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Facile synthesis of branched graft copolymers via combination of RAFT self-condensing vinyl polymerization and aldehyde-aminooxy reaction

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Abstract

A facile synthetic pathway to branched graft copolymers has been developed by combination of self-condensing vinyl polymerization (SCVP), reversible addition-fragmentation chain transfer (RAFT) polymerization and aldehyde-aminooxy reaction. RAFT polymerization of 2-(dimethylamino)ethyl methacrylate (DEM) in the presence of a newly designed aldehyde-containing chain-transfer monomer, 6-(2-formyl-4-vinylphenoxy)hex-yl-2-(dodecylthiocarbonothioylthio)-2-methylpropanate (FHDM), led to branched polyDEM (BPDEM) bearing aldehyde functionalities on its branching points. The degree of branching and average branch length of the resulting BPDEM can be readily tuned by manipulation of DEM/FHDM feed ratio. The aldehyde groups on the branching points of BPDEM were then reacted with aminooxy-terminated poly(ethylene oxide)s (PEO-ONH_2), affording structurally well-defined branched graft copolymer BPDEM-*g*-PEO with branched polyDEM backbone and PEO grafted chains. The thermo-induced micellization behavior of BPDEM-*g*-PEO in water was investigated. Opportunities are open for BPDEM-*g*-PEO to form either multimolecular micelles or unimolecular micelles via

simply adjusting the chain length of grafted PEO. Further modification of BPDEM-*g*-PEO by quaternization resulted in branched cationic polyelectrolytes which are capable of capturing negatively charged guest molecules via electrostatic complexation to form in situ self-assembled nanoparticles with simultaneous encapsulation of the guests.

Introduction

Branched polymers, a subclass of dendritic polymers, have drawn considerable interest due to their facile preparation, unique physical and chemical properties and potential applications in various fields such as supramolecular chemistry, biomaterials and nanotechnology.[1-6] One particularly versatile method to synthesize branched polymers is the polymerization of conventional vinyl monomers in the presence of a branching agent capable of both propagation and initiation, a process called self-condensing vinyl polymerization (SCVP).[7] The branching agent employed for SCVP is actually a special kind of vinyl monomer containing a group that can be activated to generate an initiating site. In order to avoid gelation and obtain branched polymers with controllable molecular parameters, SCVP methodology is generally combined with various living/controlled radical polymerization techniques,[8-10] especially reversible addition-fragmentation chain transfer (RAFT) polymerization due to its advantages such as relatively mild reaction conditions and wide range of monomers.[11] A range of branched polymers, including branched polystryrene,[5] polyacrylates,[12] poly(*N*-isopropyl acrylamide),[13] and poly(vinyl acetate),[14] have been prepared via combination of RAFT and SCVP (RAFT-SCVP) by employing a

polymerizable chain transfer agent (i.e., chain transfer monomer, CTM) as the branching agent.

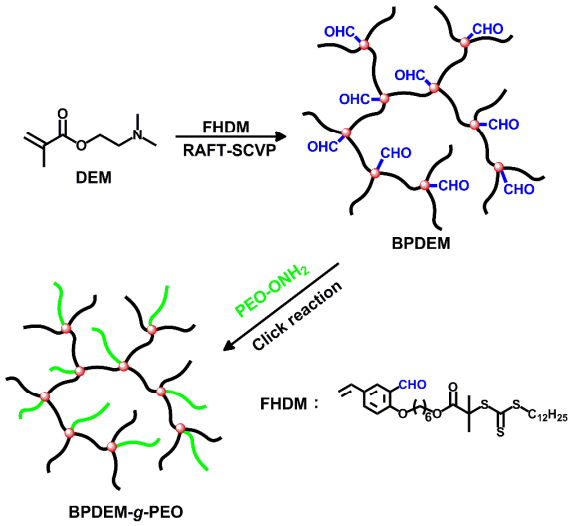
In some cases, modification of branched polymers is necessary to tailor their properties such as polarity, solubility and flexibility for a specialized purpose. Branched polymers can be modified and functionalized by grafting polymer chains onto their branched backbones, generating branched graft copolymers with tailor-made properties and more complex architectures. However, the complex architectures bring more difficulties for access to well-defined branched graft copolymers as the synthesis usually requires multistep reactions and cumbersome purifications between each step; and consequently reports concerning the synthesis and properties of branched graft copolymers are very scarce.[15-16] Therefore, to simply and controllably synthesize branched graft copolymers still remains a challenge. Recently, Gao et al. have prepared a branched graft copolymer with branched poly(tertiary amino methacrylate) backbone and poly(ethylene glycol) grafted chains via RAFT-SCVP strategy in combination with click-like Menschukin reaction (i.e., quaternarization) and copper-catalyzed azide-alkyen cycloaddition click reaction.[15] Branched graft copolymers with the same backbone but different grafts such as poly(ε-caprolactone), polystyrene and polyacrylates were also obtained via a similar route by Zhao et al..[12]

Reaction of an aldehyde with an aminooxy via formation of an oxime linkage is a highly efficient coupling reaction and shares common features with "click" chemistry.[17] This coupling reaction has the advantage that, in comparison to the most

popular copper-catalyzed alkyne-azide click reaction, no other auxiliaries including toxic metallic catalyst are required; and the reaction is tolerant to the presence of oxygen and moisture and can be conducted at ambient temperatures. Nevertheless, the potential of aldehyde-aminooxy click-like reaction in novel macromolecular architecture construction has been far less explored;[18-20] so far there are no example of the synthesis of branched copolymers using this technique.

Herein, we report on a facile synthetic pathway to branched graft copolymers by combination of SCVP-RAFT technique and aldehyde-aminooxy reaction. As shown in Scheme 1, RAFT polymerization of 2-(dimethylamino)ethyl methacrylate (DEM) was conducted using a new designed aldehyde-containing chain-transfer monomer, 6-(2-formyl-4-vinylphenoxy)hexyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoate (FHDM), as a branching RAFT agent to generate the branched polyDEM (BPDEM) with aldehyde functionalities on its branching points. The aldehyde groups on the branching points were then coupled with aminooxy-terminated poly(ethylene oxide)s (PEO-ONH_2) via aldehyde-aminooxy reaction, affording a branched graft copolymer BPDEM-*g*-PEO with branched polyDEM backbone and PEO grafted chains. Because aminooxy end groups can be easily incorporated into polymers by living radical polymerizations using an aminooxy-containing initiator or by post-modification,[21-23] this synthetic pathway can be extended to other branched graft structures. More importantly, the resulting branched graft copolymers can maintain the inherent characteristics of the branched backbone precursors because the grafted chains are attached to the branched

backbones at the position of their branching points rather than being connected with their linear chains (i.e., chains between branching points). The branched graft copolymer BPDEM-*g*-PEO, obtained in this work, may inherit the thermoresponsive features from its branched polyDEM precursor and thus exhibit thermo-induced micellization character in aqueous solution. Further modification of branched polyDEM backbone via quaternization can endow branched graft copolymer BPDEM-*g*-PEO with ability to capture anionic guest molecules via electrostatic complexation.



