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Short communication

Preparation of dextran–poly(lactide)–1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine copolymer and its micellar characteristics

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ABSTRACT

A novel amphiphilic copolymer based on dextran, poly(lactide) (PLA) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) was synthesized and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra. Their molecular weights were determined by gel permeation chromatography (GPC). The molecular weights range from 30 000 g mol^{-1} to 37 000 g mol^{-1} with a feed weight ratio of 20:1–50:1 (DPPE/activated dextran–PLA). Its micellar characteristics were investigated using fluorescence technique, transmission electron microscopy (TEM) and dynamic light scattering (DLS). It was found that dextran–PLA–DPPE copolymer could form spherical micelles. The micelle diameters ranged from 85 to 120 nm with a feed weight ratio of 20:1–50:1 (DPPE/activated dextran–PLA) in the absence of surfactant.

1. Introduction

Amphiphilic copolymers consisting of hydrophilic and hydrophobic segments can form micelle structure (Kaminski et al., 2008; Kato et al., 2006; Lee, Kim, Youn, & Bae, 2007). Moreover, through adjusting the structure of the copolymers, the size of the micelles can be easily controlled. In addition, micelles are more stable than surfactant. Thus, these micelles may be used as drug delivery vehicles, especially when the micelles are made with biodegradable polymers.

PLA is a kind of biodegradable materials with low toxicity, excellent biocompatibility and bioabsorbability in vivo (Baras, Benoit, & Gillard, 2000; Perez et al., 2001). Certain polyethylene glycol/phosphatidylethanolamine (PEG–PE) conjugates, as DPPE, may form very stable micelle in aqueous medium (Gao, Lukyanov, Anurag Singhal, Vladimir, & Torchilin, 2002; Lukyanov, Elbayoumi, Chakilam, & Torchilin, 2004). However, the low hydrophilicity and high crystallinity of PLA result in lower soft tissue compatibility (Suh, Hwang, Lee, Han, & Park, 2001). One drawback of these PEG-based copolymers is the absence of reactive groups at their molecular chains, which limits further modification or bind coupling. In contrast, dextran (Cai, Yang, Bei, & Wang, 2002; Ouchi, Saito, Kontani, & Ohya, 2004; Ouchi, Kontani, & Ohya, 2003) with good hydrophilicity, biocompatibility and biodegradability seems to be an attractive alternative to PEG hydrophilic segments for designing amphiphilic copolymers.

In this short communication, we reported the first synthesis of a novel copolymer based on dextran, PLA and DPPE and its micellar characteristics.

2. Experimental

2.1. Materials

Dextran (1500 g mol^{-1}), DL-lactide, 4-nitrophenyl chloroformate, and 4-dimethylaminopyridine (DMAP) were purchased (Alfar Aesar, a Johnson Matthey Co., Ward Hill, MA, USA). 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) and triethylamine were purchased (Sigma–Aldrich Chemical Co., St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Synthesis of dextran-PLA

The dextran–PLA (Scheme 1(a)) was synthesized by one-step method (Wu et al., 2005) with some changes. Briefly, 10 g of DLLA was added to 500 mg of dextran/DMSO solution by stirring and

