Synthesis and Characterization of Biodegradable Block Copolymer Pluronic-*b*-poly(L-Lysine)

Hui Yu,[1,2] Xiaohua Gu,[1] Xinyuan Shen,[1] Yaogang Li,[1] Yourong Duan[2]

[1]*State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 200051, China*
[2]*Cancer Institute of Shanghai Jiao Tong University, Shanghai 200032, China*

Received 4 August 2008; accepted 2 November 2008
DOI 10.1002/app.29643
Published online 6 March 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This study presented the investigations on the synthesis of a novel biodegradable block copolymer of pluronic-*b*-poly(L-lysine) (pluronic-*b*-PLL), which combined the characteristics of aliphatic polyester and poly(amino acids). The synthesis work started with end-capping of pluronic with *N*-*t*-butoxycarbonyl-L-phenylalanine using dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine, followed by a deprotection process to obtain the amino-terminated pluronic; the new primary amino group in the modified pluronic initiated ring-opening polymerization of amino acid *N*-carboxyanhydride, which afforded the pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer. Finally, removal of the side-chain N^{ε}-(carbonybenzoxy) end protecting groups yields the block copolymer of pluronic-*b*-PLL. The products were characterized by ^{1}H-NMR, FTIR, DSC, and GPC. The block copolymer micelle containing the anticancer drug paclitaxel was prepared by the double emulsion method. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 3371–3379, 2009

Key words: biodegradable; biopolymers; FTIR; gel permeation chromatography; ring-opening polymerization

INTRODUCTION

Due to their superior biocompatibility and ability to form gels in response to changes in temperature, A-B-A triblock copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) have been widely employed in the fields of controlled drug encapsulation and delivery systems.[1–3] The most interesting feature of the amphiphilic PEO-PPO-PEO is that it can self-assemble into a micellar structure with a hydrophilic shell and a hydrophobic core because of a relative difference in the hydrophobicity between PPO and PEO in aqueous solution.[4–6] Micellization in the PEO-PPO-PEO solutions is a consequence of dehydration of the PPO blocks. The hydrophobic core can accommodate hydrophobic drugs such as doxorubicin and paclitaxel (PTX), making copolymeric micelles excellent drug delivery vehicles.[7,8] In addition, the formulations of PEO-PPO-PEO block copolymers have recently attracted increasing attention to treat drug-resistant cancers.[9,10] Experimental studies have demonstrated that PEO-PPO-PEO block copolymers sensitize multidrug resistant cancer (MDR) cells with respect to various anticancer agents, especially, anthracycline antibiotics. Therefore, considerable research works were progressing in controlled release of anticancer drugs using PEO-PPO-PEO micelles.[11,12] However, because of the absence of functional groups on the copolymer backbone, PEO-PPO-PEO is limited in numerous biomaterial applications.

Polymeric materials extensively modified by cationic poly(α-amino acid)s, such as poly(L-lysine) (PLL), displayed a relatively high cationic surface charge, which caused electrostatic interactions with a negatively charged cellular membrane, e.g., malignant cell.[13,14] These cationic copolymers could be covalently or ionically combined with drugs, antibodies, or DNA's and widely used in the fields of targeting drug delivery and gene delivery.

