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Novel Polymer Micelles Prepared from Chitosan Grafted Hydrophobic Palmitoyl Groups for Drug Delivery

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Abstract: Chitosan-based polymer micelles have a splendid outlook for drug delivery owing to the interesting properties, abundance, and low cost of chitosan. A new method of preparation of water-soluble *N*-palmitoyl chitosan (PLCS) which can form micelles in water is developed in this paper. The preparation of PLCS was carried out by swollen chitosan coupling with palmitic anhydride in dimethyl sulfoxide (DMSO). The degree of substitution (DS) of PLCS was in the range of 1.2−14.2%, and the critical aggregation concentration (CAC) of PLCS micelles was in the range of 2.0×10^{-3} to 37.2×10^{-3} mg/mL. The properties of PLCS micelles such as encapsulation capacity and controlled release ability of hydrophobic model drug ibuprofen (IBU) were evaluated. Experimental results indicated that the loading capacity (LC) of PLCS was approximately 10%. The drug release strongly depended on pH and temperature: low pH and high temperature accelerated drug release markedly. Moreover, the IR, ^{1}H NMR, and TEM of PLCS, IBU-loaded PLCS, and a PLCS−IBU physical mixture have been measured to show that IBU is loaded by PLCS micelles.

Keywords: Drug delivery systems; chitosan; *N*-palmitoyl chitosan; polymer micelles; encapsulation

Introduction

Polymer micelles have many advantages in drug delivery: their hydrophobic cores can solubilize hydrophobic drug and prevent degradation of drug molecules, while their hydrophilic shells prolong drug circulation time;[1] their small sizes promote drug escape through vasculature and drug accumulating at the target site. So, polymer micelles have been recently regarded as among the most promising carriers for delivery of bioactive materials such as water-insoluble drugs, hormones, and plasmid DNA.[2]

Various block copolymer micelles have been explored for drug delivery over the past decades, especially for the solubilization of water-insoluble drugs.[3] However, the industrialization development of these systems is still limited because of the difficulty and high cost of preparation.

Chitosan, the second most abundant biomass, is a natural cationic polymer with outstanding biologic properties including biodegradability, biocompatibility, and bioactivity.[4,5] It has recently emerged as one of the most promising biopolymers for a variety of potential applications in both the biomedical and pharmaceutical fields. It is expected that

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(1) Govender, T.; Stolnik, S.; Xiong, C.; Zhang, S.; Illum, L.; Davils, S. S. Drug-polyionic block copolymer interactions for micelle formation: Physicochemical characterization. *J. Controlled Release* **2000**, *75*, 249−258.

(2) Ngimhuang, J.; Furakawa, J.; Satoch, T.; Furuike, T.; Sakairi, N. Synthesis of a novel polymeric surfactant by reductive *N*-alkylation of chitosan with 3-*O*-dodecyl-D-glucose. *Polymer* **2004**, *45*, 837−841.

(3) Yoo, S. S.; Park, T. G. Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer. *J. Controlled Release* **2001**, *70*, 63−70.

(4) Lee, K. Y.; Ha, W. S.; Park, W. H. Blood compatibility and biodegradability of partially *N*-acylated chitosan derivatives. *Biomaterials* **1995**, *16*, 1211−1216.

(5) Lee, D. W.; Powers, K.: Baney, R. Physicochemical properties and blood compatibility of acylated chitosan nanoparticles. *Carbohydr. Polym.* **2004**, *58*, 371−377.

