

Synthesis of a PEG-PNIPAm Thermosensitive Dendritic Copolymer and Investigation of Its Self-association

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Abstract A Novel thermosensitive dendritic copolymer based on polyethylene glycol (PEG) and poly(*N*-isopropylacrylamide) (PNIPAm) with a cloud point (CP) around 36 °C was successfully synthesized by preparation of a dendritic polyol and followed by atom transfer radical polymerization (ATRP) of *N*-isopropylacrylamide. The dendritic copolymer was characterized using gel-permeation chromatography (GPC), FTIR and ¹H-NMR spectroscopy. The self-association behavior of the copolymer in aqueous medium was investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). These investigations confirmed that the dendritic copolymer showed different association behaviors at various temperatures.

Keywords: Thermosensitive; Dendritic; Atom transfer radical polymerization; Poly(*N*-isopropylacrylamide); Polyethylene glycol.

INTRODUCTION

During the last decades, amphiphilic block copolymers have been extensively investigated because of their ability to self-assembling into various morphological nanosized core/shell aggregates in solutions, such as spheres, vesicles, rods, micelles, *etc*^[1–4]. In comparison with the conventional surfactant aggregates the amphiphilic macromolecules provide some distinct advantages because of their lower critical micelle concentrations and more stable aggregates^[5]. These aggregates are good vehicles for drug delivery since the drugs are loaded in the hydrophobic core and protected from possible degradation by enzymes^[6, 7]. Polymeric micelles have several advantages, such as exclusive core-shell architecture, which provides interiors that can noncovalently encapsulate guest molecules. In addition, the polymeric micelles are prepared easily, and provide design flexibility for controlling nanostructure and functionality^[8, 9]. However, traditional micelles are not able to sense a stimulus as a signal and respond by changing their structures. From the viewpoint of applications, it would be greatly beneficial if the micelles self-assembled in aqueous media may respond to the external changes. Based on this concept, many stimuli-responsive polymeric micelle systems have been prepared based on amphiphilic block copolymers, which can change their physical or chemical properties in response to external stimuli such as pH^[10], temperature^[11], ionic strength^[12], light^[13], redox potential^[14] and other environmental variables.

Among several stimuli, temperature is frequently used because it is easy to control and has practical advantages *in vitro* and *in vivo*. Especially, amphiphilic block copolymers with poly(*N*-isopropylacrylamide) (PNIPAm), as a thermosensitive block, have been developed and actively investigated^[15–17]. It is well known that

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Received August 7, 2014; Revised August 29, 2014; Accepted August 29, 2014

doi: 10.1007/s10118-015-1561-y

PNIPAm exhibits a thermo-responsive phase transition at around 32–33 °C in aqueous solutions^[16, 17], which is called a lower critical solution temperature (LCST). Below the LCST, the PNIPAm exhibits a hydrophilic and extended chain conformation, while it undergoes a phase transition into coiled and hydrophobic aggregates above the LCST. This phase transition sharply occurs in a narrow temperature range through the LCST and is reversible. Thus, this thermo-responsive property has been utilized as an “on-off” switch for drug release, attachment/detachment of cells, and so on^[18, 19].

In most cases, poly(ethylene glycol) (PEG) has been used as hydrophilic segments in these amphiphilic block copolymers mainly due to its flexibility, hydrophilicity, non-toxicity and non-immunogenicity^[20, 21].

Dendrimers are highly branched macromolecules with unique structural properties. They may be thought of as core-shell type macromolecules wherein they amplify their mass and terminal groups as a function of growth stages. Their globular shape structure and thus unique properties are allowed to be useful in many fields, including drug delivery, catalysis, gene therapy, light-harvesting antennae, chemical sensors, *etc*^[22, 23]. Furthermore, the incorporation of stimuli-sensitive character into dendrimers can significantly expand the scope of these molecules in application^[24, 25]. Recently, atom transfer radical polymerization (ATRP), a new type of controlled radical polymerization, has been developed and proven to be the most robust way to prepare well-defined homopolymers and copolymers. There have been several reports on synthesis of block copolymers by ATRP^[26, 27].

Here, we describe the multi-step synthesis of a novel water-soluble thermosensitive dendritic copolymer based on a hydrophilic PEG segment and thermosensitive PNIPAm branches. The aggregation behavior of the novel thermosensitive dendritic copolymer in aqueous solution was studied by means of DLS and TEM measurements.

EXPERIMENTAL

Materials

N-Isopropylacrylamide (NIPAm, 97 %, Sigma-Aldrich) was purified by recrystallization from a hexane/toluene (2:1) mixture. *N*-(3-Aminopropyl)diethanolamine (96%, TCI), PEG ($M_w = 6000$, Fluka), phenyl chloroformate (98%, Sigma-Aldrich), pyridine (98%, Sigma-Aldrich), L-ascorbic acid (98%, Sigma-Aldrich), copper (II) bromide (Fluka), pentamethyldiethylenetriamine (PMDETA, 98%, Sigma-Aldrich), dimethylformamide (DMF, 99%, Sigma Aldrich), 2-bromo isobutyryl bromide (98 %, Sigma-Aldrich) and dichloromethane (DCM, Baker) were used as received. Double-distilled water was used as solvent for the polymerization.