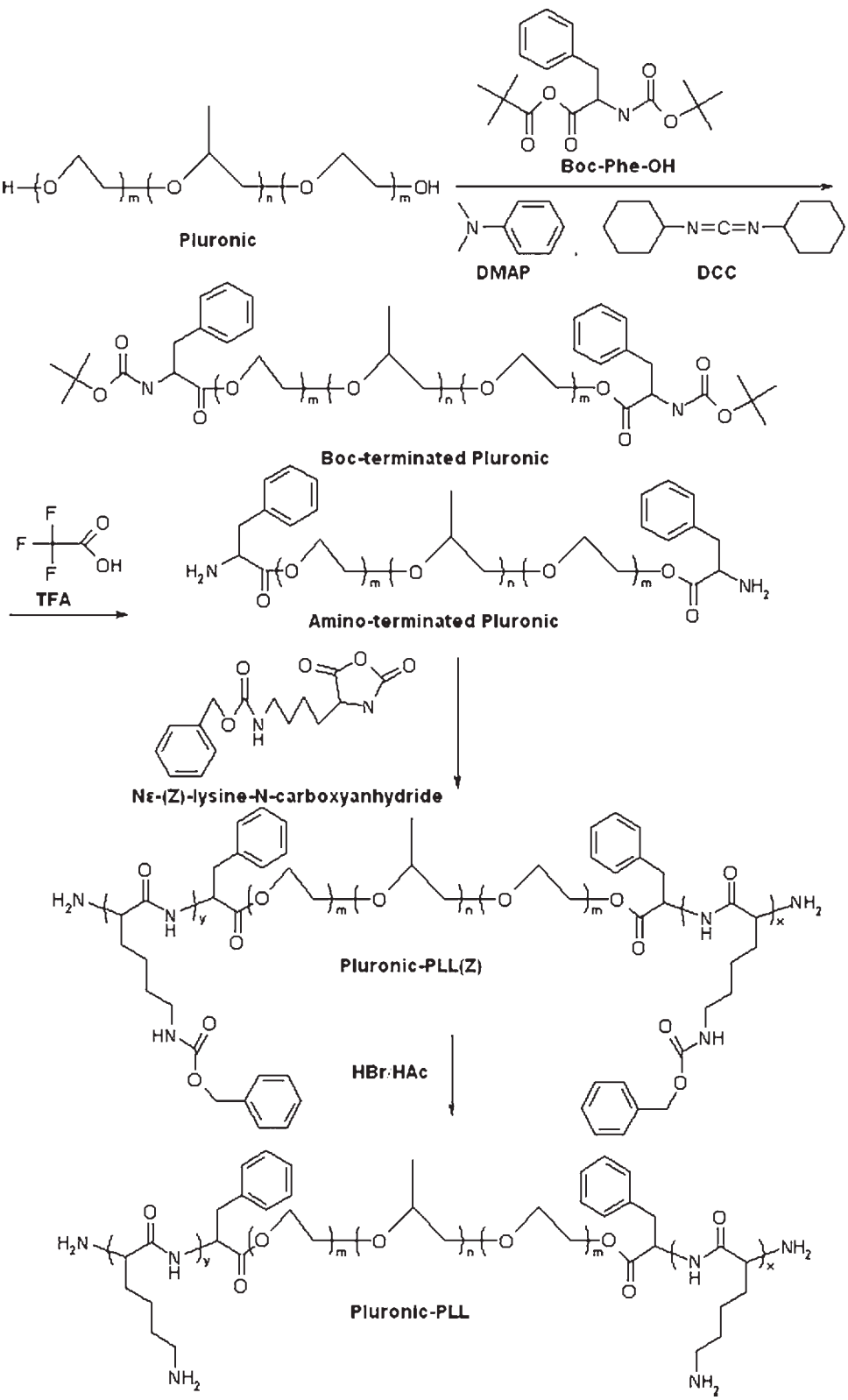
Considerable synthesis works have been published on the preparation of polymer-*b*-poly(α-amino acid)s block copolymer through the ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydride (NCA) and hydroxyl-terminated polymer. One strategy to prepare amino-terminated polymer is to initiate polymerization of aliphatic lactone using an alcohol containing a protective amino group, and then deprotect the protective group.[15] Another approach is to connect the hydroxyl end group of these polymers with agents containing a protective amino

The authors are grateful to the analysis center of Donghua University.

Correspondence to: Y. Li (yaogang_li@dhu.edu.cn) or Y. Duan (yrduan@sci.shmu.edu.cn).

Contract grant sponsor: Natural Science Foundation of China; contract grant numbers: 30572271, 50673058.

group and followed by removal of the protective group to release the amino group. Deng et al.[16] reported end-capping of poly(L-lactide) (PLLA) with *N*-*t*-butoxycarbonyl-L-phenylalanine (Boc-L-Phe) using dicyclohexylcarbodiimide (DCC) as a condensing agent. Fan et al.[17] improved this synthetic route by



Scheme 1 Synthesis of block copolymer pluronic-*b*-PLL.

using a mixed acid anhydride of Boc-L-Phe in the presence of 4-(1-pyrrolidinyl) pyridine because there was no sufficient conversion of the end hydroxyl group of PLLA into amino group by using only DCC. However, this end-capping approach is too complicated.

PTX is one of the significant anticancer agents and has provided effective treatment for a variety of tumors.[18,19] However, PTX is a hydrophobic drug with poor aqueous solubility.[20] To increase its solubility for clinical applications, many delivery systems such as liposomes, emulsions, hydrotropic polymer micelle, especially polymeric micelles have been developed.[21–24]

Therefore, temperature-sensitive and biocompatible cationic block copolymers having PEO-PPO-PEO and poly(L-lysine) segments were synthesized. The synthesis route is illustrated in Scheme 1. The copolymer pluronic-*b*-PLL combined the temperature-sensitive PEO-PPO-PEO segment and cationic PLL segment. Additionally, the introducing end-capping PLL segment not only provided safe group in undegradable PEO-PPO-PEO building block but also biodegraded by proteases or peptidases, thus enable to offer a biodegradable copolymer.[17,25] The micelle-loaded PTX using this copolymer was also prepared in this study.

EXPERIMENTAL

Materials

Pluronic (F108 was chosen as sample) was obtained from BASF (Shanghai, China). Trifluoroacetic acid (TFA) was purchased from Sinopharm (Shanghai, China), and it was distilled over phosphori pentoxidum before use. N^{ε}-(carbonybenzoxy)-L-lysine, Boc-L-Phe, and triphosgene were purchased from GL Biochem (Shanghai, China). DCC, 4-dimethylaminopyridine (DMAP), and HBr (33 wt %)/HAc were purchased from Acros (Shanghai, China). N^{ε}-(Z)-lysine-NCA was synthesized and purified referred to the method reported by Dorman et al.,[26] and then stored at -20°C *in vacuo*. Hexane, methylene dichloride, and chloroform were refluxed over calcium hydride and distilled under nitrogen. Tetrahydrofuran (THF) was dried and distilled from sodium immediately before use.