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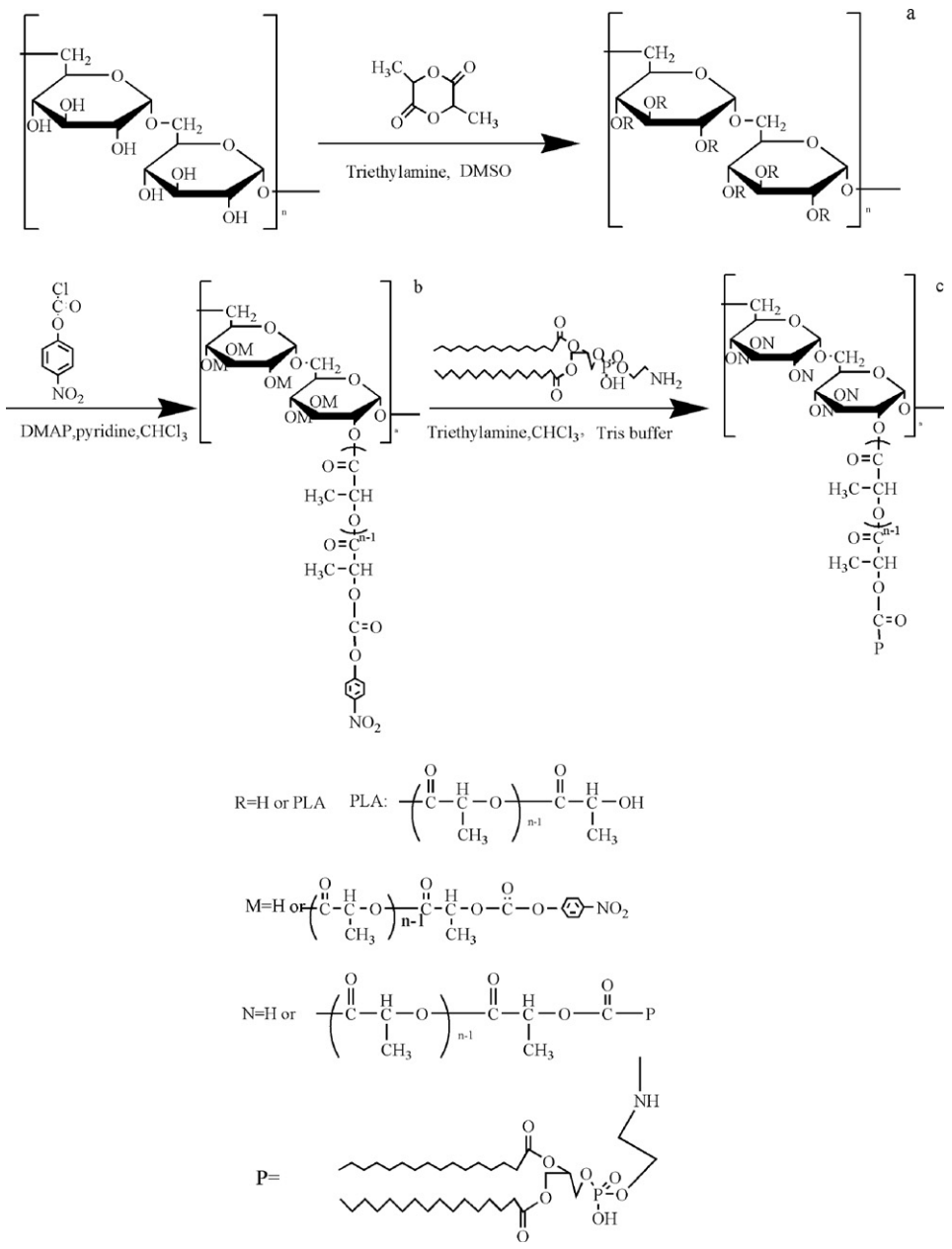

Scheme 1. Synthetic route for (a) dextran–PLA (b) dextran–PLA–pNP, and (c) dextran–PLA–DPPE.

then 0.5 mol triethylamine was added dropwise. The solution was reacted at 86 °C by magnetic stirring in N_2 atmosphere. After 12 h, the reacted solution was added to ice-water and the precipitate collected and thoroughly washed with distilled water. Finally, the obtained not purified dextran–PLA product was extracted using toluene.

