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Preparation of dendrimer-like copolymers based on polystyrene and poly(L-lactide) and formation of hollow microspheres

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Abstract

The three generations dendrimer-like copolymers $C(PSt(PLLA(PSt{-}PNAS)_2)_2)_3$ and $C(PSt(PLLA(PSt{-}PNAS)_2)_2)_4$ have been successfully synthesized by combination of ATRP and ROP. Diethanol amine and 2,2-bis(methylene-2-bromoisobutyrate) propionyl chloride (BMBIBPC) were used as divergent reagents, respectively, before preparations of the second and the third generations. The resultant polymers were characterized by GPC and ^{1}H NMR. Then the outermost PSt−PNAS in the dendrimer-like copolymers was cross-linked via substitution reaction of NAS with ethylene diamine, and at the same time, the PLLA segments in the second generation were decomposed in dilute THF or $CHCl_3$ solution, forming hollow particles. The resultant morphologies were characterized by SEM and DLS methods, and spherical particles with various sizes were observed. The hollow particles may have potential applications in catalysis and bioengineering.

Keywords: Atom transfer radical polymerization; Aminolysis; Cross-linking

1. Introduction

Dendrimers are structurally perfectly branched macromolecules with a large number of terminal groups on their surface, and they have potential applications, such as in catalysis and drug delivery, due to their unique structure [1]. In these macromolecules, two branch points are connected by a monomer unit. If a polymer chain replaces the monomer unit, a novel type of molecular architecture, named dendrimer-like polymer, can be obtained with both qualities of dendrimers and block copolymers. The structural character makes it possess some unique physical and chemical properties, which arose great interest of polymer scientists since Hedrick and Trollsås proposed the concept in 1998 [2]. At early stage, Hedrick's group investigated a series of dendrimer-like polymers consisting of poly(ε-caprolactone) [2–4] or poly(ε-caprolactone) and poly(methacrylate) [5]. Gnanou and Taton [7] and Gnanou et al. [6,8,9] synthesized dendrimer-like polymers having poly(ethylene oxide) (PEO) and polystyrene (PSt) or polyacrylic acid (PAA) subchains via combination of living anionic polymerization and atom transfer radical polymerization (ATRP). The same research group used an iterative divergent approach to synthesize dendrimer-like PEO with eight generations and 384 surface hydroxyl groups [10], and pH sensitive dendrimer-like polymers with fifth generation from PEO and PAA [11]. Dendrimer-like PSt and the copolymer having PSt and P*t*BA were also synthesized via combination of ATRP and chain end modification reactions [12,13]. In addition, the surface properties and self-assembly at the air/water interface of the dendrimer-like copolymers based on PSt and P*t*BA (or PAA) were investigated [13,14]. Except the core-first method used in the preparation of dendrimer-like polymers, Hirao et al. applied the arm-first method to synthesize dendrimer-like poly(methyl methacrylate)s (PMMAs) up to the seventh generation via an iterative divergent approach, which involved a coupling reaction of living anionic PMMA chains with terminal benzyl bromide moieties and a transformation reaction of the *tert*-butyldimethylsilyloxymethylphenyl groups into benzyl bromide [15–17].

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One interest for the preparation of dendrimer-like polymers is to study the formation of unimolecular micelles that have potential applications as "nano-reactor" or as templates for nanopore generation in organosilicates [5]. The self-assembling behavior of dendrimer-like polymers based on PEO and PAA in water was investigated [11]. In recent decade, hollow spheres with nanometer or sub-micrometer scales attracted great interests of polymer scientists due to their potential applications in drug delivery [18], catalysis [19] and various functional composite materials [20]. Among the synthetic approaches, sacrificial core approach, in which a sacrificial core particle is coated by polymer shell, and subsequently core is dissolved away, has been extensively utilized in the preparation of hollow spheres [21]. These spherical core–shell particles were prepared by surface-grafting technology [22,23], layer-by-layer assembly [24,25], emulsion or microemulsion polymerization [26–28], and self-assembly of block copolymers and star block copolymers in selective solvents [29–32]. As we mentioned above, the dendrimer-like polymers can form micelles. The spherical hollow particles might be produced after the outermost generation polymers are cross-linked, the inner polymers are decomposed and dissolved away. In our previous work, three generations dendrimer-like copolymers from the degradable poly(L-lactide) (PLLA) were prepared [33]. In this report, we report the synthesis of dendrimer-like copolymers with PSt, PLLA and PSt as the first, the second and the third generations, and the fabrication of hollow particles was studied because PLLA is easy to be hydrolyzed.

2. Experimental section

2.1. Materials and methods

Styrene (St, Shanghai Chemical Reagent Co. 99%) was washed with an aqueous solution of sodium hydroxide (5 wt%) three times and then washed with distilled water until neutralization. After dried with anhydrous calcium chloride, St was distilled under reduced pressure. L-Lactide (LLA) was purified by recrystallization from toluene. *N*,*N*-Dimethyl formamide (DMF) was distilled under reduced pressure before use. Acetonitrile was refluxed with P_2O_5 for 10 h, and then distilled. The 2,2-bis-(methylene-2-bromoisobutyrate) propionyl chloride (BMBIBPC) was synthesized in 90% yield according to the method described in reference [33]. ^1H NMR (300 MHz, $CDCl_3$), δ (TMS, ppm): 4.44 (s, 4H, $2COOCH_2$), 1.92 (s, 12H, $2C(CH_3)_2Br$), 1.50 (s, 3H, CCH_3). *N*-Acryloyloxysuccimide (NAS) was synthesized by the esterification of *N*-hydroxy succimide (NHS) with acryloyl chloride in 70% yield according to the procedure described in reference [34]. ^1H NMR (300 MHz, $CDCl_3$), δ (TMS, ppm): 6.15–6.74 (m, 3H, $CH_2{=}CH$), 2.87 (s, 4H, CH_2CH_2). 2,2′-Bipyridine (bpy) and other reagents were of analytical grade and used as received without further purification.

2.2. Measurements and instruments

^1H NMR (300 MHz) was recorded on a Bruker DRX-300 nuclear magnetic resonance (NMR) instrument at room temperature, tetramethylsilane and $CDCl_3$ were used as internal standard and solvent, respectively. The molecular weights, M_n (GPC)s and molecular weight distributions (M_w/M_n) were determined at 30 °C on a Waters 515 gel permeation chromatography (GPC) equipped with microstyragel columns (10^3, 10^4 and 10^5 Å) and a Waters 2414 refractive index detector, THF was used as eluent at a flow rate of 1 mL/min. Narrow polystyrene standards were used in the calibration of molecular weight and molecular weight distribution. Infrared spectra were recorded on a Bruker EQU55 IR spectrometer. Scanning electron microscopy (SEM) images were performed on a Hitachi X-650 instrument. All samples were dissolved in $CHCl_3$ or THF with a concentration of 1 mg/mL. SEM samples were dropped on the aluminium chips and dried in air. The size and size distribution of particles were measured by DLS carried out on a Malvern Zetasizer Nano ZS90 with a He–Ne laser (633 nm) and 90° collecting optics. All samples were tested without filtering prior to measurement. All measurements were carried out at 25 °C, and data were analyzed by Malvern Dispersion Technology Software 4.20.

2.3. Synthesis

2.3.1. Synthesis of ATRP initiator, 1,1,1-tris(methylene-2-bromoisobutyrate)propane (TMBIBP) and three-armed star PSt (C(PSt-Br)$_3$)

TMBIBP and the three-armed star PSt were prepared according to the procedure described in Ref. [33]. TMBIBP was obtained in 65% yield. ^1H NMR (300 MHz, $CDCl_3$), δ (TMS, ppm): 0.95–1.00 (t, 3*H*, $-CH_2CH_3$), 1.58–1.67 (q, 2*H*, $-CH_2CH_3$), 1.94 (s, 18*H*, $-C(CH_3)_2Br$), 4.19 (s, 6*H*, $-O-CH_2-$). The three-armed star PSt was obtained in 28% yield. $M_{n,NMR} = 10\,000$, $M_w/M_n = 1.11$.