Synthesis of the Dendritic Polyol Based on PEG with 32-terminated Hydroxyl Groups

Synthesis of PEG phenyl carbonate-terminated precursor (PEG-(OCOPh)₂, $G=0$)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG (15.00 g, 2.3 mmol, 4.6 mmol of OH), DCM (250 mL) and pyridine (5 mL) and the resulting solution was cooled to 0 °C using ice-bath. A solution of phenyl chloroformate (2.35 g, 15 mmol, 3.3 eq of OH) in 50 mL DCM was added dropwise over 20–30 min and the resulting homogeneous solution was stirred for 20 h at room temperature. Then the solution was poured into water. The layers are separated, the aqueous phase was extracted with DCM, and the combined organic layers are washed with brine, dried over MgSO₄, filtered and concentrate under reduced pressure. Diethyl ether was added to precipitate the product. The precipitate was filtered and washed thoroughly with diethyl ether and dried at room temperature to get a white solid (yield: 15.25 g). ¹H-NMR (δ , D₂O): 7.12–7.39 (10H, aromatic), 4.33 (4H, —OCH₂CH₂OCOOPh), 3.38–3.75 (PEG backbone hydrogens).

Synthesis of PEG branched hydroxyl-terminated precursor (PEG-(OH)₄, $G=0$)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OCOPh)₂ (14.5 g, 4.30 mmol of carbonate groups), DCM (250 mL) and *N*-(3-aminopropyl)diethanolamine (1.5 g, 9.25 mmol, 2.15 eq. carbonate) and the resulting solution was stirred for 24 h at 30–35 °C. Then the solution was concentrated under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with

diethyl ether and dried at room temperature to get a white solid (yield: 14.45 g). $^1\text{H-NMR}$ (δ , D_2O): 4.09 (4H, $-\text{OCH}_2\text{CH}_2\text{OCONH}-$), 3.38–3.75 (PEG backbone hydrogens and 8H, $-\text{NCH}_2\text{CH}_2\text{OH}$), 3.03 (4H, $-\text{OCONHCH}_2\text{CH}_2-$), 2.52–2.65 (12H, $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 1.6 (4H, $-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$).

Synthesis of PEG phenyl carbonate-terminated precursor (PEG-(OCOPh)₄, G=1)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OH)₄ (14 g, 8.12 mmol of OH), DCM (250 mL) and pyridine (10 mL) and the resulting solution was cooled to 0 °C using Ice-bath. A solution of phenyl chloroformate (4.4 g, 28.1 mmol, 3.45 eq. of OH) in 50 mL DCM was added dropwise over 20–30 min and the resulting homogeneous solution was stirred for 20 h at room temperature. Then the solution was poured into water containing NaHCO_3 . The layers are separated, the aqueous phase was extracted with DCM, and the combined organic layers are washed with brine, dried over MgSO_4 , filtered and concentrate under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried at room temperature to get a off-white solid (yield: 15.20 g). $^1\text{H-NMR}$ (δ , D_2O): 7.11–7.39 (20H, aromatic), 4.25 (8H, $-\text{OCH}_2\text{CH}_2\text{OCOOPh}$), 4.02 (4H, $-\text{OCH}_2\text{CH}_2\text{OCONH}-$), 3.38–3.75 (PEG backbone hydrogens), 3.04 (4H, $-\text{OCONHCH}_2\text{CH}_2-$), 2.82 (8H, $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 2.65 (4H, $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 1.58 (4H, $-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$).

Synthesis of PEG branched hydroxyl-terminated precursor (PEG-(OH)₈, G=1)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OCOPh)₄ (13.5 g, 7.32 mmol of carbonate groups), DCM (250 mL) and *N*-(3-aminopropyl)diethanolamine (2.55 g, 15.7 mmol, 2.15 eq. of carbonate) and the resulting solution stirred for 24 h at 30–35 °C. Then the solution was concentrated under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried at room temperature to get a off-white solid (yield: 13.40 g). $^1\text{H-NMR}$ (δ , D_2O): 4.03–4.09 (12H, $-\text{OCH}_2\text{CH}_2\text{OCONH}-$ and $-\text{NCH}_2\text{CH}_2\text{OCONH}-$), 3.38–3.75 (PEG backbone hydrogens and 16H, $-\text{NCH}_2\text{CH}_2\text{OH}$), 3.03 (12H, $-\text{OCONHCH}_2\text{CH}_2-$), 2.46–2.70 (36H, $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$), 1.56 (4H, $-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$).

Synthesis of PEG phenyl carbonate-terminated precursor (PEG-(OCOPh)₈, G=2)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OH)₈ (13 g, 13.60 mmol of OH), DCM (250 mL) and pyridine (15 mL) and the resulting solution was cooled to 0 °C using ice-bath. A solution of phenyl chloroformate (7.1 g, 45.34 mmol, 3.34 eq. of OH) in 70 mL DCM was added dropwise over 20–30 min and the resulting homogeneous solution was stirred for 20 h at room temperature. Then the solution was poured into water containing NaHCO_3 and the resulting solution stirred for 5 min. The layers are separated, the aqueous phase was extracted with DCM, and the combined organic layers are washed with brine, dried over MgSO_4 , filtered and concentrate under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried at room temperature to get a yellow-white solid (yield: 14.30 g). $^1\text{H-NMR}$ (δ , $\text{D}_2\text{O}/\text{DCI}$): 7.10–7.39 (40H, aromatic), 4.51 (16H, $-\text{OCH}_2\text{CH}_2\text{OCOOPh}$), 4.05–4.17 (12H, $-\text{OCH}_2\text{CH}_2\text{OCONH}-$ and $-\text{NCH}_2\text{CH}_2\text{OCONH}-$), 3.38–3.75 (PEG backbone hydrogens), 3.1–3.40 (36H, $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OCOOPh})_2$), 3.04 (12H, $-\text{OCONHCH}_2\text{CH}_2-$), 1.86 (4H, $-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OCOOPh})_2$), 1.67 (2H, $-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{OCONH}-)_2$).

