the CROP of EO monomers,⁵⁴ $R_{\text{A/C}}$ is equal to the number of EO repeating units in each PEO arm. In other words, all of the PEHO-star-PEO samples have a similar PEO arm of nearly four EO repeating units. Besides, the number-average molecular weights (M_n) of PEHO-star-PEO samples are similar.

Preparation and Characterizations of PEHO-star-PDMAEMAs with DB-Variable PEHO Cores. Four PEHOs with different DBs ranging from 0.48 to 0.07 were first synthesized by changing the reaction temperature during cationic polymerization (Table 1). The DB of PEHOs was calculated by integrating the peak areas attributed to the dendritic (D), linear (L), and terminal units (T) in the quantitative ¹³C NMR spectra through the formula $\text{DB} = 2D/(2D + L)$.^{54,61} SEC measurements show the molecular weights of PEHO samples increase slightly as the DB goes down (Table 1), which is attributed to the effect of the DB on the mean-square radius of gyration. As elaborated by Yu and Yan,⁶⁵ the mean-square radius of gyration of the polymer decreases with increasing DB, so the molecular weight of highly branched PEHO obtained by SEC is comparatively lower than that of linear PEHO.

Then, these PEHO samples were reacted with 2-bromoisobutyryl bromide by esterification to produce the ATRP macroinitiators of PEHO-Br. According to our previous work,⁶⁴ a new signal at 1.92 ppm that was attributed to the $-\text{C}(\text{CH}_3)_2\text{Br}$ groups (peak A') appeared in the spectrum of PEHO-Br, which confirmed that macroinitiator was successfully synthesized. The conversion of hydroxyl groups in all PEHO samples can be obtained from the ¹H NMR spectrum of PEHO-Br (Figure 1) by comparing the $-\text{C}(\text{CH}_3)_2\text{Br}$ signal (peak A') with the $-\text{CH}_3$ signal (peak D) in PEHO cores. The results show that all hydroxyl groups in PEHO precursors have quantitatively reacted with 2-bromoisobutyryl bromides to give a functionalization efficiency of 100%.

Finally, the PEHO-Br samples with DB-variable PEHO cores were used as the macroinitiators to initiate the ATRP of

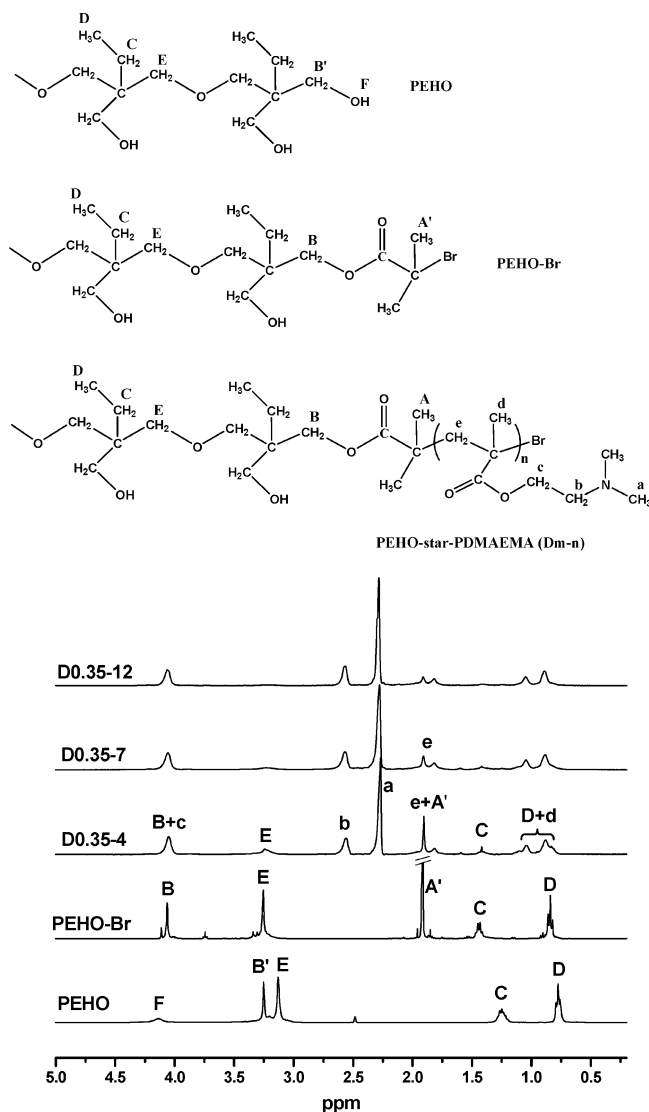


Figure 1. ¹H NMR spectra of PEHO, PEHO-Br macroinitiators, and PEHO-star-PDMAEMAs.