2.3.2. Synthesis of star PSt capped with diethanol amine (C(PSt(OH)$_2$)$_3$)

The procedure of divergent reaction is as follows. Star PSt, $C(PSt\text{-}Br)_3$ (1.00 g, 0.1 mmol, $M_{n,NMR} = 10\,000$), diethanol amine (1.05 g, 10 mmol) and DMF (10 mL) were added into a 50 mL flask. After the reaction mixture was stirred for 4 days at room temperature, the polymer was precipitated in excess of methanol. The product collected by filtration was dried under vacuum at 30 °C overnight, and the polymer with $M_{n,NMR} = 10\,000$, $M_w/M_n = 1.10$ was obtained in 90% yield.

2.3.3. Synthesis of the second generation dendrimer-like PSt-*b*-PLLA copolymer (C(PSt(PLLA-OH)$_2$)$_3$)

The dendrimer-like PSt-*b*-PLLA-OH, $C(PSt(PLLA\text{-}OH)_2)_3$, was prepared by ring-opening polymerization of LLA using $C(PSt(OH)_2)_3$ as macroinitiator, and $Sn(Oct)_2$ as catalyst. Into a 10 mL dry glass tube equipped with a magnetic stirring bar, $C(PSt(OH)_2)_3$ (0.20 g, 0.02 mmol, $M_{n,NMR} = 10\,000$), LLA (0.44 g, 3 mmol) and dry toluene (4 mL) were added. The tube was connected to a Schlenkline, and then an exhausting-refilling N_2 process was operated three times. The tube was put into an oil bath at 115 °C while stirring for 10 min. $Sn(Oct)_2$ (0.05 g, 0.1 mmol) in 2.5 mL dry toluene was added into the mixture, and the exhausting-refilling process was carried out again to remove toluene. After the polymerization was carried out for 24 h at 115 °C, the tube was cooled to room temperature rapidly. The polymer with $M_{n,NMR} = 32\,000$, $M_w/M_n = 1.04$ was obtained in 69% yield by precipitation from excess of hot cyclohexane, collected by filtration, and dried under vacuum at 30 °C overnight.

2.3.4. Divergent reaction of C(PSt(PLLA-OH)$_2$)$_3$ with BMBIBPC

Dendrimer-like copolymer, C(PSt(PLLA-OH)$_2$)$_3$ (0.320 g, 0.01 mmol, $M_{n,NMR} = 32\,000$), was dissolved in acetonitrile (10 mL), and then BMBIBPC (0.452 g, 1 mmol) was added. After the mixture was refluxed for 48 h, the polymer with $M_{n,NMR} = 32\,000$, $M_w/M_n = 1.05$ was obtained in 90% yield by precipitation in excess of methanol, filtration and drying in vacuum oven at 30 °C overnight.

2.3.5. Synthesis of the third generation dendrimer-like copolymer C(PSt(PLLA(PSt–PNAS)$_2$)$_2$)$_3$

The copolymer, C(PSt(PLLA(PSt–PNAS)$_2$)$_2$)$_3$, was prepared by ATRP with CuBr/bpy as catalyst/ligand in anisole. C(PSt(PLLABr$_2$)$_2$)$_3$ (0.320 g, 0.01 mmol, $M_{n,NMR} = 32\,000$), CuBr (0.060 g, 0.4 mmol), bpy (0.126 g, 0.8 mmol), St (3.60 g, 35 mmol), NAS (0.420 g, 2.5 mmol) and 7.0 g anisole were added into a 20 mL glass tube successively. After mixing thoroughly the polymerization tube was degassed by three freeze-vacuum-thaw cycles, and then sealed under vacuum. The sealed tube was immersed into an oil bath at 110 °C. After polymerization was carried out for 8 h, the tube was rapidly cooled to room temperature. The polymer solution was diluted with THF, and then was passed through a short column of neutral alumina for removal of metal salt. After twice precipitation by addition of the polymer solution into petroleum ether, the polymer with $M_{n,NMR} = 80\,000$, $M_w/M_n = 1.11$ was obtained (0.9 g, 21% yield) by filtration and drying in vacuum oven at 30 °C overnight.

2.3.6. Synthesis of ATRP initiator, 1,1,1,1-tetrakis(2′-bromo-2′-methylpropionyloxymethyl)methane (TBMPMM)

The synthetic procedure is as follows. The 2-bromoisobutyric acid (21 g, 126 mmol), pentaerythritol (3.4 g, 25 mmol), *p*-toluene sulfonic acid (1 g) and toluene (100 mL) were added into a 250 mL flask equipped with a Dean-Stark trap and a condenser connected to an anhydrous CaCl$_2$ tube. The reaction was carried out at 120 °C, water generated was removed by azeotropic distillation. After 3 days, the reaction was stopped. The solution was washed by dilute NaOH solution four times and water three times, and then was dried with anhydrous MgSO$_4$. After toluene was removed, the resultant crude product was recrystallized from methanol, 10.5 g of the white needle crystal product was obtained in 57% yield. ^{1}H NMR (300 MHz, CDCl$_3$), δ (TMS, ppm): 1.93 (s, 24*H*, 4 $-C(CH_3)_2Br$), 4.32 (s, 8*H*, 4 $-O-CH_2-$).

2.3.7. Synthesis of four-armed star PSt(C(PSt-Br)$_4$)

The C(PSt-Br)$_4$ with $M_{n,NMR} = 14\,800$, $M_w/M_n = 1.09$ was prepared in 35% yield by the bulk ATRP of St with TBMPMM as initiator, and CuBr/bpy as catalyst/ligand. The synthetic procedure is the same as that of C(PSt-Br)$_3$.

2.3.8. Divergent reaction of C(PSt-Br)$_4$ with diethanol amine

The similar procedure with divergent reaction of C(PSt-Br)$_3$ was adopted. The C(PSt-Br)$_4$ (2.90 g, 0.2 mmol, $M_{n,NMR} = 14\,800$) was reacted with diethanol amine (3.15 g, 30 mmol) in DMF (25 mL) at 80 °C for 24 h. The polymer with $M_{n,NMR} = 14\,800$, $M_w/M_n = 1.10$ was obtained in 90% yield after precipitation in methanol, filtration and drying in a vacuum oven at 30 °C overnight.

2.3.9. Synthesis of dendrimer-like copolymer (C(PSt(PLLA)$_2$)$_4$)

The ring-opening polymerization of LLA (1.76 g, 12 mmol) using C(St(OH)$_2$)$_4$ (0.30 g, 0.02 mmol, $M_{n,NMR} = 14\,800$) and Sn(Oct)$_2$ (0.1 g in 4 mL toluene), respectively, as macroinitiator and catalyst was performed in bulk at 115 °C for 24 h. The procedure is similar to the preparation of C(PSt(PLLA)$_2$)$_3$. The polymer with $M_{n,NMR} = 80\,000$, $M_w/M_n = 1.06$ was obtained in 80% yield.

2.3.10. Divergent reaction of C(PSt(PLLA-OH)$_2$)$_4$ with BMBIBPC

The synthetic procedure is similar to the preparation of C(PSt(PLLA-Br)$_2$)$_3$. The C(PSt(PLLA-OH)$_2$)$_4$ (1.20 g, 0.015 mmol, $M_{n,NMR} = 80\,000$) and BMBIBPC (0.54 g, 1.2 mmol) were added into 20 mL acetonitrile. After reaction was carried out at refluxing temperature for 24 h, the polymer (1.10 g) with $M_{n,NMR} = 81\,000$, $M_w/M_n = 1.06$ was obtained in 92% yield.