Synthesis of PEG branched hydroxyl-terminated precursor (PEG-(OH)₁₆, G=2)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OCOPh)₈ (12.50 g, 11.6 mmol of carbonate groups), DCM (250 mL) and *N*-(3-aminopropyl)diethanolamine (4.00 g, 24.7 mmol, 2.13 eq carbonate) and the resulting solution was stirred for 24 h at 30–35 °C. Then the solution was concentrated under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried at room temperature to get a off-white solid (yield: 12.50 g). $^1\text{H-NMR}$ (δ , D_2O): 4.03–4.08 (28H, $-\text{OCH}_2\text{CH}_2\text{OCONH}-$ and $-\text{NCH}_2\text{CH}_2\text{OCONH}-$), 3.40–3.75 (PEG backbone hydrogens and 32H, $-\text{NCH}_2\text{CH}_2\text{OH}$), 3.02 (28H, $-\text{OCONHCH}_2\text{CH}_2-$), 2.50–2.70 (84H, $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_2\text{O}-)_2$), 1.58 (28H, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{N}$).

Synthesis of PEG phenyl carbonate-terminated precursor (PEG-(OCOPh)₁₆, G=3)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OH)₁₆ (12 g, 21.02 mmol of OH), DMF (100 mL) and pyridine (25 mL) and the resulting solution was cooled to 0 °C using ice-bath. A solution of phenyl chloroformate (10.5 g, 67.05 mmol, 3.20 eq. of OH) in 80 mL DCM was added dropwise over 20–30 min and the resulting homogeneous solution was stirred for 2 h in ice-bath and 20 h at room temperature. Then the solution was poured into water containing NaHCO₃ and the resulting solution was stirred for 5 min. The layers are separated, the aqueous phase was extracted with DCM, and the combined organic layers are washed with brine, dried over MgSO₄, filtered and concentrate under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried in room temperature to get a light brown solid (yield: 14.10 g). ¹H-NMR (δ, D₂O/DCI): 7.09–7.38 (80H, aromatic), 4.52 (32H, —OCH₂CH₂OCOOPh), 4.05–4.26 (28H, —OCH₂CH₂OCONH— and —NCH₂CH₂OCONH—), 3.38–3.75 (PEG backbone hydrogens), 3.1–3.40 (84H, —CH₂CH₂N(CH₂CH₂OCOOPh)₂), 3.04 (28H, —OCONHCH₂CH₂—), 1.88 (16H, —NHCH₂CH₂CH₂N(CH₂CH₂OCOOPh)₂), 1.72 (12H, —NHCH₂CH₂CH₂N(CH₂CH₂OCONH—)₂).

Synthesis of PEG branched hydroxyl-terminated precursor (PEG-(OH)₃₂, G=3)

A 500 mL flask equipped with a magnetic stirring bar was charged with PEG-(OCOPh)₁₆ (11.5 g, 16.64 mmol of carbonate groups), DCM (250 mL) and *N*-(3-aminopropyl)diethanolamine (5.66 g, 34.9 mmol, 2.10 eq. carbonate) and the resulting solution was stirred for 24 h at 30–35 °C. Then the solution was concentrated under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried at room temperature to get a light orange solid (yield: 14.20 g).

Synthesis of multifunctional ATRP macroinitiator (PEG-(Br)₃₂)

A 200 mL flask equipped with a magnetic stirring bar was charged with PEG-(OH)₃₂ (2 g, 5.27 mmol of OH), DMF (30 mL) and pyridine (4 mL) and the resulting solution was cooled to 0 °C using ice-bath. A solution of 2-bromoisobutyryl bromide (2.7 mL, 21.84 mmol, 4.15 eq. of OH) in 15 mL DCM was added dropwise over 15–20 min and the resulting homogeneous solution was stirred for 2 h in ice-bath and 20 h at room temperature. Then the solution was poured into water containing NaHCO₃ and the resulting solution was stirred for 5 min. Dichloromethane was added, the layers are separated, the aqueous phase was extracted with DCM, and the combined organic layers are washed with brine, dried over MgSO₄, filtered and concentrate under reduced pressure. Diethyl ether was added to precipitate the product, which was filtered and washed completely with diethyl ether and dried at room temperature under vacuum to get a yellow solid (yield: 2.70 g) and stored in –20 °C.

Polymerization of NIPAm from macroinitiator and synthesis of amphiphilic dendritic copolymer, (PNIPAm)₁₆-PEG-(PNIPAm)₁₆

134 mg (0.262 mmol-Br) of macroinitiator PEG-(Br)₃₂ and 900 mg NIPAm (7.95 mmol) were stirred in 3 mL degassed ethanol to dissolve completely, then 10 mL degassed water was added and cooled in ice bath. A stock solution of the catalysts was prepared by dissolving CuBr₂ (20 mg, 0.09 mmol) and PMDETA (31 mg, 0.18 mmol) in 2 mL degassed water. A stock solution of reducing agent was prepared by dissolving ascorbic acid (100 mg, 0.57 mmol) in 2 mL degassed water.

Then 1 mL of the CuBr₂/L complex solution and 1 mL of the ascorbic acid solution were added to the reaction to start polymerization. Polymerization was continued for about 1 h in ice-bath to reach a viscous solution. Then reaction mixture exposed to air and 20 mL of water was added to stop the reaction and remove the water by freeze-drying.