Scheme 1. Synthesis of branched graft copolymer BPDEM-*g*-PEO

Experimental Section

Materials

2-(Dimethylamino)ethyl methacrylate (DEM; Aladdin, 99%) was passed through a column of basic alumina to remove the stabilizing agents. 2,2′-Azobis(isobutyronitrile) (AIBN; Sinopharm Chemical Reagent, 99%) was purified by recrystallization from

ethanol. 1,4-Dioxane and tetrahydrofuran (THF) (both from Shanghai Chemical Reagent Co., 99%) were refluxed with sodium chips under N_2 until dry and freshly distilled before use. *N*,*N*-Dimethylformamide (DMF; Shanghai Chemical Reagent Co., 99%) was distilled under reduced pressure over CaH_2 before use. Dichloromethane (Shanghai Chemical Reagent Co., 99%) was distilled over CaH_2 before use. 2-(Dodecylsulfanylthiocarbonylsulfanyl)-2-methyl propionic acid (DMPA)[24], 2-hydroxy-5-vinylbenzaldehyde (HVB)[25] and monoaminooxy end-functionalized poly(ethylene oxide)[26] (PEO-ONH_2, molecular weight 400, 1000, and 2000 g mol^{-1}) were synthesized according to literature procedures. Other reagents were all reagent grade materials, purified by standard methods if needed.

Synthesis of 2-(6-hydroxyhexyloxy)-5-vinylbenzaldehyde (HHVB)

A mixture of HVB (4.44 g, 30 mmol), K_2CO_3 (4.15 g, 30 mmol), 6-chlorohexanol (4.24 g, 30 mmol) and DMF (20 mL) was stirred at 80 ^oC under N_2 for 24 h. The reaction mixture was then poured into 200 mL of ethyl acetate, washed with brine 3 times and dried over anhydrous Na_2SO_4. After filtration, the volatiles were removed by a rotary evaporator. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (7:3) to give HHVB as a colorless viscous liquid (3.8 g, 51%). ^1H NMR($CDCl_3$), δ (TMS, ppm): 1.49 (m, 4H, -(CH_2)$_2$-), 1.63 (m, 2H, -CH_2-CH_2OH), 1.88 (m, 2H, -CH_2-CH_2O-phenyl), 3.68 (t, 2H, -CH_2-OH), 4.10 (t, 2H, -CH_2-O-phenyl), 5.21 (d, J = 10.9 Hz, 1H, *trans*-CH_2=), 5.69 (d, J = 17.4 Hz, 1H, *cis*-CH_2=), 6.66 (dd, J_1 = 10.8 Hz, J_2 = 17.4 Hz, 1H, -CH=), 6.94 (d, 2H, J = 8.8 Hz, 2-aromtic proton), 7.58 (dd, J_1 = 2.3 Hz, J_2 = 8.5 Hz, 1H, 5-aromtic proton), 7.86 (d,

J = 2.2 Hz, 1H, 6-aromtic proton), 10.5 (s, 1H, -CHO) .

Synthesis of 6-(2-formyl-4-vinylphenoxy)hexyl 2-(dodecylthiocarbonothioylthio) -2-methylpropanoate (FHDM)

HHVB (3.72 g, 15 mmol) and 4-dimethylaminopyridine (0.92 g, 7.5 mmol) and were dissolved in 10 mL dry dichloromethane and stirred for 10 min in ice-water bath. To this solution, a mixture of DMPA (6.57 g, 18 mmol) and dicyclohexylcarbodiimide (3.71 g, 18 mmol) in dichloromethane (20 mL) was added dropwise. The mixture was stirred for 24 h at room temperature. After filtration, the solvent was removed by a rotary evaporator. The crude product was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (19:1) to give FHDM as a yellow solid (8.31 g, 81%). ^{1}H NMR ($CDCl_3$), δ (TMS, ppm): 0.89 (t, 3H, CH_3CH_2-), 1.02-1.57 (22H, $CH_3(CH_2)_9$- and -OC_2H_4-$(CH_2)_2$-), 1.70 (s, 10H, -$C(CH_3)_2$ and –OCH_2-CH_2-), 1.86 (t, 2H, -SCH_2CH_2-), 3.25 (t, 2H, -SCH_2-), 4.09 (dd, J_1 = 6.4, J_2 = 14.6, 4H, -O*CH2*-), , 5.21 (d, *J* = 11.2 Hz, 1H, *trans*-CH_2=), 5.69 (d, *J* = 17.2 Hz, 1H, *cis*-CH_2=), 6.66 (dd, J_1 = 10.9 Hz, J_2 = 17.6 Hz, 1H, -*CH*=), 6.94 (d, 1H, *J* = 8.7 Hz, 2-aromtic proton), 7.58 (dd, J_1 = 2.4 Hz, J_2 = 8.6 Hz, 1H, 5-aromtic proton), 7.86 (d, *J* = 2.3 Hz, 1H, 6-aromtic proton), 10.5 (s, 1H, -CHO). Anal. Calcd for $C_{32}H_{50}O_4S_3$: C 64.60; H 8.47; S 16.17. Found: C 64.31; H 8.69; S 16.03. FAB MS: m/z calcd for $C_{32}H_{50}O_4S_3$ 594.29; found 594 (M+).

RAFT polymerization of DEM with chain transfer agent FHDM

For a typical polymerization, a stock solution in 1,4-dioxane (4.2 mL) comprising FHDM (2.40 g, 4.0 mmol), DEM (12.72 g, 80 mmol), AIBN (67.2 mg, 0.4 mmol)

was prepared in a N_2-filled dry glove box. Aliquots (1 mL) were removed and transferred to ampules, degassed by three freeze-pump-thaw cycles, sealed under N_2, and heated at 65 °C. After the predetermined intervals, the sealed ampule was cooled in an ice bath. The reaction mixture was diluted with THF and immediately analyzed by GPC. The resulting aldehyde-containing branched polyDEM (BPDEM) was isolated by precipitating into cold petroleum ether and dried under vacuum. Conversions of DEM and FHDM were determined by ^{1}H NMR spectroscopy.

Grafting of PEO onto BPDEM via aldehyde-aminooxy reaction

A typical example is given below. BPDEM (200 mg, $M_{n,\,GPC}$ = 16700 and M_w/M_n = 2.24, 54 μmol of aldehyde groups) and PEO-ONH_2 (81 mg, M_n = 1000, 81 μmol of animooxy groups) were dissolved in 5 mL of THF and stirred at room temperature for 24 h. The resulting branched graft copolymer BPDEM-*g*-PEO was purified by dialysis against THF for 24 h using a semipermeable membrane (cutoff molecular weight 3000) to remove the unreacted PEO-ONH_2.

Quaternization of DEM units of branched graft copolymer

Methyl iodide (0.3 g, 2.1 mmol, ~ 5-fold molar excess to DEM units) was added to a solution of branched graft copolymer BPDEM-*g*-PEO (100 mg) in THF (10 mL). The mixture was stirred for 24 h at room temperature, and a yellow precipitate started to form after ~1 h. Then the quaternized product was separated by centrifuge and washed with THF three times to remove the excess of methyl iodide. The resulting quaternized polymer was dried under vacuum at room temperature to give a yellow powder (135 mg, 90%).

Complexation of sodium deoxycholate with quaternized polymer

For a typical procedure, 59 μL of aqueous solution of sodium deoxycholate (5.9 μmol) was added to a solution of the quaternized polymer (25 mg, containing 6.6 μmol of quaternary ammonium groups) in water (5 mL) under vigorous stirring. The mixture was stirred overnight before characterization.

