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Short communication

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

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ABSTRACT

A novel amphiphilic copolymer based on dextran and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) was synthesized and characterized by ^{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 13 kDa to 21 kDa in the molar ratio of 3:1–10:1 (DPPE/activated dextran). Its micellar characteristics in aqueous solution were investigated by fluorescence technique, transmission electron microscopy (TEM) and dynamic light scattering (DLS). It was found that dextran/DPPE copolymer could self-assembly in water into spherical micelles with the diameters ranged from 30 to 60 nm in the absence of organic solvent or surfactant.

1. Introduction

Amphiphilic copolymers consisting of hydrophilic and hydrophobic segments can form micelle structure with the hydrophobic inner core and the hydrophilic outer shell in aqueous media. The hydrophobic inner core is surrounded by a hydrophilic outer shell, and the hydrophilic outer shell provides a stabilizing interface between the micelle core and the aqueous environment (Chen, Yu, Cheng, Yu, & Cheung, 2006; Dimitrov et al., 2006; Janes, Calvo, & Alonso, 2001). Polymeric micelles have received special attention due to their potential application and academic interest in many interdisciplinary field (Bae, Diezi, Zhao, & Kwon, 2007; Cho, Lee, Lee, Huh, & Park, 2004; Lee, Oh, Kim, Youn, & Bae, 2007; Zhou, Deng, & Yang, 2004). Moreover, through adjusting the structure of the amphiphilic copolymers, the size of the polymeric micelles can be easily controlled. In addition, polymeric micelles were also considerably more stable than surfactant micelles (Attwood, Booth, Yeates, Chaibundit, & Ricardo, 2007; Batrakova, Han, Alakhov, Miller, & Kabanov, 1998). Thus, these core–shell type micelles may be used as drug delivery vehicles, 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 (Gao, Lukyanov, Anurag Singhal, Vladimir, & Torchilin, 2002; Lukyanov, Elbayoumi, Chakilam, & Torchilin, 2004; Maedaa et al., 2004; Meijere, Brezesinski, Zschornig, Arnold, & Mohwald, 1998) also have reported about structure studies of phospholipid monolayer coupled to dextran sulphate. However, one drawback of PEG-based copolymers is the absence of reactive groups at their molecular chains, which limits further modification or ligand coupling. In contrast, naturally occurring polysaccharides with good hydrophilicity, biocompatibility and biodegradability seem to be attractive alternatives to PEG hydrophilic segments for designing amphiphilic copolymers.

In this short communication, we reported the first synthesis of a novel amphiphilic copolymer based on the combination of a natural polysaccharide (dextran) with phosphatidylethanolamine (DPPE) and its micellar characteristics in aqueous solution. In order to obtain such material, first, the activation of dextran using 4-nitrophenyl chloroformate and then activated dextran/DPPE copolymer was prepared. Final, activated dextran/DPPE copolymer was added to Tris buffer (pH 8.5) to prepare dextran/DPPE copolymer.

2. Experimental

2.1. Materials

Dextran (1500 Da), 4-nitrophenyl chloroformate, 4-dimethylaminopyridine (DMAP), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), triethylamine (TEA) and CL-4B sepharose were purchased from Sigma. All other reagents were analytical grade and used as received.

