Synthesis and Characterization of Amphiphilic Pluronic (F68)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine Copolymers and Their Micelles as a Drug Carrier

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Received 24 April 2009; accepted 30 July 2009
DOI 10.1002/app.31231
Published online 22 March 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A new Pluronic (F68)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) (Pluronic (F68)–DPPE) copolymer was synthesized with Pluronic (F68) and DPPE. The chemical structure and physical properties of copolymers were determined by FTIR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and TGA. Environmental scanning electron microscopy, fluorescence spectroscopy, and dynamic light scattering method confirmed the formation of copolymeric micelles of Pluronic (F68)-DPPE. To estimate the feasibility as novel drug carriers, the copolymer micelles were prepared by the phase separation dialysis method. Amphotericin B as a lipophilic model drug was incorporated into copolymeric micelles and the drug release behavior was investigated. It was found that the chemical composition of the micelle was a key factor in controlling micelles size, drug-loading content, and drug release behavior. As DPPE segment weight ratio increased, the micelle size and drug-loading content increased, and the drug release rate decreased. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 604–613, 2010

Key words: Pluronic (F68); 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; copolymeric micelles; amphotericin B; drug delivery

INTRODUCTION

Amphiphilic copolymers consisting of hydrophilic and hydrophobic segments can form micelle structures with hydrophobic inner core and hydrophilic outer shell in aqueous media.[1–5] Polymeric micelles have received special attention because of their potential application and academic interest in many interdisciplinary fields.[6–8] These core-shell type micelles may be used as drug delivery vehicles for poorly water-soluble drugs, especially when the micelles are made with suitable biodegradable polymers.

As has been known, in aqueous media, certain polyethylene glycol/phosphatidylethanolamine (PEG-PE) conjugates form very stable micelles. The PEG-based corona makes these micelles long circulating, whereas the lipid hydrophobic core may be used as a cargo space for poorly soluble compounds, including many anti-cancer drugs.[9–11] The characteristic size, stability, and the longevity in the systemic circulation make PEG-PE micelles as a promising carrier for the delivery of drugs to the ill site via the enhanced permeability and retention effect.[12–15] But the PEG-PE copolymers are expensive, so this might obstruct its application.

Pluronics, water-soluble ABA triblock copolymers of poly ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO), are commercially available nonionic macromolecular surface active agents. They contain hydroxyl functional groups at the end of the chains.[16–19] PEO-PPO-PEO block copolymers are an important class of surfactants. Depending on the ratio of PEO to PPO and the molecular weight of block copolymer, Pluronics have been used for specialized applications such as in pharmaceutics for the solubilization and controlled release of drug. Illum et al. demonstrated that it was possible to alter significantly the *in vitro* interaction with isolated macrophages and the biodistribution of model polystyrene nanospheres after coating the particles surface with PEO-PPO-PEO block copolymers.[17,18] In addition, the presence of PEO on the surface of nanospheres reduced the extent of phagocytosis by mouse peritoneal macrophage cells *in vitro*. Furthermore, Pluronics series also have found interests because of their temperature-dependent

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Contract grant sponsor: Major Program for Fundamental Research of the Chinese Academy of Sciences, China; contract grant number: KJCX2-YW-M02.
Contract grant sponsor: State Key Development Program for Basic Research of China (973); contract grant number: 2009CB930200.

micellization and gel formation of aqueous PEO-PPO-PEO block copolymer solutions. Therefore, many studies have been conducted about the driving force or mechanism of the phenomena.[19] But the information of the micelles prepared by Pluronic-DPPE to deliver lipophilic drug has not been published.

The aim of the present work was to assess the merits of Pluronic (F68)-DPPE copolymeric micelles as drug carriers. For this purpose, the copolymer Pluronic (F68)-DPPE was synthesized with Pluronic (F68) and DPPE. The chemical structure and physical properties of the copolymers were characterized and the micellar formation of the copolymers was investigated. Finally, the lipophilic drug amphotericin B, first-line treatment for intraocular fungal infections,[20,21] was selected as a model drug to incorporate into the potential of the copolymeric micelles. The drug release behavior of the Pluronic (F68)-DPPE copolymeric micelles was investigated.

EXPERIMENTAL

Chemicals and materials

Pluronic (F68), 4-nitrophenyl chloroformate, 4-dimethylaminopyridine (DMAP), 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), triethylamine (TEA), and CL-4B Sepharose were purchased from Sigma–Aldrich and used without further purification. Amphotericin B was purchased from Shanghai Xinxianfeng Pharmaceutical. (Shanghai, China). All other reagents and solvents were of analytical grade.

Synthesis of Pluronic (F68)–DPPE copolymer

The activation of Pluronic (F68) using 4-nitrophenyl chloroformate (Pluronic (F68)-pNP) was performed as follows [Scheme 1(a)]: 2 g of Pluronic (F68) and 43 mg of DMAP were dissolved in DMSO pyridine solution (1 : 1, v/v) at 0°C with magnetic stirring. 4-Nitrophenyl chloroformate (3 g) was added to the solution and allowed to react at −10°C with magnetic stirring. After 8 h, the reaction solution was added to ethanol and the precipitate was collected and extensively washed with ethanol. The structure of Pluronic (F68)-pNP was also checked by Fourier transform infrared spectroscopy (FTIR). The activated Pluronic (F68) was 43% activated. The degree of carbonate substitution could be determined by analysis after alkaline hydrolysis.