Characterization

^{1}H NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. FTIR-Raman spectra were measured using a Nicolet NEXUS-670 Fourier transform infrared spectrometer. GPC measurements were carried out on a Polymer PL-GPC 50 gel permeation chromatograph system equipped with an RI detector. Differential scanning calorimetry (DSC) experiments were performed using a TA-2910 MDSC. Nitrogen was used as the purge gas at a flow rate of 40 mL/min for the DSC cell.

The size distribution of micelle was performed by dynamic light scattering (DLS; NICOMP^{TM} 380ZLS Santa Barbara, CA) at 25°C. The scattered light of a vertically polarized He-Ne laser (633 nm) was measured at the angle of 90°. Transmission electron microscopy (TEM) image was obtained using HITACHI (H-800, Japan), operating at the acceleration voltage of 200 kV.

End-capping of pluronic with Boc-L-Phe

The end-capping of pluronic with Boc-L-Phe was performed as follows: A nitrogen-purged flask containing 10.0 g (0.68 mmol) pluronic (F108) and 1.5 g (4.3 mmol) Boc-L-Phe dissolved in 80 mL anhydrous methylene dichloride was cooled to -10°C and treated with a solution of 1.2 g (5.7 mmol) DCC and

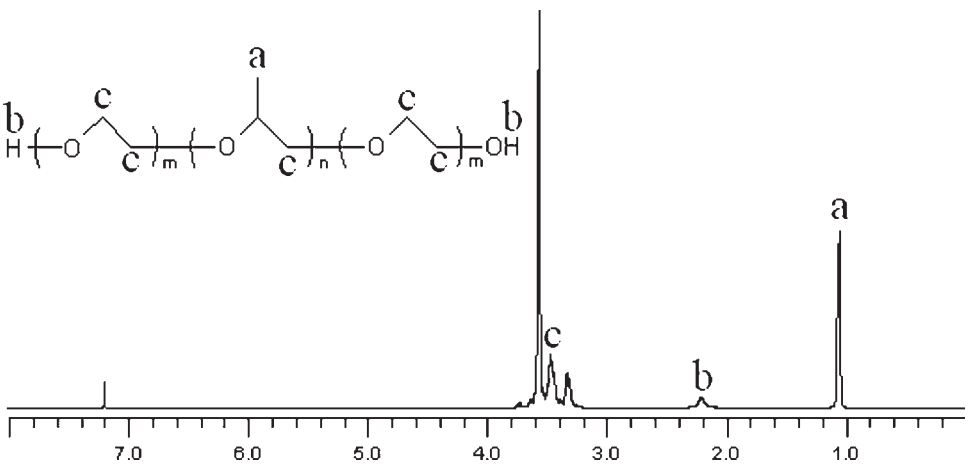


Figure 1 The ^{1}H-NMR spectrum of pluronic (F108) in $CDCl_3$.

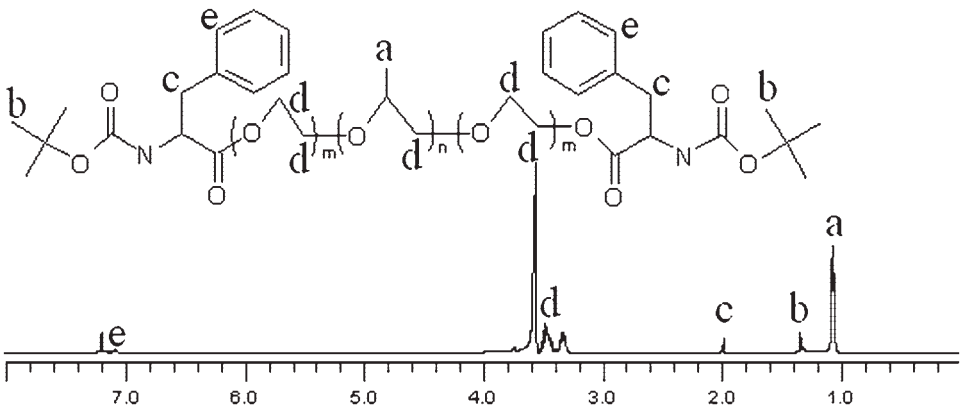


Figure 2 The ^{1}H-NMR spectrum of Boc-L-Phe end-capped pluronic in $CDCl_3$.