2.3.11. Synthesis of the third generation dendrimer-like copolymer (C(PSt(PLLA(PSt–PNAS)$_2$)$_2$)$_4$)

The macroinitiator C(PSt(PLLABr$_2$)$_2$)$_4$ (0.40 g, 0.005 mmol, $M_{n,NMR} = 81\,000$), CuBr (0.045 g, 0.3 mmol), bpy (0.095 g, 0.6 mmol), St (2.08 g, 20 mmol) and NAS (0.20 g, 1.2 mmol) were successively added into 4.0 g anisole in 10 mL polymerization tube. After three freeze-vacuum-thaw cycles, the tube was sealed under reduced pressure, and then the polymerization was carried out at 110 °C for 8 h. After precipitated in an excess of methanol, separated by filtration and dried in a vacuum oven at 30 °C overnight, the target polymer (0.92 g) with $M_{n,NMR} = 150\,000$, $M_w/M_n = 1.15$ was obtained in 34% yield.

2.3.12. Preparation of the spherical hollow particles

A typical procedure is as follows. The dendrimer-like copolymer C(PSt(PLLA(PSt–PNAS)$_2$)$_2$)$_3$ (200 mg) [or C(PSt(PLLA (PSt–PNAS)$_2$)$_2$)$_4$ (200 mg)] was dissolved in THF (200 mL) or CHCl$_3$ (200 mL) completely. The concentration of the resultant polymer solution was 1 mg/mL. Into this solution, the ethylene diamine (120 mg, 0.2 mmol) was added. After the cross-linking reaction was carried out at room temperature for 7 days, the reaction solution was dialyzed against THF for 3 days for removal of the small molecules produced. One part of the solution was taken for characterization of SEM and DLS, the other part of the solution was concentrated, and the precipitate from petroleum ether was collected by filtration, and then dried in vacuum oven at room temperature overnight.

3. Results and discussion

3.1. Synthesis of the three generations dendrimer-like copolymers, $C(PSt(PLLA(PSt-PNAS)_2)_2)_3$ and $C(PSt(PLLA(PSt-PNAS)_2)_2)_4$

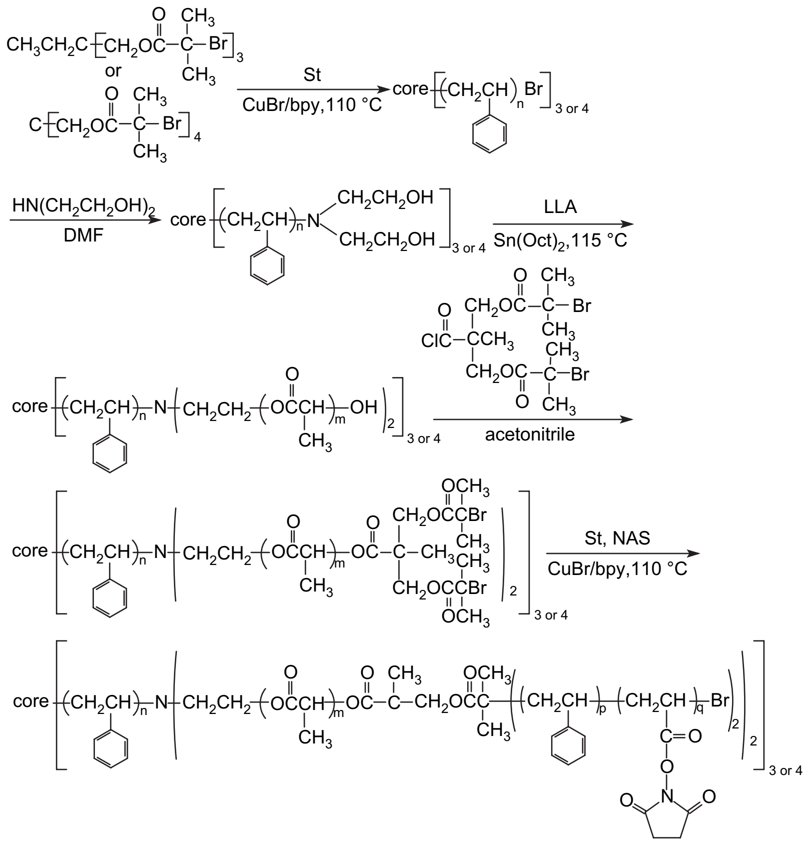
These two dendrimer-like copolymers were synthesized according to Scheme 1. For discussion convenience, the $C(PSt(PLLA(PSt-PNAS)_2)_2)_4$ is used as an example. The four-armed star polymer, the second and the third generation were prepared by ATRP of St, ring-opening polymerization of LLA and ATRP of St, respectively. The divergent reaction similar to the approach reported by Gnanou's group [13] was used in transformation of four bromine groups in $C(PSt\text{-}Br)_4$ to eight hydroxyl groups ($C(PSt(OH)_2)_4$). The transformation of eight hydroxyl groups in $C(PSt(PLLA\text{-}OH)_2)_4$ to 16 bromine groups in $C(PSt(PLLABr_2)_2)_4$ was achieved by esterification reaction of $C(PSt(PLLA\text{-}OH)_2)_4$ with BMBIBPC. The conditions and results of the polymerizations are listed in Tables 1–3. The traces of GPC and ^1H NMR spectra of the first, the second and the third generation dendrimer-like polymers are presented in Fig. 1 and Figs. 2–4, respectively. Fig. 1 shows unimodal and symmetrical GPC curves and narrow molecular weight distributions for all resultant polymers, which indicates the high divergent reaction efficiency of $C(PSt\text{-}Br)_4$ with diethanol amine, and $C(PSt(PLLA\text{-}OH)_2)_4$ with BMBIBPC, since incomplete divergent reactions will result in the different initiating sites in different polymer molecules, leading to the broaden molecular weight distributions of the second and the third generation dendrimer-like copolymers obtained.

Table 1
Synthesis of three-armed and four-armed star PSt[a]

No.	$[M]_0/[I]_0$ (molar ratio)	Time (min)	Conversion[d] (%)	$M_{n,GPC}$[e]	$M_{n,NMR}$[f]	M_w/M_n[e]
1[b]	300	110	17	5900	6400	1.18
2[b]	300	150	28	9400	10 000	1.11
3[c]	400	200	35	14 500	14 800	1.09
4[c]	400	300	52	24 000	24 500	1.05

[a] ATRPs of St were carried out at 110 °C; the feed molar ratio of TMBIBP/CuBr/bpy = 1/3/9, TBMPMM/CuBr/bpy = 1/4/12.
[b] TMBIBP was used as initiator.
[c] TBMPMM was used as initiator.
[d] Measured by gravimetric method.
[e] Measured by GPC.
[f] $M_{n,NMR} = 104\ (nI_{Ar}/5I_{4.5}) + M_I$, where $n = 3$ or 4 based on initiator used, M_I is the molar mass of initiator.

Figs. 2 and 3 show ^1H NMR spectra of the polymers before and after two divergent reactions, one from three (or four) bromine groups to six (or eight) hydroxyl groups and the other from six hydroxyl groups to 12 bromine groups, respectively. Their conversions can be estimated from their ^1H NMR spectra. A signal at $\delta = 4.5$ ppm (a) corresponding to the methine proton next to terminal bromine in Fig. 2A and C is absent in the ^1H NMR



Scheme 1. The synthetic route of the three generations dendrimer-like copolymers of styrene and L-lactide.