Then dissolve the freeze-dried product in DCM and pass throw a neutral alumina column to remove the copper catalyst. Then the solution was concentrated using an evaporator to about 5 mL then add diethyl ether to precipitate the product. Filter the product and wash it completely with diethyl ether and dried at room temperature to get a white solid (yield ~0.85 g).

Characterization Methods

^1H -NMR (400 MHz) spectra were recorded on a Bruker DPX-400 NMR spectrometer in the deuterated water (D_2O). FTIR spectra of samples were taken in KBr pellets using an ABB Bomem MB-100 FTIR spectrophotometer.

The molecular weights and molecular weight distributions of polymers were estimated by a gel-permeation chromatographic (GPC) system equipped with a Waters 2690D separations module and Waters 2410 refractive index detector. *N*-Methylpyrrolidone (NMP) was used as the eluent at a flow rate of 0.3 mL/min. Waters millennium module software was used to calculate molecular weight on the basis of a universal calibration curve generated by polyethylene glycol standards with narrow molecular weight distribution.

Transmission electron microscopy (TEM) measurements were carried out by a JEOL JEM-1200 electron microscope operated at an acceleration voltage of 60 kV. TEM samples were prepared as following: the aqueous solutions of the dendritic copolymer (1 mg/mL), phosphotungstic acid (PTA) and Formvar-coated copper grids were placed in an oven with preset temperature, *i.e.*, 25 °C and 40 °C, respectively, for a half-hour to reach temperature equilibrium. A droplet of sample solution was then dripped onto the copper grids, which were allowed to dry for another half-hour in the oven. Afterward, the samples were stained with PTA. The stained TEM samples were again allowed to dry in the oven before observation.

Average hydrodynamic radius and distribution of the aggregates in deionized water at various temperatures was determined by a dynamic light scattering instrument (DLS) at a fixed angle of 90° with a Nano-ZS90 laser nano-granulometric analysis instrument (Malvern Instrument, Watts, U) using the cumulant analysis method. Before each measurement, the solution was allowed to equilibrate until a stable reading of the intensity was obtained.

Cloud Point

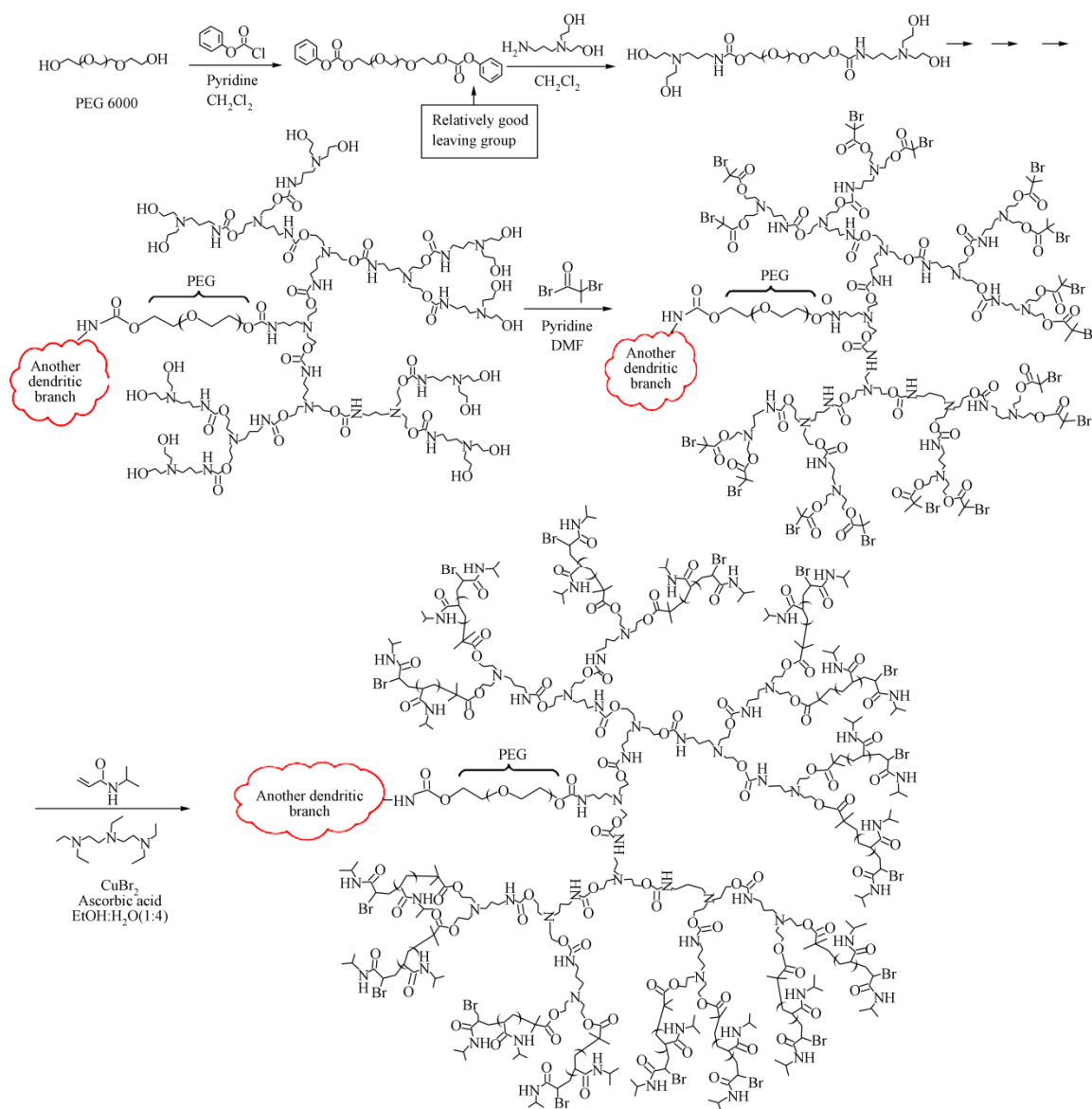
Transition temperature of the dendrimer was determined using a UV-Vis spectrophotometer at 500 nm. The polymer concentration was 1 mg/mL in distilled water. The cloud point was defined as the temperature at which 50% of the transmittance of the solution was observed.