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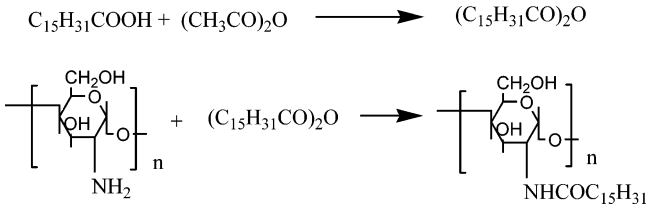
chitosan can be used as a starting material to prepare polymer micelles as drug carries; then the drug delivery systems will be boosted dramatically, since chitosan is so abundant and cheap. Fortunately, polymer micelles obtained by modification of chitosan, including chitosan coupling with both hydrophobic groups and hydrophilic groups, has been reported recently.[6−13] For example, Zhang et al.[9] reported how to obtain polymer micelles from *N*-alkyl-*O*-sulfate chitosan derivatives, however, Zhang did not mention the degree of substitution (DS) of their derivatives and the critical aggregation concentration (CAC) of the obtained micelles, but CAC is a measure describing the physical properties and the stability of micelle.[14] In addition, another disadvantage accompanied with the synthesis route is that the introduction of sulfate groups must be performed under N_2 as the reaction proceeded vigorously. Kwon et al.[10] obtained micelles based on glycol chitosan modified by 5β-cholanic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbordiimide (EDC), but the low DS of the derivatives (1.1−8.7%) and the great values of CAC (4.7×10^{-2} to 21.9×10^{-2} mg/mL) may result in poor stability of the micelles in dilute solution.[2] The same situation happened to micelles of linolenic acid modified chitosan (DS 1.8%, CAC 5×10^{-2} mg/mL) reported by Liu et al.[13] In addition, considering deoxycholic acid has hydroxy groups at 3α and 12α positions and may form self-assemblies in water, Lee et al.[11,12] also synthesized deoxycholic acid modified chitosan. However, they found that derivatives with higher DS could not be obtained because of the limited solubility of deoxycholic acid in the acidic reaction medium,[11] and the CACs (1.7×10^{-2} to 4.1×10^{-2} mg/mL) of their products were still high. Although Li and Kwon[15] have pointed out that increasing the level of hydrophobic attachment leads to the high stability of polymer micelles whereas low hydrophobic DS may result in weakening stability of micelles, few chitosan-based micelles with tolerable properties have been reported up to now, not to mention emergence of stable chitosan-based micelles which catch up with those block copolymer micelles and are suitable for loading drug.

Scheme 1. The Synthesis of *N*-Palmitoyl Chitosan (PLCS)



On the basis of these considerations, we used ordinary fatty acid to modify chitosan and avoided the introduction of hydrophilic groups. The present paper reports that stable micelles based on water-soluble *N*-palmitoyl chitosan (PLCS) were prepared and the hydrophobic model drug ibuprofen (IBU) was encapsulated by the PLCS micelles, through a simple and convenient route. In addition, the drug release behavior of PLCS micelles was investigated.

Experimental Section

Materials. Chitosan (M_w 190 kDa, 90−95% deacetylated) was obtained from Shanghai Boao Biotechnology Co. (Shanghai, China). Palmitic acid and acetic anhydride were purchased from Guangzhou Chemical Factory (Guangzhou, China). Ibuprofen (IBU) was purchased from Sigma (MO, USA), pyrene was purchased from Aldrich Chemical Co. (USA). All reagents were analytical grade and used without further purification.

Synthesis of PLCS. Synthesis was carried out as shown in Scheme 1. The chitosan (3.0 g, 0.016 mol) was dissolved in 100 mL of acetic acid (0.1 M), precipitated with 55 mL of NaOH (0.2 M), then collected by filtration, and washed with water to pH 7. The incompact or swollen chitosan obtained from above was dispersed in 100 mL of dimethyl sulfoxide (DMSO) with magnetic stirring, and palmitic anhydride (0.032−0.064 mol, obtained through palmitic acid reacting with acetic anhydride as in the previous method[16]) was added dropwise to the mixture and allowed to react at 60 °C for 8 h. The precipitate obtained by pouring the solution into acetone was collected by filtration, washed with acetone five times, and dried at 60 °C under vacuum. The

