

THE AMPHIPHILIC MULTIARM COPOLYMERS BASED ON HYPERBRANCHED POLYESTER AND LYSINE: SYNTHESIS AND SELF-ASSEMBLY*

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Abstract The amphiphilic multiarm copolymers were synthesized through the modification of commercially available hyperbranched polyesters (Boltorn H40) with *N*- ϵ -carbobenzoxy-L-Lysine *N*-carboxyanhydride (ZLys-NCA). After being condensed with *N*-Boc-phenylalanine (Boc-^NPhe) and deprotected the Boc-groups in trifluoroacetic acid (TFA), the original terminal hydroxyl groups were transformed into the amino groups and then initiated the ring-opening polymerization of ZLys-NCA. The hydrophilic poly(L-lysine) was grafted to the surface of Boltorn H40 successfully after the protecting benzyl groups were removed by the HBr solution in glacial acetic acid (33 wt%). The resulting multiarm copolymers were characterized by the ¹H-NMR, GPC and FTIR. The arm length calculated by NMR and GPC analysis was about 3 and 13 lysine-units for H40-Phe-PLys1 and H40-Phe-PLys2 respectively. Due to the amphiphilic molecular structure, they displayed ability to self-assemble into spherical micelles in aqueous solution with the average diameter in the range from 70 nm to 250 nm. The CMC of H40-Phe-PLys1 and H40-Phe-PLys2 was 0.013 mg/mL and 0.028 mg/mL, respectively, indicating that H40-Phe-PLys1 with shorter arm length is easier to self-assemble than H40-Phe-PLys2 with longer arm length.

Keywords: *N*- ϵ -carbobenzoxy-L-Lysine *N*-carboxyanhydride; Amphiphilic multiarm copolymer; Hyperbranched polyester; Modification; Self-assembly.

INTRODUCTION

The Boltorn Hx is a series of commercially available hyperbranched polyesters which was first reported by Malmström and then industrialized by Perstorp Group Co^[1]. Due to the large number of terminal hydroxyl groups, Boltorn polyesters usually display some excellent physical/chemical properties and are widely applied in many fields. For example, Hu *et al.* reported Boltorn H20 was used as hyperbranched segments to prepare aliphatic polyester-based polyurethane elastomers^[2]. Zhu *et al.* adopted crosslinked Boltorn H30 as the active separation layer in nanofiltration membranes^[3]. Recently, Boltorn H40 has received much attention in designing multiarm amphiphilic copolymers due to its biodegradability, biocompatibility and ease of synthesis comparing with dendrimers. Klok *et al.* reported Boltorn H40 was used as a macroinitiator to prepare some water soluble multiarm amphiphilic block copolymers as unimolecular containers by sequential atom transfer radical polymerization of *n*-butyl methacrylate and poly(ethylene glycol) methyl ether methacrylate^[4]. In another work, they also used Boltorn H40 as a macroinitiator to synthesize multiarm amphiphilic copolymers by ring-opening

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polymerization of ϵ -caprolactone and then by ATRP of tertbutyl acrylate for the encapsulation of fragrance molecules^[5]. Chen *et al.* reported folate functionalized nanoparticles based on Boltorn H40, poly(ϵ -caprolactone) and poly(ethylene glycol)^[6]. Gong *et al.* recently reported a unimolecular micelle system based on Boltorn H40, poly(L-lactide) and methoxy poly(ethylene glycol) as drug delivery carriers^[7]. In recent years, our group also reported some polymeric vesicles on the basis of the modification of Boltorn H40 with succinic anhydride^[8,9].

Amino acids, as natural materials, are a good choice for the design of novel biomaterials. Recently, much interest has been concentrated on the self-assembly behavior of the block copolymers containing hydrophilic block and poly(amino acid)s such as poly(lysine), poly(aspartic acid) and poly(γ -benzyl-glutamic acid). The functional side groups in the block of poly(amino acid)s, such as the carboxyl groups in aspartic and glutamic acids and the amino groups in lysine, can help to improve the affinity of copolymers to proteins and cells, or to covalently or ionically combine with drugs, antibodies or DNAs^[10,11]. Their self-assembled structures have thus been used as carriers of drug delivery, or constructing hydrogels for tissue engineering and other biomedical applications^[12–14]. So we naturally consider if amino acids are attached to the surface of Boltorn Hx, some multiarm amphiphilic copolymers based on hyperbranched polyester and linear poly(amino acid)s will be obtained. To the best of our knowledge, such potential biomaterials have not been reported before.