DMAEMA monomers to obtain PEHO-star-PDMAEMA copolymers (Scheme 2). The molar feed ratio of DMAEMA monomer to the initiator site of macroinitiator (R_{feed}) was changed from 5 to 16 to get copolymers with different PDMAEMA chain lengths. Typical ¹H NMR spectra of PEHO-star-PDMAEMAs with assignments are also presented in Figure 1.⁶⁴ Both PEHO (peaks B–E) and PDMAEMA (peaks a–e) signals can be found in the spectra, and the signals of $-\text{C}(\text{CH}_3)_2\text{Br}$ groups (peak A') in PEHO-Br decreased quickly after initiating the polymerization of DMAEMA monomers to form the $-\text{C}(\text{CH}_3)_2-$ (peak A). These results confirm PDMAEMA arms were successfully grafted on the PEHO cores. The detailed characterizations of the obtained PEHO-star-PDMAEMAs are shown in Table 3. In the Table, f_i means the initiation efficiency of PEHO-Br macroinitiators calculated from ¹H NMR measurements (Supporting Information), and the results indicate all of the initiator groups have participated in ATRP when more DMAEMA monomers were added ($R_{\text{feed}} \geq 7$). $R_{\text{A/C}}$ means the molar ratio of the DMAEMA units in PDMAEMA arms to the EHO units in the PEHO core, and is calculated from ¹H NMR measurements (Supporting Information). The PDMAEMA arm length can be obtained by the ratio $R_{\text{A/C}}/f_i$, and thus, $R_{\text{A/C}}$ is equal to the number of DMAEMA repeating units in each PDMAEMA arm. To better recognize

TABLE 3: Characterizations of PEHO-star-PDMAEMAs

sample (Dm-n)	DB _{core} (%)	R_{feed}^a	$R_{\text{A/C}}^b$	f_i^c (%)	M_n^d	polydispersity ^d
D0.48-4	48	5	4	70	10 800	1.73
D0.35-4	35	5	4	75	16 700	1.78
D0.07-4	7	5	4	71	21 600	1.83
D0.48-7	48	8	7	100	19 500	1.75
D0.35-7	35	8	7	100	22 700	1.79
D0.07-7	7	8	7	100	30 800	1.77
D0.48-12	48	16	12	100	40 300	1.68
D0.35-12	35	16	12	100	40 700	1.78
D0.23-12	23	16	12	100	43 800	1.85
D0.07-12	7	16	12	100	47 800	1.76

^a R_{feed} is the feed ratio of DMAEMA to the initiation site of macroinitiator. ^b $R_{\text{A/C}}$ is determined by ^1H NMR, elaborated in the Supporting Information. ^c Initiation efficiency, determined by ^1H NMR spectra, elaborated in the Supporting Information. ^d Determined by SEC.

the samples, an abbreviation of Dm-n was used as the sample name (*m* means the DB of PEHO cores, and *n* means $R_{\text{A/C}}$). According to Table 3, the M_n values detected by SEC increase with the increase of $R_{\text{A/C}}$, which further supports the successful copolymerization process. However, it should be noted that, since the hydrodynamic volumes of highly branched macromolecules are smaller than the linear analogues, their molecular weight measured by SEC may be smaller than the truth.⁶⁶