(6) Kjøniksen, A. L.; Iversen, C.; Nystrõm, B.; Nakken, T.; Palmgren, O. Light scattering study of semidilute aqueous systems of chitosan and hydrophobically modified chitosans. *Macromolecules* **1998**, *31*, 8142−8148.
(7) Miwa, A.; Ishibe, A.; Nakano, M.; Yamahira, T.; Itai, S.; Jinno, S.; Kawahara, H. Development of novel chitosan derivatives as micellar carries of Taxol. *Pharm. Res.* **1998**, *15* (12), 1844−1850.
(8) Liu, W. G.; Zhang, X.; Sun, S. J.; Sun, G. J.; Yao, K. D.; Liang, D. C.; Guo, G.; Zhang, J. Y. N-alkylated chitosan as a potential nonviral vector for gene transfection. *Bioconjugate Chem.* **2003**, *14*, 782−789.
(9) Zhang, C.; Ping, Q.; Zhang, H.; Shen, J. Preparation of *N*-alky-*O*-sulfate chitosan derivatives and micellar solubilization of Taxol. *Carbohydr. Polym.* **2003**, *54*, 137−141.
(10) Kwon, S. K.; Park, J. H.; Chung, H.; Kwon, I. C.; Jeong, S. Y. Physicochemical characteristic of self-assembled nanoparticles based on glycol chitosan bearing 5β-cholanic acid. *Langmuir* **2003**, *19*, 10188−10193.
(11) Lee, K. Y.; Jo. W. H.; Kwon, I. C.; Kim, Y.-H.; Jeong, S. Y. Physicochemical characteristics of self-aggregates of hydrophobically modified chitosan. *Langmuir* **1998**, *14*, 2329−2332.
(12) Lee, K. Y.; Jo. W. H.; Kwon, I. C.; Kim, Y.-H.; Jeong, S. Y. Structural determination and interior polarity of self-aggregates prepared from deoxycholic acid-modified chitosan in water. *Macromolecules* **1998**, *31*, 378−383.
(13) Liu, C. G.; Desai, K. G. H.; Chen, X. G.; Park, H. J. Linoleic acid-modified chitosan for formation of self-assembled nanoparticles. *J. Agric. Food Chem.* **2005**, *53* (2), 437−441.
(14) Nagasaki, Y.; Okada, T.; Scholz, C.; Iijima, M.; Kato, M.; Kataoka, K. The reactive polymeric micelle based on an aldehyde-ended poly (ethylene glycol)/poly (lactide) block copolymer. *Macromolecules* **1998**, *31*, 1473−1479.
(15) Li, Y.; Kwon, G. S. Methotrexate esters of poly (ethylene oxide)-*block*-poly (2-hydroxyethyl-L-aspartamide). Part 1: effects of the level of Methotrexate conjugation on the stability of micelles and drug release. *Pharm. Res.* **2000**, *17* (5), 607−611.
(16) Xing, Q. Y.; Xv, R. Q.; Zhou, Z. *Basic organic chemistry*; Higher education press: Beijing, 1980; p 469.

DS, defined as the number of palmitoyl groups per 100 sugar residues of chitosan, was calculated by ^{1}H NMR using the ratio of palmitoyl methyl protons (δ = 0.86 ppm) to H-2 protons (δ = 2.89 ppm) of chitosan. The number behind the sample code DS indicates the degree of substitution (DS) of the sample.

Preparation of Polymer Micelles and IBU Loading. PLCS can form polymeric micelles via self-assembling in dilute aqueous solution. Water-insoluble drug IBU was loaded by PLCS micelles through the following steps: 200 mg of IBU in a beaker was dissolved in 5−10 mL of organic solvent such as CH_2Cl_2, acetone, and ethanol, and then 200−1000 mg of PLCS was added into the organic solution, and at this moment PLCS was yet insoluble. However, when pure water was dropped slowly into the organic solution with magnetic stirring, PLCS was dissolved and self-aggregated slowly, at the same time IBU was precipitated gradually, and a small portion of precipitated IBU was encapsulated into the hydrophobic cores of PLCS micelles and was solubilized. After 4 h of continuous stirring to evaporate the organic solvent, the IBU deposit was filtered out and adhered to the filter paper. The beaker was washed three times with water, and the eluate was used to rinse the IBU deposit adhered to the filter paper. The filtrate was freeze-dried to obtain IBU-loaded PLCS powder, and the powder was washed with ethanol three times to get rid of the IBU adhering to the shell surface of the micelles. The washed down IBU was collected, dried, and incorporated with the IBU deposit adhered to the filter paper. The total IBU, regarded as the free drug, was dissolved in 50% ethanol solution, and its amount was determined by UV spectrophotometer. The washed PLCS powder was then dried, weighed, and used for further investigations.

The drug loading capacity (LC) and encapsulation efficiency (EE) of PLCS were calculated by using eqs 1 and 2, respectively,[17]

$$\mathrm{LC} = (A - B)/C \quad (1)$$

$$\mathrm{EE} = (A - B)/A \quad (2)$$

where A = total amount of added IBU; B = free amount of IBU; C = weight of PLCS.

Characterization and Identification. The FT-IR spectra were obtained on KBr disks using a Nicolet 670 FT-IR spectrometer (Thermo Nicolet, USA).