In this paper, the terminal hydroxyl groups in Boltorn H40 were first transformed into amino groups and then initiated the ring-opening polymerization of ZLys-NCA. After removing the protecting benzyl groups, the hydrophilic poly(L-lysine)s were grafted to the surface of Boltorn H40. The resulting multiarm copolymers were characterized by the ¹H-NMR, GPC and FTIR in detail. The self-assembly behaviors of these amphiphilic multiarm copolymers in aqueous solution were also investigated.

EXPERIMENTAL

Materials

Boltorn H40 was obtained from Perstorp AB, Sweden. Tetrahydrofuran (THF) was dried over calcium hydride (CaH₂) for more than 1 week and purified by refluxing over fresh sodium-benzophenone complex, and a deep purple color indicated an oxygen- and moisture-free solvent. Ethyl acetate and petroleum ether were dried over calcium hydride (CaH₂) and distilled before use. All drying procedures were performed under nitrogen atmosphere, and dried chemicals were stored and handled under nitrogen at all times. All other chemicals were used as received.

Methods

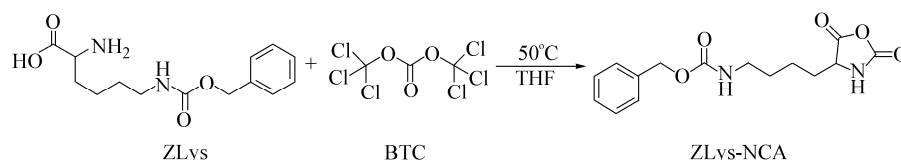
¹H-NMR spectra of the monomers and polymers in CDCl₃ or DMSO-d₆ were measured by using an AV-400 MHz NMR spectrometer at room temperature. Molecular weight and molecular weight distribution were determined by gel permeation chromatography (GPC). The GPC measurement was conducted on a Waters 410 GPC with DMF as eluent (flow rate: 1 mL/min) at 35°C, and the molecular weights were calibrated against polyethylene glycol (PEG) standards. The FTIR spectra of the polymers was measured on a PE Paragon 1000 infrared spectrometer by using samples prepared in potassium bromide (KBr). The transmission electron microscopy (TEM) measurements were performed on a JEOL JEM-2100F transmission electron microscope at an accelerating voltage of 200 kV. The samples prepared as follows: 5 μ L aqueous solution of self-assembled aggregates was dropped onto a carbon-coated copper grid, then dried naturally at room temperature. Dynamic light scattering (DLS) measurements were performed in aqueous solution at 25°C by using a Malvern Zetasizer Nano S apparatus equipped with a 4.0 mW laser operating at $\lambda = 633$ nm. Both samples were measured at a scattering angle of 173° with the concentration of 0.5 mg/mL.

Synthesis

N- ϵ -carbobenzoxy-L-Lysine *N*-carboxyanhydride (ZLys-NCA)

The synthesis route of ZLys-NCA is shown in Scheme 1. The ZLys-NCA was synthesized according to the previous references^[14]. 20 g dried and grinded ZLys and 10 g triphosgene were suspended in 200 mL of tetrahydrofuran (THF) with stirring at 50–60°C for 1 h. Nitrogen was bubbled into the mixture to remove the

byproduct HCl and excess phosgene. The mixture was poured into 500 mL petroleum ether and white solid appeared. The crude ZLys-NCA was filtered and dissolved in ethyl acetate. After being washed by 0°C cold saturated aqueous NaHCO₃ and water in turn to remove remaining HCl, and dried by anhydrous MgSO₄, the product was recrystallized three times by mixed solvent of anhydrous ethyl acetate/petroleum ether (2/5, *V/V*). The dried product weighed 8.32 g, and the final yield of ZLys-NCA was 38.12%. ¹H-NMR (CDCl₃, Fig. 1): δ = 7.21–7.29 (phenyl group, a and b), 5.12 (–CH₂–, c), 5.08 (–NH–, i), 4.25 (–CH–, d), 1.50–3.23 (–CH₂–, e, f, g, h).



Scheme 1 The synthesis route of ZLys-NCA

Amino-terminated Boltorn H40 (H40-NH₂)

5.07 g (0.69 mmol) Boltorn H40 was suspended in 120 mL DMF with stirring until it was clear, and 12.37 g (46.67 mmol) Boc-protected phenylalanine (Boc-^NPhe) and 9.61 g (46.67 mmol) *N,N'*-dicyclohexylcarbodiimide (DCC) were slowly added into the solution respectively with stirring of 24 h. After filtration, DMF was removed under vacuum evaporation. The Boc-^NPhe grafted Boltorn H40 was dissolved in CHCl₃ and washed with the saturated NaHCO₃ aqueous solution and deionized water for 2 times respectively. Anhydrous MgSO₄ was used to remove the remaining water, and the anhydrous solution was concentrated to about 30 mL under vacuum evaporation. In the deprotection process, the solution was suspended in 16 mL trifluoroacetic acid (TFA) with stirring at 0°C for 2 h. The crude production was precipitated in 300 mL anhydrous ethyl ether and washed by the saturated NaHCO₃ aqueous solution to neutralize the TFA. The product was dialyzed against DMF and deionized water by the membrane (MWCO = 7000) for 48 h each. The H40-NH₂ was obtained by the lyophilization for 2 days.