The Pluronic (F68)-pNP-DPPE copolymer was synthesized as follows. DPPE was dissolved in chloroform to obtain a 50 mg/mL of solution. A mixture of Pluronic (F68)-pNP and DPPE (contain 0.5 mol TEA) (in the molar ratio of 3 : 1–15 : 1 (DPPE/Pluronic (F68)-pNP)) was suspended in 20 mL of chloroform and polymerized under magnetic stirring at room temperature under argon. After a further 12 h continuous stirring, the organic solvents were removed using a rotary evaporator. The Pluronic (F68)-pNP- DPPE copolymer was purified by RP-HPLC preparative column using methanol/0.01*M* HCl (70/30, v/v) as a mobile phase, and the mobile phase was removed using a vacuum evaporator. The Pluronic (F68)-pNP-DPPE copolymer was stored as a powder at −20°C.

To prepare Pluronic (F68)-DPPE copolymer [Scheme 1(b)] and remove the pNP group, the Pluronic (F68)-pNP-DPPE copolymer was added to Tris buffer (pH 9.0), then mixed and incubated overnight at 4°C under an argon atmosphere. The obtained Pluronic (F68)-DPPE copolymer was purified by the overnight dialysis against distilled water at 4°C using a dialysis bag (MWCO : 8000 g/mol), after which samples were freeze-dried. Pluronic (F68)-DPPE copolymer was identified by NMR and stored as a powder at −20°C.

Characterization of Pluronic (F68)-DPPE copolymer

The structure of Pluronic (F68)-pNP copolymer was confirmed by FTIR (FTIR, spectrum One, Perkin-Elmer, America). For FTIR analysis, the polymer samples were mixed with KBr and pressed to a plate for FTIR measurement.

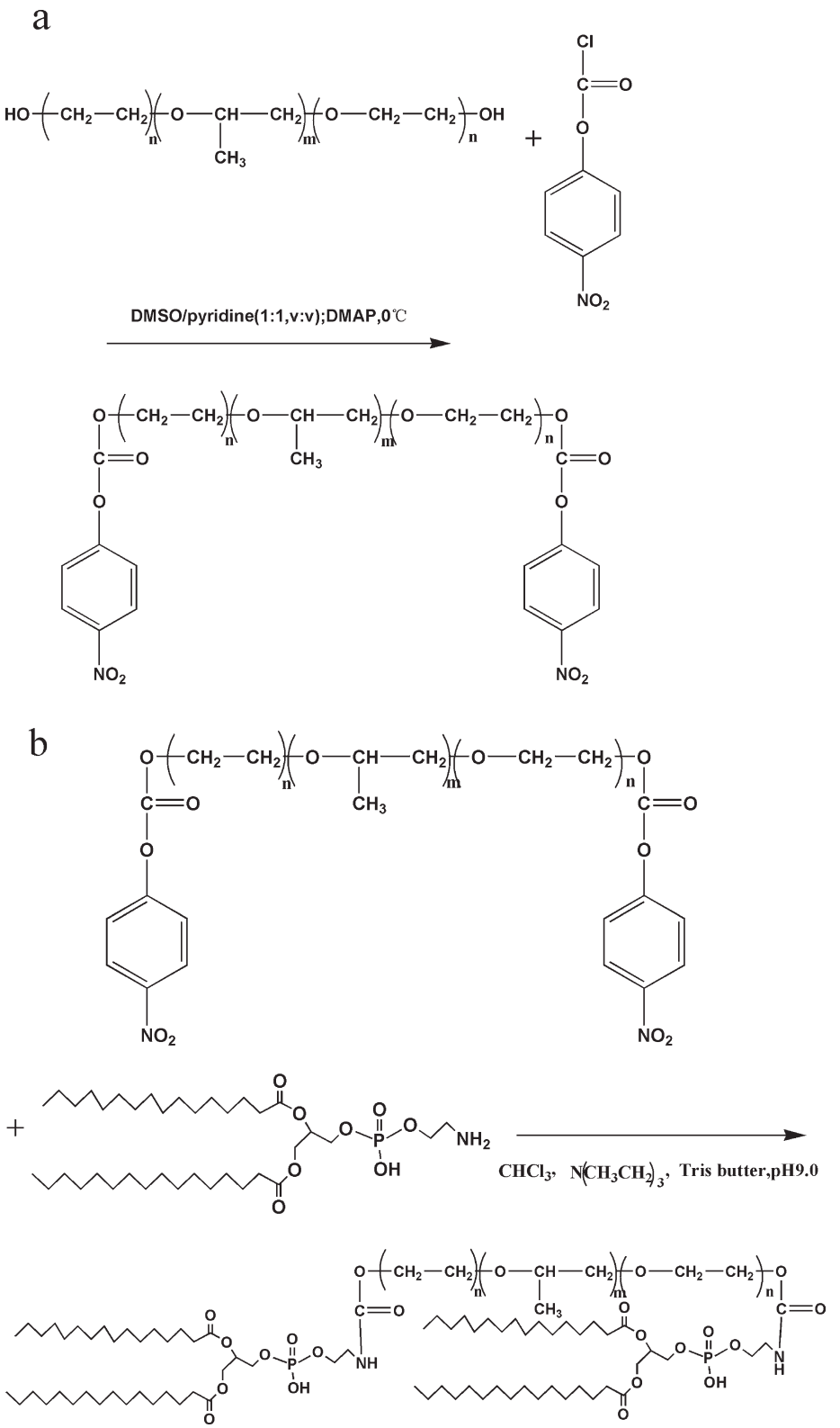
The spectra of ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR of Pluronic (F68) and Pluronic (F68)-DPPE copolymer was recorded on a (Bruker AVANCE 400) NMR spectrometer. Pluronic (F68) and Pluronic (F68)-DPPE copolymer were dissolved in $CDCl_3$.