Table 2
ROP of LLA using star $C(PSt(OH)_2)_n$ as macroinitiators, $n = 3$ or 4[a]

No.	$[M]_0/[I]_0$ (molar ratio)	Conversion[d] (%)	$M_{n,GPC}$[e]	$M_{n,NMR}$[f]	M_w/M_n[e]
1[b]	150	69	32 000	32 000	1.04
2[c]	600	80	40 000	80 000	1.06

[a] ROP of LLA was performed at 115 °C for 24 h; feed molar ratio of LLA/$C(PSt(OH)_2)_n$ ($n = 3$ or 4)/$Sn(Oct)_2$ = 150/1/5 ($n = 3$) or 600/1/10 ($n = 4$).

[b] The $C(PSt(OH)_2)_3$ ($M_{n,NMR} = 10\,000$) was used as macroinitiator.

[c] The $(PSt(OH)_2)_4$ ($M_{n,NMR} = 14\,800$) was used as macroinitiator.

[d] Measured by gravimetric method.

[e] Measured by GPC.

[f] $M_{n,NMR} = (144/104)(5I_{5.2}/2I_{Ar})\ M_{n(PSt)} + M_{n(PSt)}$, where $I_{5.2}$ and I_{Ar} are integral values of signals at $\delta = 5.2$ and 6–7 ppm, and 144, 104 and $M_{n(PSt)}$ are molar masses of LLA, St and three- or four-armed star $C(PSt(OH)_2)_n$.

spectra of $C(PSt(OH)_2)_3$ and $C(PSt(OH)_2)_4$ of Fig. 2B and D. Two new signals at $\delta = 2.5$ (g) and 3.4 ppm (h), which correspond to the protons of two methylene groups, one next to the nitrogen and the other next to the terminal hydroxyl groups in the diethanol amine, appear in Fig. 2B and D. The conversion of bromine was calculated based on the integration ratio of signal h and the phenyl protons' signals. Under the conditions mentioned in Section 2, the conversion was almost completed. The molecular weights of $C(PSt)_3$ and $C(PSt)_4$ were calculated based on the integration ratio of the signals of phenyl protons to that of methine proton next to terminal bromine, and the results are listed in Table 1. The assignment of protons in $C(PSt(PLLA\text{-}OH)_2)_3$ and $C(PSt(PLLABr_2)_2)_3$ is shown in Fig. 3. The signal at $\delta = 4.35$ ppm (a) in Fig. 3A and the signals at $\delta = 4.4$ (b) in Fig. 3B correspond the methine proton next to terminal hydroxyl group, the ester methylene protons, respectively, and the integration ratio of signals b to a was about 12 when phenyl protons were used as standard and then the integral values of the signals a and b were compared to that of their respective phenyl protons. This indicates that the complete divergent reaction was achieved. The number-average molecular weight of $C(PSt(PLLA\text{-}OH)_2)_3$ can be calculated based on the integration ratio of the signal at $\delta = 5.2$ ppm to that of phenyl protons and molecular weight of PSt; the results calculated are listed in Table 2.

In synthesis of the third generation dendrimer-like copolymer, the atom transfer radical copolymerization (ATRCP) of St and NAS was performed because the NAS units in the copolymers are reactive, and can be easily substituted by alkyl diamines such as ethylene diamine, resulting in cross-linking shell. The results and polymerization conditions for the copolymerization of St and NAS using $C(PSt(PLLABr_2)_2)_3$ and $C(PSt(PLLABr_2)_2)_4$ as initiators are listed in Table 3. Their structures can be verified by ^1H NMR spectra, and a typical ^1H NMR spectrum of the $C(PSt(PLLA(PSt\text{-}co\text{-}PNAS)_2)_2)_3$ is shown in Fig. 4. The signals at $\delta = 6.4$–7.4 (p), 5.2 (b) and 2.72 ppm (a) are attributed to the phenyl protons of St units, methine proton of LLA units and two methylene protons of NAS units, respectively. GPC traces of the two dendrimer-like copolymers, $C(PSt(PLLA(PSt\text{-}co\text{-}PNAS)_2)_2)_3$ and $C(PSt(PLLA(PSt\text{-}co\text{-}PNAS)_2)_2)_4$ are shown in Fig. 1, and we can observe that these two traces are shifted to higher molecular weight completely, and they are unimodal and symmetrical. All the facts indicate the formation of dendrimer-like copolymers with three generations. Based on the ^1H NMR data, the number-average molecular weight $M_{n,NMR}$ can be calculated according to Eq. (1).

$$M_{n,NMR} = \left[(104/144)\left(2I_p/5I_{5.2}\right) + (169/144)\left(2I_{2.7}/4I_{5.2}\right)\right] \times M_{n(PLLA)} + M_{n(PLLA)} \qquad (1)$$

Here, I_p, $I_{2.7}$ and $I_{5.2}$ are the intensities of signals at $\delta = 6.4$–7.4 (p), 2.72 (a) and 5.2 ppm (b); 104, 144, 169 and $M_{n(PLLA)}$ are the molecular weights of St, LLA, NAS and PLLA blocks.

In the polymerization, the content of NAS units in the copolymer can be adjusted by the feed molar ratio of NAS to St. The numbers of the monomer units, St, NAS and LLA in the dendrimer-like copolymers obtained can be calculated by Eq. (1). The last term $M_{n(PLLA)}$ of Eq. (1) can be obtained based on the integration ratio of the signal at $\delta = 5.2$ ppm to that of phenyl protons and molecular weight of the first generation PSt (Fig. 3). The numbers of St and NAS units were calculated based on the first and the second terms of Eq. (1). The results are listed in Table 3. For two samples listed in Table 3, the feed molar ratios of St/NAS are 14 and 16.7, but the molar ratios of St/NAS in the third generation PSts are 1.85 and 2.30, respectively, indicating higher reactivity of NAS than that of St.

On comparing $M_{n,GPC}$ with $M_{n,NMR}$ listed in Table 3, big difference ($M_{n,GPC} = 43\,000$ vs $M_{n,NMR} = 80\,000$ for No. 1 and $M_{n,GPC} = 70\,000$ vs $M_{n,NMR} = 150\,000$ for No. 2) can be observed. The reason for this phenomenon is the compact structure of the dendrimer-like copolymers with three generations in solution.

Table 3
Copolymerization of St and NAS using $C(PSt(PLLABr_2)_2)_3$ or $C(PSt(PLLABr_2)_2)_4$ as macroinitiators[a]

No.	$[St]_0/[NAS]_0/[I]_0$ (molar ratio)	Conversion[d] (%)	$M_{n,GPC}$[e]	$M_{n,NMR}$[f]	M_w/M_n[e]	$(St)_n(LLA)_m(NAS)_p$		
						n	m	p
1[b]	3500/250/1	21	43 000	80 000	1.11	342	153	133
2[c]	4000/240/1	34	70 000	150 000	1.15	618	453	203

[a] Polymerization conditions: St/anisole (weight) = 1/2; temperature: 110 °C; time: 8 h; the feed molar ratio of $[I]_0$/CuBr/bpy = 1/40/80 (No. 1) and 1/60/120 (No. 2).

[b] Dendrimer-like $C(PSt(PLLABr_2)_2)_3$ ($M_{n,NMR} = 32\,000$) was used as macroinitiator.

[c] Dendrimer-like $C(PSt(PLLABr_2)_2)_4$ ($M_{n,NMR} = 80\,000$) was used as macroinitiator.

[d] Measured by gravimetric method.

[e] Measured by GPC.

[f] Calculated based on Eq. (1).