Amphiphilic multiarm copolymers

The multiarm copolymers were synthesized by using H40-NH₂ as macroinitiator to initiate the ring-opening polymerization of ZLys-NCA. The typical polymerization process is as follows: The H40-NH₂ and ZLys-NCA with a certain molar ratio were suspended in anhydrous chloroform under stirring at room temperature for 72 h. After being precipitated in ethyl ether and dried under vacuum, the H40-Phe-poly(ϵ -carbobenzoxyl-L-lysine) (H40-Phe-PZLys) was obtained. Then the H40-Phe-PZLys was deprotected by 33% HBr/acetic acid trifluoroacetate and neutralized by the NaHCO₃ aqueous solution. After being dialyzed in deionized water by the membrane (MWCO = 7000) and freeze-dried, the pure amphiphilic multiarm copolymer H40-Phe-PLys was obtained.

Self-assembly of amphiphilic multiarm copolymers in aqueous solution

At room temperature, 10 mL ultrapure water was added dropwise into 20 mg amphiphilic multiarm copolymer with the mild stirring to form a translucent solution^[8, 10]. The morphology and size of the self-assembled aggregates was characterized by TEM and DLS.

The critical micelle concentration (CMC) of H40-Phe-PLys

Here pyrene was used as the fluorescence probe to investigate the CMC of H40-Phe-PLys. First some H40-Phe-PLys were dissolved in the aqueous solution of pyrene. Then the solution was diluted to various desired concentrations of H40-Phe-PLys (from 5 mg/mL to 1.0×10^{-4} mg/mL) with a constant pyrene concentration of 6.0×10^{-7} mol/L. The fluorescence spectra of all solutions were recorded on a LS-50B luminescence spectrometer (Perkin Elmer Co.). The excitation wavelength was set at 335 nm. The I_3/I_1 ratio values of all solutions were recorded.

RESULTS AND DISCUSSION

The Synthesis and Characterization of ZLys-NCA

Up to now, the triphosgene method^[15] is still the general synthetic method to prepare NCA because of the mild reaction condition and the low toxicity. Here we also used this effective method to synthesize ZLys-NCA. The ¹H-NMR spectrum of the resulting NCAs is shown in Fig. 1. The signals were assigned and labeled by the letters to confirm the chemical structure of the resulting NCAs.

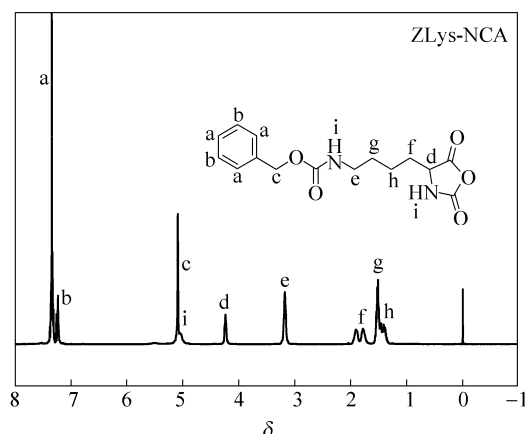


Fig. 1 The ¹H-NMR spectrum of ZLys-NCA in CDCl₃

Synthesis and Characterization of H40-NH₂

Because the ring-opening polymerization of NCA must be initiated by the amino groups, the terminal hydroxyls of Boltorn H40 must be transformed into amino groups. Here the Boc-^NPhe was condensed with the hydroxyls of Boltorn H40 using DCC as the dehydration agent. After the Boc- was deprotected, a part of the terminal hydroxyls in Boltorn H40 were transformed into amino groups. Considering the easy hydrolyzing of the ester bonds in Boltorn H40, the deprotecting procedure with TFA was conducted in the ice-water bath.

The ¹H-NMR spectra of H40-NH₂ and Boltorn H40 are shown in Fig. 2. Comparing both of them, three new peaks appear at $\delta = 1.29$, 2.95 and 7.21 in the spectrum of H40-NH₂, which are all assigned to Boc-^NPhe. Furthermore, on the basis of the peak area of methyl in Boltorn H40 (which was not changed in the reaction process), the peak area of methylene in CH₂OH of Boltorn H40 was decreased with the reaction time increasing and indicated that Boc-^NPhe had been grafted to Boltorn H40 successfully. By GPC measurements, the number-average molecular weight of H40-NH₂ was larger than that of Boltorn H40 which also proved the Boc-^NPhe was grafted to the Boltorn H40. The similar polydispersity index (M_w/M_n) of H40-NH₂ (PDI = 2.18) and Boltorn H40 (PDI = 2.38) confirmed the hyperbranched structure of Boltorn H40 was still kept in H40-NH₂.