^{1}H NMR spectra were recorded on a Mercure Plus 300 MHz spectrometer (Varian, USA).

The hydrodynamic radii and polydispersity of PLCS micelles were determined by dynamic light scattering (DLS) measurement at 25 °C, using a BI-200SM goniometer light scattering spectrophotometer (Brookhaven, USA). Distilled water solutions of PLCS in glass cells were measured with a vertically polarized incident beam at 532 nm at a detector angle of 90°.

Table 1. Influence of DS of PLCS, Organic Solvents,[a] and the Weight Ratios of IBU[b] to Polymer on Encapsulation Efficiency and Loading Capacity

samples of PLCS	organic soln vol (mL)	wt ratios (IBU:PLCS)	encapsulation efficiency (%)	loading capacity (%)
DS5.6	5 mL of CH_2Cl_2	1:4	23.6	5.9
DS5.6	5 mL of acetone	1:4	32.4	8.1
DS5.6	5 mL of ethanol	1:4	38.4	9.6
DS5.6	10 mL of ethanol	1:1	11.5	11.5
DS5.6	10 mL of ethanol	1:4	42.8	10.7
DS5.6	10 mL of ethanol	1:5	46.0	9.2
DS1.2	10 mL of ethanol	1:4	22.8	5.7
DS14.2	10 mL of ethanol	1:4	55.2	13.8

[a] The organic solvents were used to dissolve 200 mg of IBU. [b] The weight of IBU was 200 mg, and the added water volume was 100 mL.

The morphology and size distribution were observed by TEM using a JEM-2010 electron microscope (JEOL, Japan). Samples with negative staining were performed as follows: a drop of sample solution was placed onto a 200 mesh copper grid coated with carbon, taped with a filter paper to remove surface water, and air-dried for 5 min. These self-aggregates were deposited on the grid, followed by the application of methylamine tungstate negative stain for 2 min.

Fluorescence spectra were recorded on a FLS920 fluorescence spectrometer (Edinburgh instruments Ltd, UK). A sample solution containing 6.0×10^{-7} M pyrene was excited using a xenon lamp. The concentration of sample solution was varied from 1.28×10^{-5} to 1 mg/mL. For measurement of the intensity ratio of the first and the third highest energy bands in the emission spectra of pyrene, the slit openings for excitation and emission were both set at 0.5 mm, the excitation wavelength (λ_{ex}) was 334 nm, and the spectra were accumulated with an integration time of 1 s/nm.

In Vitro Drug Release Studies. Micelles powder (10 mg) containing known IBU and 5 mL of phosphate buffered saline (PBS, 0.1 M, pH 7.4 and containing 1% ethanol) or 5 mL of dilute hydrochloric acid (pH 3.5, containing 1% ethanol) were put into a dialysis bag (cutoff molecular weight: 35 kDa). Then the dialysis bag was introduced into a vial containing 50 mL of PBS or 50 mL of dilute hydrochloric acid (the same as the solution added in the bag). The systems were immersed in a thermostatic bath (37 or 4 °C) and kept at constant 100 rpm stirring. At appropriate intervals, 1 mL samples of solution were withdrawn from the vials and assayed for drug release by UV spectrophotometer at 261 nm and replaced by 1 mL of fresh PBS or dilute hydrochloric acid.

Results

Drug Encapsulation Efficiency (EE) and Loading Capacity (LC) of PLCS. The loading capacity (LC) of the chitosan derivatives was affected markedly by their degree of substitution (DS). Table 1 shows that the LC of DS14.2

(17) Gupta, K. C.; Ravkumar, M. N. V. J. pH dependent hydrolysis and drug release behavior of chitosan/poly (ethylene glycol) polymer network microspheres. *Mater. Sci.: Mater. Med.* **2001**, *12*, 753−759.