2.3. Activated dextran-PLA

The activation of dextran–PLA (Scheme 1(b)) was performed according to Wang, Chui, and Ho (2009) with a few modifications. 1.1 g dextran–PLA were dissolved in 6 mL of chloroform by stirring and then 0.5 g of 4-nitrophenyl chloroformate (pNP), 40 mg of DMAP (dissolved in 6 mL of chloroform) and 1 mL pyridine was added to the solution. This reagent was allowed at 0 °C for 6 h and at room temperature for 12 h by magnetic stirring. The resulting product dextran–PLA–pNP was added to ether/petroleum ether (2:1, v/v) and the precipitate collected and washed for three times.

2.4. Synthesis of dextran-PLA-DPPE

The activated dextran–PLA/DPPE was synthesized as follows. A mixture of activated dextran–PLA and DPPE (contain 0.1 mol triethylamine) with a feed ratio of 20:1–50:1 (w/w, DPPE/dextran–PLA–pNP)) was suspended in 20 mL of chloroform with a magnetic stirrer at room temperature in the absence of light. After being stirred continuously for 12 h, the resulting product was added to ether/petroleum ether (2:1, v/v) and the precipitate collected and washed for three times. The dextran–PLA–pNP–DPPE was dried at 25 °C, 0.1 MPa under vacuum (DZF-6050 vacuum oven, Beifang Co., Beijing, China) for 48 h.

To prepare dextran–PLA–DPPE copolymer (Scheme 1(c)) and remove the *p*-nitrophenyl carbonate group, the dextran–PLA–pNP–DPPE above was added to Tris buffer (pH 8.5), then mixed and incubated overnight at 4 °C under N_2 atmosphere. The obtained dextran–PLA–DPPE copolymer was purified through the dialysis against distilled water at 4 °C using a dialysis

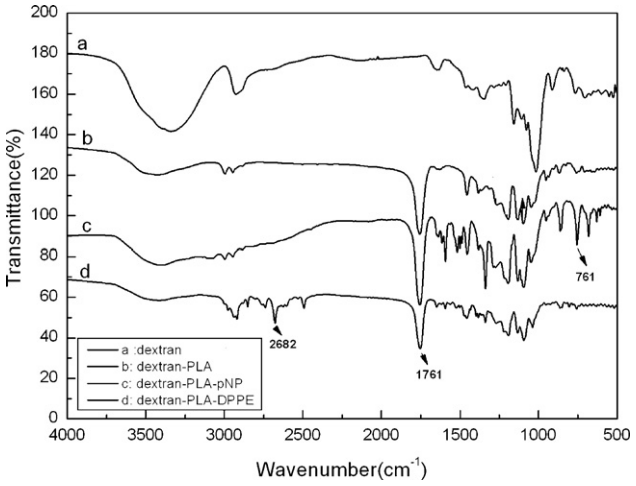

Fig. 1. FT-IR spectra of dextran (a), dextran–PLA (b), dextran–PLA–PNP (c) and dextran–PLA–DPPE (d).

bag (Sigma–Aldrich Chemical Co., St. Louis, MO, USA, molecular-weight cutoff (MWCO), 3500 g mol^{-1}) for 24 h, after which samples were freeze-dried and stored as a powder at −20 °C.

2.5. Preparation of copolymer micelle

The copolymer micelle was prepared by nanoprecipitation method (Govender, Stolnik, Garnett, Illum, & Davis, 1999). 6 mL of the polymer in acetone (10 mg mL^{-1}) was added into 12 mL of distilled water under magnetic stirring for 30 min. The acetone was eliminated use a rotary evaporator and the micelle were obtained.

2.6. Analysis

The structure of dextran and its copolymers was recorded using Fourier-transform infrared (FT-IR) spectrometer (spectrum one, Perkin Elmer Instruments, Waltham, MA, USA). Dextran and its copolymers were mixed with KBr pressed to a plate for measurement.