According to the ¹H-NMR spectra of H40-NH₂, we calculated the percent conversion of hydroxyls to amino groups in Boltorn H40. Theoretically, the molecular weight of Boltorn H40 is 7300 with 64 terminal hydroxyls. The peak of methyls in Boltorn H40 was not changed in the reaction process so that it can be used as the internal standard. Relative to the internal standard, the peak area of methylene (CH₂OH) in the terminal unit of Boltorn H40 was decreased and indicated that the partial hydroxyls of them had reacted with Boc-^NPhe. The number of hydroxyls reacted with Boc-^NPhe was about 15.2 for each Boltorn H40 macromolecule according to Fig. 2. After the deprotection, the number of undeprotected Phe could be calculated by the ratio of peak area of phenyl in Phe to the remaining methyl in Boc. Here the deprotection percent of Boc- was 59.2%, which means each H40-NH₂ macromolecule had 9.00 terminal amino groups. On the whole, the ratio of terminal hydroxyls transformed into terminal amino groups was 14.1%. The lower percent conversion of hydroxyls to amino groups was adopted here because the hydrophobic ability of Boltorn H40 is not very strong. If overmany hydroxyls were transformed into amino groups, it will result in many hydrophilic poly(amino acid) arms attached to the

surface of Boltorn H40. The self-assembled aggregates may not be obtained due to the over strong hydrophilic ability of arms. Furthermore, it should be noted that the peak of CH₂OH in terminal units of H40-NH₂ is partly overlapped with the peak of water in deuterated DMSO. So we removed the water peak by NMR software and this may also bring some negative effects on the calculation of the percent conversion of hydroxyls to amino groups.

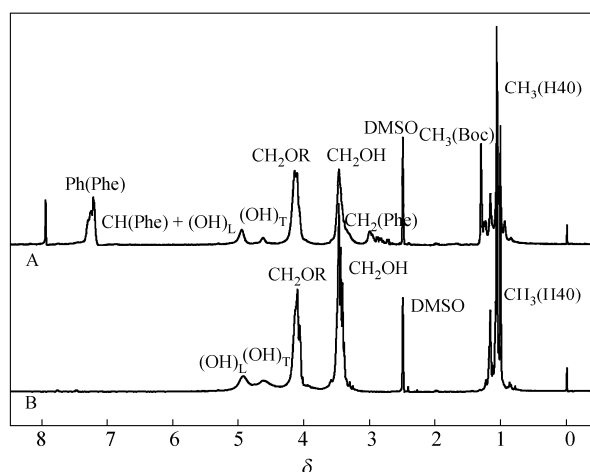
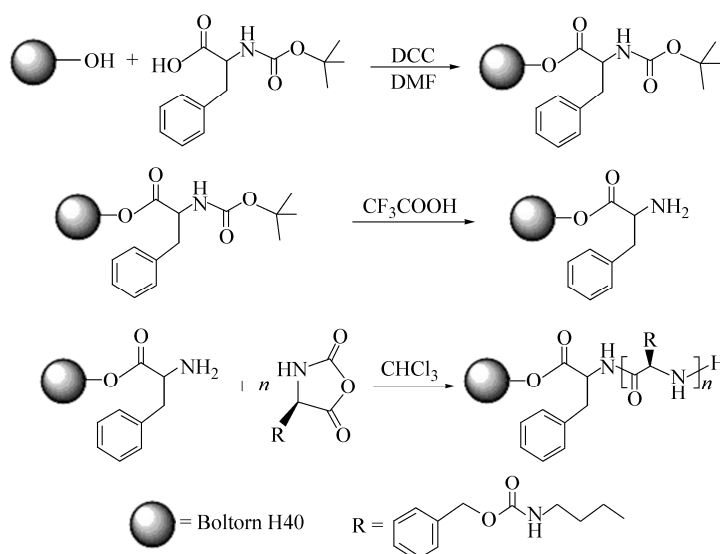


Fig. 2 ¹H-NMR spectra of H40-NH₂ (A) and Boltorn H40 (B)

Synthesis and Characterization of Amphiphilic Multiarm Copolymers

H40-Phe-PLys

The primary amine is a common nucleophilic initiator in the ring-opening polymerization of NCA to prepare poly(amino acid)s and the molecular weight of resulting products is controllable when the molar ratio of NCA/initiator is in the definite range^[16–18]. The synthesized H40-NH₂ was used as the macroinitiator to initiate the ring-opening polymerization of ZLys-NCA and then deprotected the Z-group to produce amphiphilic multiarm copolymer H40-Phe-PLys. The synthesis route is shown in Scheme 2.