Gel permeation chromatography (GPC) was performed on a Waters 2410 GPC apparatus (USA). Molecular weight and molecular weight distribution of the copolymer were calculated using polystyrene as the standard.

The thermal stability of Pluronic (F68)-DPPE samples was measured by TGA (Perkin-Elmer, USA). The temperature range was carried out from 25 to 900°C under nitrogen flow and the heating rate is 20°C /min.

Preparation of amphotericin B- loaded Pluronic (F68)-DPPE copolymeric micelle

The Pluronic (F68)-DPPE copolymeric micelle was prepared as reported previously with slight modification.[22] Amphotericin B and Pluronic (F68)-DPPE copolymer were dissolved in 5 mL of dimethyl sulfoxide (DMSO). This solution was slowly dropped into 20 mL of deionized water for 10 min and then stirred 5 min additionally to form copolymeric micelle. This micelle solution was introduced into dialysis tube (MWCO 8000 g/mol) and dialyzed against deionized water for 24 h to remove solvent. The deionized water was exchanged intervals of 1 h for



Scheme 1 Synthetic route of the copolymer Pluronic (F68)-DPPE: (a) synthetic route of the of Pluronic (F68)-pNP and (b) Pluronic (F68)-DPPE.

3 h and then exchanged 3 h intervals for 21 h. The dialyzed solution was filtered with 1.0 μm syringe filter to sterilize it and then lyophilized or analyzed.

Plain copolymeric micelle of Pluronic (F68)-DPPE copolymer was prepared by same procedure described earlier with the exception of amphotericin B.

TABLE I
Composition and Molecular Weight Distribution of Pluronic (F68)-DPPE[a]

Sample	Copolymer	Molecualr weight of copolymer M_w (kDa)	M_n (kDa)	Polydispersity (M_w/M_n)
1	Pluronic (F68)- DPPE(1/3)	12846	9806	1.31
2	Pluronic (F68)- DPPE(1/6)	13411	10316	1.30
3	Pluronic (F68)- DPPE (1/10)	15403	11669	1.32
4	Pluronic (F68)- DPPE (1/15)	18317	13772	1.33

[a] Measured by GPC.

Characterizations of the Pluronic (F68)-DPPE copolymeric micelle

ESEM (Environmental SEM, Quauta 200FEG, FEI) was used to observe the morphology of the micelle.

The size and distribution of copolymeric micelle were measured by dynamic light scattering (DLS) (Zetasizer Nano series ZEN 3600Malvern Instruments, England). All DLS measurements were carried out at an wavelength of 532 nm at 25°C with an angle detection of 90°.

Steady-state fluorescence spectra were recorded on a spectrofluorophotometer (FL-920 England). A solution of Pluronic (F68)-DPPE copolymer containing $6 \times 10^{-7}M$ of pyrene was placed in a square cell and the fluorescence spectrum was obtained with a fluorometer. The concentrations of sample solution were varied from 1.0×10^{-4} to 1.0 mg/mL. The excitation wavelength (λex) was 336 nm.

Measurement of drug content

For evaluation of drug content and loading efficiency, 5 mg of amphotericin B- loaded Pluronic (F68)-DPPE copolymeric micelle was dissolved in 10 mL of DMSO and diluted it with DMSO. Amphotericin B concentration was evaluated using an UV-spectrophotometer (Perkin Elmer Lambda850) at 415 nm. Empty copolymeric micelle of Pluronic (F68)-DPPE copolymer was used as a blank test. The amphotericin B encapsulation efficiency (EE) and the loading capacity (LC) of the micelle are as follows:

EE% = 100 wt % of amphotericin B in micelles/ weight of Amphotericin B initially.

LC% = 100 wt % of amphotericin B in micelles/ weight of micelles. Each sample was assayed in triplicate.

In vitro release

In *in vitro* release studies, 5 mg of lyophilized copolymeric micelle of Pluronic (F68)-DPPE copolymer was reconstituted into 5 mL of PBS and then introduced into dialysis tube (MWCO: 8000 g/mol). The dialysis tube was placed in 200 mL bottle with 95 mL of PBS and the media stirred at 100 rpm and 37°C. At specific time intervals, the medium was taken for the analysis of drug concentration. After that whole media was replaced with fresh PBS to prevent drug saturation. The medium was diluted 10–100 times with DMSO and the concentration of the amphotericin B released was determined using an UV-spectrophotometer (Perkin-Elmer Lambda850) at 415 nm. The properties of UV spectrum of amphotericin B in DMSO were not changed at the range between 0.1 and 10 μg/mL. Therefore, we