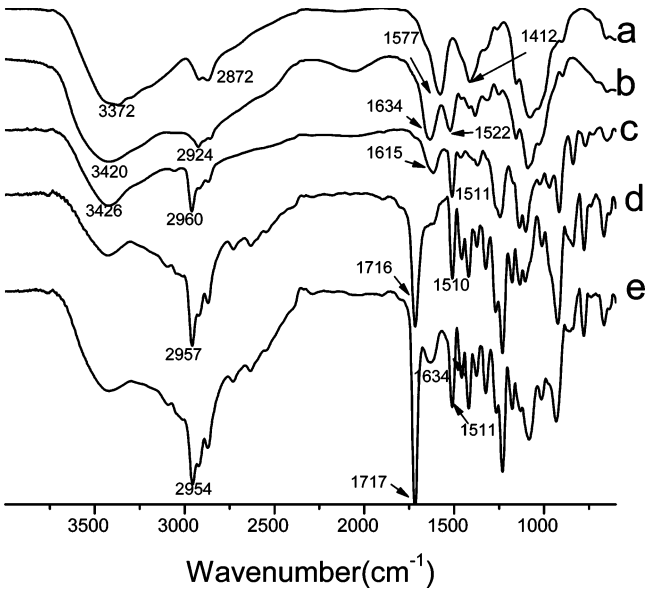


Figure 1. IR spectra: (a) chitosan; (b) PLCS (DS14.2); (c) IBU-loaded PLCS (DS14.2, LC = 13.8%); (d) IBU; (e) IBU−PLCS mixture (14% w/w).

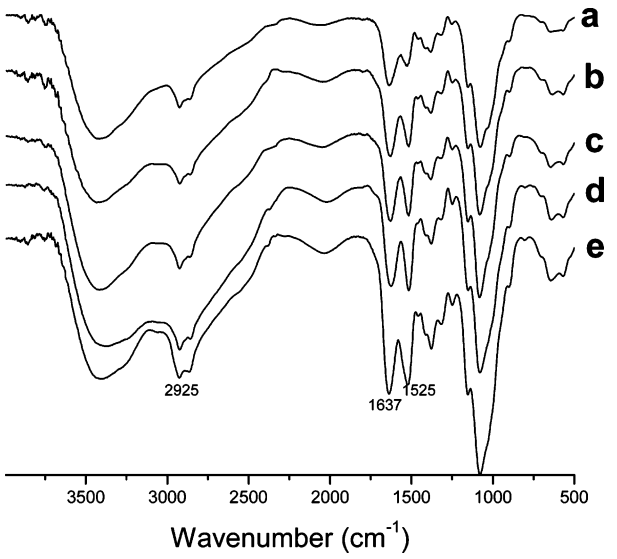


Figure 2. IR spectra: (a) PLCS (DS1.2); (b) PLCS (DS9.4); (c) PLCS (DS5.6); (d) *N*-stearoyl chitosan (DS 8.2); (e) *N*-octanoyl chitosan (DS29.6).

is much higher than that of DS1.2, perhaps because the micelles become more stable and the cores of the micelles become more hydrophobic as the DS increases. Organic solvents and drug concentrations also affect the LC of IBU. The low LC in CH_2Cl_2 solvent can be explained as follows: because only nanoparticle IBU has a chance of being encapsulated into the cores of PLCS micelles, since CH_2Cl_2 is a poor mutual solvent of water and it volatilizes more quickly than ethanol, so the IBU dissolved in CH_2Cl_2 precipitates too quickly and cannot be encapsulated by PLCS micelles; when PLCS was dissolved in water and formed micelles, the precipitated IBU nanoparticle had accumulated into large particles and deposited down. More ethanol can prolong the precipitating time of IBU and increase the amount of IBU encapsulated by the hydrophobic cores of PLCS micelles; therefore, more ethanol can improve the EE and LC.