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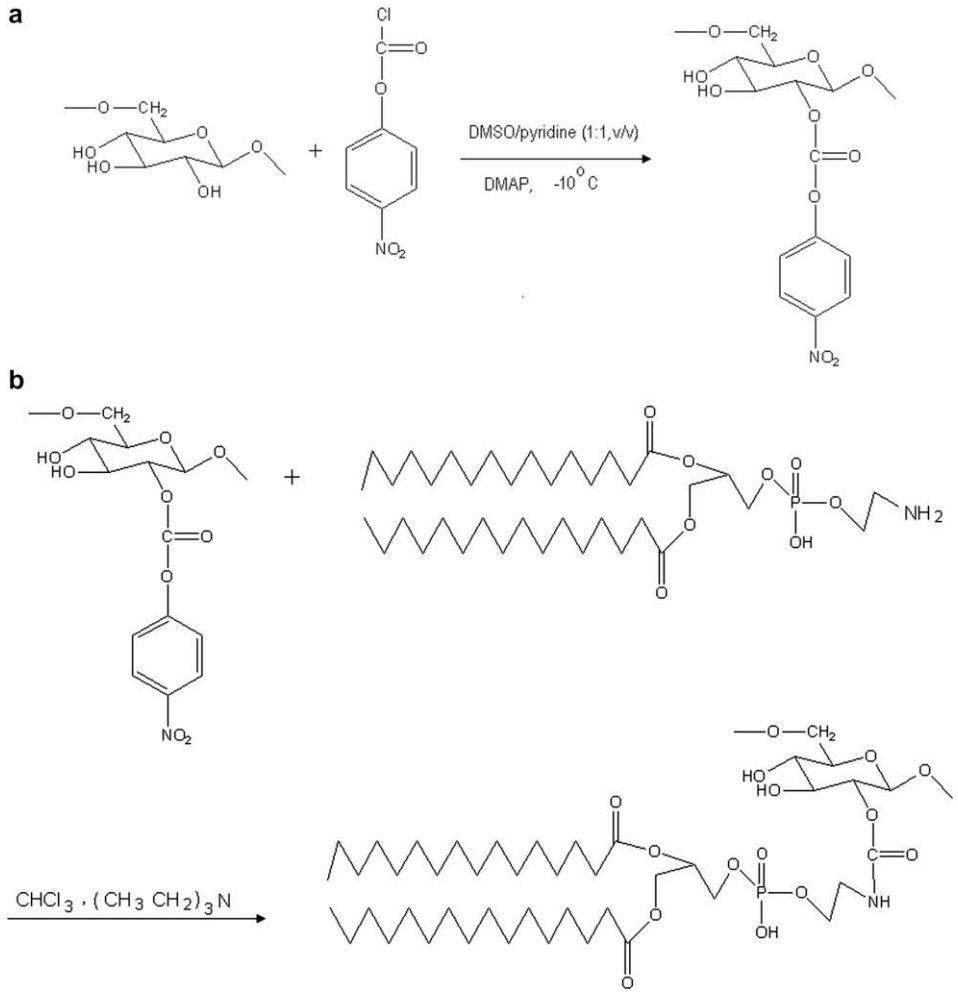

Scheme 1. Synthetic route of the copolymer dextran/DPPE: (a) synthetic route of the activation of dextran and (b) dextran/DPPE copolymer.

2.2. Synthesis of activated dextran

The activation of dextran was performed as follows (Scheme 1a): 2 g of dextran and 43 mg of DMAP were dissolved in DMSO pyridine solution (1:1, v/v) with stirring and then 4-nitrophenyl chloroformate (3 g) was added to the solution and allowed to react at −10 °C with stirring. After 6 h, the reaction solution was added to ethanol and the precipitate collected and extensively washed with ethanol.

2.3. Synthesis of dextran/DPPE copolymer

The activated dextran/DPPE was synthesized as follows. A mixture of activated dextran and DPPE (contain 0.5 mol triethylamine) (in the molar ratio of 3:1–10:1 (DPPE/activated dextran) was suspended in 20 ml of chloroform with magnetic stirring at room under argon. After a further 12 h continuous stirring, the organic solvents were removed using a rotary evaporator. The activated dextran/DPPE 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 activate dextran/DPPE was stored as a powder at −20 °C.

To prepare dextran/DPPE copolymer (Scheme 1b) and remove the *p*-nitrophenyl carbonate group, the above activated dextran/DPPE was added to Tris buffer (pH 8.5), then mixed and incubated overnight at 4 °C under an argon atmosphere. The dextran/DPPE copolymer was purified by the overnight dialysis against distilled water at 4 °C using a dialysis bag (MWCO of 3500 Da), after which samples were freeze-dried and stored as a powder at −20 °C.