Thermoresponsibility of PEHO-star-PEOs (Series A) with DB-Variable PEHO Cores. As elaborated previously,⁴⁴ for the PEHO-star-PEOs with a hyperbranched PEHO core, we have found the copolymers are thermosensitive and the LCST transition occurs in a broad temperature range from 8 to 81 °C as $R_{\text{A/C}}$ changes from 2 to 20. However, we have not investigated the effect of the DB of PEHO on the thermoresponsive behaviors of the copolymers. Herein, we synthesized a series of PEHO-star-PEOs (series A, samples HP1–HP4) with DB-variable but molecular-weight-analogous PEHO cores, and similar $R_{\text{A/C}}$ being close to 4, and the LCST transitions as well as the corresponding molecular mechanism have been studied by variable temperature UV–vis spectrometry and ^1H NMR. The PEHO-star-PEO samples with $R_{\text{A/C}} = 4$ are selected for two reasons: first, they have good water solubility; second, their LCST behaviors will be significantly affected by the structure of PEHO core due to such short PEO arms.

LCST Transition. Variable temperature UV–vis spectrometry was used to characterize the thermoresponsive transition of series A. For convenience, the experiments were directly conducted in aqueous solutions with a constant polymer concentration of 2 mg/mL and a constant heating rate of 1 °C/min. As shown in Figure 2, the transmittance decreases sharply in all solutions (except HP4 sample solution) at a specific temperature on heating, indicating a LCST transition. The HP4 sample solution displays a rather broad transmittance transition compared to other sample solutions. In addition, comparing the transmittance curves with one another, it is evident that the copolymer having a higher DB of PEHO core possesses a higher LCST (the LCST is determined from the first decline point on the curve) in water, and the increase of DB from 0.05 to 0.44 causes a notable increase of the LCST from 18 to 38 °C (the inset of Figure 2).

Molecular-Level Investigation of the LCST Transition. A quantitative variable temperature NMR analysis was performed to investigate the molecular mechanism of the LCST transition of series A. Potassium hydrogen phthalate was added as the external standard, and its signals were kept in the same intensity in the ^1H NMR spectra. Signals of D_2O were kept in the same

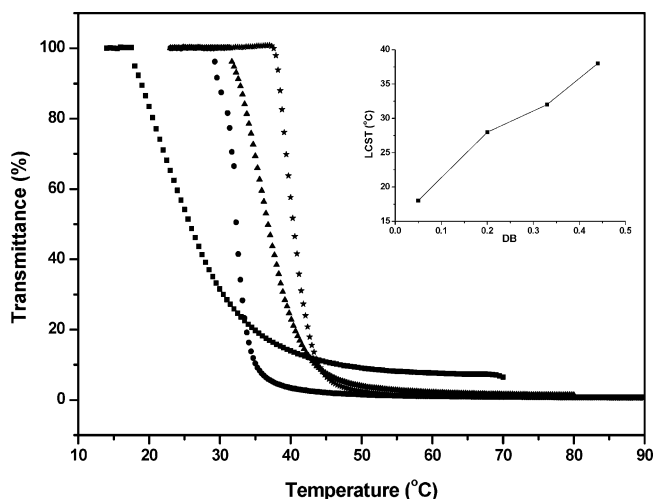


Figure 2. Temperature dependence of optical transmittance at 500 nm for aqueous solution of series A: HP1 (★); HP2 (▲); HP3 (●); HP4 (■). The inset shows the dependence of the LCST on the DB of the PEHO core for copolymers in series A.