Measurements

The number-average molecular weight (M_n), and molecular weight distribution (M_w/M_n) were measured by gel permeation chromatography (GPC) against polystyrene standard in THF at a flow rate of 1.0 mL min^{-1} at 35 °C on three Waters Styragel columns (measurable molecular weight range: 100–5000, 500–30000, and 5000–600000) connected to a Waters 1515 pump and a Waters 2414 refractive index detector. The absolute weight-average molecular weight (M_w) of the polymers was determined by laser light scattering (LLS) in THF at 40 °C on the chromatograph system equipped with a Waters 1515 pump, two PL gel 10 μm MIXED-B columns, a Waters 2414 refractive index detector and a Viscotek 270 laser light scattering detector (dual laser light scattering, λ = 670 nm). The M_w values were obtained using the sample mass injected into the GPC-LLS instrument.

^{1}H NMR (300 MHz) spectra was recorded on a Varian Unity Inova 300 spectrometer. Polymer samples for ^{1}H NMR and LLS analysis were fractionated by preparative GPC (column: Ultrastyragel 10^4 Å, in THF at a flow rate of 3.0 mL min^{-1} at 35 °C).

Differential scanning calorimetric analysis (DSC) was performed using a TA

DSC Q20 at a heating rate of 10 ^{o}C /min under nitrogen.

Dynamic light scattering (DLS) measurements were conducted at 25 ^{o}C on a Brookhaven BI-200SM apparatus with a BI-9000AT digital correlator and a He-Ne laser at 532 nm. Prior to the measurement, the sample solutions were filtered through nylon filters (13-HV, Millipore, 0.45 μm pore size). The data were analyzed by CONTIN algorithm.

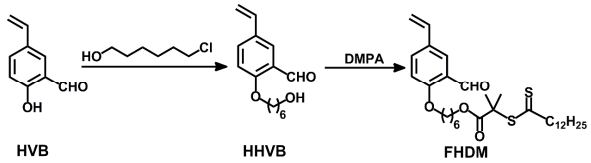
Atomic force microscopy (AFM) experiments were performed in ScanAyst mode in air at room temperature with a Bruker Multimode 8 AFM (Bruker Nano Inc). Measurements were performed using non-conductive silicon nitride AFM probes (Bruker Nano Inc). For sample of thermo-induced BPDEM-g-PEO micelles, a hot polymer aqueous solution (0.5 mg mL^{-1}, 70 ^{o}C) was dropped on a mica wafer in a hot air circulation oven and then dried in the oven at 70 ^{o}C for 3h. For sample of quaternized polymer/sodium deoxycholate complexes, a complex aqueous solution (0.5 mg mL^{-1}) was dropped on a mica wafer and dried at room temperature.

Results and Discussion

Synthesis of aldehyde functionalized chain-transfer monomer FHDM

To introduce the aldehyde groups into the branching points of branched polymer, a novel aldehyde functionalized chain-transfer monomer FHDM was designed and synthesized. FHDM was synthesized by reaction of HVB with 6-chlorohexanol, followed by esterification with DMPA (Scheme 2). The structure of FHDM was confirmed on the basic of the elemental analysis, MS, and NMR spectroscopy. The ^{1}H NMR spectrum (Fig. 1A) exhibits the characteristic signals corresponding to vinyl

(a,b,c), aldehyde (e) and DMPA moiety (i,j,k); the other signals were assigned as shown in Fig. 1A. Carbon signals in ^{13}C NMR spectrum were also clearly classified as depicted in Fig. 1B.



Scheme 2. Synthesis of aldehyde functionalized chain-transfer monomer FHDM

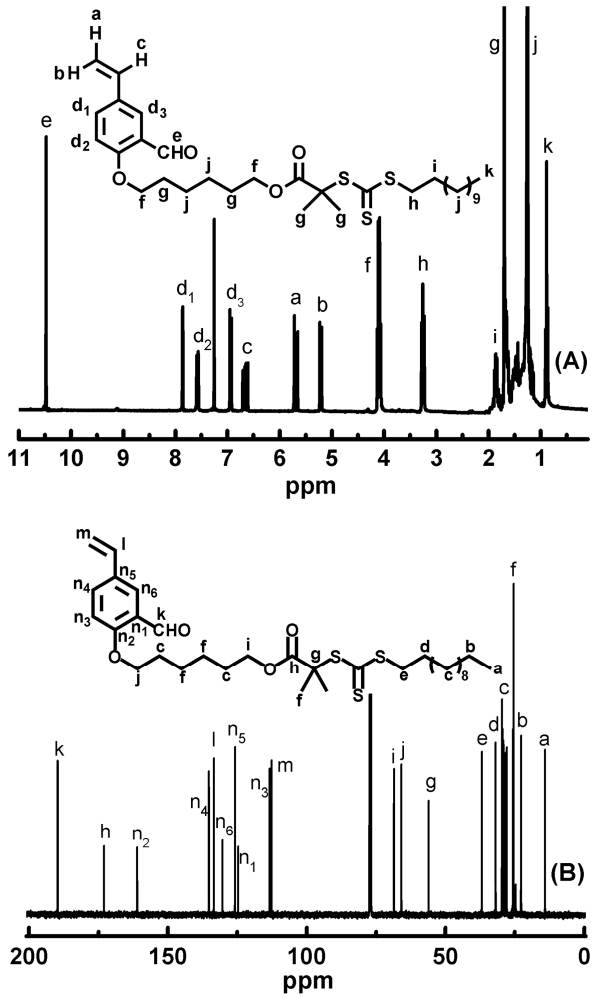


Fig. 1 ^{1}H NMR spectrum (A) and ^{13}C NMR spectrum (B) of chain-transfer monomer FHDM in $CDCl_3$.

RAFT polymerization of DEM with chain transfer monomer FHDM

RAFT polymerizations of DEM were attempted at 65 °C using AIBN as initiator and 1,4-dioxane as solvent in the presence of chain transfer monomer FHDM to synthesize branched polyDEM (BPDEM) with aldehyde functionalities via SCVP approach. As an example, Fig. 2 shows the GPC trace as a function of time for the polymerization with a feed ratio of $[DEM]_0/[FHDM]_0 = 20$. As the polymerization proceeded, the molecular weight grew larger as indicated by shift of the GPC trace toward higher molecular weight region. The weight distributions were multicomponent and thus broad, and the M_w/M_n value increased with polymerization time. Broad and polymodal molecular weight distribution is usually observed in SCVP due to its hybrid chain/step growth mechanism, and has been proven as the result of polymers with branched structures.[15,27]

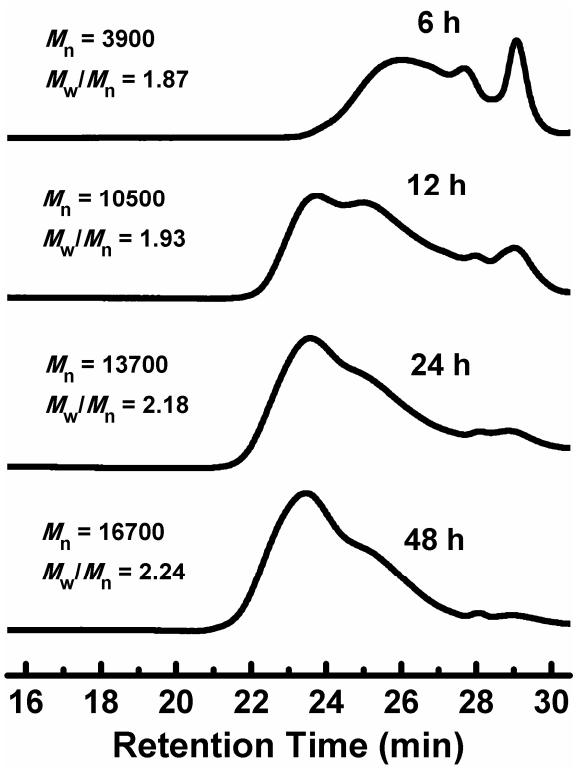


Fig. 2 GPC traces as a function of time for RAFT polymerization of DEM with chain-transfer monomer FHDM at $[DEM]_0/[FHDM]_0 = 20$ in 1,4-dioxane at 65 °C.