Infrared Analysis. Chitosan, PLCS, IBU-loaded PLCS, and a PLCS−IBU physical mixture (abbreviated ph-mixture) were characterized by FT-IR to identify the changes of structure (Figure 1). The chitosan shows a distinct primary amino groups bending vibration at 1577 cm^{-1}; N−H stretching and O−H stretching vibrations can be characterized by the broad peak in the region of 3200−3500 cm^{-1} (Figure 1a). When palmitoyl groups were grafted onto the amine groups of chitosan, the absorption peak of 1577 cm^{-1} almost disappeared, while prominent bands at 1634 and 1522 cm^{-1} were observed (Figure 1b), which were assigned to the carbonyl stretching of amide I band and amide II band, respectively. As DS increased, the intensity of carbonyl stretching of amide I and amide II increased obviously. These results clearly revealed that *N*-acylated derivatives were obtained and the reaction is highly selective toward *N*-acylation, as it can be confirmed by the absence of a band present at ∼1750 cm^{-1}, diagnostic of the presence of *O*-acyl ester groups. This phenomenon is consistent with the report of Félix,[18] and the acylated derivatives differ from the products of Badaway[19] and Zong,[20] who used acyl chloride coupling with dissolved chitosan or swollen chitosan respectively, and obtained *N*,*O*-acyl chitosan with very high DS. In addition, peaks at 2860−2972 cm^{-1} were ascribed to $-CH_2$. It is noticeable that their intensity was not obviously proportional to the length of acyl chain, which is different from the previous report of Tien et al.[21] The reason is probably that the degrees of substitution of all PLCS were not high (the DS values here were only 1.2−14.2%, while those of Tien were in the range of 40−50%), and the degree of substitution of acylated derivatives affected the peak intensity as well, which can be observed in Figure 2. Another interesting phenomenon is that the IR spectrum of IBU-loaded PLCS was utterly different from that of the ph-mixture. Pure IBU (Figure 1d) shows strong absorption of the carbonyl group at 1717 cm^{-1}, while the absorption peak at 1717 cm^{-1} almost cannot be observed after IBU was encapsulated by PLCS (Figure 1c); moreover, many absorption peaks are significantly weaker than those of IBU alone, and the ph-mixture system is only a simple coalition of IBU

(18) Félix, L.; Hernández, J.; Argüelles-Monal, W. M.; Goycoolea, F. M. Kinetics of Gelation and Thermal Sensitivity of N-Isobutyryl Chitosan Hydrogels. *Biomacromolecules* **2005**, *6*, 2408−2415.

(19) Badawy, M. E. I.; Rabea, E. I.; Rogge, T. M.; Stevens, C. V.; Smagghe, G.; Steurbaut, W.; Höfte, M. Synthesis and fungicidal activity of new N,O-acyl chitosan derivatives. *Biomacromolecules* **2004**, *5*, 589−595.

(20) Zong, Z.; Kimura, Y.; Takahashi, M.; Yamane, H. Characterization of chemical and solid state structures of acylated chitosan. *Polymer* **2000**, *41*, 899−906.

(21) Tien, C. L.; Lacroix, M.; Ispas-Szabo, P.; Mateescu, M. A. *N*-acylated chitosan: hydrophobic matrices for controlled drug release. *J. Controlled Release* **2003**, *93*, 1−13.

and PLCS (Figure 1e). All of these observation reveal that some bond interaction, such as hydrogen bonds of C(2)NH···O═C(IBU) and C(6)OH···O═C(IBU),[21,22] perhaps exist between IBU and PLCS when IBU is encapsulated by the PLCS micelles. At least, a conclusion can be deduced that IBU is loaded by PLCS.

^{1}H NMR Spectra Studies. The ^{1}H NMR spectra of PLCS and IBU-loaded PLCS complex systems were performed in D_2O and DMSO-d_6, respectively, as shown in Figure 3. Proton assignments in PLCS in DMSO-d_6 (Figure 3a): $\delta_{0.85}$ = CH_3 (palmitoyl), $\delta_{1.24}$ = CH_2 (palmitoyl), $\delta_{1.46}$ = CH_2 (palmitoyl deshielded by carbonyl, β to carbonyl), $\delta_{1.82}$ = CH_3 (acetyl), $\delta_{2.07}$ = CH_2 (palmitoyl deshielded by carbonyl, α to carbonyl), $\delta_{2.89}$ to H-2 proton, $\delta_{3.14-4.02}$ to the ring protons (H-3,4,5,6,6′) overlapped with H_2O, $\delta_{4.76-5.53}$ to the H-1 protons, $\delta_{8.52}$ to N−H. Proton assignments for PLCS in D_2O (Figure 3b, relative to HDO = $\delta_{4.70}$): $\delta_{0.80}$ = CH_3 (palmitoyl), $\delta_{1.22}$ = CH_2 (palmitoyl), $\delta_{1.53}$ = CH_2 (palmitoyl deshielded by carbonyl, β to carbonyl), $\delta_{2.01}$ = CH_2 (palmitoyl deshielded by carbonyl, α to carbonyl), $\delta_{3.10}$ = H-2, $\delta_{3.56-4.07}$ to the ring protons (H-3,4,5,6,6′). $\delta_{4.59-5.05}$ were signs of H-1 overlapped with the sign of HDO. As clearly shown in Figure 3b, in D_2O only small signals to palmitoyl moiety at $\delta_{0.70}$ (to CH_3 of palmitoyl) and $\delta_{1.22}$ (to CH_2 of palmitoyl) were observed, suggesting that PLCS molecules formed hydrophobic cores to minimize their interaction with D_2O,[23] which indicated that PLCS formed micelles via self-aggregation in an aqueous phase.[23,24] In DMSO-d_6, the ^{1}H NMR spectrum of IBU-loaded PLCS was similar to that of the derivative itself as presented in Figure 3c, which revealed that IBU was utterly encapsulated by the PLCS, and PLCS can self-aggregate in DMSO to some extent; however, after heating at 50 °C for 15 min, the IBU signals emerged (Figure 3d), which revealed that the IBU was released from the polymer micelles after being heated. However, the ^{1}H NMR spectra of IBU-loaded PLCS in D_2O always did not have the IBU signals, no matter whether IBU-loaded PLCS was heated or not (the two spectra were not shown in Figure 3, however they were the same as Figure 3b), which revealed that the PLCS micelles were stable in D_2O.