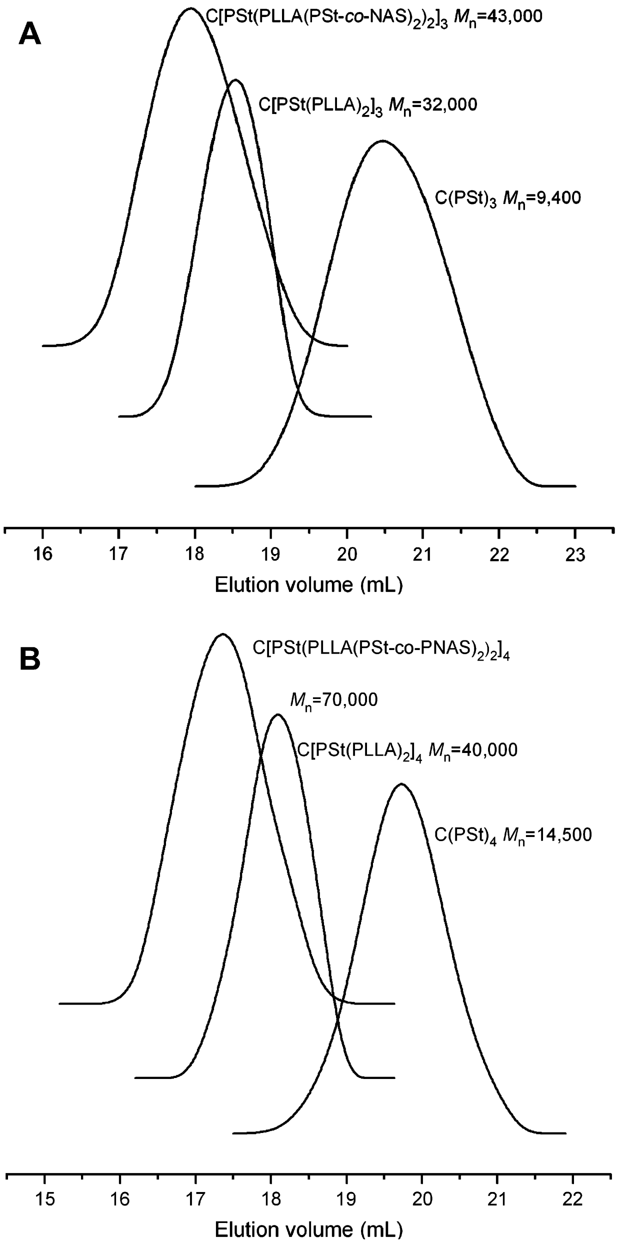


Fig. 1. GPC traces of the first to third generation dendrimer-like copolymers prepared from St, LLA and three functional groups initiator (A); and that from St, LLA and four functional groups initiator (B).

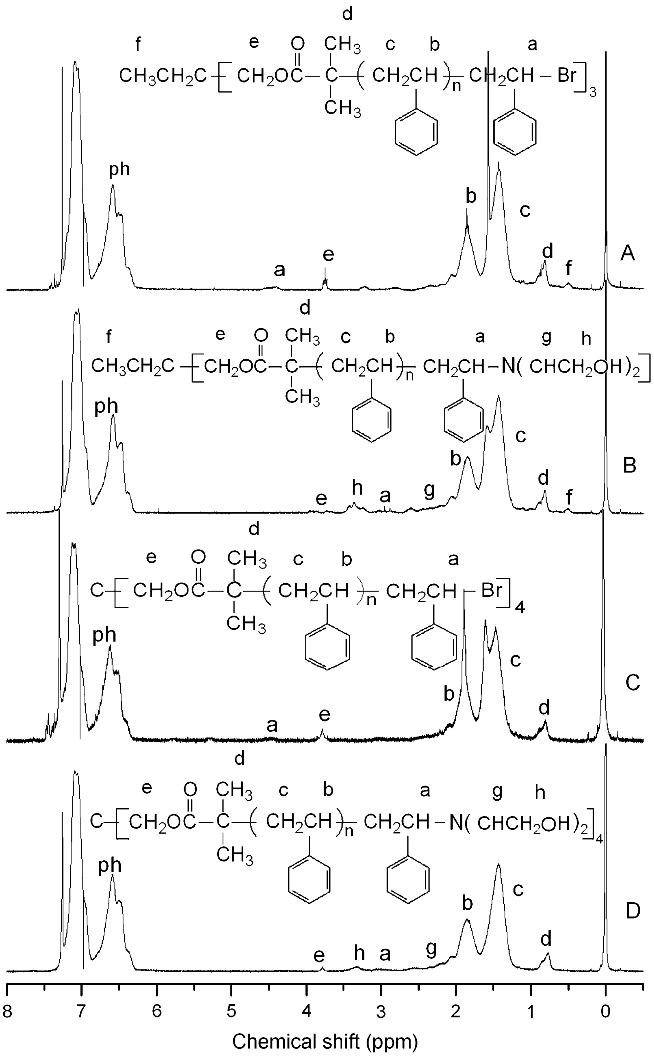


Fig. 2. ^{1}H NMR spectra of three-armed star PSt, C(PSt-Br)$_3$ (A); C(PStOH$_2$)$_3$ (B); four-armed star PSt, C(PSt-Br)$_4$ (C) and C(PStOH$_2$)$_4$ (D).

3.2. Shell cross-linking reaction and aminolysis of PLLA segments

The cross-linking reaction of shell polymer in the dendrimer-like copolymers can be achieved by NAS groups of PNAS segments with ethylene diamine, and this reaction was carried out in THF for 3 and 7 days, respectively, and the two products were obtained. Their ^{1}H NMR spectra are shown in Fig. 5. We can find that the signals at $\delta = 1.55$ (e), 5.2 ppm (b) corresponding to methyl and methine protons of PLLA block and the signal at $\delta = 2.77$ ppm (a) belonging to two methylene protons of PNAS decreased greatly (Fig. 5A), and disappeared completely after 7 days reaction (Fig. 5B). Thus the second generation PLLA blocks were hydrolyzed during the shell cross-linking reaction. The result was also confirmed by FT-IR spectra (Fig. 6). Fig. 6A and B showed FT-IR spectra of the third generation dendrimer-like copolymer C(PSt(PLLA(PSt−PNAS)$_2$)$_2$)$_3$ before and after cross-linking reaction, respectively. On comparison with Fig. 6A, the characteristic absorption band of ester carbonyl at $\nu_{C=O} = 1742$ cm^{-1} disappeared completely, and a new characteristic carbonyl-stretching band of acylamino group at 1654 cm^{-1} appeared in Fig. 6B. At the same time, the signals of two methylene protons of ethylene diamine substituted in the copolymers, one next to NH$_2$ group (g) and the other next to amide (f), appear at $\delta = 2.8$−3.0 and 3.3−3.5 ppm, respectively, in Fig. 5. Based on the integration ratio of the signals f to g, the ratio of those substituted ethylene diamine groups having one free amine group to that having two imine groups can be calculated. The values for the products obtained from 3 and 7 days reactions were 42% and 45%, respectively. This value is related not only with the reaction time, but also with the ratios of NAS units in copolymers to ethylene diamine added in the reaction mixture. Considering 35% NAS

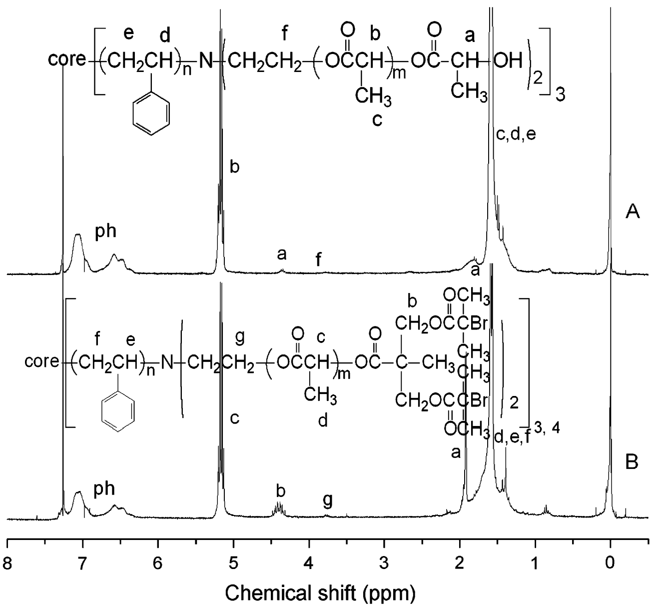


Fig. 3. The second generation dendrimer-like copolymers C(PSt(PLLA-OH)$_2$)$_3$ (A) and C(PSt(PLLABr$_2$)$_2$)$_3$ (B).

units in the third generation PSt-*co*-PNAS, and assuming that the reaction of one ethylene diamine molecule with two NAS units in the same chain does not occur, the cross-linking degree of shell is 22%, that is to say, 22% monomer units in shell are cross-linked by ethylene diamine.