2.4. Measurements

The structure of activated dextran copolymer was confirmed by FT-IR (Perkin–Elmer, America) and pressed to a plate with KBr. The ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a (Bruker AVANCE 400) NMR spectrometer and DMSO-*d*6 as a solvent. The GPC measurement was performed on a Waters 515-410 gel permeation chromatograph. Micellar sizes and size distribution were determined by dynamic light scattering (DLS) (Zetasizer Nano series ZEN 3600 analyzer, Malvern Instruments Ltd., England). The experiment was performed at 25 °C using the samples appropriately diluted with distilled water. The morphological examination of micellar was performed using a transmission electron microscope (TEM, Hitachi, H-600) following negative staining with sodium phosphotungstate solution (2%, w/w). Excitation spectra were monitored at 335 nm. The slit widths for both excitation and emission sides were maintained at 0.5 nm. Sample solutions were prepared by dissolving a predetermined amount of copolymer in an aqueous pyrene solution of known concentration, and the solutions were allowed to stand for 48 h for equilibration.

3. Results and discussion

Fig. 1a and b showed the FT-IR spectra of dextran and activation of dextran. Compared with dextran (Fig. 1a), the FT-IR spectra of

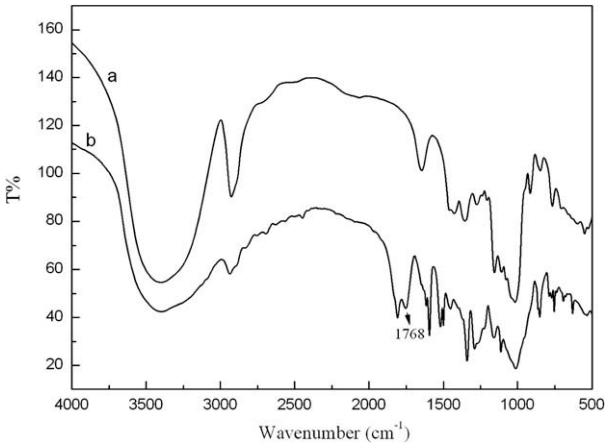

Fig. 1. FT-IR spectra of dextran (a) and activated dextran (b).

activation of dextran (Fig. 1b) showed that the absorption peak at 1768 cm^{-1} was attributed to 4-nitrophenyl carbonates (Chiu, Hsiue, Lee, & Huang, 1999).

Fig. 2a and b showed the ^{1}H NMR spectrum of dextran and dextran/DPPE copolymer. Compared with dextran (Fig. 2a) (Shi & Zhang, 2006), the ^{1}H NMR spectrum of dextran/DPPE copolymer (Fig. 2b) showed that the signal at ~0.9 ppm resulted from the terminal methyl proton of the DPPE moiety. The signals at ~1.2–1.6 ppm were attributed to the methenyl protons of the DPPE moiety. All other absorption peaks were attributed to the protons of the DPPE moiety (Percot et al., 2004).

Fig. 3a and b showed the ^{13}C NMR spectra of dextran and the dextran/DPPE copolymer. Compared with dextran (Fig. 3a), the ^{13}C NMR spectra of the dextran/DPPE copolymer (Fig. 3b) showed that the peak at ~14 ppm was attributed to $-CH_3$ group carbon peak of the DPPE moiety located at the terminal group. The signals at ~22 and ~31 ppm were assigned to $-CH_2$ group carbon peak of the DPPE moiety. The signals at ~160 and ~175 ppm were assigned to –COO group carbon peak of the DPPE moiety.

Furthermore the typical ^{31}P NMR spectra of DPPE and dextran/DPPE copolymer was recorded and shown in Fig. 4a and b. Compared with DPPE (Fig. 4a), the ^{31}P NMR spectra of the dextran/DPPE copolymer (Fig. 4b) showed that the peak at −0.91 ppm was generally expected for ^{31}P functionalities (Sabesan & Neria, 1992; Lebouc, Dez, & Madec, 2005). The ^{31}P NMR spectra confirmed that phosphate groups were chemically bonded to the material. All above these results evidenced that the copolymer contained DPPE side chains.