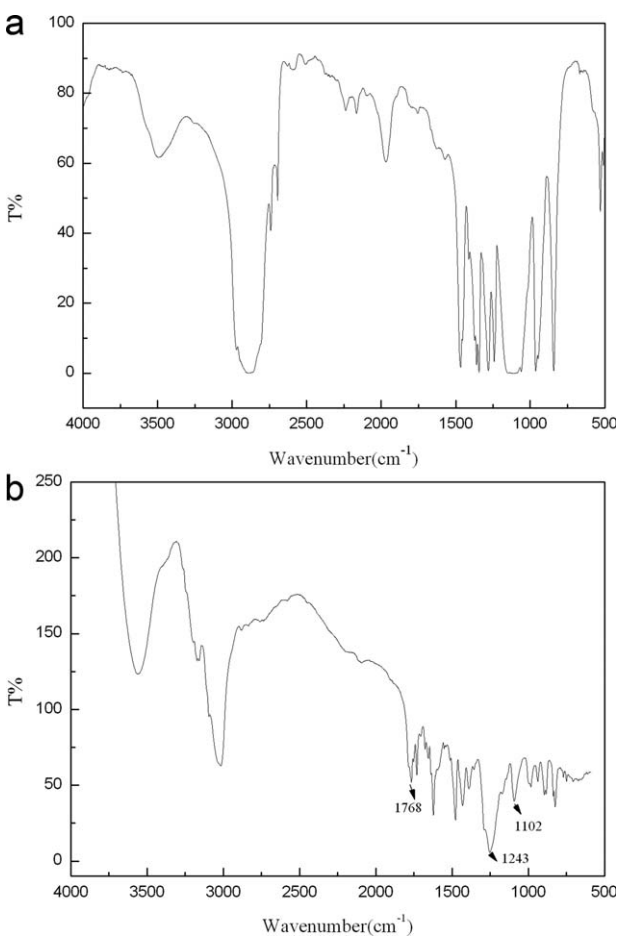


Figure 1 IR spectra of Pluronic (F68) (a) and Pluronic (F68)-pNP copolymer (b).

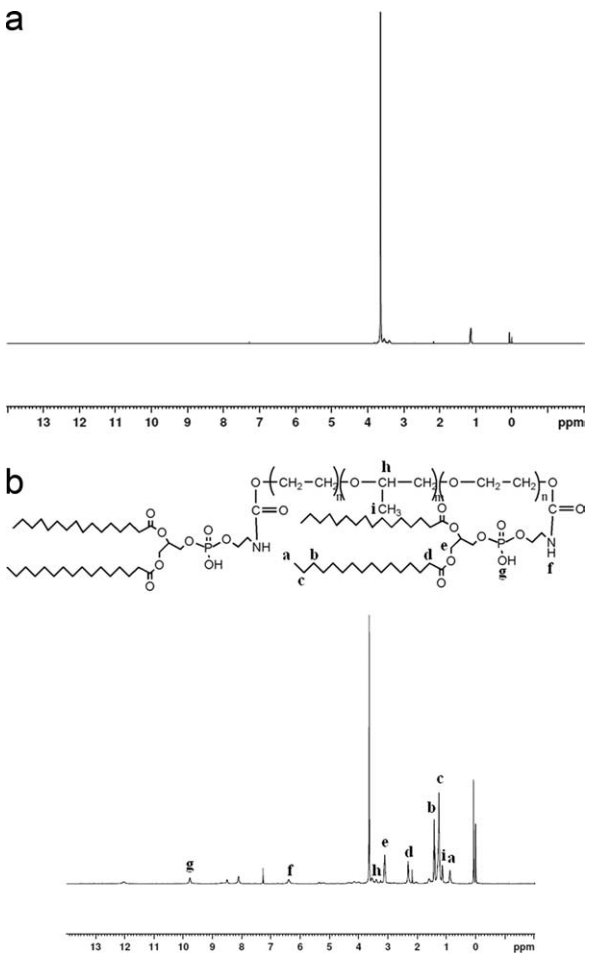


Figure 2 [1]HNMR spectrum of the Pluronic (F68) (a) and Pluronic (F68)-DPPE copolymer (b).

diluted the release medium with DMSO to this range for drug concentration estimation using UV-spectrophotometer.

RESULTS AND DISCUSSION

Synthesis and characterization of Pluronic (F68)-DPPE copolymer

Several activation methods for polymer contained hydroxyl have been considered: tosylation,[23,24] esterification using 4-nitrophenyl chloroformate,[25–27] carbonyl diimidazole,[28] cyanogen halide,[29] and so on. In our work, 4-nitrophenyl chloroformate was selected for the activation of hydroxyl groups of Pluronic (F68) in copolymer [Scheme 1(a)]. The degree of carbonate substitution could be determined easily by UV analysis after alkaline hydrolysis. The content of 4-nitrophenyl carbonate moieties was determined during the course of the reaction. The degree of 4-nitrophenyl carbonate substitution was controlled by adjusting the amount of chloroformate added. The degree of substitution initially increased to reach a maximum value at 8 h (43 %).

Pluronic (F68)-DPPE copolymer was synthesized. The polymerization route was shown in Scheme 1(b). The M_W of the copolymers was controlled by the molar feed ratio of the DPPE to Pluronic (F68)-pNP. The different samples named Pluronic (F68)-DPPE 1/3 (feed ratio of Pluronic (F68)-pNP with DPPE), Pluronic (F68)-DPPE 1/6, Pluronic (F68)-DPPE 1/10, and Pluronic (F68)-DPPE 1/15, respectively, were synthesized. The final products of Pluronic (F68)-DPPE have good solubility in $CHCl_3$, DMSO, and tetrahydrofuran.

The molecular weights and polydispersity index of the Pluronic (F68)-DPPE copolymers were shown in Table I. The amount of DPPE introduced to Pluronic (F68)-pNP increased with the molar ratio of DPPE to Pluronic (F68)-pNP. This indicated that higher the content of DPPE, higher the opportunity for the DPPE to react with Pluronic (F68)-pNP reactive center.