As aforementioned, the aminolysis reaction of PLLA and cross-linking reaction of PNAS with ethylene diamine happened at the same time. However, the reaction rates are different. By comparison of signal b with a in Fig. 5A, 60% of LLA units were hydrolyzed, but 80% NAS units were reacted with ethylene diamine after 3 days reaction. So the cross-linking reaction was faster than hydrolysis reaction of PLLA, thus the particles' shape was kept.

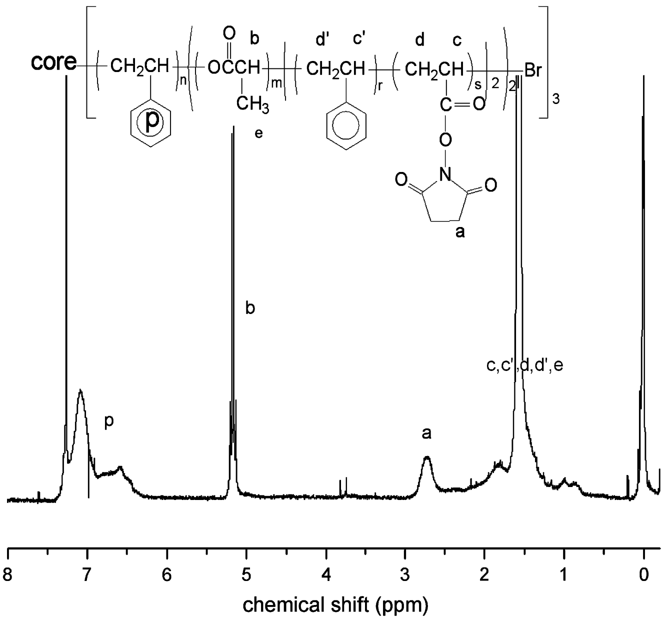


Fig. 4. ^{1}H NMR spectrum of the third generation dendrimer-like copolymer C[PSt(PLLA(PSt-*co*-PNAS)$_2$)$_2$]$_3$.

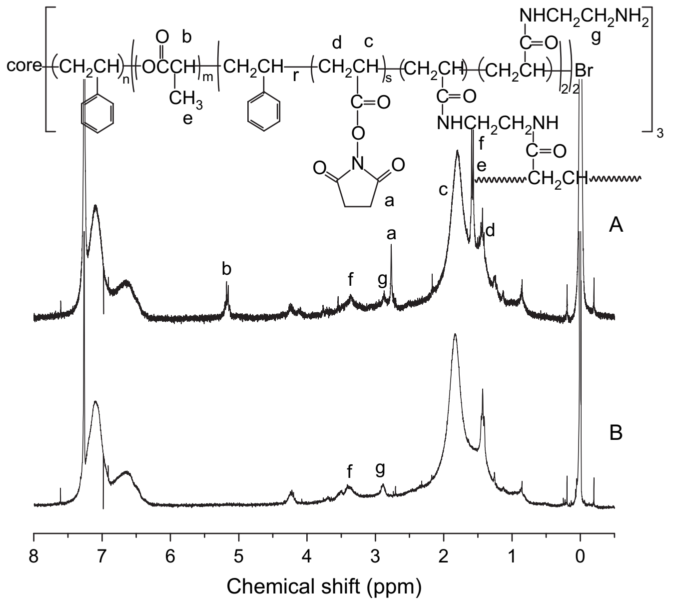


Fig. 5. ^{1}H NMR spectra of dendrimer-like copolymer C(PSt(PLLA(PSt–PNAS)$_2$)$_2$)$_3$ obtained from aminolysis in THF by ethylene diamine for different reaction times: (A) 3 days; (B) 7 days.

3.3. Morphology of the aggregates formed from the third generation dendrimer-like copolymers in selective solvents

In the previous report, the seventh generation dendrimer-like PEO formed loose spherical particle with about 40 nm diameter in water, and the size distribution of the particles was very narrow [10]. The particles can be considered as unimolecular particles since their size is in accordance with their molecular weight. Encouraged by these results we studied the morphologies of the dendrimer-like copolymers with

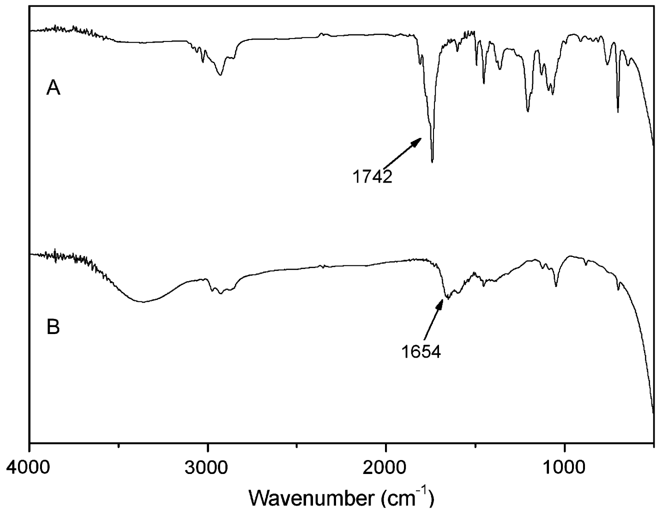


Fig. 6. FT-IR spectra of dendrimer-like copolymer C(PSt(PLLA(PSt–PNAS)$_2$)$_2$)$_3$ before (A) and after (B) cross-linking and aminolysis reactions in THF.

three generations, which was performed by dissolving $C(PSt(PLLA(PSt-PNAS)_2)_2)_3$ and $C(PSt(PLLA(PSt-PNAS)_2)_2)_4$ in THF or $CHCl_3$, a solution with the concentration of 1 mg/mL was afforded, and then EDA was added into half of this solution for cross-linking and aminolysis reactions. The SEM images of the products obtained before and after the reactions are shown in Fig. 7, and all of them show spherical particles, the size distribution is extremely broad, ranging from less than 100 nm to about 900 nm in diameter. Furthermore, the size of particles is far larger than that of unimolecular particles because the diameter of unimolecular particle estimated based on their molecular weights should be about 10–20 nm based on the data of $M_{w,SLS}$ (9.69×10^4) and average diameters of PSt particles (12 nm) in toluene [35]. So the resultant spherical particles are composed of various numbers of macromolecules. Similar results were obtained for use of $CHCl_3$ as solvent. To make sure the size of the particles in solution, DLS measurements were performed, and the results are shown in Fig. 8. The average diameter of the particles obtained by DLS is 295 nm, and the size distribution is also extremely broad, ranging from about 60 nm to about 1000 nm in diameter. This result is accordant with that obtained from SEM images. Both results showed spherical particles and their size is bigger than that of one macromolecule. Probably, the particles were formed via an aggregation process as shown in Scheme 2. At first, a few macromolecules aggregate to form initial spherical cores, and then more macromolecules deposit on the surface of the cores, resulting in the size increase of microspheres. Those big microspheres with large surface absorb more small particles. So some particles increase rapidly, leading to broad size distribution as we mentioned. In Fig. 7C and inset, we can see clearly that some particles showed chapped and/or desquamating shell, the layered structure of the microspheres can be seen clearly;