To investigate the relative reactivity of DEM and FHDM, ^{1}H NMR spectroscopy was utilized to follow the conversion of both monomers by monitoring the intensity change of their respective vinyl signals during the polymerization ($[DEM]_0/[FHDM]_0$ = 20). As shown in Figure 3, the vinyl resonance signals of DEM (a) and FHDM (b) gradually became weaker as the polymerization proceeded; and FHDM was consumed with a rate comparable to that of DEM (inset in Fig. 3), suggesting the similar reactivity of DEM and FHDM. The similar reactivity between monomer and chain transfer monomer is critical for generating polymers with highly branched structure via SCVP approach. [15]

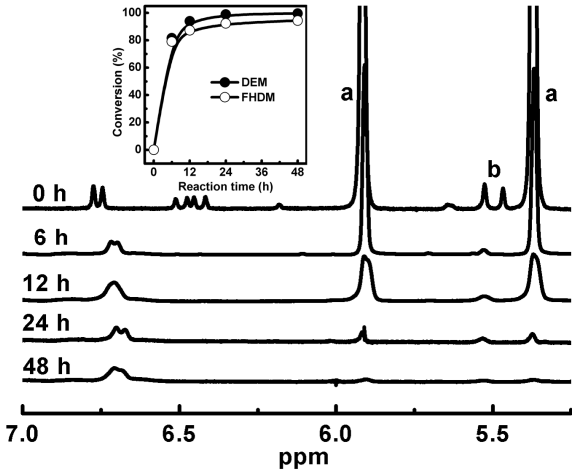


Fig. 3 ^{1}H NMR spectra of vinyl signals as a function of time for RAFT polymerization of DEM with chain-transfer monomer FHDM at $[DEM]_0/[FHDM]_0$ = 20. Inset: conversion of DEM and FHDM vs polymerization time.

After 48 h polymerization, the conversions of DEM and FHDM reached ~100% and ~ 94.3%, respectively; and the M_w expanded to 37400 (M_w/M_n =2.24). The resulting polymer was fractionated by preparative GPC and then analyzed by the ^{1}HNMR spectroscopy (Fig. 4A). Apart from the characteristic proton signals of

-$N(CH_3)_2$ (g，2.2 ~ 2.4 ppm) and -CH_2N- (f, 2.5 ~ 2.7 ppm) groups from PDEM monomer units, the peaks of aldehyde proton (j, 10.4 ppm) and aromatic protons (h_1, h_2 and h_3, 6.8 ~ 7.8 ppm) derived from chain transfer monomer FHDM were also distinctly observed, indicating the copolymerization of DEM with chain transfer monomer FHDM. The integration ratio of aldehyde protons vs aromatic protons in the polymer was 1:3, indicating the essential absence of side reactions on the aldehyde functionality of FHDM during the RAFT-SCVP. Moreover, the molar ratio of DEM to FHDM in the polymer can be determined from the peak intensity ratio of aldehyde proton (j) to -CH_2N- protons (f). The value is 21.3, which is quite close to the feed ratio ($[DEM]_0/[FHDM]_0$ = 20) due to the high conversions of DEM and FHDM (~100% and ~ 94.3%, respectively).

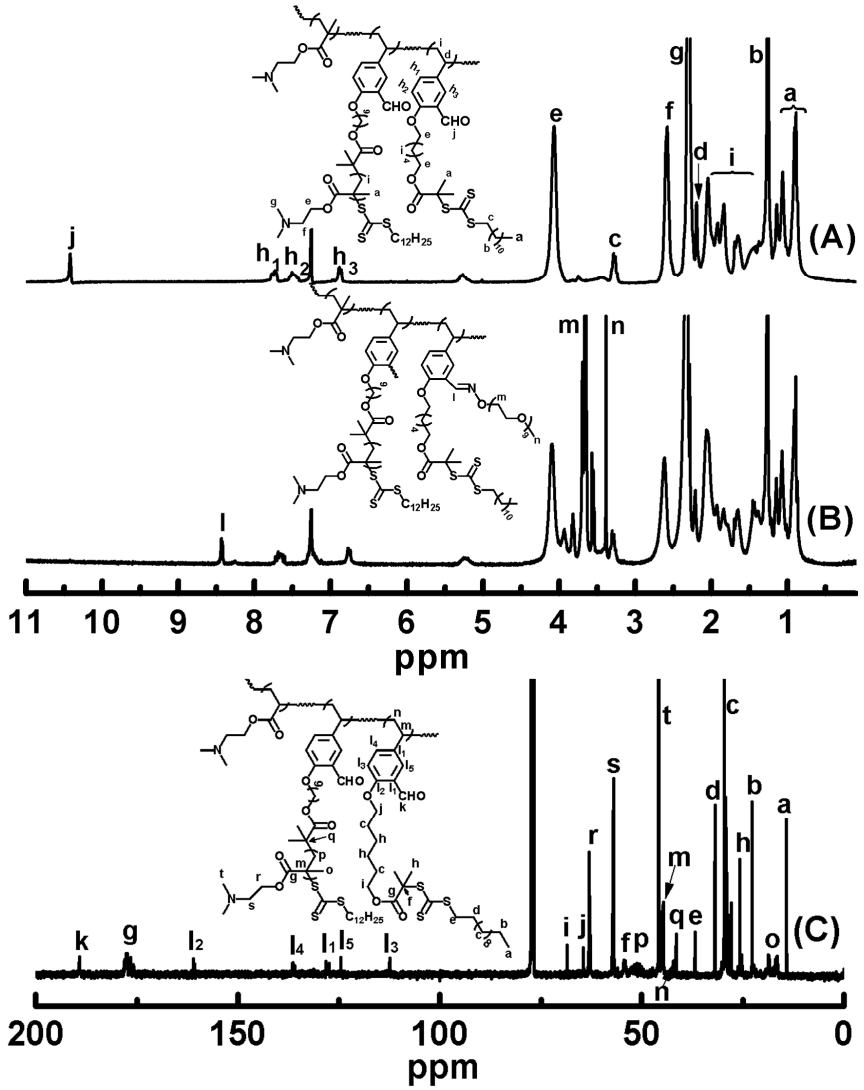


Fig. 4 ^{1}H NMR spectra in $CDCl_3$ of branched polymer BPDEM (A) and branched graft copolymer BPDEM-*g*-PEO (B) and ^{13}C NMR spectra of BPDEM in $CDCl_3$ (C) .