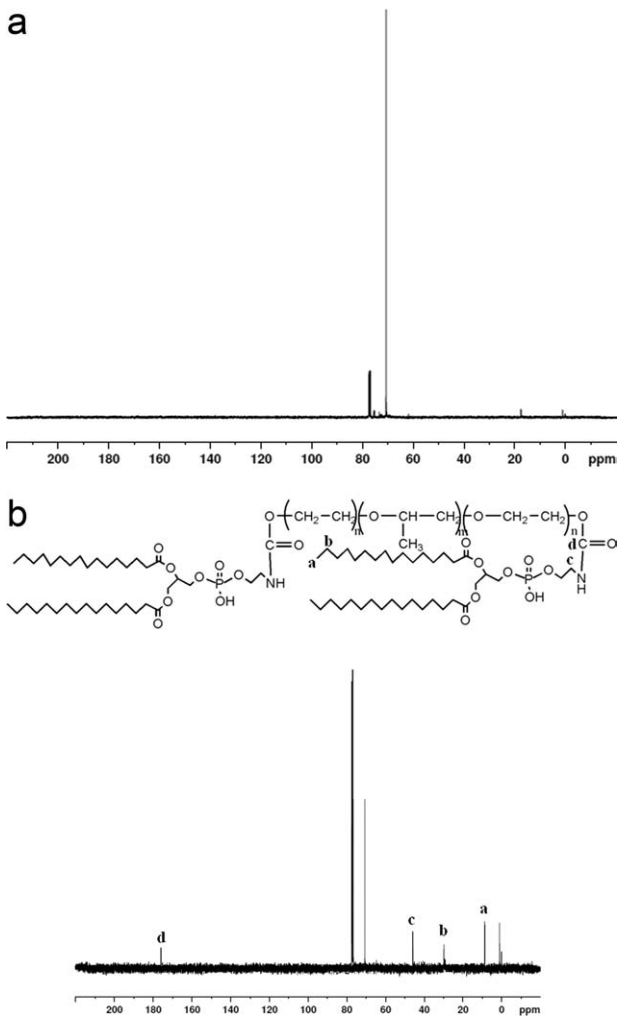


Figure 3 [13]C NMR spectrum of the Pluronic (F68) (a) and Pluronic (F68)-DPPE copolymer (b).

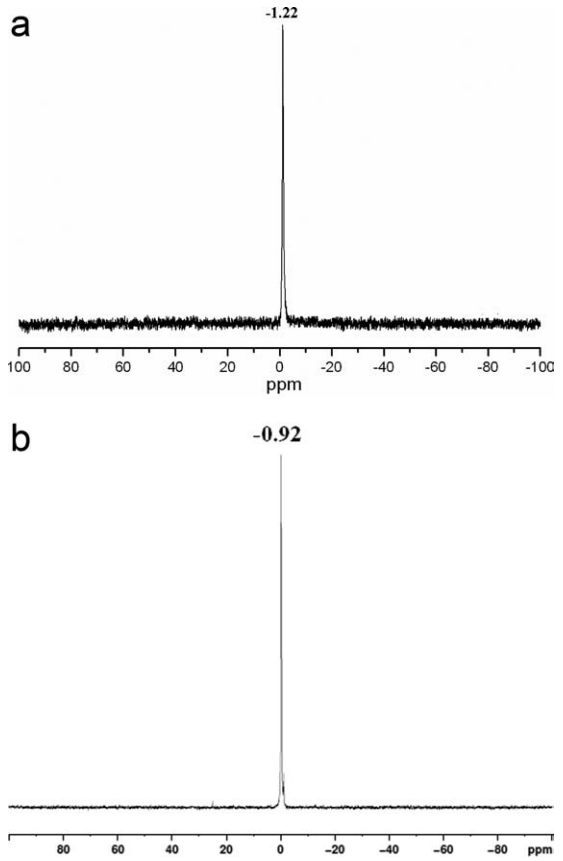


Figure 4 ^{31}P NMR spectrum of the DPPE (a) and Pluronic (F68)-DPPE copolymer (b).

Characterization of Pluronic (F68)-DPPE copolymer

FTIR and NMR were used to characterize the obtained copolymers. Figure 1(a,b) showed the FTIR spectrum of Pluronic (F68) and Pluronic (F68)-pNP copolymer. A new absorption band at 1768 cm^{-1} was attributed to 4-nitrophenyl carbonate [in Fig. 1(b)]. The absorption bands at 1102 cm^{-1} and 1243 cm^{-1} were attributed to the characteristic C—O—C stretching vibrations of repeated —OCH_2CH_2 units of Pluronic (F68) and the —COO— bands stretching vibrations, respectively.

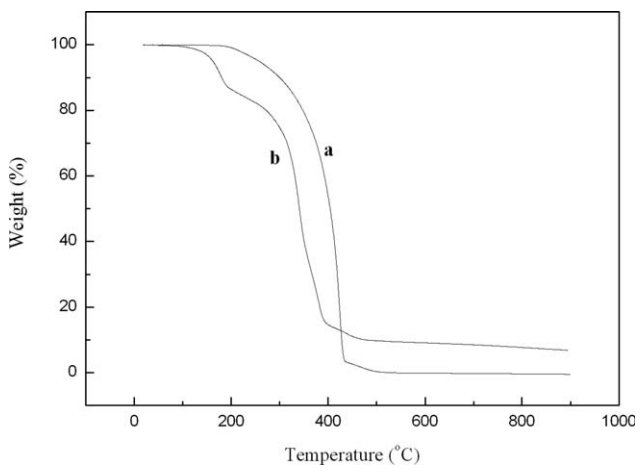


Figure 5 TGA graphs of Pluronic (F68) (a) and Pluronic (F68)-DPPE copolymer (b).