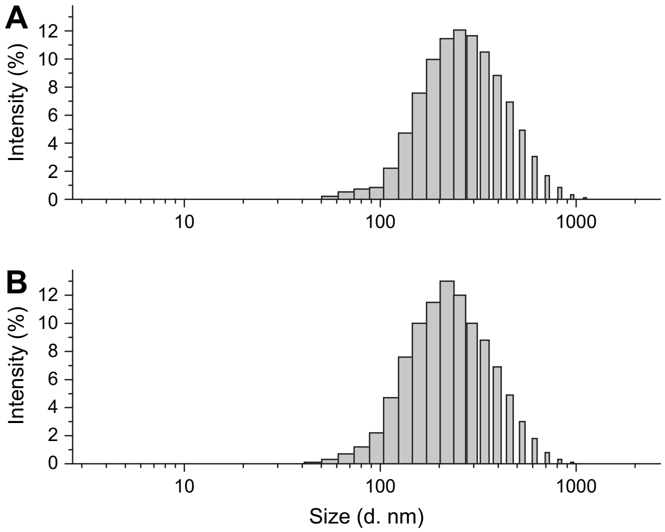


Fig. 8. DLS of the third generation dendrimer-like copolymer $C(PSt(PLLA(PSt\text{-}co\text{-}PNAS)_2)_2)_3$ (No. 1 in Table 3) in THF solution before (A) and after (B) cross-linking and aminolysis for 7 days.

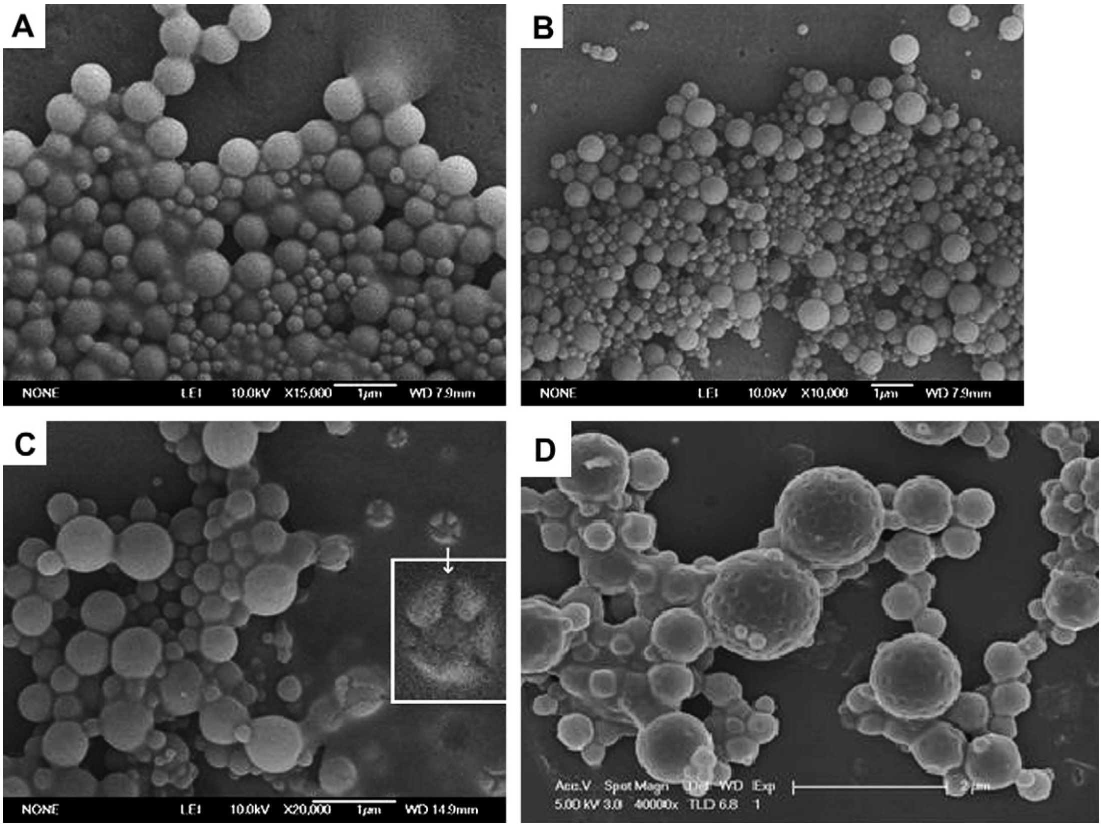
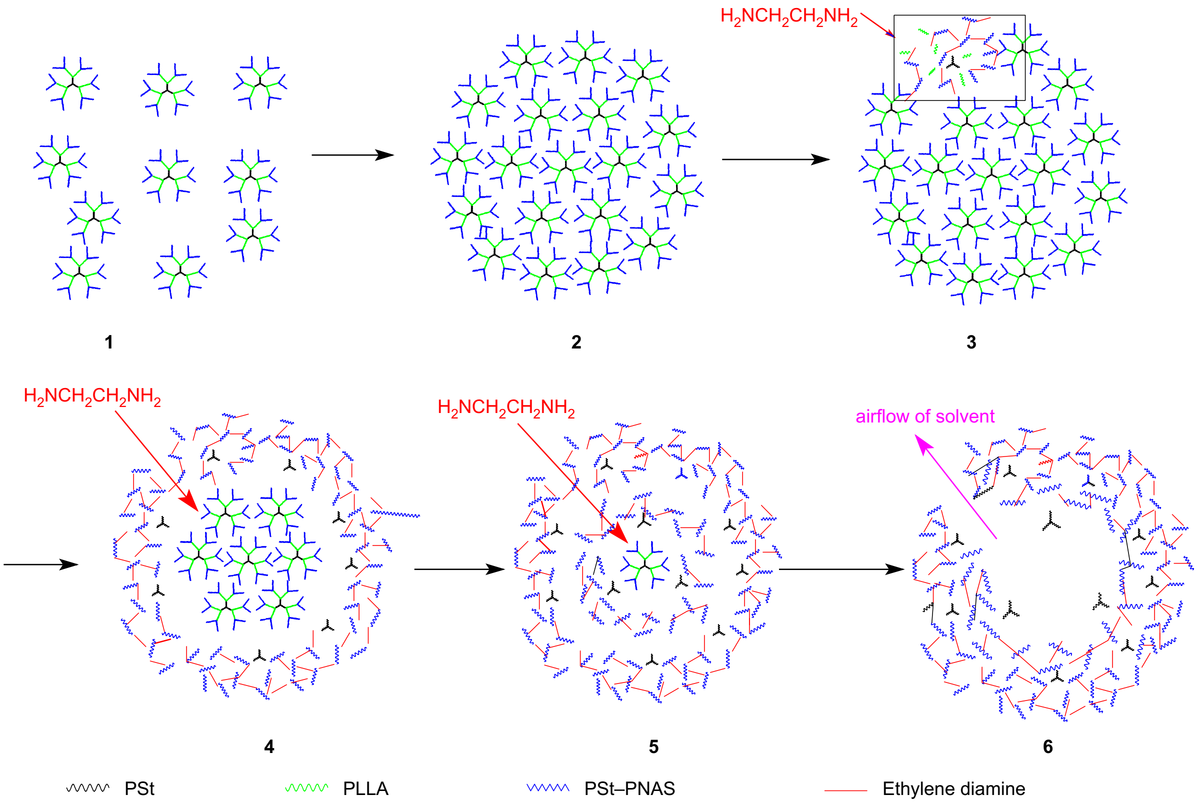


Fig. 7. SEM images of the dendrimer-like copolymer $C(PSt(PLLA(PSt-PNAS)_2)_2)_3$ (No. 1, Table 3) in two solvents: (A) $CHCl_3$; (B) and (C) THF; (D) THF after cross-linking and aminolysis reactions for 7 days, but before precipitation.