Scheme 2 The synthetic route of H40-Phe-PLys copolymer

Two kind of amphiphilic multiarm copolymers (H40-Phe-PLys) were synthesized with the different arm length by adjusting the molar ratio of macroinitiator (H40-NH₂) and monomer (ZLys-NCA). The resulting products with the different arm length were named as H40-Phe-PLys1 and H40-Phe-PLys2 respectively. Before deprotection, the intermediates were named as H40-Phe-PZLys1 and H40-Phe-PZLys2 respectively. The ¹H-NMR spectra of H40-Phe-PZLys1 and H40-Phe-PZLys2 in CDCl₃ are shown in Fig. 3. The signals belong to the peptide arms were labeled by small letters. The strong peak a ($\delta = 7.31$) is assigned to the protons of phenyl group in Phe and Z-Lysine. The peak b ($\delta = 5.49$) is assigned to the protons of methylidyne in the main chain of peptide. The peak c ($\delta = 4.98$) is assigned to the protons of methylene in the carbobenzoxy group. The other four peaks d, e, f and g ($\delta = 3.05, 1.93, 1.49$ and 1.32) are assigned to the protons of methylene in the side group of peptide. In addition, the other small peaks are assigned to the protons of Boltorn H40. Thereinto, the peaks at $\delta = 4.25$ and 3.70 belong to the methylene in $-\text{CH}_2\text{OR}-$ and $-\text{CH}_2\text{OH}-$ units. The peaks at $\delta = 1.05-1.35$ belong to the methyl. The chemical structure of H40-Phe-PZLys1 and H40-Phe-PZLys2 was confirmed.

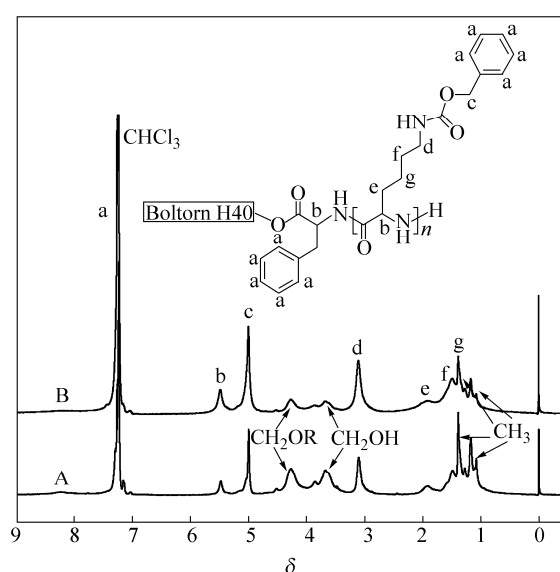


Fig. 3 ¹H-NMR spectra of H40-Phe-PZLys1 (A) and H40-Phe-PZLys2 (B)

After deprotection, the ¹H-NMR spectra of H40-Phe-PLys1 and H40-Phe-PLys2 in D₂O are shown in Fig. 4 and the signals belong to the peptide arms were also labeled by small letters. Obviously, the peak a ($\delta = 7.35$) became very weak due to the Z-groups left and assigned to the protons of phenyl group in Phe. The strong peak b ($\delta = 4.08$) is assigned to the protons of methylidyne in the main chain of peptide. The other four peaks c, d, e and f ($\delta = 2.76, 1.50, 1.48$ and 1.24) are assigned to the protons of methylene in the side group of peptide. Furthermore, the other small peaks are also assigned to the protons of Boltorn H40. Thereinto, the peaks at $\delta = 3.50$ and 4.15 belong to the methylene in $-\text{CH}_2\text{OR}-$ and $-\text{CH}_2\text{OH}-$ units. The peaks at $\delta = 0.90-1.10$ belong to the methyl. Owing to the solvent effect, the location of each peak in D₂O moved $\delta = 0.1-1.4$ to the higher field comparing to that in CDCl₃. On the other hand, the integral area of signals belong to Boltorn H40 in H40-Phe-PLys was lower than that in the corresponding H40-Phe-PZLys. This is attributed to the different deuterium solvent and the amphiphilic structure of H40-Phe-PLys. CDCl₃ is a good solvent for Boltorn H40 and PZLys so that H40-Phe-PZLys can be soluble fully in CDCl₃. The integral area for each peak reflects the relative content of various protons in H40-Phe-PZLys. However, D₂O is a good solvent only for PLys but not for Boltorn H40 so that signals belong to the protons of Boltorn H40 became weak due to the shielding. This result also indicates the self-assembly of the products could form in H₂O. The above results confirmed the chemical structure of H40-Phe-PLys1 and H40-Phe-PLys2.