RESULTS AND DISCUSSION

Design, Synthesis and Characterization of the Dendritic Copolymer

The process for synthesis of this copolymer was accomplished in three main steps: (i) synthesis of dendritic polyol based on PEG using stepwise terminal hydroxyl group increment, (ii) converting of these terminal OH groups into the ATRP initiator active sites, and (iii) atom transfer radical polymerization of NIPAm using of this multifunctional macroinitiator. The general synthetic routes toward the polyol, the multifunctional macroinitiator and the block copolymer are outlined in Scheme 1. Hydroxyl groups of PEG were activated by reaction with phenyl chloroformate to form an active carbonate ester for subsequent modification. Then, these carbonate groups were reacted with *N*-(3-aminopropyl)diethanolamine to increase the hydroxyl groups. A dendritic polyol with 16 terminal hydroxyl groups at each end was obtained by reiteration of these two steps for two more times. The hydroxyl groups of this dendritic polyol were allowed to react with the 2-bromo isobutyryl bromide to yielding a multifunctional dendritic ATRP macroinitiator with 32 initiation sites. Finally, the macroinitiator was used for polymerizations of NIPAm under ATRP conditions.

The FTIR spectra of PEG, polyol, macroinitiator and amphiphilic dendritic copolymer are shown in Fig. 1. In the spectrum of polyol as compared to the PEG, an additional peak appears at 1705 cm^{-1} which is related to the carbamate group. Moreover, the intensity of OH band around 3500 cm^{-1} has increased significantly because the numbers of hydroxyl groups have increased. In the spectrum of macroinitiator, the band around 1730 cm^{-1} is related to the ester groups which overlaps with the peak of carbamate group at 1705 cm^{-1} . Additionally, the broad band of hydroxyl groups in polyol has been eliminated approximately because the hydroxyl groups are converted to ester groups. Finally, at the spectra of the dendrimer the band at 1650 cm^{-1} is related to the amide group of PNIPAm and the shoulder at $1700\text{--}1730\text{ cm}^{-1}$ is due to the ester and carbamate groups.



Scheme 1 Synthesis route for preparation of the polyol, the multifunctional macroinitiator and the final dendritic copolymer

The ^1H -NMR spectra of polyol, macroinitiator and amphiphilic dendritic copolymer in D_2O are shown in Fig. 2. The protons in the all three structures are assigned completely in the figure. These results demonstrate the successful formation of the final dendritic copolymer with PNIPAm brushes.

The average molecular weights and polydispersity indexes (PDI) were also measured by GPC using NMP as elution solvent and monodisperse polyethylene glycol as standard. The GPC results (Table 1) clearly show an increase in molecular weight from PEG to the final dendritic copolymer which is another confirmation of successful synthesis of the copolymer.

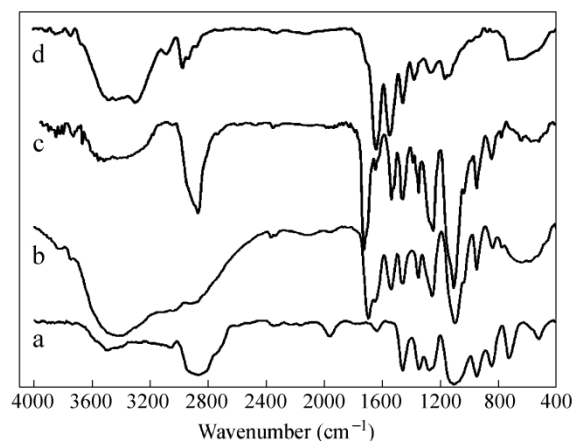
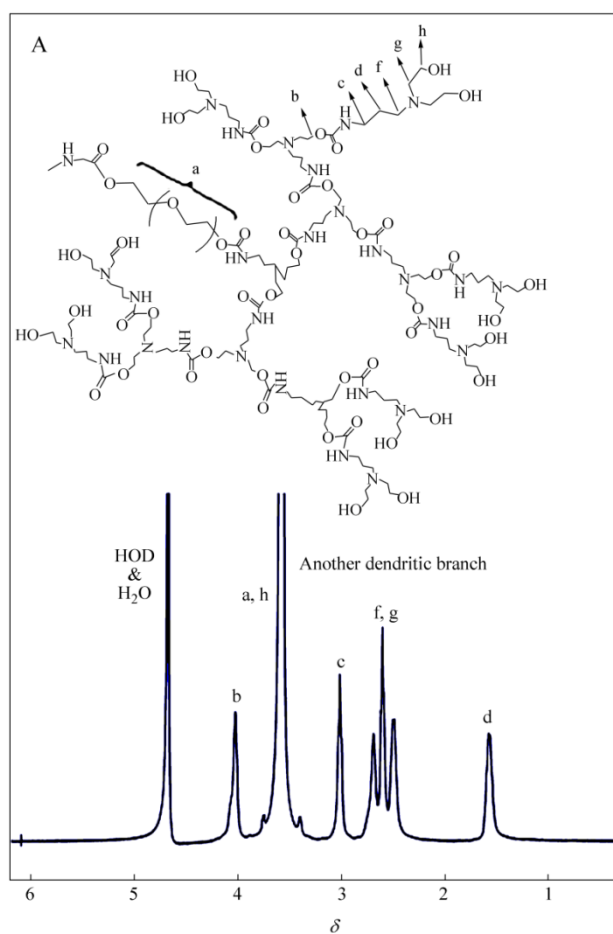