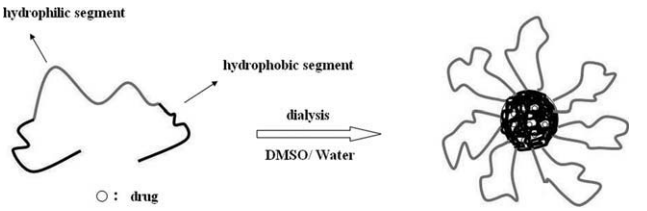


Figure 6 The schematic illustration of Pluronic (F68)-DPPE copolymeric micelle formation and drug loading. (Pluronic (F68) as a hydrophilic segment and DPPE as the hydrophobic segment in the Pluronic (F68)-DPPE copolymer).

Figure 2 showed the ^1H NMR spectrum of Pluronic (F68) and Pluronic (F68)-DPPE copolymer. The characteristic absorption peaks were also indicated in the figure. Compared with Pluronic (F68) [Fig. 2(a)], the ^1H NMR spectra of the Pluronic (F68)-DPPE copolymer [Fig. 2(b)] showed that the signal at $\sim$ 0.9 ppm was attributed to the terminal methyl proton of the DPPE moiety. Peaks at 2.1–2.3 ppm were attributed to the methenyl protons (connected with —COO— group) of the DPPE moiety. The signals at $\sim$ 1.2, 1.6, 2.3, and 3.1 ppm were attributed to the methenyl protons of —CH_2—, —$OOCCH_2$—, —CH_2OOC— in DPPE moiety, respectively. The ^1H NMR spectra of the Pluronic (F68)-DPPE copolymer [Fig. 2(b)] showed that signal at $\sim$ 5.1 ppm was assigned to the proton of —NH_2 group in the DPPE moiety. The peak at $\sim$ 9.8 ppm was attributed to the protons of the phosphate group in the DPPE moiety. The sharp peak at $\sim$ 3.65 ppm was attributed to methylene protons units of PEO

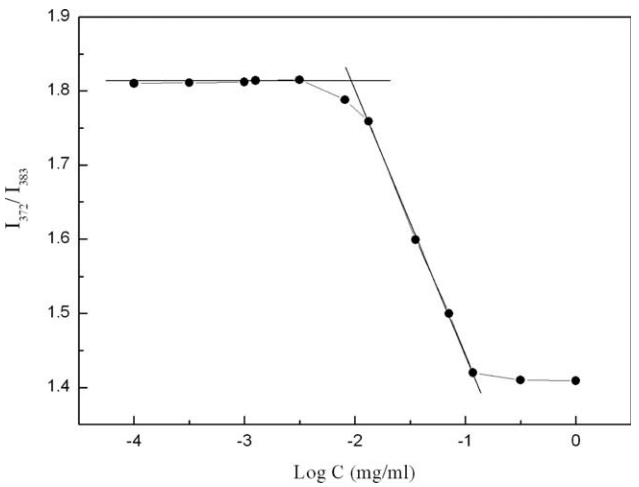


Figure 7 Change of the intensity ratio (I_{372}/I_{383}) versus the concentration of the Pluronic (F68)-DPPE (1/10) copolymer at 20°C.

TABLE II
Effect of Different Composition of Copolymer on the Properties of Polymeric Micelles and Amphotericin B-Loaded Polymeric Micelles[a]

Sample	Copolymer	Mean diameter (nm)	Polydispersity	CMC × 10^2 (mg/mL)
1	Pluronic (F68)-DPPE (1/3)	76	0.05	5.11
2	Pluronic (F68)-DPPE (1/6)	84	0.06	4.36
3	Pluronic (F68)-DPPE (1/10)	106	0.07	1.92
4	Pluronic (F68)-DPPE (1/15)	123	0.05	0.88
AmNP3	Pluronic (F68)-DPPE (1/3)	91	0.06	
AmNP6	Pluronic (F68)-DPPE (1/6)	105	0.05	
AmNP10	Pluronic (F68)-DPPE (1/10)	126	0.08	
AmNP15	Pluronic (F68)-DPPE (1/15)	143	0.07	

[a] Amphotericin B content 10% (w/w).

block in Pluronic (F68) moiety in Pluronic (F68)-DPPE copolymer. The signal at ~ 1.13 ppm belongs to the —CH_3— protons in the PPO block in Pluronic (F68) moiety in Pluronic (F68)-DPPE copolymer. Each characteristic peak at ~ 3.4 ppm was attributed to the —CH and —CH_2 units of PPO block in Pluronic (F68) moiety in Pluronic (F68)-DPPE copolymer.

To further confirm the formation of Pluronic (F68)-DPPE copolymer, ^{13}C-NMR spectrum was also recorded and shown in Figure 3. Compared with Pluronic (F68) [Fig. 3(a)], the ^{13}C-NMR spectra of the Pluronic (F68)-DPPE copolymer [Fig. 3(b)] showed that the peak at ~ 14 ppm was attributed to the —CH_3 group carbon peak of the DPPE moiety located at the terminal groups. The signal at ~ 30 ppm was assigned to —CH_2 group carbon peak of the DPPE moiety. The signals at 170–175 ppm were assigned to —COO group carbon peak of the DPPE moiety. These results evidenced that the copolymer contained DPPE side chains.

Furthermore the typical ^{31}P-NMR spectra of DPPE and Pluronic (F68)-DPPE copolymer were recorded and shown in Figure 4. Compared with DPPE [Fig. 4(a)], the ^{31}P-NMR spectra of the Pluronic (F68)-DPPE copolymer [Fig. 4(b)] showed that the peak at ~ 0.92 ppm was generally expected for ^{31}P functionalities.[30–32] The ^{31}P-NMR spectra confirmed that phosphate groups were chemically bonded to the material.