The NMR spectra were recorded with a NMR spectrometer (Bruker Avance 400, Bruker Corporation, Switzerland) and $CDCl_3$ or DMSO-*d6* as the solvent. The GPC measurement was performed with a Waters 515-410 gel permeation chromatograph. Micellar sizes and size distribution were determined through dynamic light scattering (DLS) (Zetasizer Nano series ZEN 3600 analyzer, Malvern Instruments Ltd., England). The morphological examination of micellar was performed using a transmission electron microscope (TEM, FEI, Tecnai G2 20 S-TWIN). Samples were stained with uranyl acetate before measurements. Steady-state fluorescence spectra were recorded on a spectrofluorophotometer (Perkin Elmer Instruments LS-55, Perkin Elmer Instruments, Waltham, MA, USA). Excitation spectra were monitored at 335 nm. The slit widths for both excitation and emission sides were maintained at 5 nm.

3. Results and discussion

Fig. 1(a–d) showed the FT-IR spectrum of dextran, dextran–PLA, dextran–PLA–pNP, and dextran–PLA–DPPE, respectively. In Fig. 1(b), the peak at ~1761 cm^{-1} was attributed to carbonyl group of the polylactide. In Fig. 1(c), the peak around ~1761 cm^{-1}was attributed to 4-nitrophenyl carbonates (overlapped with carbonyl group peak). The absorption peak at ~761 cm^{-1} indicated phenyl group existing. In Fig. 1(d), the peak at ~2682 cm^{-1} was attributed to P–OH stretching vibration.

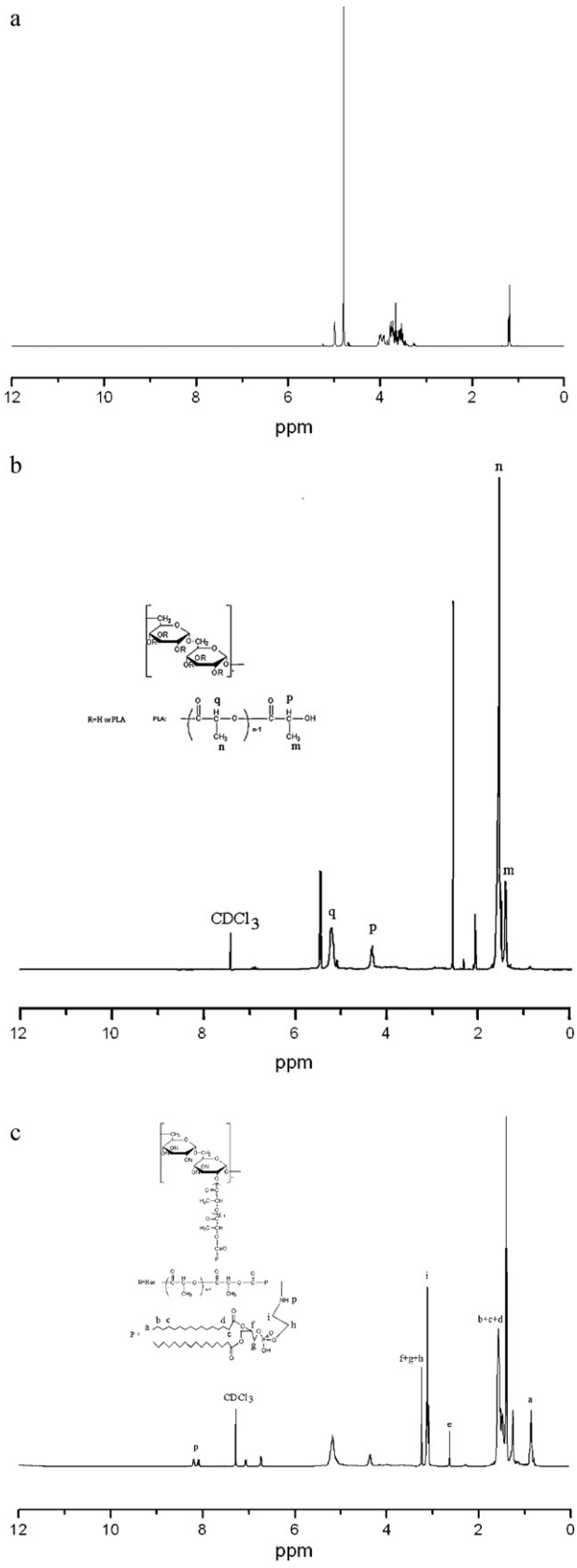

Fig. 2. ^{1}H NMR spectra of dextran (a), dextran–PLA (b), and dextran–PLA–DPPE(c).