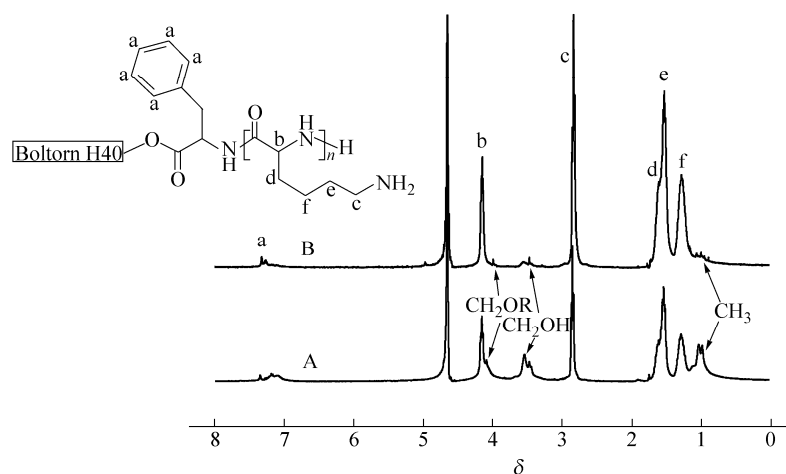


Fig. 4 ^1H -NMR spectra of H40-Phe-PLys1 (A) and H40-Phe-PLys2 (B)

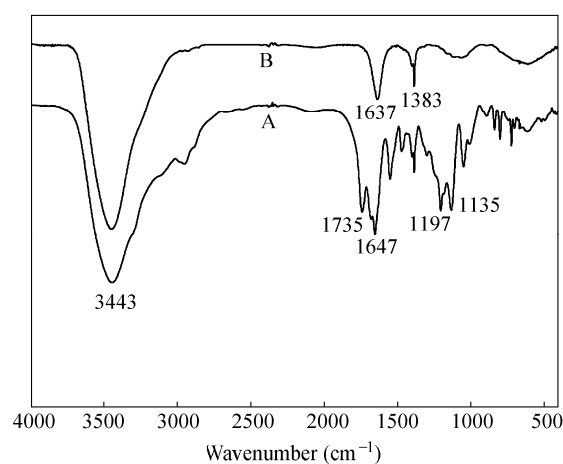


Fig. 5 FTIR spectra of H40-Phe-PLys1 (A) and H40-Phe-PLys2 (B)

The FTIR spectra of H40-Phe-PLys1 and H40-Phe-PLys2 are shown in Fig. 5. The characteristic peaks belong to the Boltorn H40 core and the PLys arms can be observed clearly in the spectrum of H40-Phe-PLys1. For example, the signals at 1735 and 1197 cm^{-1} are the stretching vibration of $\text{C}=\text{O}$ and $\text{C}-\text{O}$ in the ester group of Boltorn H40. The signals at 1647, 1550 and 1383 cm^{-1} are assigned to various amide groups in PLys. However, the characteristic peaks belong to the Boltorn H40 core nearly disappeared in the spectrum of H40-Phe-PLys2. This may be caused by the low content of Boltorn H40 in H40-Phe-PLys2 and its characteristic signals cannot be detected.

The Molecular Weights and the Peptide Arm Lengths of the Resulting Copolymers

The molecular weights of the resulting amphiphilic multiarm copolymers were measured by GPC and ^1H -NMR spectrum. The peptide arm lengths of the resulting amphiphilic multiarm copolymers were also calculated. All the results including the data of intermediates are shown in Table 1.

According to the difference of number-average molecular weight between Boltorn H40 and H40-NH_2 by GPC, and the formular weight of Phe unit, the average number of Phe grafted to each Boltorn H40 is 12.3. So each H40-NH_2 macromolecule had 7.3 terminal amino groups because the deprotection percent of Boc- was 59.2%. Consequently, each peptide arm in H40-Phe-PZLys1 has 2.5 lysine-units on the basis of its average-number molecular weight. After further deprotection, the average-number molecular weight of H40-Phe-PLys1

can be calculated as 7010. In the same way, they are 13.6 and 17400 for H40-Phe-PLys2. On the other hand, the molecular weights and the peptide arm lengths also can be calculated through analysis of $^1\text{H-NMR}$. Apparently the M_n s for H40-Phe-PLys calculated by GPC were smaller than those by $^1\text{H-NMR}$, but the difference decreased with the increasing of M_n s. This can be attributed to the following two reasons. One is the torispherical molecular structure of the copolymers makes the GPC results smaller than their real values. For example, Zagar *et al.* also presented the M_n of Boltorn H40 as 2580 measured by SEC-MALS, which is also smaller than its theoretical M_n ($= 7300$)^[19]. The other is M_n s calculated by the $^1\text{H-NMR}$ method are on the basis of theoretical M_n of Boltorn H40 given by Perstorp Co. Furthermore, there was only small difference in the peptide arm lengths calculated from the GPC and $^1\text{H-NMR}$ methods, which indicated that both of them are suitable for characterization of these amphiphilic multi-armed copolymers.