Thermal properties

TG curves of Pluronic (F68) and Pluronic (F68)-DPPE copolymer were shown in Figure 5 (a,b). It could be seen that all copolymer samples exhibited a weight loss during the heating process. Compared to Pluronic (F68) [Fig. 5(a)], Pluronic (F68)-DPPE copolymer [Fig. 5(b)] has lower thermal degradation temperature. A fast process of weight loss appears in the TG curves response for the copolymer in thermal degradation ranges. The thermo decomposed rate increased with increase of the rate of DPPE in the copolymers. These results indicated that the thermal stability of the copolymer was decreased with increase of DPPE chains.

Characterization of copolymeric micelle

It is well known that amphiphilic copolymers with a suitable hydrophilic/hydrophobic balance can form a micellar structure when exposed to a selective solvent. Pluronic (F68) is an amphiphilic block copolymer and can form a micelle. But the critical micelle concentration (CMC) of Pluronic (F68) is higher. The hydrophobic property of PPO segment of Pluronic (F68) is not strong, so it is difficult for encapsulation of lipophilic drug into Pluronic (F68) micelle. For

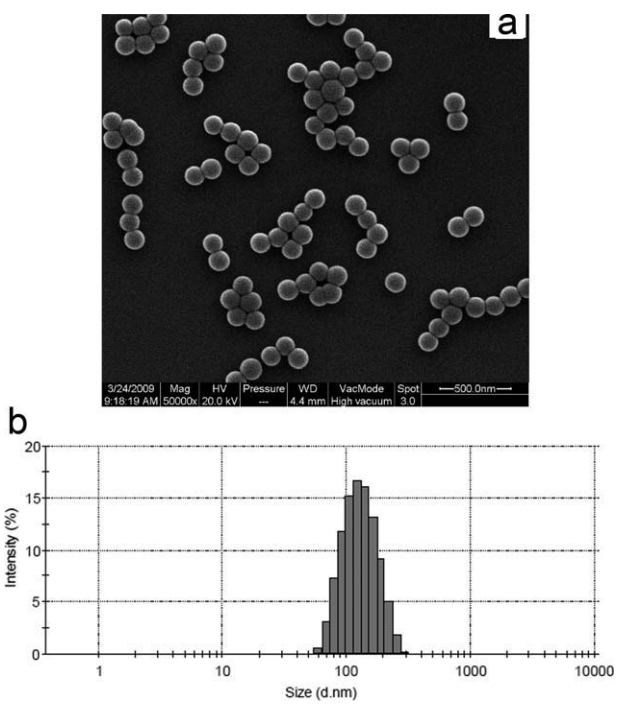


Figure 8 ESEM images (a) and the size distribution (b) of amphotericin B-loaded Pluronic (F68)-DPPE copolymeric micelle in water (Pluronic (F68)-DPPE (1/10)).

TABLE III
Drug Loading Efficiency, Drug Entrapment Efficiency and Micelle Yield of amphotericin B-Loaded Copolymeric Micelles[a]

Sample	Copolymer	Entrapment efficiency (%)	Drug loading (%)	Micelle yield (%)
1	Pluronic (F68)-DPPE (1/3)	63.1	5.4	54.1
2	Pluronic (F68)-DPPE (1/6)	69.6	7.6	57.3
3	Pluronic (F68)-DPPE (1/10)	75.3	8.7	70.2
4	Pluronic (F68)-DPPE (1/15)	84.7	11.2	86.7

[a] The mass of amphotericin B used was 20% (w/w) in relation to polymer mass.

improvement of the hydrophobic property of Pluronic (F68) and for better encapsulation of lipophilic drug, the objective of this study was to prepare amphiphilic block copolymers composed of Pluronic (F68), ABA triblock copolymers of PEO–PPO–PEO, as a hydrophilic segment, and DPPE, as the hydrophobic segment. The Pluronic (F68)-DPPE copolymer, consisting of hydrophilic Pluronic (F68) and hydrophobic DPPE segments, provided an opportunity to form micelle in water (Fig. 6). The micelle behavior of Pluronic (F68)-DPPE copolymer in aqueous media was monitored by fluorometry in the presence of pyrene as a fluorescence probe. In studying the formation of micelle from hydrophobically modified copolymer in aqueous solution, pyrene is generally used as a molecular probe, and the variation in the ratio of intensity of first (372 nm) to third (383 nm) vibronic peaks I_{372}/I_{383}, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where pyrene is located. The change of the intensity ratio (I_{372}/I_{383}) was shown in Figure 7. For Pluronic (F68)-DPPE copolymer, at lower concentration, I_{372}/I_{383} values remain nearly unchanged. Further increasing concentration, the intensity ratio start to decrease, implying the onset of micelle from Pluronic (F68)-DPPE copolymer. The CMC was determined by the interception of two straight lines.

The CMC values of Pluronic (F68)-DPPE copolymer were listed in Table II. From the Table II, it could be seen that the CMC values of copolymer are lower than the CMC of low molecular weight surfactants, indicating the stability of micelles from Pluronic (F68)-DPPE copolymer at dilute conditions. The increasing hydrophobicity by introduction of a large amount of hydrophobic groups further reduces the CMC values (Table II). These results indicated that the longer the hydrophobic segment, the easier the forming of micelle-like nanoparticles, which was in a good agreement with those by other researches.[33,34]

To characterize the morphology and size distribution of the amphotericin B-loaded copolymeric micelles, ESEM and DLS measurement were carried out. Figure 8 (a,b) showed the photographs and size distribution of the amphotericin B-loaded copolymeric micelle. It could be seen that copolymeric micelles had a regular spherical shape and narrow size distribution.