0.11 g (0.91 mmol) DMAP for 48 h at 0°C, and then removed the white precipitate by vacuum filtration. The solution was washed with 2 × 100 mL saturated aqueous sodium hydrogen carbonate and 2 × 100 mL distilled water. The copolymer in methylene dichloride solution was precipitated when poured into an excess of chilled methanol. The white precipitate Boc-L-Phe end-capped pluronic was recovered by vacuum filtration and dried *in vacuo* at room temperature.

Removal of the protective *t*-butoxycarbonyl end group from Boc-L-Phe end-capped pluronic

Amino-terminated pluronic was synthesized by removing the protective *t*-butoxycarbonyl end group from Boc-L-Phe end-capped pluronic. Generally, 6 g (0.41 mmol) of Boc-L-Phe end-capped pluronic was dissolved in 70 mL dry methylene dichloride, and then the solution was cooled to 0°C and treated with 15 mL (mmol) TFA for 2 h under nitrogen atmosphere. TFA and methylene dichloride were then removed in vacuum, and the residue redissolved in dry chloroform, washed with 2 × 100 mL saturated aqueous sodium hydrogen carbonate, and 2 × 100 mL distilled water and then dehydrated by sodium sulfate. Finally, the copolymer was precipitated when its chloroform solution was poured into an excess of chilled methanol, and then collected by vacuum filtration and dried *in vacuo* at room temperature.

Synthesis of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer

Copolymerization of N^{ε}-(Z)-lysine-NCA and amino-terminated pluronic was carried out to obtain pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) as follows: 2.0 g (6.6 mmol) N^{ε}-(Z)-lysine-NCA and 1.5 g (0.1 mmol) amino-terminated pluronic were charged into an oven-dried flask and dissolved in 80 mL dry chloroform. The flask was purged with nitrogen three times and the solution was stirred for 72 h at room temperature. The reaction solution was concentrated to 30 mL by rotary evaporator and poured into an excess of chilled diethyl ether to precipitate the pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer. The copolymer was collected through vacuum filtration and dried *in vacuo* at room temperature.

Deprotection of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer

Pluronic-*b*-PLL was obtained by the removal of the side-chain protecting groups of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine): 1.0 g pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) was charged into an oven-dried flask, and then 30 mL of hydrobromic acid/acetic acid was added

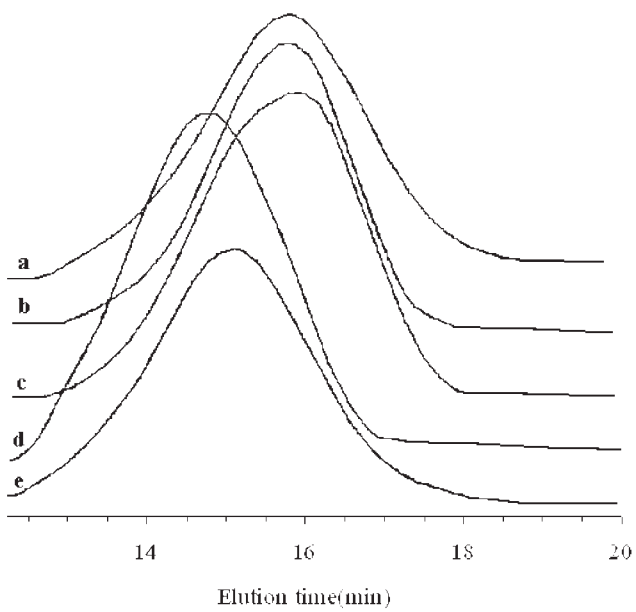


Figure 3 The GPC traces of pluronic (a), Boc-L-Phe end-capped pluronic (b), amino-terminated pluronic (c), pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) (d), pluronic-*b*-PLL (e).

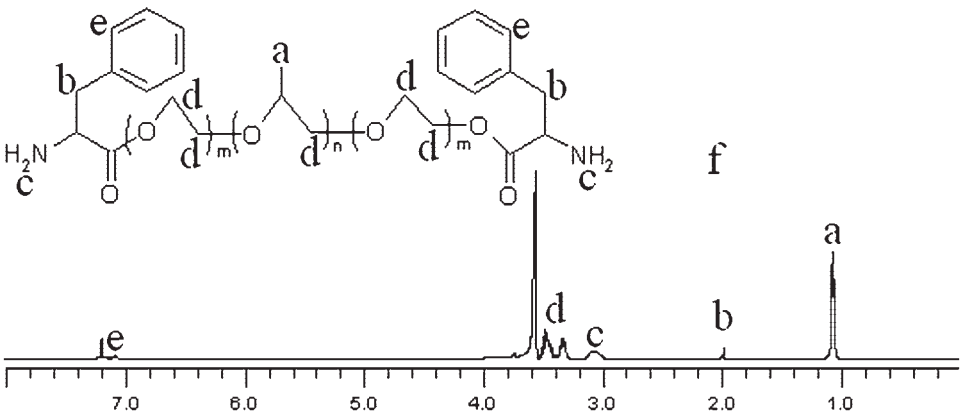


Figure 4 The ^{1}H-NMR spectrum of amino-terminated pluronic in $CDCl_3$.

to the flask through a syringe to dissolve the solid. The flask was purged with nitrogen three times and the slurry was stirred for 1 h at room temperature. Finally, the precipitate was obtained by pouring the reaction solution into an excess of chilled diethyl ether. The block copolymer pluronic-*b*-PLL was collected by vacuum filtration and dried *in vacuo* at room temperature.