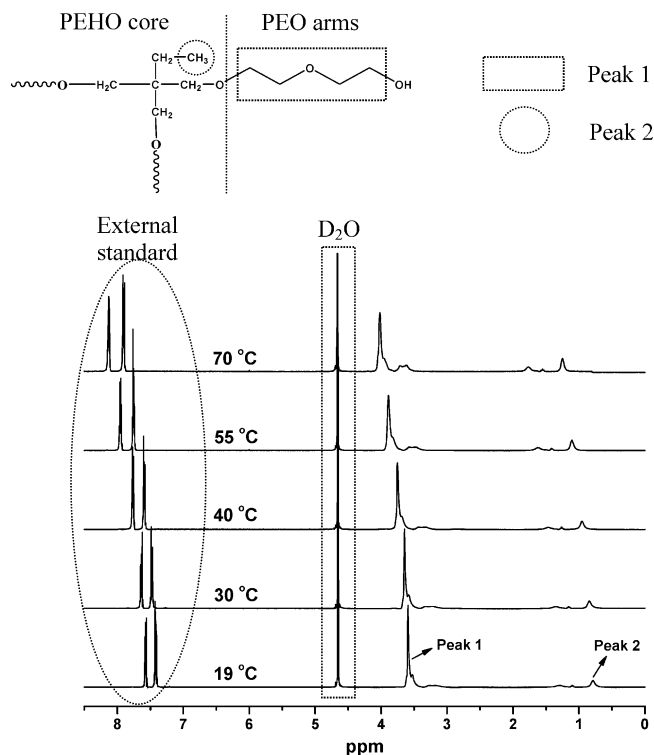


Figure 3. ^1H NMR spectra of sample HP2 in D_2O conducted at 19, 30, 40, 55, and 70 °C.

position (4.64 ppm) for calibration. Figure 3 shows typical variable temperature ^1H NMR spectra from the HP2 sample. All of the proton signals of PEO arms are attributed to $-\text{CH}_2-\text{O}-$ groups, and are denoted as peak 1. Peak 2 is attributed to $-\text{CH}_3$ groups in the PEHO core. It is not easy to directly judge the change of these peaks with increasing temperature from the figure, so quantitative information is necessary.

Since we have added an external standard, we can calculate the relative ratio (J_{arm}) of PEO arm signal and potassium hydrogen phthalate signal by comparing the integral of peak 1 with the integral of peaks (7.0–8.5 ppm) of external standard (Figure 3), and the expression is $J_{\text{arm}} = S_1/S_{\text{es}}$. Thus, we can quantitatively explore the mobility change of PEO chains in

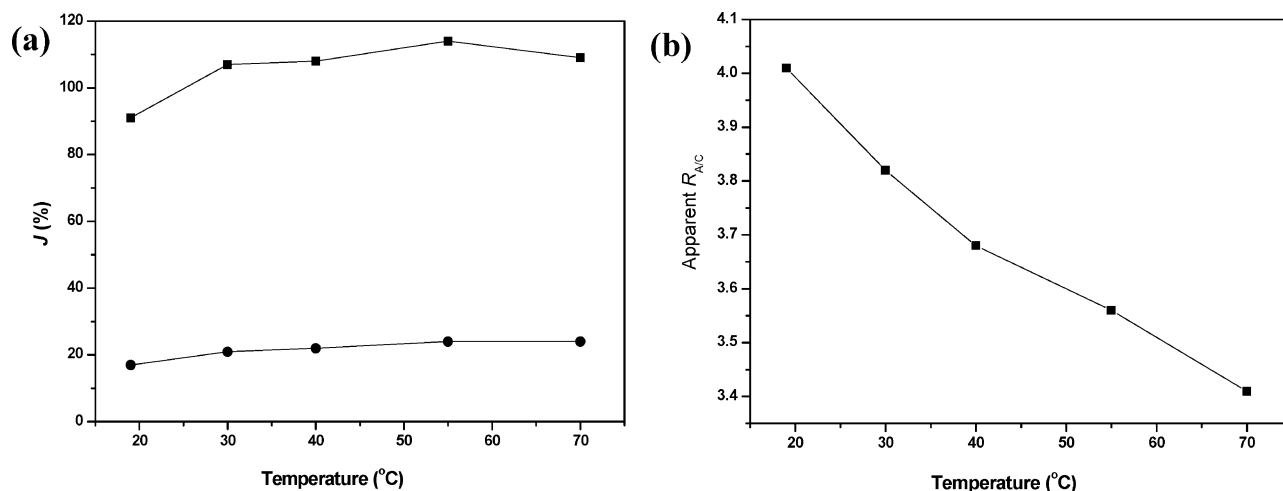


Figure 4. (a) Relative ratio (J) from ^1H NMR spectra with increasing temperature, J_{arm} (■) and J_{core} (●). (b) Apparent $R_{\text{A/C}}$ dependence on temperature.