Fig. 1 FTIR spectra of (a) PEG, (b) polyol, (c) macroinitiator and (d) PEG-*b*-PNIPAm dendrimer

Table 1. Molecular weights and polydispersities of PEG, polyol, macroinitiator and its block copolymer with NIPAm

Sample	M_n	M_w	PDI
PEG	5537	6529	1.18
Polyol	10151	12385	1.22
Macroinitiator	15308	17839	1.17
Amphiphilic dendritic copolymer*	89840	143648	1.60

* Initiation sites: NIPAm = 1:30; Ratio of NIPAm to ethylene glycol in final polymer: 6:1 (at conversion 75%)



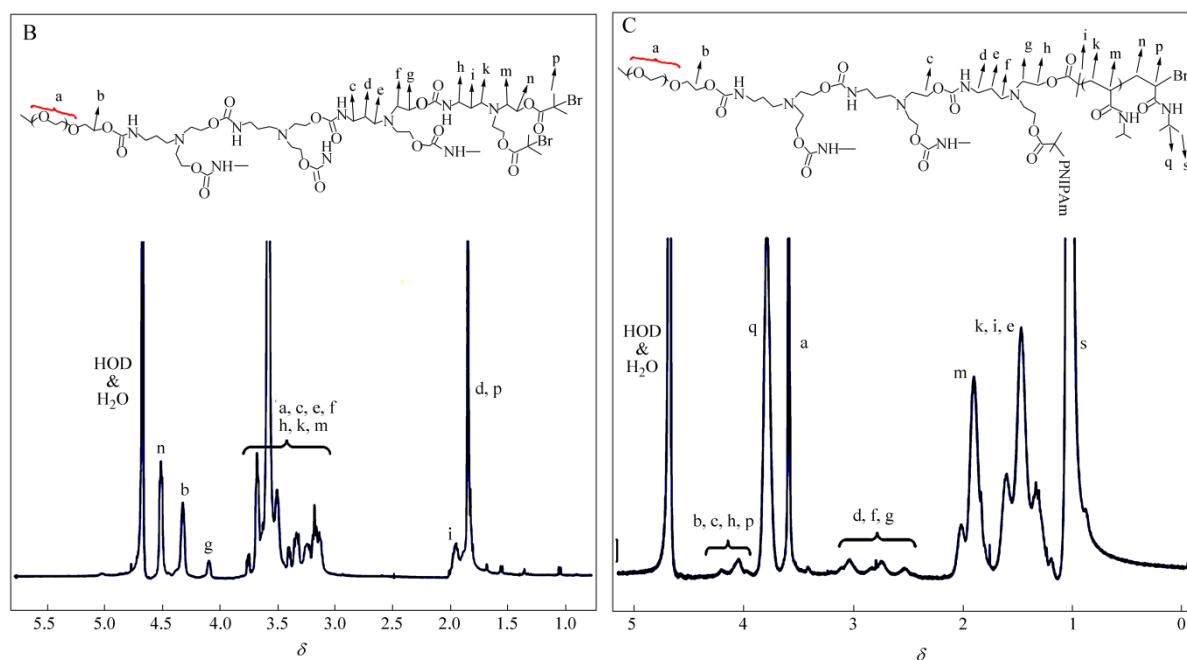


Fig. 2 ^1H -NMR spectra of (A) polyol, (B) multifunctional macroinitiator and (C) final dendritic copolymer

Aggregation and Thermosensitive Behavior of the Dendritic Block Copolymer

Polyethylene glycol is highly hydrophilic and dissolves in aqueous solution over a wide temperature range. In contrast, the solubility of the PNIPAm in aqueous solutions is temperature dependent: it dissolves molecularly at 20 °C but precipitates above its lower critical solution temperature (LCST) of approximately 32 °C. In dendritic PEG-*b*-PNIPAm copolymer, the poly-NIPAm part is presented as hydrophilic segment. If this hydrophilic component is converted to a hydrophobic one in response to temperature, then the copolymer should precipitate out. The cloud point of the dendritic copolymer was determined by a UV-Visible spectrophotometer. Figure 3 shows the dependence of the transmittance for a solution of dendritic PEG-*b*-PNIPAm (1 mg/mL) in water as a function of temperature at 500 nm. Formation of a precipitate was observed with increase in temperature, and this process was thermally reversible. It was recognized that the incorporating of hydrophilic units into poly-NIPAm would result in the increased cloud point to 36 °C which was upper than that of PNIPAm (*i.e.* 32 °C).