Preparation of micelle

Pluronic-*b*-PLL micelle loaded with PTX was prepared using the double emulsion method as follows: A solution of PTX (V = 0.05 mL, C = 2 mg/mL) in acetone was emulsified in 0.5 mL chloroform, in which 4.5 mg of copolymer had been dissolved, using probe sonication (Scientz, Ningbo, China) at 500 W for 1 min. The w/o emulsion was transferred to pluronic F68 (V = 4 mL, C = 1 wt %) solution, and the mixture was again probe sonicated at 500 W for 1 min. The formed w/o/w emulsion was stirred at room temperature until the organic phase was completely evaporated. Finally, the micelles were filtered through a 0.45-μm filter (Millipore, Shanghai, China) to eliminate the aggregate micelles.

RESULTS AND DISCUSSION

Preparation of Boc-L-Phe end-capped pluronic

Boc-L-Phe end-capped pluronic was obtained by the reaction of *tert*-butoxycarbonyl-protective phenylalanine with pluronic using DCC in the presence DMAP. Compared with the ^{1}H-NMR of pluronic in Figure 1, three new peaks distinctly appeared at 1.35 ppm (b, $(CH_3)_3C$—), 1.99 ppm (c, —CH_2—), and 7.11 ppm (e, singlet, C_6H_5—) in Figure 2; one peak disappeared at 2.22 ppm (b, —OH) in Figure 1, indicating the existence of methyl from the *tert*-butoxycarbonyl group and the phenyl proton in the phenylalanine, respectively.

Kang and Leroux[27] reported the reaction of end-capping of PLLA with amino acid, only using DCC

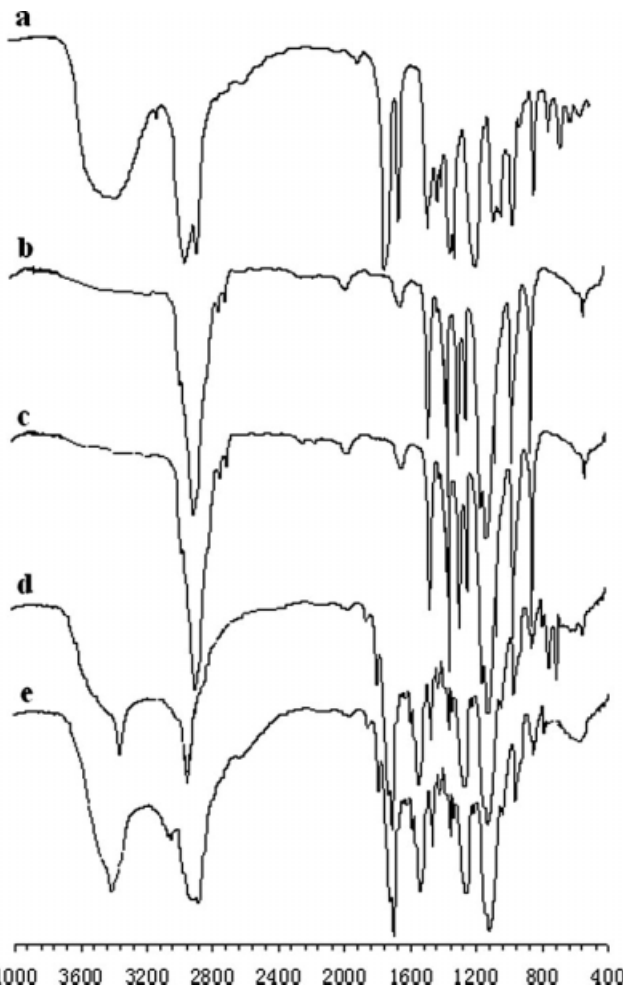


Figure 5 The IR spectra of pluronic (a), Boc-L-Phe end-capped pluronic (b), amino-terminated pluronic (c), pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) (d), pluronic-*b*-PLL (e).

as a condensing agent was inadequate. To promote this type of reaction, the amino pyridine catalyst (DMAP) was applied in this study. Based on the intensity ratio of *tert*-butoxycarbonyl to unchangeable groups (CH_3— for pluronic-Boc), the terminal hydroxyl group of pluronic was nearly completely capped. The molecular weight resulting from GPC in Figure 3(a,b) showed the similar shape and the same position of retention time before and after the end-capping treatment of pluronic, revealing that the end-capping of the terminal hydroxyl group of pluronic was successfully completed through a simple one-step reaction, simultaneously maintaining the polymeric backbone unchanged.