Branched polymers are characterized by their degree of branching (DB), a measure of branching density. DB can be calculated by $DB = (D+T)/(D+T+L)$, where D, T and L represent the number for branching unit, terminal group and linear unit, respectively. NMR spectroscopy is commonly used to determine the DB of branched polymers. In the present case, it is difficult to calculate DB of the polymer by ^{1}H NMR spectrum owing to the overlapping of typical signals. Thus ^{13}C NMR

measurement was also conducted for the analysis of the branched structure. Fig. 4C presents the ^{13}C NMR spectrum of the as-prepared polymer ($[DEM]_0/[FHDM]_0$ = 20, 48 h). The signals of methyl carbon (a) of trithiocarbonate end groups (terminal groups), quaternary carbon (q) of FHDM moiety connecting with DEM units (branching units), and *N*-methyl carbon (t) of DEM repeat units (linear units) were clearly observed at 14.1, 41.5 and 45.8 ppm, respectively. Thus, the DB was calculated to be 0.07 from the equation DB = $(I_{36.9} + I_{14.1})/(I_{36.9} + I_{14.1} + 0.5I_{45.8})$, where $I_{36.9}$, $I_{14.1}$, and $I_{45.8}$ mean the integrated intensity of carbon signals at 36.9, 14.1, and 45.8 ppm, respectively. The average branch length, that is, the average number of repeat units per branch (RB), was deduced as ~14 according to $RB = DB^{-1}$.[13]

Various branched polyDEMs were prepared with different feed ratios of DEM to FHDM ($[DEM]_0/[FHDM]_0$ = 5-50) via the above RAFT-SCVP process. The characterization data on the resulting branched polymers are listed in Table 1. In all cases, the conversions of DEM and FHDM were almost full in 48 h (~100% for DEM and above 92% for FHDM), implying the high efficiency of the RAFT-SCVP. Increasing the feed ratio of DEM to FHDM increased the molecular weight of the branched polymer, and molecular weight up to $M_{w,GPC}$ = 45200 could be obtained at $[DEM]_0/[FHDM]_0$ = 50. The same tendency was also observe for other branched polymers using RAFT-SCVP.[12,13] The degree of branching (DB) of the resulting polymer increased with the amount of chain transfer monomer FHDM used in the RAFT-SCVP since the chain transfer monomer played the role of branching agent.[28]

For example, DB increased from 0.02 to 0.22 as the feed ratio of DEM to FHDM changed from 50 to 5. Therefore, the degree of branching and thus average branch length of the resulting branched polyDEM could be readily tuned by manipulation of DEM/FHDM feed ratio. Glass transition temperatures (T_g) of branched copolymers were measured by DSC, and the results are also listed in Table 1. Polymers with more branches have enhanced free volumes due to increased chain ends, which will reduce T_g.[12]

Table 1. Synthesis of branched polyDEM by RAFT polymerization of DEM (M_1) with chain-transfer monomer FHDM (M_2)[a]

run	$[M_1]_0/[M_2]_0$	C_{M1} (%)[b]	C_{M2} (%)[b]	M_w[c]	M_w/M_n[c]	$M_{w,LLS}$[d]	DB[e]	RB[f]	T_g (^oC)[g]
1	5	100	92.3	17200	1.93	39600	0.22	5	1.4
2	10	100	93.0	25100	2.28	48600	0.12	8	3.0
3	20	100	94.3	37400	2.24	64300	0.07	14	9.5
4	50	100	95.2	45200	2.38	71900	0.02	41	15.8

[a] In 1,4-dioxane at 65 ^{o}C for 48 h. [b] Conversions of the FHDM and DEM determined by ^{1}H NMR. [c] Determined by GPC. [d] Determined by GPC-LLS. [e] Average degree of branching determined by ^{13}C NMR spectroscopy, DB = $(I_{36.9} + I_{14.1})/(I_{36.9} + I_{14.1} + 0.5I_{45.8})$ where I means peak at different chemical shifts. [f] Average number of repeat units per branch, RB = DB^{-1}. [g] Glass transition temperatures determined by DSC.

The weight-average molecular weights (M_w) were also evaluated by GPC with laser light scattering (LLS) detector. For all samples, the molecular weights

determined by light scattering were higher than these by GPC (Table 1), suggesting that the resulting polymers demonstrate a higher molecular density because of their branched structures.[13]

Synthesis of branched graft copolymers BPDEM-*g*-PEO and their thermo-induced micellization in aqueous solution

Using FHDM as a chain transfer monomer for the RAFT-SCVP of DEM not only constructed a branched structure but also enabled the resulting branched polyDEM to be functionalized quantitatively with reactive aldehyde groups on its branching points (Scheme 1). Aldehydes can react readily with aminooxy groups via the formation of a stable oxime linkage under mild conditions in a near-quantitative yield.[17] Thus the aldehyde-containing branched polyDEM (M_w = 37400 and M_w/M_w = 2.24; obtained at $[DEM]_0/[FHDM]_0$ = 20 in 48 h) was then allowed to react with aminooxy-terminated PEO (PEO-ONH_2) to generate a branched graft copolymer BPDEM-*g*-PEO with branched polyDEM backbone and PEO grafted chains (Scheme 1). To adjust relative proportion of PEO and PDEM in BPDEM-*g*-PEO, aminooxy-terminated PEOs with different chain lengths, PEO_9-ONH_2, PEO_{22}-ONH_2 and PEO_{45}-ONH_2 (degree of polymerization of PEO = 9, 22 and 45, respectively), were used. The reactions were carried out in THF at room temperature for 24 h. Excess PEO-ONH_2 (1.5 equiv. to –CHO group of BPDEM) was used to ensure the complete reaction of the aldehyde groups in BPDEM. After removing the ungrafted PEO-ONH_2 by dialysis against THF, the products were analyzed by GPC (Fig. 5). The successful grafting of PEOs onto BPDEM was confirmed by the shifting of GPC traces to higher molecular weight than the starting BPDEM. As expected, the molecular weight of the resulting

BPDEM-*g*-PEO increased with the increase of chain length of PEO-ONH_2 used.

To further confirm the formation of the branched graft copolymers, the products were fractionated by preparative GPC and then analyzed using ^{1}H NMR spectroscopy. Fig. 4B shows the ^{1}H NMR spectrum of a typical sample BPDEM-*g*-PEO_9 obtained with grafting of PEO_9-ONH_2. Compared with the spectrum of BPDEM precursor (Fig. 4A), new signals of methylene protons (a, 3.5 ~ 3.7 ppm) for PEO was observed, indicating the successful introducing of PEO chains. Concomitantly, a characteristic signal corresponding to oxime proton **(*b*,** ~8.32 ppm**)** appeared in consequence of aldehyde-aminooxy coupling reaction. Importantly, the signal of aldehyde protons completely disappeared, demonstrating the complete reaction of aldehyde groups.

The molar ratio of ethylene oxide (EO) to DEM units in the branched graft copolymers can be estimated on the basis of the integration areas of methylene protons (m) from PEO and -CH_2N- protons (f) from PDEM. The values were 0.8, 1.9 and 4.1 for BPDEM-*g*-PEO_9, BPDEM-*g*-PEO_{22} and BPDEM-*g*-PEO_{44}, respectively, which are close to the calculated values assuming 100% consumption of –CHO groups. Obviously, the longer the length of grafted PEO chain, the higher the PEO proportion in BPDEM-*g*-PEO.