The microscopic characteristics of resultant amphiphilic copolymer in aqueous medium were investigated using a fluorometer in the presence of pyrene as a fluorescent probe. It is known that the

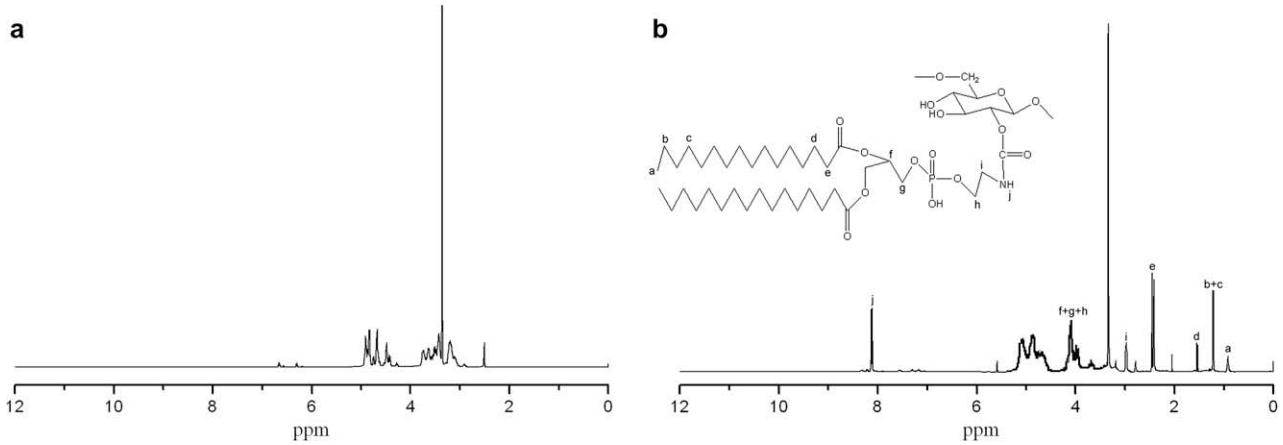

Fig. 2. ^{1}H NMR spectrum of dextran (a) and dextran/DPPE copolymer (b).

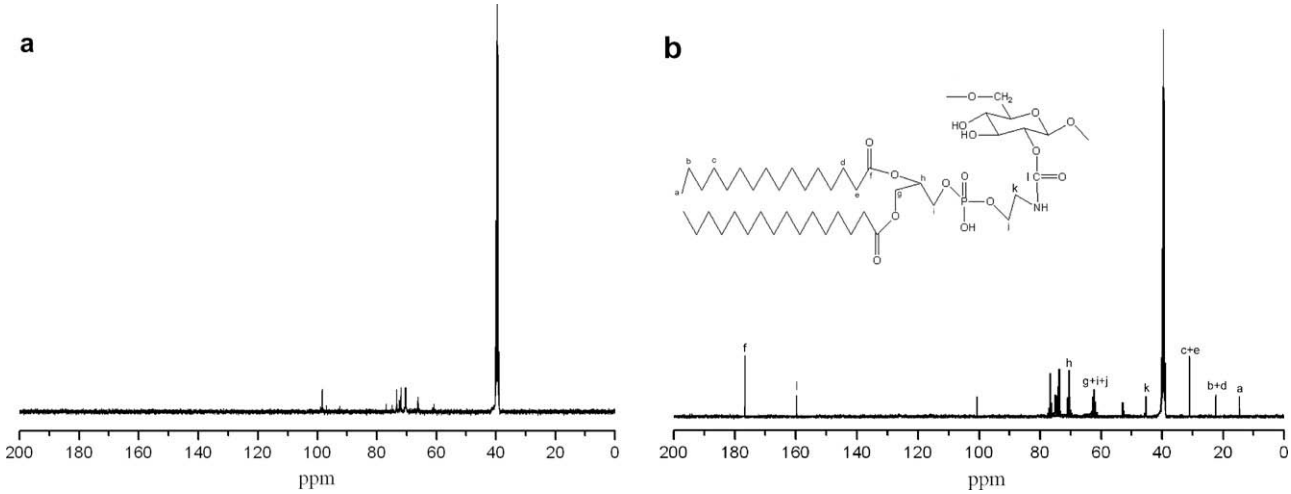

Fig. 3. ^{13}C NMR spectrum of dextran (a) and dextran/DPPE copolymer (b).