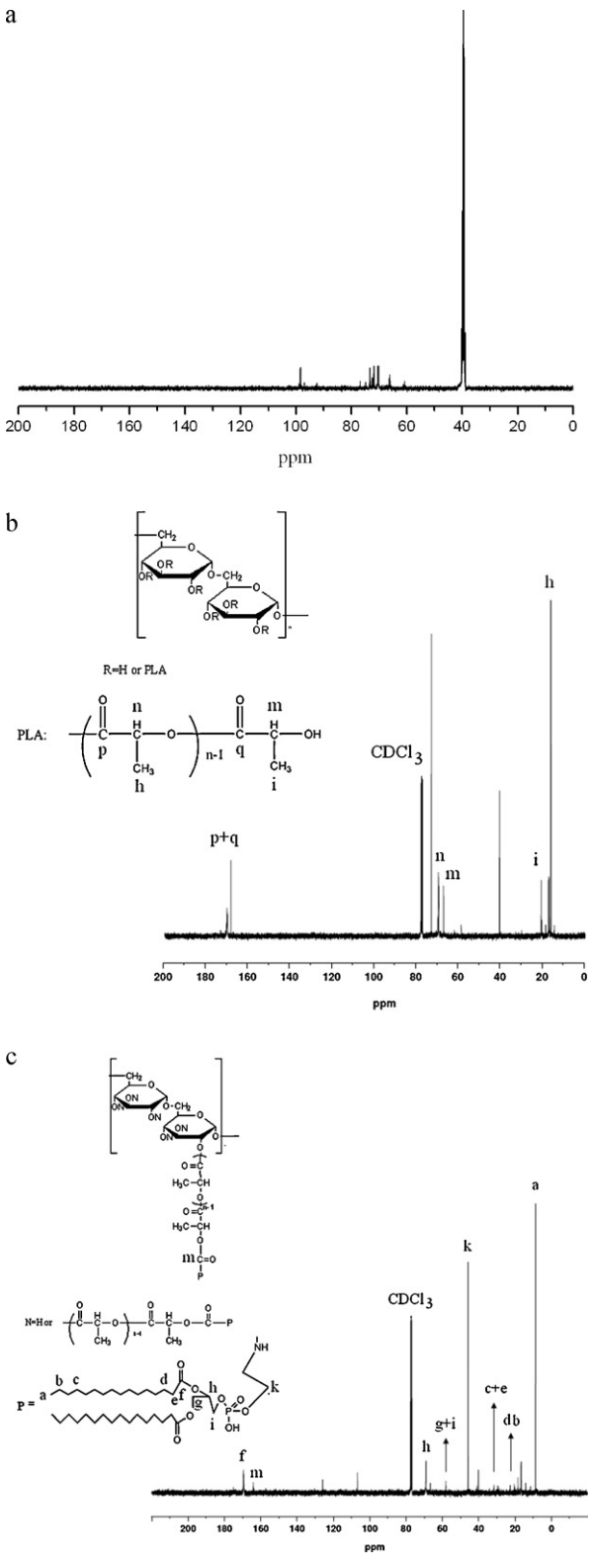

Fig. 3. ^{13}C NMR spectra of dextran (a), dextran–PLA (b), and dextran–PLA–DPPE (c).