aqueous solution with increasing temperature. In the same way, the relative ratio (J_{core}) of PEHO core signal and potassium phthalate signal can be calculated by comparing the integral of peak 2 with the integral of peaks of external standard, and the expression is $J_{\text{core}} = S_2/S_{\text{es}}$. As shown in Figure 4a, the mobility changes of both PEO arms and PEHO core with increasing temperature are extremely tiny. However, since we want to know the cocontributions of the PEO arms and PEHO core to the LCST transition, we should explore the temperature-dependent change of a factor both related to PEO and PEHO segments. Evidently, $R_{\text{A/C}}$, as denoted by the molar ratio of the EO units in PEO arms to the EHO units in the PEHO core, is a good quantitative factor. Herein, since $R_{\text{A/C}}$ is measured at a certain temperature, we denoted it as the “apparent $R_{\text{A/C}}$ ”. Figure 4b shows the apparent $R_{\text{A/C}}$ of the HP2 sample as a function of temperature. To our great surprise, the apparent $R_{\text{A/C}}$ clearly decreases with the increase of temperature. However, we have not observed the clear inflection around the LCST of sample HP2 (31 $^{\circ}\text{C}$).

The quantitative information as mentioned above is very useful to disclose the LCST transition mechanism. As indicated in Figure 4a, the mobility of PEO arms of copolymers does not change a lot upon heating, so the LCST phase transition of series A is not caused by the coil-to-globule transition of PEO arms. Besides, the apparent $R_{\text{A/C}}$ characterizing the hydrophilic–hydrophobic ratio of series A decreases upon heating (Figure 4b). Furthermore, we have previously found that the larger the $R_{\text{A/C}}$, the higher the LCST of HBPO-star-PEO (HBPO means the hyperbranched PEHO).⁴⁴ All of these results clearly point out that the LCST phase transition of series A is based on the breakage of hydrophilic–hydrophobic balance with increasing temperature.

Discussion on the DB-Dependent LCST Transition of Series A. As described above, the LCST phase transition of series A based on PEO chains is based on the hydrophilic–hydrophobic balance. For this kind of thermoresponsive polymer system, the solubility of polymers can significantly affect the LCST, and a better solubility causes a higher LCST. In series A, as shown in Scheme 1, PEHO-star-PEO with a higher DB in the PEHO core is more core–shell-like, and thus, the interactions between the PEHO core and water are enhanced by the PEO arms. In other words, the PEHO-star-PEOs with a higher DB are more water-soluble than those with a lower DB, which leads to an increased LCST depending on the DB.³¹ Therefore, we deduce that the LCST phase transition for the thermoresponsive polymer

system based on hydrophilic–hydrophobic balance is dependent on the polymer topology, and the adjustment of any factors related to the polymer topology such as the DB will lead to a notable change in the LCST transition.

Thermoresponsibility of PEHO-star-PDMAEMAs (Series B) with DB-Variable PEHO Cores. Now, our attention is concentrated on another thermosensitive polymer of PEHO-star-PDMAEMA. Here, we hope to study the effect of DB as well as the PDMAEMA arm length on the thermoresponsibility. For this purpose, we have synthesized a series of PEHO-star-PDMAEMAs (series B) with three different $R_{\text{A/C}}$ values (4, 7, and 12) by ATRP. In addition, for a copolymer with a certain $R_{\text{A/C}}$, we have synthesized three or four samples with DB-variable but molecular-weight-analogous PEHO cores.

LCST Transition. Variable temperature UV–vis spectrometry was also used to characterize the thermoresponsive transition of series B. Since PDMAEMA is a weak polybase, the solution pH will change during heating in pure water, which will exert a pronounced effect on the LCST behavior.^{64,67–70} Thus, we have investigated the LCST behaviors of series B in PBS (0.2 M), and this kept the solution pH more constant over the whole temperature range. The buffer pH values were set at 7.5 ± 0.03 , 8.0 ± 0.03 , and 8.5 ± 0.03 , respectively. Besides, the polymer concentration was kept at 10 mg/mL. Figure 5 shows the transmittance–temperature curves of three series of PEHO-star-PDMAEMA with $R_{\text{A/C}}$ values of 4 (Figure 5a), 7 (Figure 5b), and 12 (Figure 5c) at pH 8.0 ± 0.03 , respectively. In addition, for each $R_{\text{A/C}}$, three or four polymer samples with DB-variable PEHO cores were studied. Every sample solution undergoes a rapid decrease of transmittance above a critical temperature around 65 $^{\circ}\text{C}$, indicating a LCST transition. Evidently, only very small changes on the LCST transition were observed by altering the DB of PEHO cores as well as the $R_{\text{A/C}}$ of the copolymers. The LCST transition curves of series B at pH 7.5 ± 0.03 or 8.5 ± 0.03 (Figures S1 and S2, Supporting Information) also show the same trend.