Scheme 2. The formation process of hollow microspheres from the dendrimer-like polymers.

this indicates further that they are formed by aggregation of the dendrimer-like macromolecules.

When cross-linking and aminolysis reactions of the PSt-*co*-PNAS in the third generation dendrimer-like copolymers were carried out, the morphology of the microspheres changed slightly (Figs. 7 and 8). Probably, cross-linking of the outermost PSt in the microspheres makes the original morphology remained, but causes the particles size decreased. The average diameter measured by DLS is 264 nm, slightly smaller than that (295 nm) before cross-linking and aminolysis reactions. In addition, we can see a great number of dents on surface of the microspheres in the SEM image of the samples obtained after cross-linking and aminolysis reactions (Fig. 7D). Different from assembly of the block copolymers, the microspheres are formed by aggregation of many spherical macromolecules, and the PLLA segments are sandwiched between two layered PSt segments. When PLLA segments were hydrolyzed and some of the hydrolyzed products were removed, the solid microspheres became porous, and dents on their surfaces were observed. Based on those facts, we can propose the cross-linking and aminolysis process as follows. At first crosslinker EDA reacted with NAS units on the surface of the microspheres, a few EDA molecules are formed as cross-linkages, others reacted with one NHS group to form 2-aminoethylacylamide groups considering the reactivity of the second amino group lower than that of the first amino group in EDA as aforementioned. With proceeding of cross-linking and substitution reactions, the shell copolymers of dendrimer-like macromolecules on the surface of particles react with each other, forming cross-linked shell of the whole particles. As EDA is infiltrated into the inner of dendrimer-like copolymers, the second generation PLLA was decomposed, and the core PSt and PSt-*co*-PNAS with some free amine aggregate to the surface; further reaction of NAS units with amine increases the wall thickness of the particles. The same process occurs for the dendrimer-like copolymers in the inner of particles (2–5 in Scheme 2). During dialysis against THF, small molecules formed from hydrolysis diffuse through cross-linked shell slowly, resulted in a pore of every particle.

When the samples obtained after cross-linking and aminolysis reactions in THF were precipitated three times from petroleum ether and dried in a vacuum oven, the SEM image was measured. As shown in Fig. 9A, we can see the hollow spheres, and interestingly, there is one hole and seldom two holes on the surface of every microsphere. Similar hollow particles prepared by other methods were observed by Im et al. [36] and Han et al. [37]. During the precipitation in petroleum ether, the small molecules produced by hydrolysis inside the particles were diffused into petroleum ether solution, and fast evaporation of solvents inside the particles towards outside produced a pore on the weakest point of the PSt shell (6 in Scheme 2). When the microspheres were preserved in desiccator for longer time, such as 3 months, their SEM images are shown in Fig. 9B and C. On comparison with Fig. 9A, the holes on the surface became bigger, and even some of the particles became hemispheres. Further reaction of the free secondary amines with NHS groups on the shell polymers yields contraction of shell, even strain, as a result, the holes expanded, even the particles were broken. The wall thickness of the spheres is about 80–90 nm, larger than the

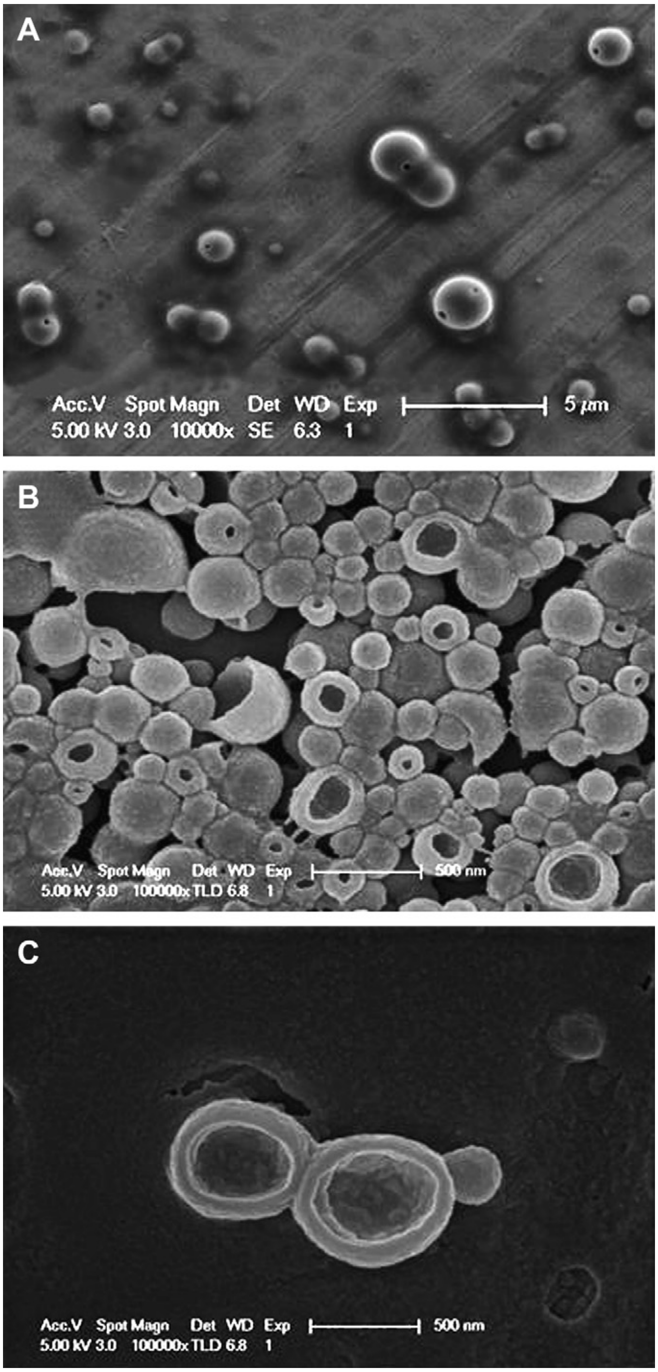


Fig. 9. SEM images of the third generation dendrimer-like copolymer $C(PSt(PLLA(PSt-PNAS)_2)_2)_3$ (No. 1 in Table 3) measured after (A) 3 days and (B) and (C) 3 months after cross-linking and aminolysis reactions in THF and precipitation in petroleum ether.

thickness of a polymer layer (∼25 nm). It should be the consequence that inner macromolecules were extruded to the surface of the spheres.

4. Conclusions

The third generation dendrimer-like copolymers, $C(PSt(PLLA(PSt-PNAS)_2)_2)_3$ and $C(PSt(PLLA(PSt-PNAS)_2)_2)_4$ have been successively prepared by combination of ATRP and ring-opening polymerization. The divergent reactions for transformation of three (or four) bromines into six (or eight) hydroxyl groups and six (or eight) OH groups into 12 (or 16) ATRP initiating sites have been achieved by the reaction of PSt-Br with diethanol amine and PLLA-OH with BMBIBPC, respectively. The solution of the dendrimer-like copolymers in THF with concentration of 1 mg/mL form microspheres with average diameter of 295 nm measured by DLS, and their SEM images show the layered structure and broad size distribution of the spheres. The shell of the particles can be cross-linked via substituted reaction of NAS in the shell polymer with ethylene diamine. After cross-linking and aminolysis reactions, precipitation in petroleum ether and drying, hollow microspheres with one hole and seldom two holes on the surface of every particle were obtained due to removal of hydrolyzed small molecules and evaporation of solvents inside the particles. After the microspheres were preserved for long time, the hole on the surface became big. The hollow particles should have potential application in catalysis and bioengineering.

Acknowledgements

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