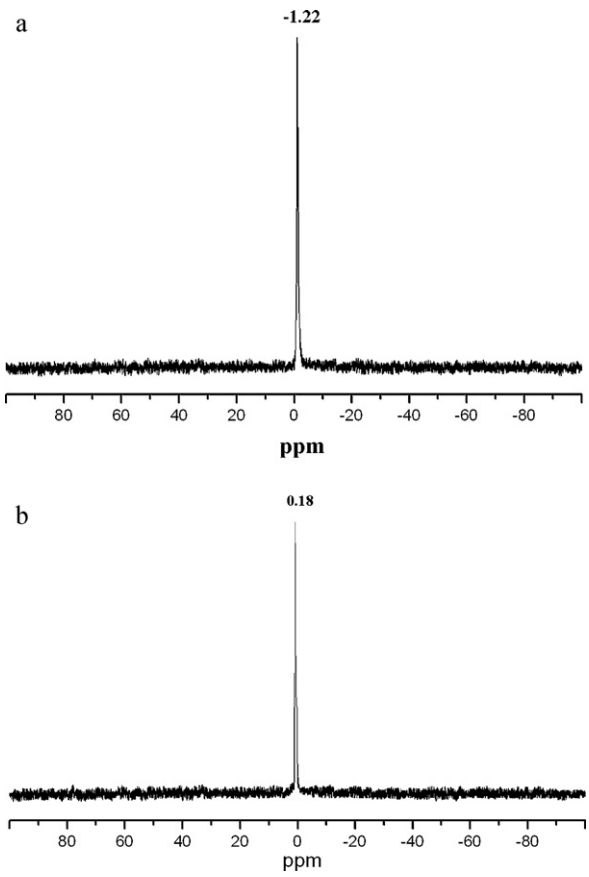

Fig. 4. ^{31}P NMR spectrum of the DPPE (a) and dextran–PLA–DPPE copolymer (b).

Fig. 2(a–c) showed the ^{1}H NMR spectrum of dextran, dextran-PLA, and dextran–PLA–DPPE copolymer, respectively. The signals at 3.4–3.9 and 4.8 ppm were attributed to the protons in methylene groups and other methine groups of dextran (Fig. 2(a)) (Shi & Zhang, 2006). In Fig. 2(b), the signals at ~4.2 and 5.1 ppm were assigned to terminal methenyl protons of branched PLA and repeat units of it in the chain, respectively. The signals at ~1.2 and 1.4 ppm were attributed to methyl protons of PLA moiety located at terminal groups and backbones (Wu et al., 2005). In Fig. 2(c), the signal at ~0.9 ppm was attributed to terminal methyl proton of DPPE moiety. The signal at ~8.3–8.7 ppm was assigned to the proton of –NH in the DPPE moiety. All other absorption peaks are attributed to protons of DPPE moiety (Percot et al., 2004).

Fig. 3(a–c) showed the ^{13}C NMR spectrum of dextran, dextran–PLA, and dextran–PLA–DPPE copolymer, respectively. Fig. 3(a) showed the ^{13}C NMR spectra of dextran. It could be seen that the C-1 glucose ring carbon absorbed at ~98, C-2 at ~72, C-3 at ~74, C-4 at ~70, C-5 at ~71 and C-6 at ~66 ppm (Ramirez, Sanchez-Chaves, & Arranz, 1994). In Fig. 3(b), the signal at ~170 ppm was attributed to C=O carbon peak of polylactide. In Fig. 3(c), the peak at ~9.0 ppm was attributed to $-CH_3$ group carbon peak of DPPE moiety located at the terminal group. The signals at ~165 and ~175 ppm were assigned to C=O carbon peak of dextran–PLA–DPPE.

Furthermore the typical ^{31}P NMR spectra of DPPE and dextran–PLA–DPPE copolymer were shown in Fig. 4(a and b). In Fig. 4(a), the signal at −1.22 ppm was attributed to the phosphate group of DPPE. In Fig. 4(b), the peak at 0.18 ppm was generally expected for ^{31}P functionalities (Lebouc, Dez, & Madec, 2005). The ^{31}P NMR spectra confirmed that phosphate groups were chemically bonded to the material.