Table 1. The molecular weights, arm lengths and CMCs of amphiphilic multiarm copolymers

Sample name	M_n^a (NMR)	M_n^c (GPC)	PDI (M_w/M_n)	CMC (mg/mL)	Arm length (NMR)	Arm length (GPC)
Boltorn H40	7300 ^b	2880	2.38	—	—	—
H40-NH ₂	10200	4700	2.18	—	—	—
H40-Phe-PZLys1	17200	9420	2.28	—	2.9	2.5
H40-Phe-PZLys2	39500	30600	3.54	—	12.4	13.6
H40-Phe-PLys1	13600	7010 ^d	—	0.013	—	—
H40-Phe-PLys2	24500	17400 ^d	—	0.028	—	—

^a Calculated from $^1\text{H-NMR}$ spectra; ^b Theoretical M_n given by Perstorp Group Co; ^c Measured by GPC with PEG as the standard and DMF with 0.5% LiBr as the eluent; ^d Calculated from the GPC data of H40-Phe-PZLys

Self-assembly of Amphiphilic Multi-arm Copolymers

Generally, an amphiphilic copolymer forms a core/shell structure in water, and so do H40-Phe-PLys. The amphiphilic nature of H40-Phe-PLys provides an opportunity to self-assemble into micelles in the selective solvent. Here in water, H40-Phe-PLys molecules spontaneously self-assemble into micelles driven by the strong hydrophobic/hydrophilic interaction of Boltorn H40 core and peptide arms. The cores of micelles consist of the hydrophobic Boltorn H40 and the shells of micelles compose of the hydrophilic peptide arms.

The morphologies and sizes of micelles were studied by TEM and DLS measurements respectively. The TEM results in Fig. 6 show that all H40-Phe-PLys aggregated into approximate spherical micelles in water with the diameter from about 70 nm to 250 nm. Apparently, the average diameter of the micelles increased with the peptide arm length decreased. In other words, the shorter the peptide arm, the larger the aggregates.

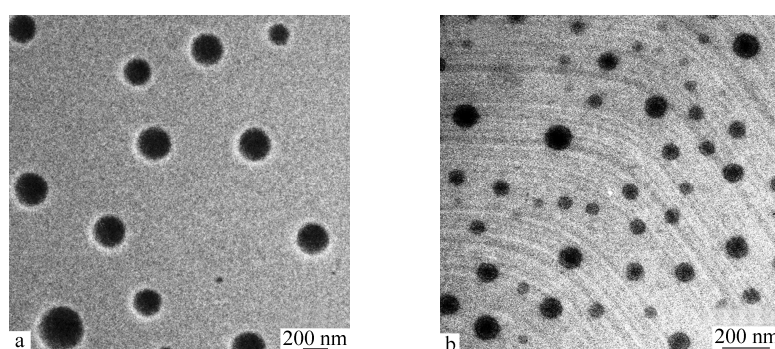


Fig. 6 TEM images of H40-Phe-PLys micelles: (a) H40-Phe-PLys1 and (b) H40-Phe-PLys2

The DLS results are shown in Fig. 7. The Z-average radiuses of the H40-Phe-PLys1 and H40-Phe-PLys2 micelles were 127.5 nm and 87.3 nm respectively, so the average diameters of the micelles were 255.0 nm and 174.7 nm correspondingly. The size distributions of H40-Phe-PLys1 and H40-Phe-PLys2 were 0.155 and 0.306. These results are almost consistent with the results measured by TEM.