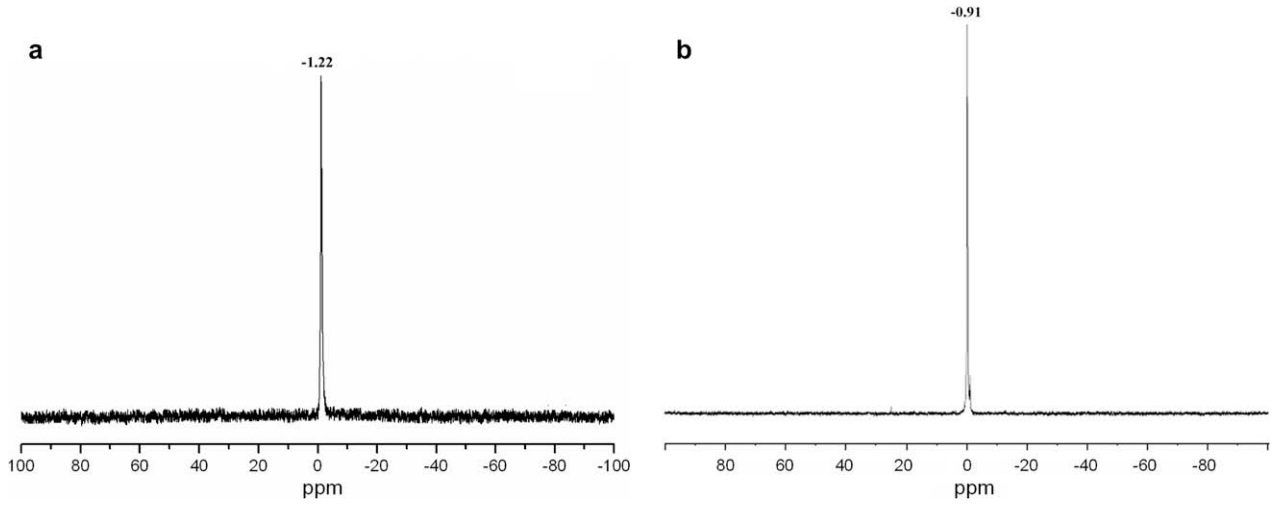

Fig. 4. ^{31}P NMR spectrum of the DPPE (a) and dextran/DPPE copolymer (b).

variation in the ratio I_1/I_3 of intensity of first (372 nm) to the third (383 nm) vibronic peaks, the so-called polarity parameter, is quite sensitive to the polarity of microenvironment where the pyrene is located (Chen et al., 2006; Zhang, Lam, & Tan, 2005). Fig. 5a and b showed the emission spectra of pyrene in its aqueous solutions with various concentrations and the change of I_1/I_3 with the concentration. At lower concentrations, the I_1/I_3 values remain nearly unchanged. Further increasing concentration, the intensity ratio starts to decrease, implying the micelle formation. The critical micelle concentration (*cmc*) was determined to be 6.42×10^{-2} mg/ml by the interception of two straight lines. Compared with low molecular weight surfactants (Zhang, 2001), the resultant amphiphilic copolymer has a lower *cmc* value, indicating the stability of the micelles from this dextran/DPPE copolymer at aqueous solution. Further work was carried out on the morphology of the formed micelles by the transmission electron microscopy (TEM) technique. From Fig. 6, it can be confirmed that the resulting polymeric micelles in water are spherical in shape, with the diameters ranged from 30 to 60 nm. The size distribution of the micelles was also investigated by the dynamic light scattering (DLS) technique. As shown in Fig. 7, a relative narrow size distribution was obtained.