Measurement of Fluorescence Spectroscopy. PLCS was composed of chitosan backbone and pendant palmitoyl group. The chitosan backbone became water-soluble as long as the appropriate introduction of acylated group destroyed the crystalline structure of chitosan, whereas the palmitoyl groups were water-insoluble segments, which formed the tightly packed cores via hydrophobic attraction and were surrounded by diffuse outer shells (corona) formed from the soluble chitosan backbone. The aggregation behavior of PLCS in aqueous media was determined by fluorescence spectroscopy using pyrene as a fluorescence probe. The fluorescence emission spectra of pyrene in the presence of various

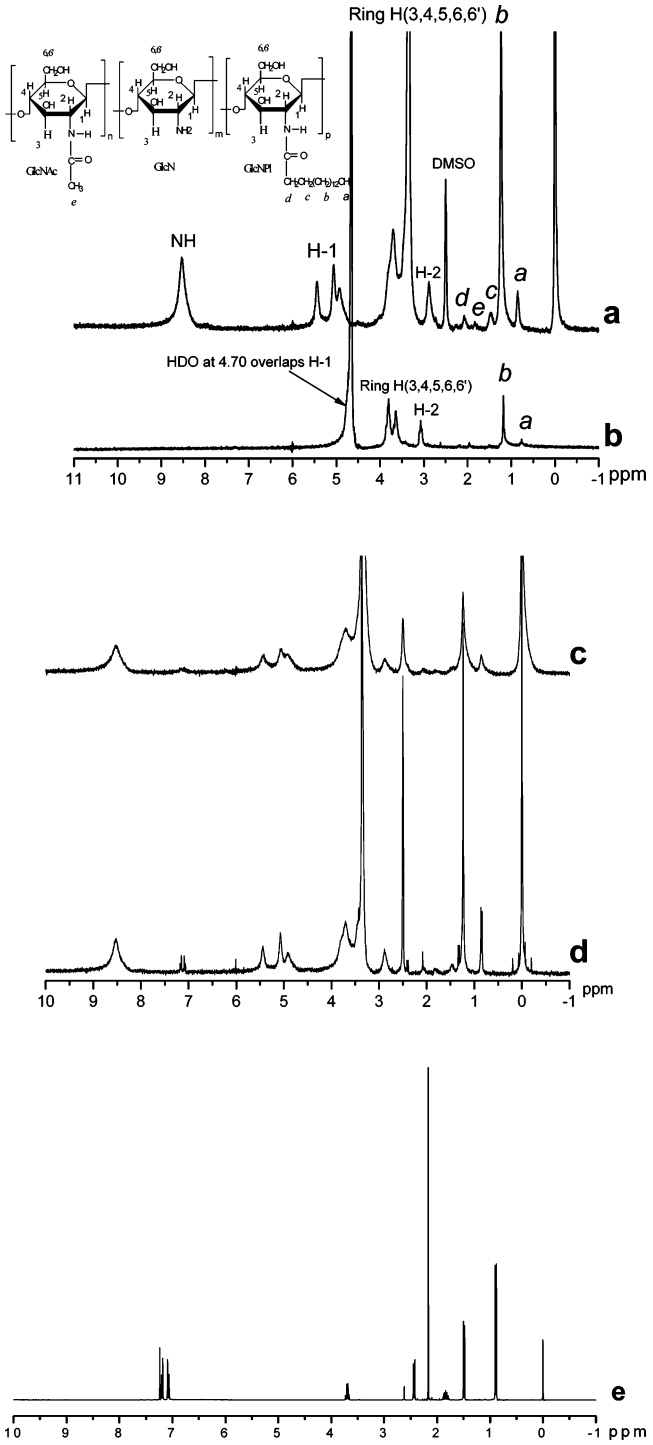


Figure 3. ^{1}H NMR spectra: (a) PLCS (DS14.2) in DMSO; (b) PLCS (DS14.2) in D_2O; (c) IBU-loaded PLCS (DS14.2; LC = 13.8%) in DMSO; (d) IBU-loaded PLCS (DS14.2; LC = 13.8%) in DMSO after being heated to 50 °C for 15 min; (e) IBU in $CDCl_3$.

(22) Cho, Y. W.; Jang, J.; Park, C. R.; Ko, S. W. Preparation and solubility on acid and water of partially deacetylated chitosan. *Biomacromolecules* **2000**, *1*, 609−614.

(23) Park, K.; Kim, K.; Kwon, I. C.; Kim, S. K.; Lee, S.; Lee, D. Y.; Byun, Y. Preparation and characterization of self-assembled nanoparticles of heparin-deoxycholic acid conjugates. *Langmuir* **2004**, *20*, 11726−11731.