The size and its size distribution of copolymeric micelles and amphotericin B-loaded copolymeric micelles (AmNP3, AmNP6, AmNP 10, AmNP15) were measured by DLS (Table II). The size of these plain copolymeric micelles was 76–123 nm in water. For these micelles, the preparation conditions are same, so the chemical composition of the micelles might be the main reason for difference of the mean diameter. According to the formation mechanism of micelles, the mean diameter of micelles was determined by the hydrophobic property of the core. The DLS data demonstrate that the micelle sizes get larger as the DPPE molar ratio increase, suggesting the elongation of hydrophobic DPPE side chain facilitated the growth of the hydrophobic core of copolymeric micelles. These results indicated that the micelle size was dependent on the ratio of hydrophobic DPPE segment to hydrophilic Pluronic (F68) segment in the chain. This result was also in agreement with the characteristic of amphiphilic copolymer micelles that fewer the hydrophobic

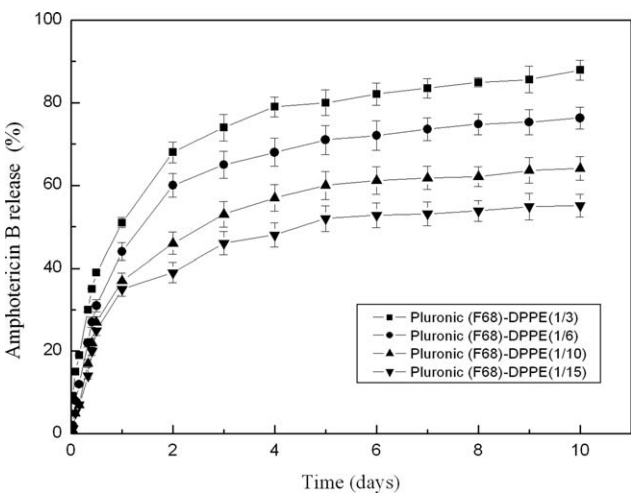


Figure 9 Release profiles of amphotericin B from Pluronic (F68)-DPPE copolymeric micelles.

component, the smaller the micelles.[33] The amphotericin B-loaded copolymeric micelles showed a larger size than the plain copolymeric micelles (Table II). It suggested that amphotericin B was incorporated into the copolymeric micelles effectively.

Drug content studies

Table III demonstrated that the entrapment efficiency and drug loading of Pluronic (F68)-DPPE copolymeric micelles. The entrapment efficiency and drug loading depended mainly on the copolymer composition ratio of DPPE to Pluronic (F68). The drug-loading content in copolymeric micelles increased from 5.4 to 11.2% with increasing the molar ratio of DPPE/Pluronic (F68). This result could be explained by the amphotericin B having a hydrophobic character. Therefore, the higher the DPPE content in copolymer, the more easily the drug was entrapped in copolymeric micelles. It suggested that the elongation of hydrophobic DPPE side chain facilitated the compatible of the hydrophobic core of copolymeric micelles and hydrophobic drug amphotericin B.

In vitro release

Figure 9 showed release profiles of amphotericin B from Pluronic (F68)-DPPE copolymeric micelles with various DPPE/Pluronic (F68) ratios. For all copolymeric micelles, amphotericin B release both showed an initial burst release and after amphotericin B release profiles displayed a sustained fashion. In an initial burst release, a significant amount of amphotericin B was release within 12 h, 31.56% for Pluronic (F68)-DPPE copolymeric micelles. After the initial burst, Pluronic (F68)-DPPE release profiles displayed a sustained release. The amount of cumulated Pluronic (F68)-DPPE release over 10 days was 76.5% for Pluronic (F68)-DPPE copolymeric micelles (amphotericin B content 20%). This sustained release could result from diffusion of amphotericin B into the polymer wall and the drug through polymer wall as well as the erosion of the polymers. The release of a drug from the copolymeric micelles is rather complicated process. It can be affected by many factors such as the polymer degradation, molecular weight, crystallinity, the binding affinity between the polymer and the drug, and so on. In this study, the drug release rate might be mainly determined by the diffusion of the drug through the polymer matrix. The initial burst might be attributed to the rapid release of drugs in the microchannels probably existing in micelles. Amphotericin B, because of its lipophilic character, was physically entrapped in the hydrophobic core of a micelle. Accordingly, the *in vitro* release behaviors of a lipophilic compound from these copolymeric micellar systems were largely affected by its inner core with hydrophobic properties.[34] Therefore, as the DPPE content of the copolymer increased, the hydrophobic segment in the copolymer increased resulting in the increase of the binding affinity between amphotericin B and hydrophobic DPPE side chain. Consequently, in this copolymeric micelle system, the drug release rate was inversely proportional to the hydrophobic DPPE content of the copolymer. Another reason for the fast release rate of Pluronic (F68)-DPPE copolymeric micelle was their small particle size with relatively high surface area.

CONCLUSIONS

Amphiphilic Pluronic (F68)-DPPE copolymers that can form the copolymeric micelles were prepared. The copolymers with controlled structure were obtained by adjusting the molar ratio of DPPE to Pluronic (F68) unit. The amphotericin B- loaded copolymeric micelles were prepared by the phase separation dialysis method. The preliminary investigations for the novel copolymeric micelle system have shown that the composition of the copolymer made a large influence on the micelle size, size distribution, and drug release behavior. Control of the micelle size, drug-loading content, and drug release behavior could be achieved by optimizing the DPPE to Pluronic (F68) ratio of the copolymer. These results showed that Pluronic (F68)-DPPE copolymeric micelles could be promising as a new drug delivery system for lipophilic drug.

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