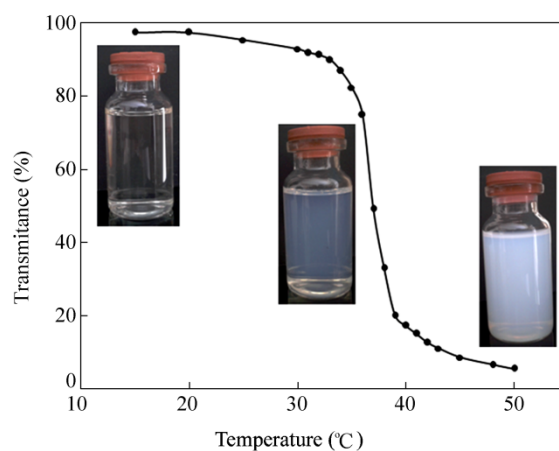
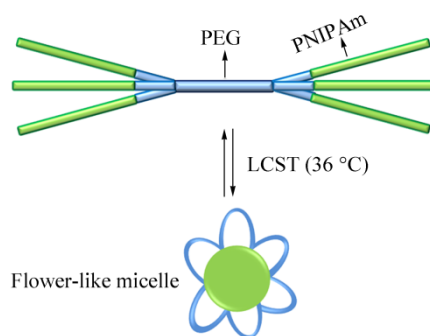


Fig. 3 Temperature-dependence of transmittance for aqueous solution of dendritic copolymer at 500 nm ($c = 1 \text{ mg/mL}$)

The micellization of BAB triblock copolymers in solvents that preferentially dissolve the middle block has been well documented in literature^[28]. Spherical micelles in these systems consist of a core containing B blocks, surrounded by loops of A block. Most likely, these are the *flowerlike micelles*. Recently, micellization of dendron-like/linear/dendron-like poly(ϵ -caprolactone)-*b*-poly(ethylene glycol)-*b*-poly(ϵ -caprolactone) triblock copolymers was investigated by Yang *et al.*^[29]. They observed that these triblock copolymers also mainly self-assembled into spherical flower-like micelles in aqueous solution. The schematic illustration of the formation of flower-like micelles from the synthesized triblock copolymer in aqueous solution is shown in Scheme 2.



Scheme 2 Schematic illustration of the formation of micelles from dendritic PEG-*b*-PNIPAm triblock copolymer in aqueous solution

The micellization behavior of the dendritic PEG-*b*-PNIPAm dendritic copolymer was investigated by DLS and TEM. The DLS diagrams at 25 °C and 40 °C are shown in Fig. 4. At 25 °C all parts of the dendritic copolymer is hydrophilic and so there is no micelle formation, the copolymer is dissolved molecularly with a low D_h around 15 nm at 25 °C, but at 40 °C, PEG chains act as hydrophilic sections and PNIPAm chains act as hydrophobic sections, hence, a flower-like micelle can be formed in this condition which the PNIPAm chains make the core and the PEG ones make the shell of this micelle. The outer shell of these micelles are completely hydrophilic PEGs so no aggregation between single micelles can be formed at 40 °C, therefore only one size distribution ($D_h \sim 110$ nm) was observed at 40 °C. Also the TEM images at 25 °C and 40 °C are shown in Fig. 5. As it is obvious, TEM images confirm the DLS results. The diameters observed from TEM images were slightly smaller than the DLS diameters which is because of the dehydration and shrinkage of the micelles during drying.

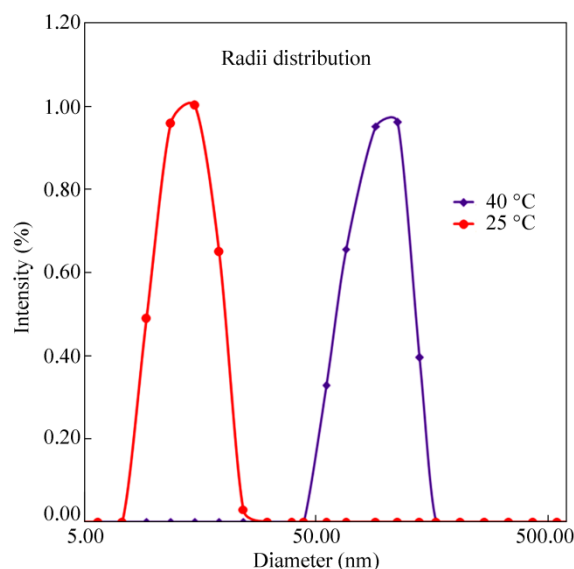


Fig. 4 DLS results in 25 °C and 40 °C of the dendritic copolymer in water

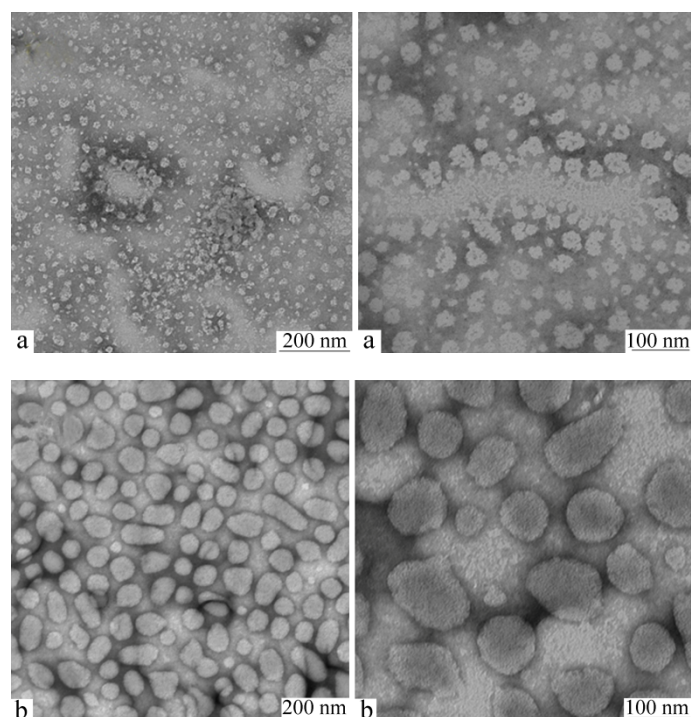


Fig. 5 TEM results of the dendritic copolymer in water at (a) 25 °C and (b) 40 °C