The microscopic characteristics of resultant amphiphilic copolymer in aqueous medium were investigated using a fluorometer with pyrene as fluorescent probe. It is known that the variation in the ratio I_{372}/I_{391} of intensity, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where the pyrene is located (Chen, Yu, Cheng, Yu, & Cheung, 2006). Fig. 5(a and b) showed the emission spectra of pyrene in its aqueous solutions

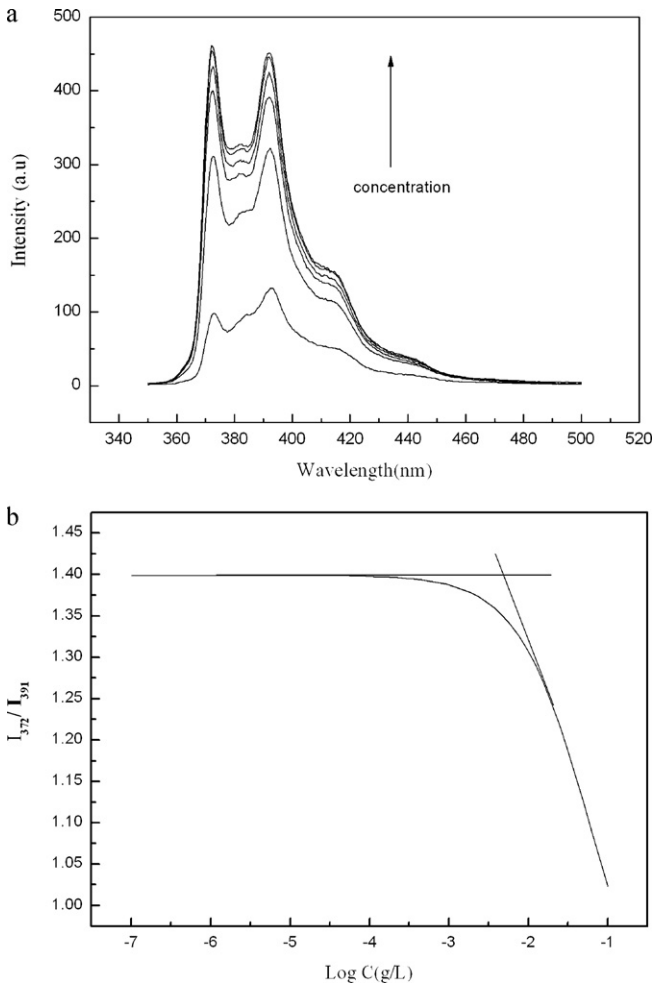

Fig. 5. (a) Fluorescence emission spectra of pyrene in water in the presence of the dextran–PLA–DPPE copolymer at 20 °C (copolymer concentration 10^{-1}, 10^{-2}, 10^{-3}, 10^{-5}, 10^{-6}, 10^{-7} mg mL^{-1}); (b) change of the intensity ratio (I_{372}/I_{391}) versus the concentration of the dextran–PLA–DPPE copolymer at 20 °C.