To better understand the influences of the PEHO DB, $R_{\text{A/C}}$, and solution pH on the LCST transition of series B, we have summarized the LCST of the copolymers with linear or highly branched PEHO core at different pH and $R_{\text{A/C}}$ values in Figure 6. The LCST is determined from the first decline point on the transmittance curves of Figure 5. From Figure 6, we can make two conclusions: one is that the LCST of PEHO-star-PDMAEMA is highly dependent on the solution pH, and the higher

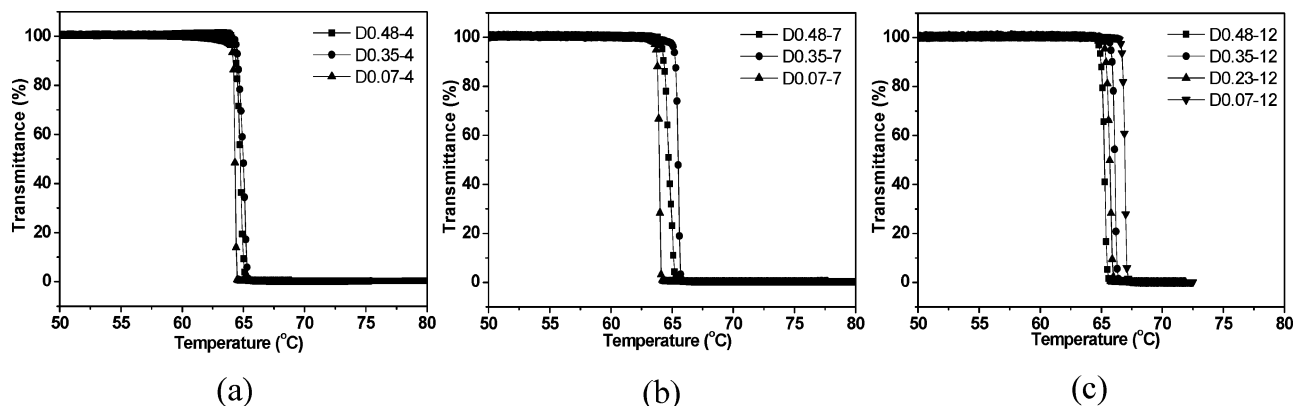


Figure 5. Temperature dependence of optical transmittance at 500 nm for series B solved in pH 8.0 ± 0.03 PBS. The legends in the figures mean the sample $Dm-n$, where m is the PEHO DB and n is $R_{A/C}$.

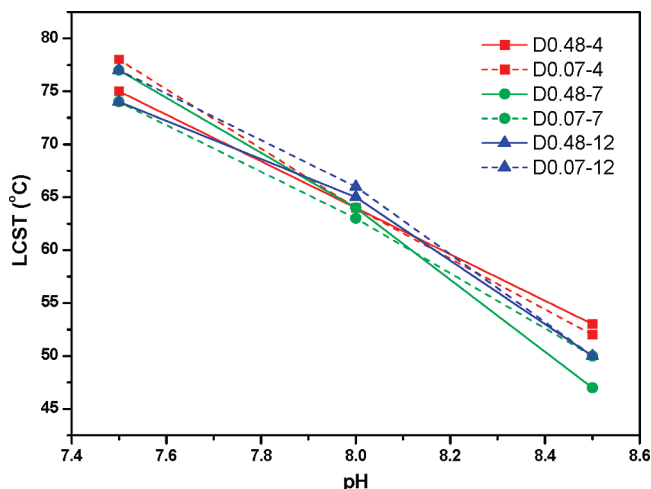


Figure 6. Dependence of LCST on solution pH for copolymers with linear or highly branched PEHO core and different $R_{A/C}$ in series B.

the pH, the lower the LCST; the other is that the effect of the PEHO DB and $R_{A/C}$ on the LCST is small (most of the alternations are within 2 or 3 °C, and the highest alternation is 6 °C between the samples of D0.48-4 and D0.48-7).