CONCLUSIONS

A novel dendritic polymer with $CP = 36\text{ }^{\circ}\text{C}$ was successfully synthesized by preparation of a dendritic polyol based on PEG and converting it into an ATRP macroinitiator. Subsequently, the dendritic macroinitiator was used for polymerization of NIPAm *via* ATRP to provide the dendritic PNIPAm-*b*-PEG-*b*-PNIPAm copolymer. The self association of the final dendritic copolymer was temperature reliant because of the thermosensitive nature of the PNIPAm blocks. Finally, the association behavior and self-assembly of the dendritic copolymer was investigated by TEM and DLS techniques. The results showed that the copolymer chains dissolve without any aggregation at room temperature but make flower-like micelles above $36\text{ }^{\circ}\text{C}$.

REFERENCES

- 1 Huang, X., Sevimli, S.I. and Bulmus, V., *Eur. Polym. J.*, 2013, 49: 2895
- 2 Wang, J., Yao, Y., Ji, B., Huang, W. and Yan, D., *Chinese J. Polym. Sci.*, 2011, 29(2): 241
- 3 Deng, J.N., Wang, W.B., Zheng, Z.H., Ding, X.B. and Peng, Y.X., *Chinese J. Polym. Sci.*, 2014, 32(7): 817
- 4 Bagheri, M. and Bigdeli, E., *J. Polym. Res.*, 2013, 20: 84
- 5 Stefani, M., Coudane, J. and Vert, M., *Polym. Degrad. Stab.*, 2006, 91: 2554
- 6 Bencini, M., Ranucci, E., Ferruti, P., Manfredi, A., Trotta, F. and Cavalli, R., *J. Polym. Sci., Part A: Polym. Chem.*, 2008, 46: 1607
- 7 Zou, T., Li, S.L., Zhang, X.Z., Wu, X.J. and Cheng, S.X., *J. Polym. Sci., Part A: Polym. Chem.*, 2007, 45: 5256
- 8 Hawker, C.J. and Wooley, K.L., *Science*, 2005, 309: 1200
- 9 Gohy, J.F., Willet, N., Varshney, S., Zhang, J. and Jérôme, R., *Angew. Chem. Int. Ed.*, 2001, 40: 3214
- 10 Mitsukami, Y., Hashidzume, A., Yusa, S., Morishima, Y. and Lowe, A.B., *Polymer*, 2006, 47: 4333
- 11 Wei, H., Cheng, S.X., Zhang, X.Z. and Zhuo, R.X., *Prog. Polym. Sci.*, 2009, 34: 893
- 12 Wang, D., Wu, T., Wan, X.J., Wang, X.F. and Liu, S.Y., *Langmuir*, 2007, 23: 11866

- 13 Wang, Y.P., Zhang, M., Moers, C., Chen, S.L. and Xu, H.P., *Polymer*, 2009, 50: 4821
- 14 Ma, N., Li, Y., Xu, H.P., Wang, Z.Q. and Zhang, X., *J. Am. Chem. Soc.*, 2010, 132: 442
- 15 Tanaka, F., Koga, T. and Kojima, H., *Chinese J. Polym. Sci.*, 2011, 29(1): 13
- 16 Yan, J., Ji, W., Chen, E., Li, Z. and Liang, D., *Chinese J. Polym. Sci.*, 2010, 28(3): 437
- 17 Zakir, M.R., Dincer, S. and Piskin, E., *Prog. Polym. Sci.*, 2007, 32: 534
- 18 Wei, H., Zhang, X.Z., Cheng, C., Cheng, S.X. and Zhuo, R.X., *Biomaterials*, 2007, 28: 99
- 19 Li, X.W., Liu, W.G., Ye, G.X., Zhang, B.Q. and Zhu, D.W., *Biomaterials*, 2005, 26: 7002
- 20 Herold, D.A., Keil, K. and Bruns, D.E., *Biochem. Pharmacol.*, 1989, 38: 73
- 21 Castelletto, V., Gouveia, R.J., Connon, C.J. and Hamley, I.W., *Eur. Polym. J.*, 2013, 49: 2961
- 22 Zou, J.H., Shi, W.F. and Wang, J., *Macromol. Biosci.*, 2005, 5: 662
- 23 Knapen, J.W.J., van der Made, A.W., de Wilde, J.C. and van Leeuwen, P., *Nature (London)*, 1997, 372: 659
- 24 Gillies, E.R., Jonsson, T.B. and Frechet, J.M.J., *J. Am. Chem. Soc.*, 2004, 126: 11936
- 25 Kojima, C., Haba, Y., Fukui, T., Kono, K. and Takagishi, T., *Macromolecules*, 2003, 36: 2183
- 26 Davis, K.A. and Matyjaszewski, K., *Macromolecules*, 2000, 33: 4039
- 27 Boyes, S.G., Brittain, W.J., Weng, X. and Cheng, S.Z.D., *Macromolecules*, 2002, 35: 4960
- 28 Xu, B., Yuan, J., Ding, T. and Gao, Q., *Polym. Bull.*, 2010, 64: 537
- 29 Yang, Y., Hua, C. and Dong, C., *Biomacromolecules*, 2009, 10: 2310