Synthesis of amino-terminated pluronic

The amino-terminated pluronic was successfully prepared by removing the protective *tert*-butoxycarbonyl group from Boc-L-Phe end-capped pluronic. The deprotection was carried out with TFA under an anhydrous condition at 0°C. The ^{1}H-NMR spectrum of amino-terminated pluronic was illustrated in Figure 4. The signal at 1.36 ppm disappeared completely, demonstrating the elimination of *tert*-butoxycarbonyl group from the amino group at the end of the pluronic. The GPC trace of pluronic-NH_2 remained unchanged in Figure 3(c), indicating that the polymeric backbone did not change much before and after the deprotection process. As shown in Figure 5(a–c), the IR spectra of pluronic-NH_2 and Boc-L-Phe end-capped pluronic had no obvious differences. The absorption peak at 2900 cm^{-1} was assigned to γ_{CH3} stretch vibration and 1088 cm^{-1} was attributed to γ_{C-O-C} stretch vibration of the pluronic segment. However, the absorption peak at 3440 cm^{-1} (γ_{OH}) in Figure 5(a), a characteristic peak of pluronic, disappeared in Figure 5(b,c), further indicating a complete conversion of pluronic.

Copolymerization of amino-terminated pluronic and N^{ε}-(Z)-lysine-*N*-carboxyanhydride

Block copolymer of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) was prepared by the ROP of N^{ε}-(Z)-lysine-NCA initiated by amino-terminated pluronic. A representative ^{1}H-NMR spectrum of the block copolymer was shown in Figure 6. Compared with the ^{1}H-NMR of pluronic-NH_2, an obvious peak at 7.38 ppm was attributed to the benzene ring from the protecting group of (Z)-lysine. The peaks at 5.0 (g, —CH_2—), 4.19 (f, —CH—), 2.95 (d, —CH_2—), 1.71 (c, —CH_2—), and 1.37 (b, —CH_2—) ppm were assigned to protons of the lysine segment. Figure 5(d) illustrated the IR spectrum of the pluronic-*b*-poly(N^{ε}-(Z)-L-lysine). Two amide bands at 1650 and 1700 cm^{-1} and one

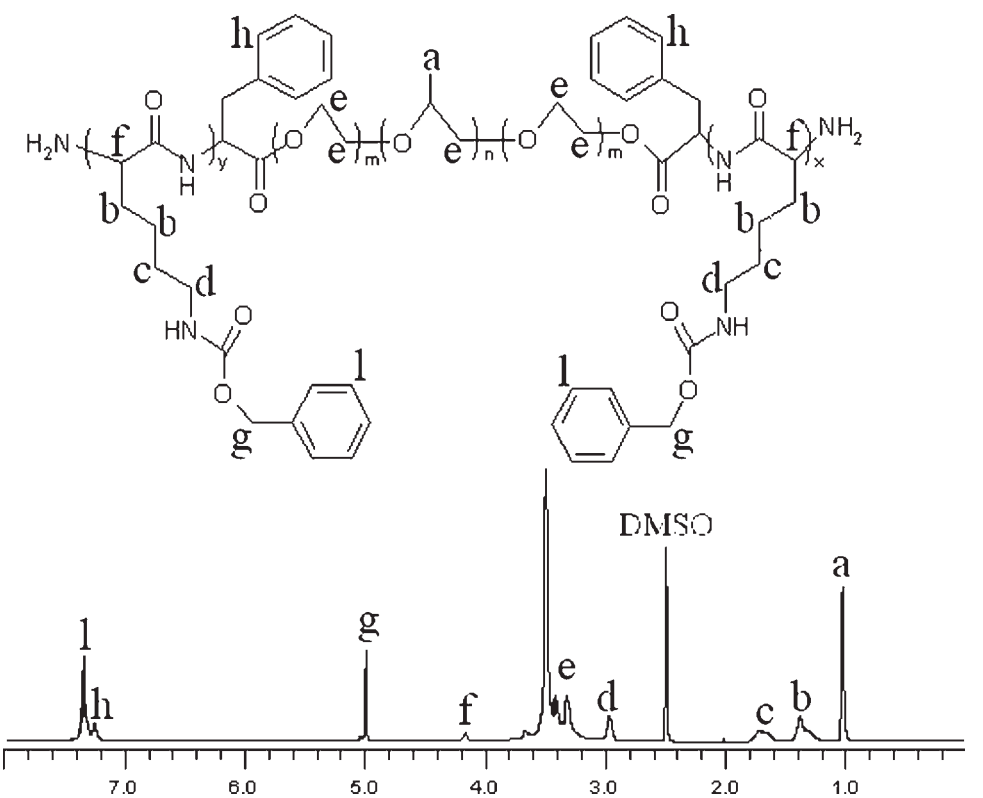


Figure 6 The ^{1}H-NMR spectrum of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) in DMSO-d_6.