Molecular-Level Investigation of the LCST Transition. Quantitative variable temperature NMR analysis was also performed to investigate the molecular mechanism of the LCST transition of series B. The same as series A, potassium hydrogen phthalate was added as the external standard, and its signals were kept in the same intensity in the ^1H NMR spectra. Signals of D_2O were kept in the same position (4.64 ppm) for calibration in the ^1H NMR spectra. Figure 7 shows typical variable temperature ^1H NMR spectra of the D0.35-4 sample in pD 8.5 *d*-PBS. Peak 1 is attributed to $-\text{N}(\text{CH}_3)_2$ groups in PDMAEMA arms. Obviously, the signals of peak 1 became weaker and weaker on heating, indicating the mobility of PDMAEMA arms decreases gradually.

Quantitative information was also obtained to directly evaluate the change of peak 1 with increasing temperature. Being consistent with series A, the relative ratio (J_{arm}) of the PDMAEMA arm signal and potassium hydrogen phthalate signal was calculated by comparing the integral of peak 1 with the integral of peaks (7.0–8.5 ppm) of external standard (Figure 7), and the expression is $J_{\text{arm}} = S_1/S_{\text{es}}$. The results are shown in Figure 8. J_{arm} undergoes a sigmoid transition with the increase of temperature. It changes slightly before 40 °C, then decreases rapidly when temperature rises from 40 to 50 °C, and levels off again above 50 °C. The clear transition zone spanning from 40 to 50 °C is related to the LCST transition.⁶⁴

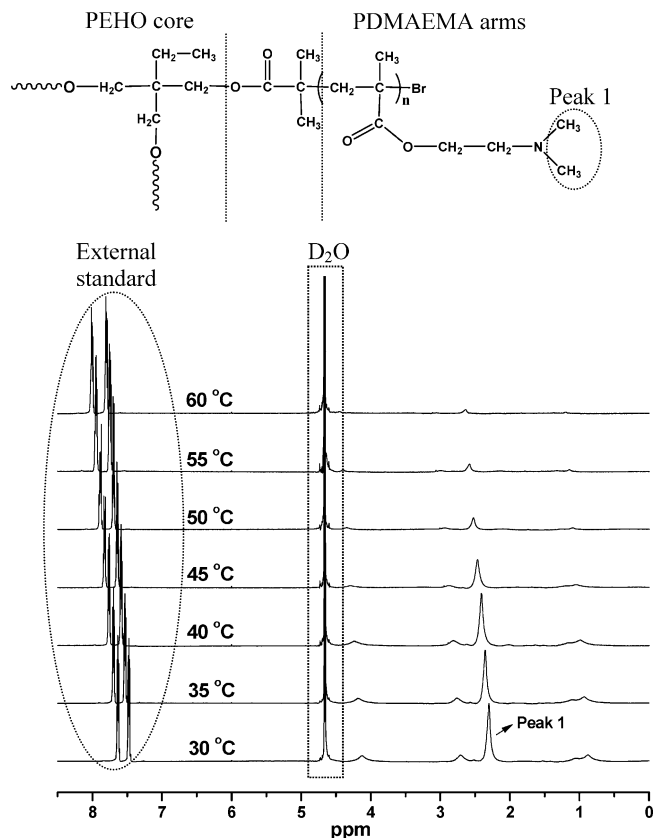


Figure 7. ^1H NMR spectra of sample D0.35-4 in pD 8.5 *d*-PBS conducted at 30, 35, 40, 45, 50, 55, and 60 °C.