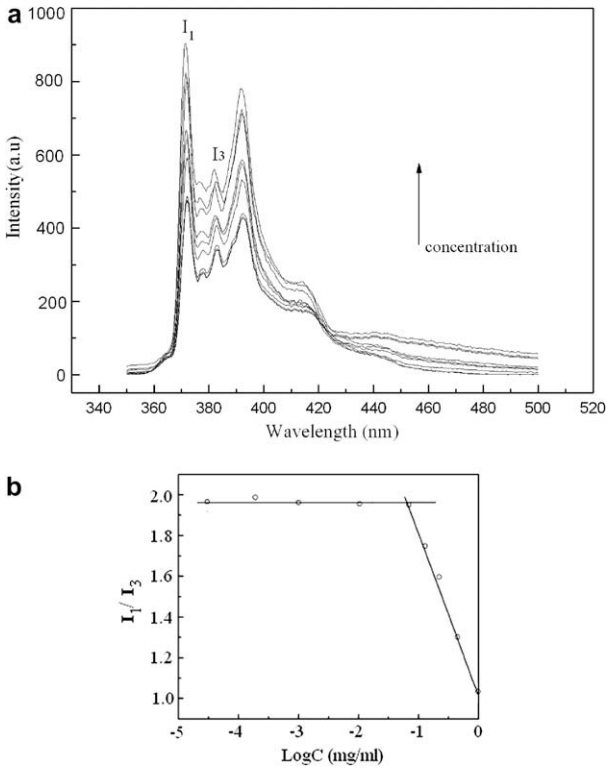

Fig. 5. (a) Fluorescence emission spectra of pyrene in water in the presence of the dextran/DPPE copolymer at 20 °C (copolymer concentration 0.001, 0.003, 0.006, 0.01, 0.03, 0.06, 0.1, 0.25, 0.5 mg/ml); (b) change of the intensity ratio (I_1/I_3) versus the concentration of the dextran/DPPE copolymer at 20 °C.

4. Conclusion

In conclusion, a novel amphiphilic copolymer was synthesized for the first time by the reaction between activate dextran and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine. It could self-assembly in water into polymeric micelles without any organic solvent or surfactant. The copolymers with controlled structure can be obtained by adjusting the molar ratio of DPPE to activate dex-

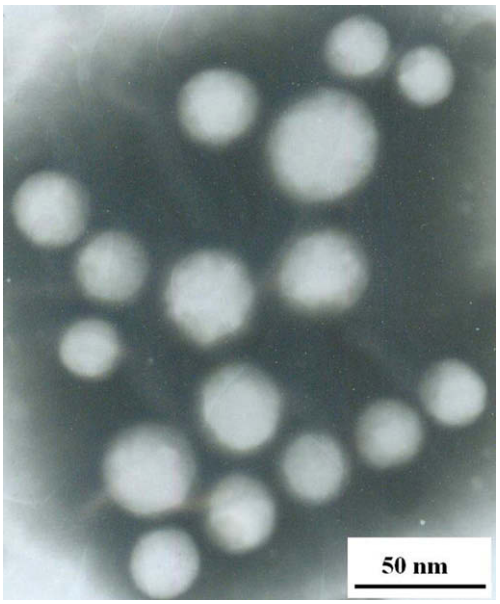

Fig. 6. TEM images of dextran/DPPE copolymer micelles.

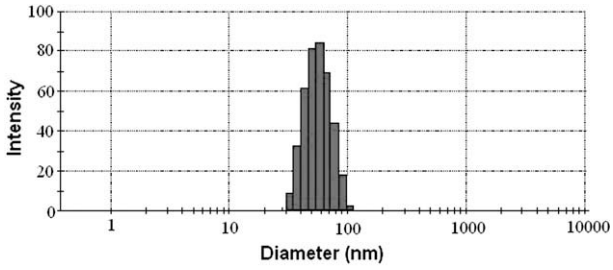

Fig. 7. The size distribution of dextran/DPPE copolymer micelles in water.

tran unit. Due to good biocompatibility of dextran and DPPE, and multifunctional conjugation capability of used dextran, such polysaccharide derivative (dextran/DPPE) will hold greater advantages as the nanoscale container for hydrophobic drugs and genes when compared with widely used amphiphilic block copolymer compose of polyethylene oxide or polyethylene glycol.

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References

Attwood, D., Booth, C., Yeates, S. G., Chaibundit, C., & Ricardo, N. M. P. S. (2007). Block copolymers for drug solubilisation: Relative hydrophobicities of polyether and polyester micelle-core-forming blocks. *International Journal of Pharmaceutics, 345*, 35–41.

Bae, Y., Diezi, T. A., Zhao, A., & Kwon, G. S. (2007). Mixed polymeric micelles for combination cancer chemotherapy through the concurrent delivery of multiple chemotherapeutic agents. *Journal of Controlled Release, 122*, 324–330.

Batrakova, E. V., Han, H. Y., Alakhov, V. Y., Miller, D. W., & Kabanov, A. V. (1998). Effects of pluronic block copolymers on drug absorption in Caco-2-cell monolayers. *Pharmaceutical Research, 15*, 850–855.