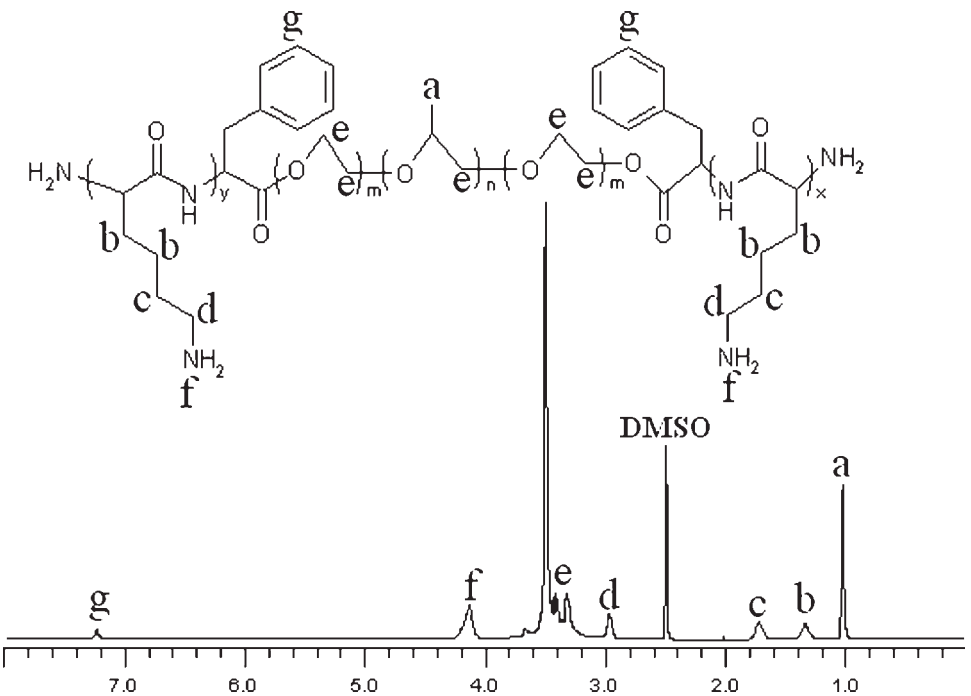


Figure 7 The ^{1}H-NMR spectrum of pluronic-*b*-PLL in DMSO-d_6.

amide band at 1540 cm^{-1} were observed; the absorption peak at 3340 cm^{-1} was assigned to —NH— stretch vibration; the absorption peaks at 690 and 745 cm^{-1} were attributed to phenyl group of poly (N^{ε}-(Z)-L-lysine) segment. The degree of polymerization (DP) of the block copolymer was calculated from the integral ratio of CH_3— (a at 1.02 ppm) to —$C_6H_5CH_2OCO$— (g at 5.0 ppm) in the ^{1}H-NMR spectrum of the block copolymer. The obtained DP of the pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) was 30, which was consistent with the feed ratio of NCA monomer to the macroinitiator.

The GPC trace of the block copolymer is shown in Figure 3(d). The shift of the peak to a shorter retention time indicated an increasing molecular weight. Only a single peak in the GPC trace was found, which revealed that the copolymerization was completed successfully and there was no homopolymer in the copolymer.

Deprotection of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer

It is well known that the benzyloxycarbonyl group (Z-group) of the polymer can be removed in HBr/acetic acid solution.[28] The deprotection of pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) was carried out and confirmed by the ^{1}H-NMR spectrum and IR as shown in Figures 7 and 5(e), respectively. In the ^{1}H-NMR spectrum, the signals of the benzyloxycarbonyl group at 5.0 and 7.38 ppm disappeared, which clearly confirmed the completion of deprotection. In IR spectra, the increasing absorption peaks at 3340, 1700, 1650, and 1540 cm^{-1} indicated a block copolymer structure; the γ_{CH} vibration of the benzyl at 745 and 690 cm^{-1} disappeared, which also manifested that the benzyl groups had been removed completely. The GPC trace of the deprotective copolymer is shown in Figure 3(e). After deprotection, a decrease in the molecular weight was found and no other signal was detected simultaneously, demonstrating that the deprotection was successfully

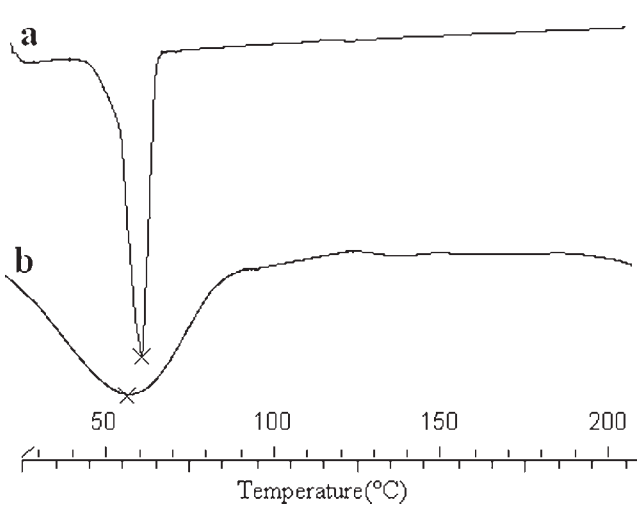


Figure 8 The DSC thermograms of pluronic (a) and pluronic-*b*-PLL block copolymers (b).