Evidently, the LCST transition of series B is highly different from that of series A. A remarkable decrease of PDMAEMA mobility with increasing temperature was observed (Figure 8), which indicates PDMAEMA arms dehydrate and collapse on heating. Thus, it can be deduced that the LCST phase transition of series B is based on coil-to-globule transition of PDMAEMA arms. Such a result agrees well with the previous publications.¹⁵

Discussion on the DB-Independent LCST Transition of Series B. To sum up, the LCST of series B is independent of the DB of the PEHO core in copolymers, and also independent of $R_{A/C}$ denoting hydrophilic–hydrophobic ratio, but significantly dependent on pH. As mentioned above, the LCST transition of series B is only related to the coil-to-globule transition of PDMAEMA arms. The PEHO core is hydrophobic, and its topology changes have little effect on such a conformation transition of PDMAEMA arms, which leads to the DB-

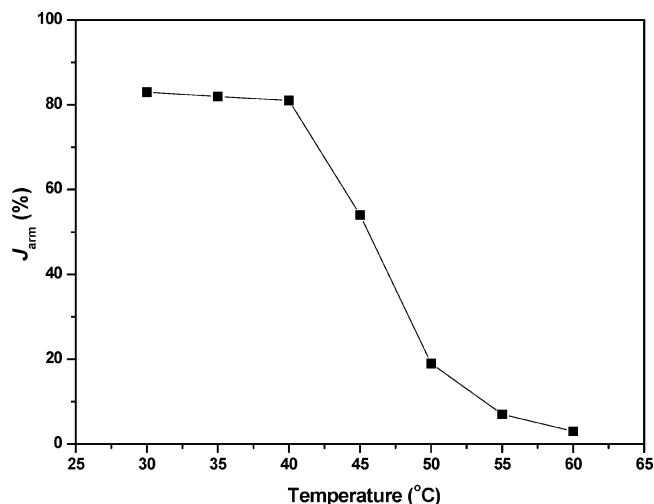


Figure 8. Relative ratio (J_{arm}) from ^1H NMR spectra with increasing temperature.

independent LCST transition. We think such a conclusion may fit all of the thermoresponsive polymer systems based on highly branched hydrophobic cores and thermosensitive arms with the coil-to-globule transition. However, it could not be extrapolated to the thermoresponsive polymer systems with highly branched hydrophilic cores. The R_{AC} -independent LCST transition behaviors indicate that the coil-to-globule conformation transition of PDMAEMA is decided not by whole polymer chains but by the repeating units. The pH dependence on the LCST transition is understandable. The solution pH will lead to the protonation or deprotonation of PDMAEMA arms, which will certainly influence the intermolecular or intramolecular interactions as well as the conformation transitions of PDMAEMA arms.

Conclusion

In conclusion, two series of hyperbranched multiarm copolymers with hydrophobic DB-variable hyperbranched PEHO cores and different kinds of linear arms (using PEO as arms in series A and PDMAEMA as arms in series B) were successfully synthesized. The quantitative information from variable temperature ^1H NMR measurements proves that series A belongs to the thermoresponsive polymer system with LCST transition based on hydrophilic–hydrophobic balance while series B belongs to the thermoresponsive polymer system with LCST transition based on intramolecular coil-to-globule transition. For series A, the LCST phase transition is highly dependent on the DB of the PEHO core in copolymers. For series B, the LCST phase transition is independent of the DB of the PEHO core as well as the arm length but is dependent on the solution pH. The findings will contribute to a fundamental understanding of the relationship between the molecular topology and the thermoresponsive phase transition behaviors of HBPs.

Acknowledgment. The authors thank the National Basic Research Program (2007CB808000, 2009CB930400), the National Natural Science Foundation of China (50633010, 20774057, 20874060, and 50873058), the Program for New Century Excellent Talents in University (NCET-07-0558), the Zhejiang Provincial Natural Science Foundation of China (Y4080421, Y405411), the Foundation for the Author of National Excellent Doctoral Dissertation of China, the Fok Ying Tung Education Foundation (No. 114029), and the Shanghai Leading Academic Discipline Project (B202) for financial support.

Supporting Information Available: Calculation of R_{AC} and f_i of series B and the LCST transition curves of series B at pH 7.5 ± 0.03 and 8.5 ± 0.03 . This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JP100714J