Chen, C., Yu, C. H., Cheng, Y. C., Yu, P. H. F., & Cheung, M. K. (2006). Biodegradable nanoparticles of amphiphilic triblock copolymers based on poly(3-hydroxybutyrate) and poly(ethylene glycol) as drug carriers. *Biomaterials, 27*, 4804–4814.

Chiu, H. C., Hsiue, G. H., Lee, Y. P., & Huang, L. W. (1999). Synthesis and characterization of pH-sensitive dextran hydrogels as a potential colon-specific drug delivery system. *Journal of Biomaterials Science Polymer Edition, 10*, 591–608.

Cho, Y. W., Lee, J., Lee, S. C., Huh, K. M., & Park, K. (2004). Hydrotropic agents for study of in vitro paclitaxel release from polymeric micelles. *Journal of Controlled Release, 97*, 249–257.

Dimitrov, P., Utrata-Wesołek, A., Rangelov, S., Wałach, W., Trzebicka, B., & Dworak, A. (2006). Synthesis and self-association in aqueous media of poly(ethylene oxide)/poly(ethyl glycidyl carbamate) amphiphilic block copolymers. *Polymer, 47*, 4905–4915.

Gao, Z., Lukyanov, A. N., Anurag Singhal, A., Vladimir, P., & Torchilin, V. P. (2002). Diacyllipid-polymer micelles as nanocarriers for poorly soluble anticancer drugs. *Nano Letters, 2*, 979–987.

Janes, K. A., Calvo, P., & Alonso, M. J. (2001). Polysaccharide colloidal particles as delivery systems for macromolecules. *Advanced Drug Delivery Review, 47*, 83–97.

Lebouc, F., Dez, I., & Madec, P. J. (2005). NMR study of the phosphonomethylation reaction on chitosan. *Polymer, 46*, 319–325.

Lee, E. S., Oh, K. T., Kim, D., Youn, Y. S., & Bae, Y. H. (2007). Tumor pH-responsive flower-like micelles of poly(L-lactic acid)-b-poly(ethylene glycol)-b-poly(L-histidine). *Journal of Controlled Release, 123*, 19–26.

Lukyanov, A. N., Elbayoumi, T. A., Chakilam, A. R., & Torchilin, V. P. (2004). Tumor-targeted liposomes: Doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody. *Journal of Controlled Release, 100*, 135–144.

Maedaa, N., Takeuchia, Y., Takada, M., Sadzuka, Y., Namba, Y., & Oku, N. (2004). Anti-neovascular therapy by use of tumor neovasculature-targeted long-circulating liposome. *Journal of Controlled Release, 100*, 41–52.

Meijere, K., Brezesinski, G., Zschornig, O., Arnold, K., & Mohwald, H. (1998). Structure studies of phospholipid monolayer coupled to dextran sulphate. *Physical B: Condensed Matter, 248*, 269–273.

Percot, A., Briane, D., Coudert, R., Reynier, P., Bouchemal, N., Lièvre, N., et al. (2004). A hydroxyethylated cholesterol-based cationic lipid for DNA delivery: Effect of conditioning. *International Journal of Pharmaceutics, 278*, 143–163.

Sabesan, S., & Neria, S. (1992). Synthesis of glycosyl phosphates and azides. *Carbohydrate Research, 223*, 169–185.

Shi, H. Y., & Zhang, L. M. (2006). Phase-transition and aggregation characteristics of a thermoresponsive dextran derivative in aqueous solutions. *Carbohydrate Research, 341*, 2414–2419.

Zhang, Y., Lam, Y. M., & Tan, W. S. (2005). Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide)-g-poly(vinylpyrrolidone): Association behavior in aqueous solution and interaction with anionic surfactants. *Journal of Colloid and Interface Science, 285*, 74–79.

Zhang, L. M. (2001). Cellulosic associative thickeners. *Carbohydrate Polymers, 45*, 1–10.

Zhou, S., Deng, X., & Yang, H. (2004). Biodegradable poly(e-caprolactone)–poly(ethylene glycol) block copolymers: Characterization and their use as drug carriers for a controlled delivery system. *Biomaterials, 24*, 3563–3570.