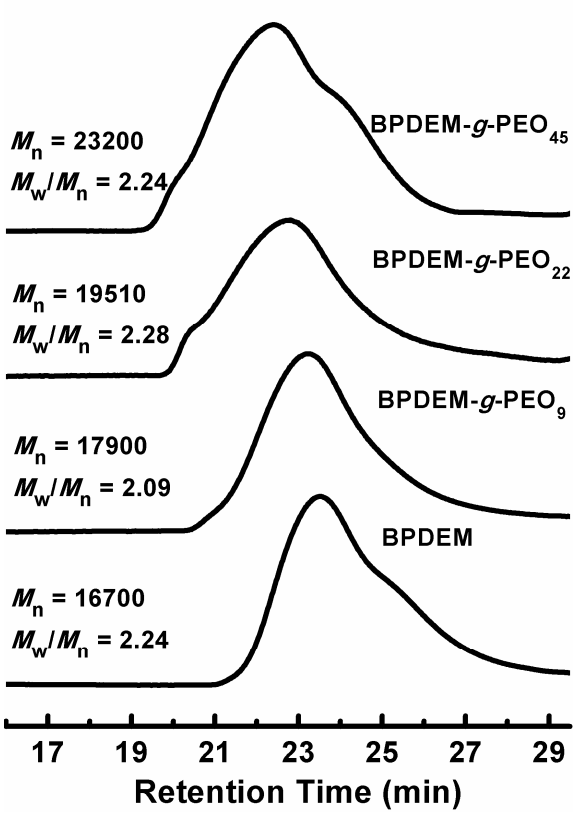


Fig. 5 GPC traces of branched graft copolymer BPDEM-*g*-PEO_m (m = 9, 22 and 45) prepared via coupling BPDEM with PEO_9-ONH_2, PEO_{22}-ONH_2 and PEO_{45}-ONH_2, respectively.

The as-prepared branched graft copolymers BPDEM-*g*-PEO contain permanently hydrophilic PEO chains and thermoresponsive PDEM segments. PDEM homopolymer exhibits a low critical solution temperature (LCST) between 20 and 50 ^oC.[29-31] Below the LCST, PDEM is hydrophilic and soluble in water owing to the intermolecular hydrogen bonding between PDEM segments and water molecules. At temperature above the LCST, chain dehydratation and interchain aggregation occur, and therefore PDEM become hydrophobic and insoluble in water. Thus, BPDEM-*g*-PEO might exhibit thermo-induced micellization character in aqueous solution. Compared to linear analogues (block copolymers), branched copolymers

would exhibit more complex phase-separation behavior in solution due to their complex macromolecular topologies.[32]

A change in copolymer composition in BPDEM-*g*-PEO may alter their hydrophilic-hydrophobic balance, which in turn affects their thermo-induced micellization behavior. Thus, the thermal phase transition of BPDEM-*g*-PEOs with varying PEO proportion was examined using the turbidimetric assay by UV-Vis spectroscopy. Fig. 6 shows the temperature-dependent transmittance at 550 nm for aqueous solutions (5 mg mL^{-1}) of BPDEM-*g*-PEO_9, BPDEM-*g*-PEO_{22} and BPDEM-*g*-PEO_{45}, respectively. For BPDEM-*g*-PEO_9 (molar ratio of EO to DEM units ~ 0.8), a thermo-induced phase transition occurred as evidenced by a decrease of light transmittance upon heating, and accordingly the LCST was evaluated as ~ 34 ^oC (Fig. 6A). Along with the decrease of light transmittance, the solution changed from clear to turbid at elevated temperature, suggesting the occurrence of thermo-induced micellization. At temperature above the LCST, the thermo-responsive PDEM segments in BPDEM-*g*-PEO_9 turned hydrophobic and subsequently inter- and intra-molecular aggregation (intermolecular micellization) took place, forming multimolecular micelles with collapsed PDEM core and soluble PEO corona. To confirm the formation of micelles, the hydrodynamic diameters (D_h) of BPDEM-*g*-PEO_9 in aqueous solution at 25 and 60 ^oC were measured by DLS (Fig. 7 C). At 25 ^oC (below the LCST), BPDEM-*g*-PEO_9 molecularly dissolved in water with a D_h of ~ 11 nm. At 60 ^oC (above the LCST), intermolecular micellizaion occurred to form multimolecular micelles with an increased D_h of ~101 nm. Similar results were

obtained for BPDEM-*g*-PEO_{22} (molar ratio of EO to DEM units ~ 1.9). It also demonstrated an LCST-type thermal responsiveness and exhibited an LCST of ~ 39 ^{o}C (Fig. 6B). DLS analysis (Fig. 7B) indicated an increase in the D_h from ~ 15 nm for the unimers (at 25 ^{o}C, below the LCST) to ~42 nm for the multimolecular micelles (at 60 ^{o}C, above the LCST). As compared to BPDEM-*g*-PEO_9, BPDEM-*g*-PEO_{22} exhibits a higher LCST and yields smaller thermo-induced multimolecular micelles since there are more PEO hydrophilic segments in the latter.

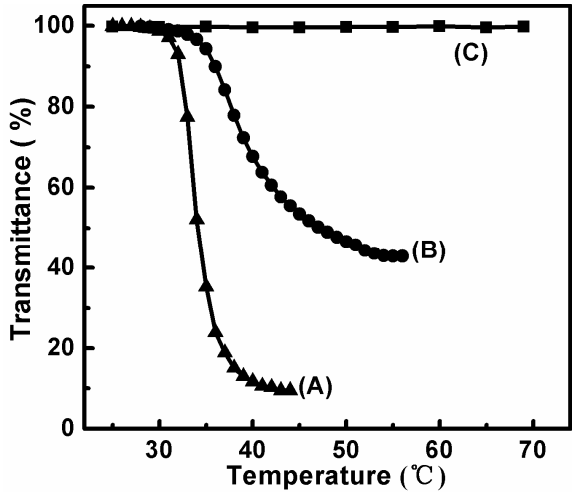


Fig. 6 Temperature dependences of optical transmittance at 550 nm obtained for 5 mg mL^{-1} aqueous solutions of BPDEM-*g*-PEO_9 (A), BPDEM-*g*-PEO_{22} (B) and BPDEM-*g*-PEO_{45} (C).