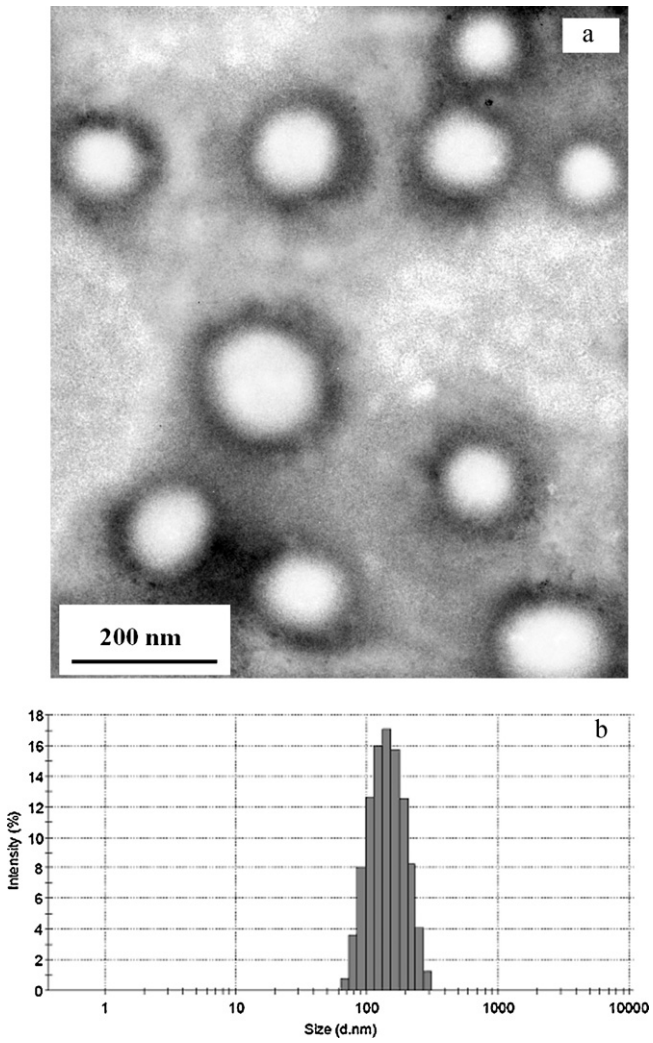

Fig. 6. Transmission electron microscopy (TEM) images of dextran–PLA–DPPE copolymer (50:1) (w/w, DPPE/dextran–PLA–pNP)) micelles (a) and the size distribution of dextran–PLA–DPPE copolymer (50:1) (w/w, DPPE/dextran–PLA–pNP)) micelles in water (b).

with different concentrations and the change of I_{372}/I_{391} with the concentration. Fig. 5(a) showed that there is an increase in the emission intensity with an increase in the polymer concentration in an aqueous solution. As shown in Fig. 5(b), at lower concentrations, the I_{372}/I_{391} values remain nearly unchanged. With the concentration increased further, the intensity ratio starts to decrease, implying the micelle formation. The critical micelle concentration (*cmc*) was determined to be 2.45×10^{-3} mg mL^{-1} through the interception of two straight lines. Compared with surfactants of low molecular weight (Zhang, 2001), the resultant amphiphilic copolymer has a lower cmc value, indicating the stability of the micelles from this dextran–PLA–DPPE copolymer in aqueous solution. Further work was carried out on the morphology of the formed micelles using TEM (transmission electron microscopy). From Fig. 6(a), it can be confirmed that the resulting polymeric micelles are of spherical shapes. The size distribution of the micelles was also investigated using the dynamic light scattering (DLS) technique. As shown in Fig. 6(b), a relatively narrow size distribution was obtained. The DLS data demonstrated that the micelle sizes get larger as the DPPE/activated dextran–PLA feed weight ratio increase, suggesting the elongation of hydrophobic DPPE segment facilitates the growth of the hydrophobic core of polymeric micelles (data not shown).

4. Conclusion

A novel dextran–PLA–DPPE copolymer was synthesized by the reaction between activate dextran–PLA and DPPE. It could self-assembly in water into micelles without any surfactant. The copolymers with controlled structure can be obtained by adjusting the ratio of DLLA to dextran and DPPE to activate dextran–PLA. Owing to good biocompatibility of dextran, PLA and DPPE, and multifunctional conjugation ability of used dextran, the dextran–PLA–DPPE copolymer can keep more advantages as a nanoscale container for hydrophobic drugs when compared with widely used amphiphilic copolymer composed of polyethylene oxide or polyethylene glycol.

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