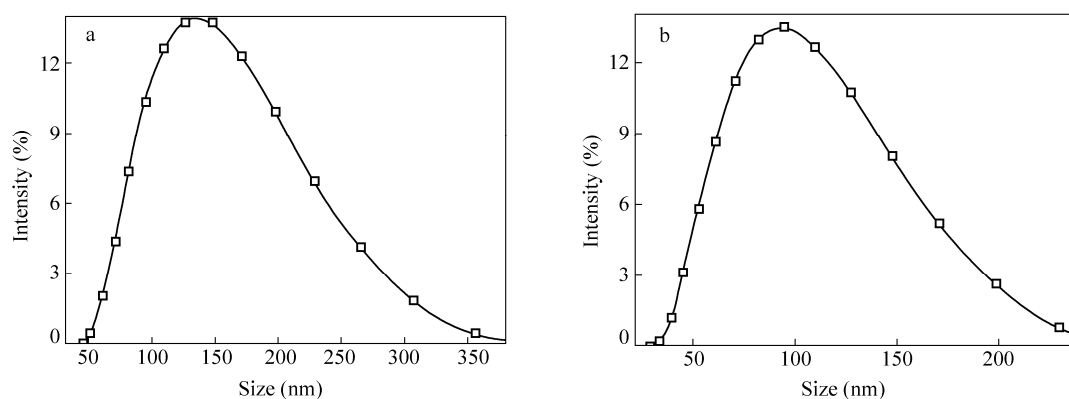


Fig. 7 The micelles size distribution of the H40-Phe-PLys measured by DLS: (a) H40-Phe-PLys1 and (b) H40-Phe-PLys2

The CMC is a key parameter describing the physical properties of micelles and relating to the thermodynamic stability of micelles^[20]. The pyrene probe fluorescence spectrometry is a recognized method to determine the CMC value^[21, 22]. Here we evaluate the CMC of amphiphilic H40-Phe-PLys in water by this method. I_1 and I_3 are the emission intensity of the first and third bands in the fluorescence spectrum of pyrene respectively (signed 1 and 3 in Fig. 8). The emission intensity ratio of I_3/I_1 is very sensitive to the polarity of the medium surrounding pyrene molecules. The smaller the ratio is, the bigger the polarity of the medium.

The relationships of the I_3/I_1 and the concentration of amphiphilic H40-Phe-PLys with different peptide arm length are shown in Fig. 8. All of them exhibited a distinct inflexion. At low concentration of H40-Phe-PLys, the intensity ratio of I_3/I_1 remained nearly unchanged, indicating the characteristic of pyrene in water environment. As the concentration of H40-Phe-PLys increased, the intensity ratio of I_3/I_1 started to increase dramatically and reached the characteristic level of pyrene in completely hydrophobic environment at a certain concentration of H40-Phe-PLys. This demonstrates the whole process of micellization. From the distinct inflexion, the CMC of H40-Phe-PLys1 and H40-Phe-PLys2 are 0.013 mg/mL and 0.028 mg/mL respectively. The results indicated that the amphiphilic multiarm copolymer with the shorter arm length was easier to self-assemble in water than that with longer arm length.

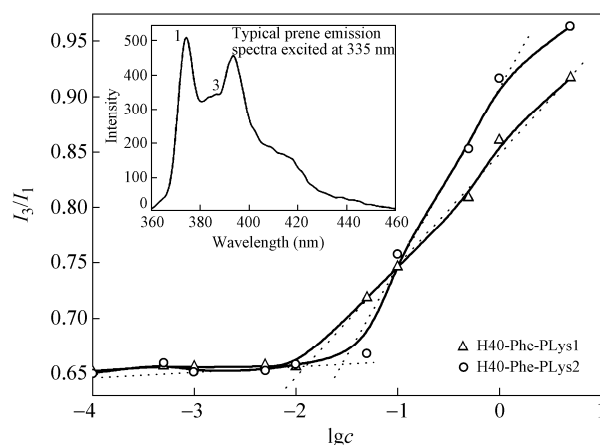


Fig. 8 The relationship of the intensity ratio (I_3/I_1) and the concentration of amphiphilic multiarm copolymer solutions

CONCLUSIONS

In summary, the amphiphilic multiarm copolymers were synthesized by the modification of commercial Boltorn H40 with ZLys-NCA. The terminal hydroxyl groups in Boltorn H40 were first condensed with Boc-^NPhe and then the Boc-groups were deprotected in TFA. As a result, the original terminal hydroxyl groups were transformed into the amino groups to initiate the ring-opening polymerization of ZLys-NCA. After the HBr solution in glacial acetic acid (33 wt%) were used to remove the protecting benzyl groups, the hydrophilic poly(L-lysine) was grafted to the surface of Boltorn H40 successfully. The chemical structure of resulting multiarm copolymers was confirmed by the ¹H-NMR, GPC and FTIR. Due to the amphiphilic molecular structure, they displayed ability to self-assemble into spherical micelles in aqueous solutions. The average diameters were in the range from 70 nm to 250 nm which were observed clearly by TEM and also confirmed by DLS. The CMC of H40-Phe-PLys1 and H40-Phe-PLys2 was 0.013 mg/mL and 0.028 mg/mL, respectively. They will have some potential applications in biomaterial fields.

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