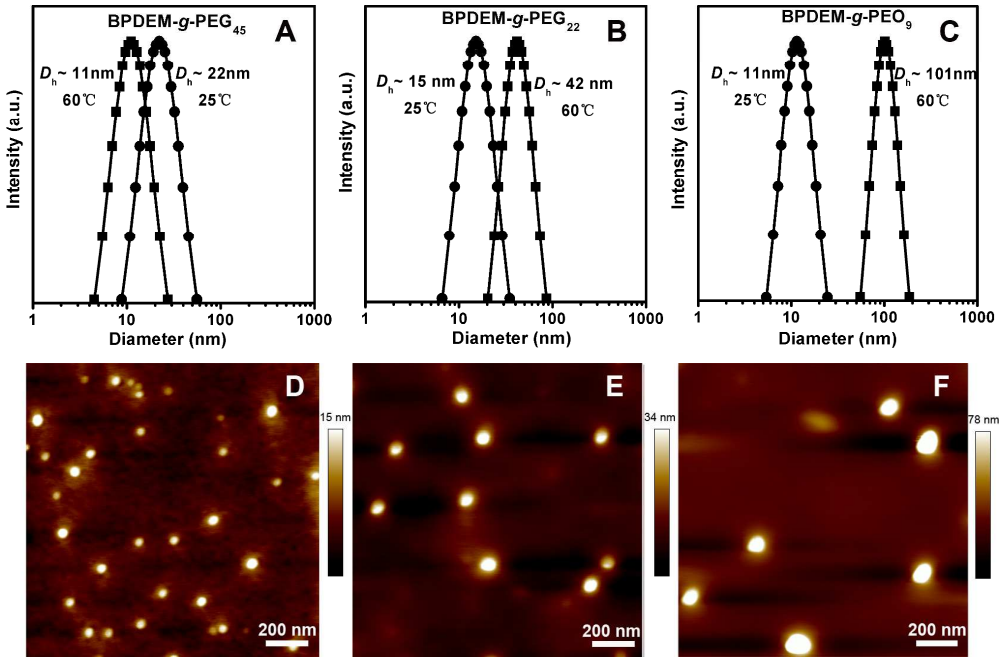


Fig. 7 Hydrodynamic diameter distribution of (A) BPDEM-*g*-PEO_9, (B) BPDEM-*g*-PEO_{22} and (C) BPDEM-*g*-PEO_{45} aqueous solution. AFM images of thermo-induced micelles of (D) BPDEM-*g*-PEO_9, (E) BPDEM-*g*-PEO_{22} and (F) BPDEM-*g*-PEO_{45}.

In the case of BPDEM-*g*-PEO_{45} with much higher PEO proportion (molar ratio of EO to DEM units ~ 4.1), no detectable changes in optical transmittance were observed over the experimental temperature range (from 25 to 70 °C, Fig. 6C). DLS analysis revealed a decrease in D_h from ~ 22 nm at 25 °C to ~ 11 nm at 60 °C (Fig. 7A). These results implied that BPDEM-*g*-PEO_{45} should undergo intramolecular, instead of intermolecular, micellization at elevated temperature, forming unimolecular micelles with a collapsed and compact PDEM core. In order to find out whether the PDEM segments collapse at elevated temperature, ^{1}H NMR was employed to check the thermo-responsive behavior of BPDEM-*g*-PEO_{45}. Fig. 8 shows the temperature-dependent ^{1}H NMR spectra of BPDEM-*g*-PEO_{45} in D_2O solution (10 mg

mL^{-1}) in the temperature range from 30 to 60 ^{o}C. The signal intensity of PEO methylene protons (d) remained constant over the experimental temperature range, whereas the signals ascribed to the methyl protons (a) of PDEM decreased at elevated temperatures, indicating that thermo-induced collapse of PDEM segments did occur. In contrast to the case for BPDEM-*g*-PEO_9 or BPDEM-*g*-PEO_{22}, the content of hydrophilic PEO in BPDEM-*g*-PEO_{45} is sufficient to protect the collapsed PDEM segments (being hydrophobic) against intermolecular aggregation at the temperature even above the LCST, leading to unimolecular micelles. Thus, for BPDEM-*g*-PEO opportunities are open to form either multimolecular micelles or unimolecular micelles via simply adjusting the chain length of grafted PEO.

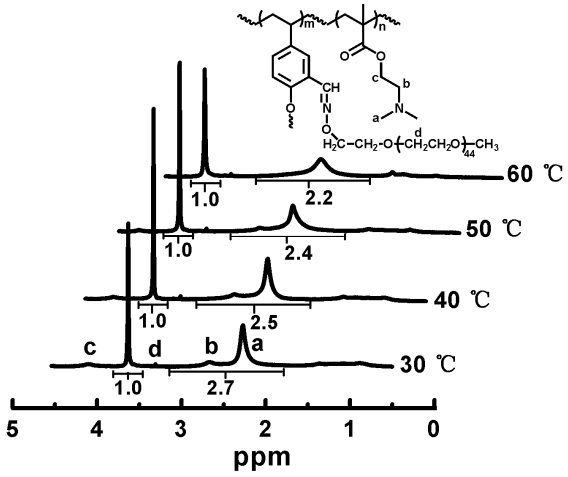


Fig 8. Temperature-dependent ^{1}H NMR spectra of BPDEM-*g*-PEO_{45} in D_2O (10 mg mL^{-1}).

The thermo-induced micelles could be visualized after drying from the hot polymer aqueous solution (0.5 mg mL^{-1}, 70 ^{o}C) onto a mica substrate using AFM. As

shown in Fig. 7D-F, the micelles were observed as spherical objects with diameters of ~ 105 nm and ~ 60 nm for BPDEM-*g*-PEO_9 and BPDEM-*g*-PEO_{22} multimolecular micelles, and ~ 31 nm for BPDEM-*g*-PEO_{45} unimolecular micelles. The diameters obtained from AFM were slightly bigger than these determined by DLS analysis, which might be ascribed to the particles being flexible and somewhat flattened on the mica in the AFM experiments.[33]

Quaternization of BPDEM-*g*-PEO and encapsulation of sodium deoxycholate

The as-prepared branched graft polymer BPDEM-*g*-PEO can be further functionalized by quaternization of its DEM units via highly efficient Menschutkin reaction,[34] resulting in branched cationic polyelectrolytes capable of complexing negatively charged guest molecules for application in, e.g., drug encapsulation. Thus the quaternization of BPDEM-*g*-PEO_{22} was carried out using a 5-fold molar excess of methyl iodide in THF at room temperature to afford its quaternary ammonium salt, *q*BPDEM-*g*-PEO_{22}. Fig. 9 presents the ^{1}H NMR spectrum of the quaternized product in D_2O. By comparing the ^{1}H NMR spectrum of BPDEM-*g*-PEO_{22} precursor (Fig. 8, spectrum for 30 ^oC), it can be seen that the proton signals of -$N(CH_3)_2$ at 2.25 ppm completely disappeared with the emergence of a new signal ascribed to -$N^+(CH_3)_3$ at 3.19 ppm, revealing a ~ 100% degree of quaternization.

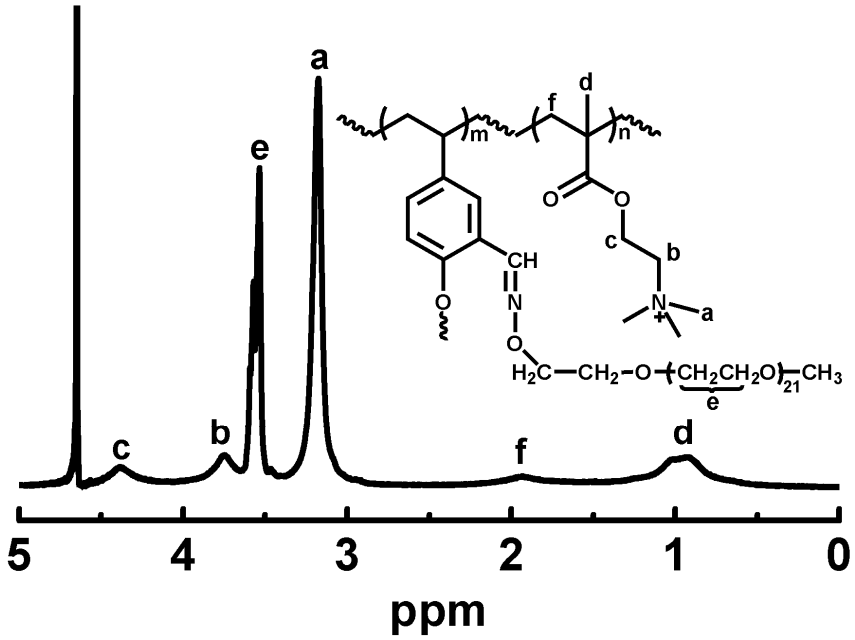


Fig. 9 ^{1}H NMR spectrum of *q*BPDEM-*g*-PEO_{22} in D_2O.