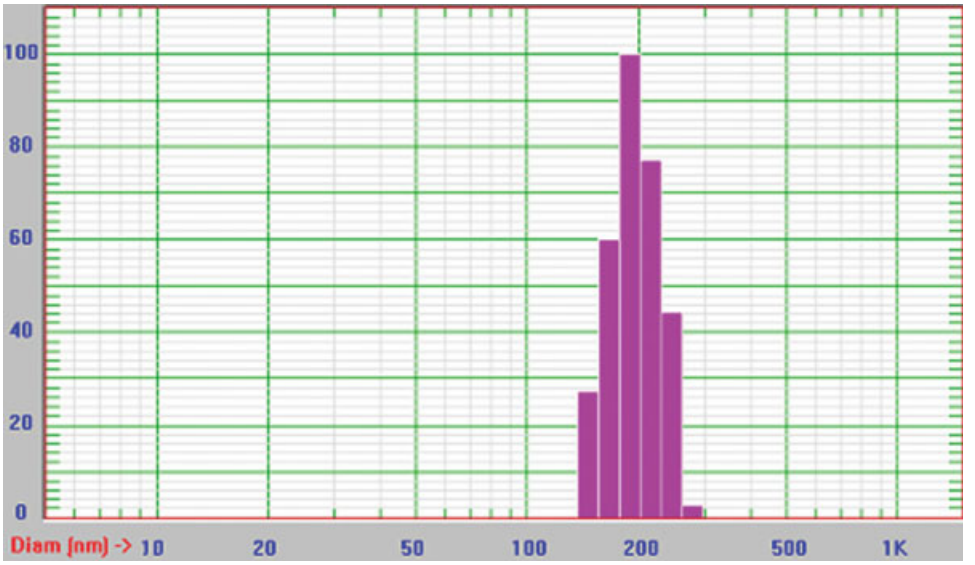


Figure 9 Particle size distribution of micelle in aqueous solution determined by DLS measurement. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

processed and no occurrence of backbone cleavage was found.

Thermal characteristics of pluronic and pluronic-*b*-PLL block copolymer were studied using a DSC. The DSC results are shown in Figure 8. There is only one confined peak at 63°C for pluronic, whereas a broad single peak at 52°C for pluronic-*b*-PLL block copolymer.

Characterization of micelle

The micelle-loaded anticancer PTX was prepared using the double emulsion method in this study. The size distribution of micelle was measured by DLS as shown in Figure 9. The mean diameter of micelle was 190.4 nm. Figure 10 shows the micelle observed by TEM, the result showed a well spherical morphology and the micelle size about 170 nm, slightly less than that determined by DLS. As well known to all, the micelle diameter determined by DLS represents its hydrodynamics diameter whereas that obtained by TEM is related to the collapsed micelle after water evaporation.[29]

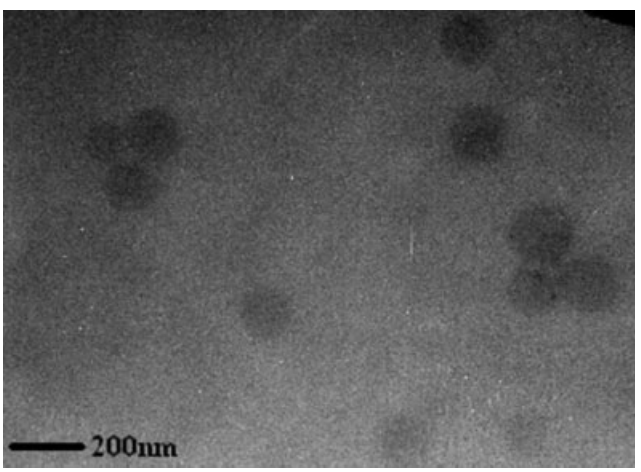


Figure 10 TEM micrograph of the micelle.

CONCLUSIONS

The novel block copolymer pluronic-*b*-PLL was successfully prepared through a relatively simple synthesis route in this study. First, the end-capping of pluronic with Boc-L-Phe was carried out by using DCC in the presence of DMAP; subsequently, the pluronic-NH_2 was obtained by the removal of the *tert*-butoxycarbonyl group; finally, pluronic-*b*-PLL was received through ROP of the NCA using amino-terminated pluronic and deprotected the N^{ε}-(carbonybenzoxy) end group from pluronic-*b*-poly(N^{ε}-(Z)-L-lysine) block copolymer in a mixed acid solution. The structure of the block copolymer and intermediates were supported by ^{1}H-NMR, FTIR, DSC, and GPC. The resultant copolymer could self-assemble into micelle-loaded hydrophobic anticancer agent PTX in an aqueous solution.

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