Complexation of the resulting branched cationic polyelectrolyte *q*BPDEM-*g*-PEO_{22} with negatively charged molecules was demonstrated using sodium deoxycholate (NaDC), a kind of cholagogic drug, as a model guest molecule. The complexation was carried out by stepwise addition of NaDC solution to *q*BPDEM-*g*-PEO_{22} solution under stirring. It should be noted that NaDC is an amphipathic molecule and thus self-associates into aggregates at a concentration above its critical micelle concentration (CMC, 2.4 mg mL^{-1}).[35] For the complexation experiments in this study, the final concentration of NaDC (typically between 0.13 and 1.18 mg mL^{-1}) was always lower than its CMC, in order to ensure NaDC existed as free molecules in the solutions.

To confirm the formation of complexes between *q*BPDEM-*g*-PEO_{22} and NaDC, the variation of hydrodynamic diameter D_h with different molar ratio of NaDC to

dimethylammonium group in *q*BPDEM-*g*-PEO_{22} (hereafter, NaDC/ammonium ratio) was tracked by DLS measurement (Fig. 10). In the case of NaDC/ammonium ratio < 0.3, a decreasing trend of D_h was observed as more NaDC was added. The D_h values in the presence of NaDC were smaller than the diameter of pure *q*BPDEM-*g*-PEO_{22}, that is, some shrinking of the initial *q*BPDEM-*g*-PEO_{22} structure may took place as a result of charge neutralization caused by NaDC complexation. When NaDC/ammonium ratio was over 0.3, however, the D_h increased rapidly with the amount of added NaDC; and the D_h increased from ~16 to ~ 250 nm as NaDC/ammonium ratio expanded from 0.3 to 1.0. Presumably, as more NaDC is added in the system the hydrophobic character of the formed complexes is enhanced due to the charge neutralization and the increase in the amount of hydrophobic tails NaDC attached to the complexes, leading to further aggregation of the complexes into structures having larger dimensions. Because the resulting complexes were decorated with hydrophilic PEO chains originating from *q*BPDEM-*g*-PEO_{22}, no precipitation was found even at a high NaDC/ammonium ratio of 1.0; instead, the solution displayed a bluish tint, indicating the formation of stable *q*BPDEM-*g*-PEO/NaDC nano-ensembles stabilized in aqueous solution by the soluble PEO chains. Additional information on the shape and size of the resulted complex nano-ensembles was acquired by AFM. Representative images of the complex obtained at NaDC/ammonium ratio = 0.3 and 1.0 are shown in Fig. 11. The complex nano-ensembles have a spherical morphology; and the diameters are ~ 20 nm and ~ 280 nm for the complexes obtained at NaDC/ammonium ratio = 0.3 and 1.0,

respectively, which agreed with D_h values determined by DLS measurements.

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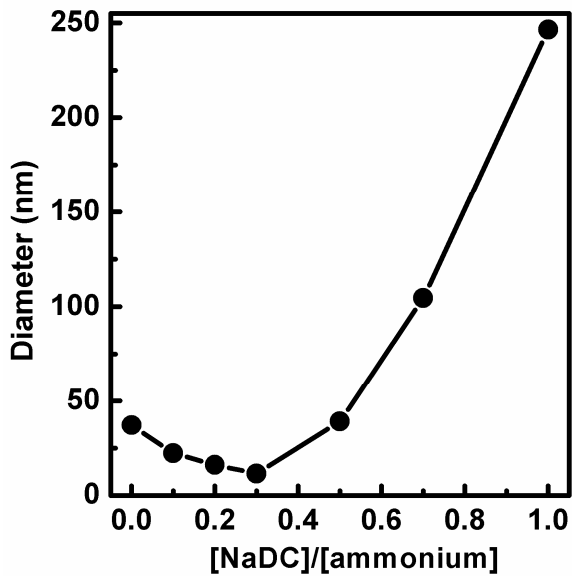

Fig. 10 D_h of qBPDEM-g-PEO_{22}/NaDC complexes (in 0.5mg mL^{-1} aqueous solution) obtained with versus molar ratio of NaDC to dimethylammonium group in qBPDEM-g-PEO_{22}.

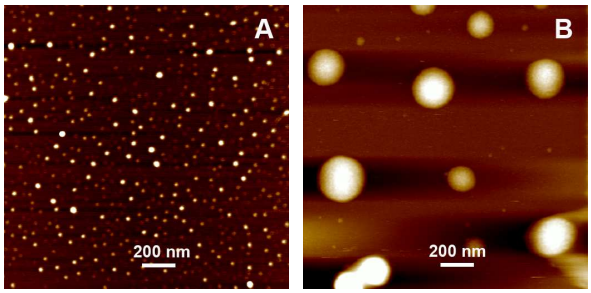

Fig. 11 AFM images of qBPDEM-g-PEO_{22}/NaDC complex obtained with molar ratio of NaDC to dimethylammonium group in qBPDEM-g-PEO_{22} = 0.3 (A) and 1.0 (B).

All of these results suggest that the as-prepared branched cationic polyelectrolyte qBPDEM-g-PEO_{22} can act as an intriguing scaffold for capture of anionic guest molecules via electrostatic complexation to form in situ self-assembled

nanoparticles with simultaneous encapsulation of guest molecules.

Conclusion

In summary, a facile synthetic pathway to branched graft copolymers has been developed by combination of SCVP-RAFT technique and aldehyde-aminooxy reaction. Branched polyDEM (BPDEM) bearing aldehyde functionalities on its branching points was initially prepared by RAFT polymerization of DEM using a newly designed aldehyde-containing chain-transfer monomer FHDM. The aldehyde groups were then allowed to simply react with aminooxy-terminated PEG to form well-defined branched graft copolymer BPDEM-*g*-PEO with branched polyDEM backbone and PEO grafted chains. By simply adjusting the chain length of grafted PEO, BPDEM-*g*-PEO may form multimolecular or unimolecular micelles via thermo-induced micellization. Further modification of BPDEM-*g*-PEO by quaternization afford branched cationic polyelectrolytes which can act as an intriguing scaffold for capture of anionic guest molecules via electrostatic complexation to form in situ self-assembled nanoparticles with simultaneous encapsulation of the guest molecules.

Acknowledgment

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Notes and References

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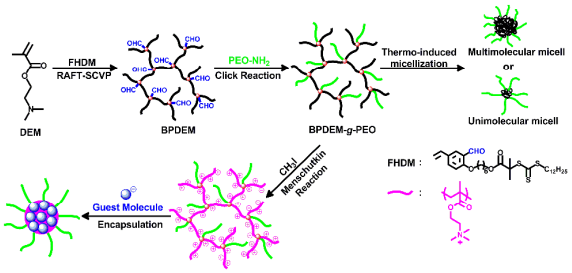

A facile synthetic pathway to branched graft copolymer BPDEM-*g*-PEO has been developed by combination of RAFT-SCVP technique and aldehyde-aminooxy reaction.