(24) Kim, C.; Lee, S. C.; Kang, S. W.; Kwon, I. K.; Kim, Y.-H.; Jeong, S. Y. Synthesis and the micellar characteristics of poly (ethylene oxide)-deoxycholic acid conjugates. *Langmuir* **2000**, *16*, 4792−4797.

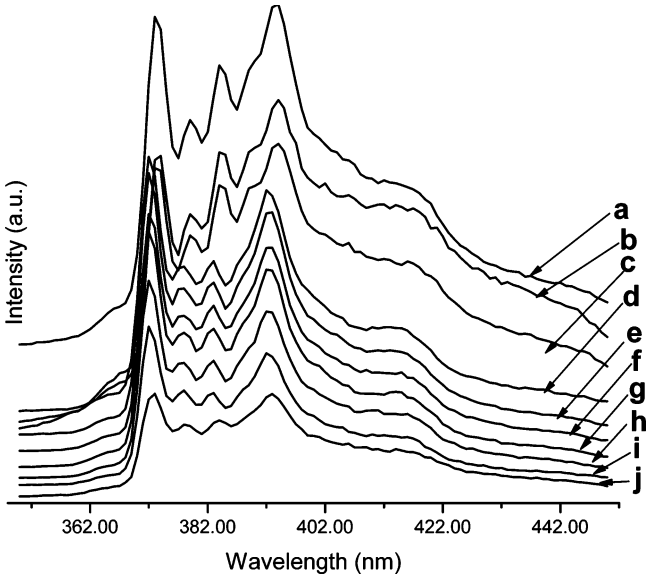


Figure 4. Emission spectra of pyrene (6.0×10^{-7} mg/mL) as a function of DS14.2 concentration: (a) 1, (b) 0.5, (c) 0.2, (d) 0.04, (e) 0.008, (f) 0.0016, (g) 0.00032, (h) 0.000064, (i) 0.000012, and (j) 0.00000256 mg/mL in water.

concentrations of DS9.4 at 25 °C were recorded, and the results are shown in Figure 4. While the concentration of polymeric amphiphile in dilute aqueous solution increases, the total emission intensity increases. Especially when the concentration reaches the critical aggregation concentration (CAC), the third highest vibrational band at 383 nm (I_3) increases drastically; however, the first highest vibrational band at 372 nm (I_1) increases nonobviously, so the intensity ratio I_{372}/I_{383} can be used to determine the CAC.[25] Figure 5 shows the CAC determined by the interception of two straight lines (I_{372}/I_{383}). The CAC values of DS14.2−DS5.6 (2.0×10^{-3} to 3.7×10^{-2} mg/mL) are approximately 2 orders of magnitude lower than the CAC values of lower molecular weight surfactants (e.g., 0.52 mg/mL for cetyltrimethylammonium bromide) and are roughly 10-fold of that of other chitosan derivative micelles previous reported, e.g., values for deoxycholic acid modified chitosan were 1.7×10^{-2} to 4.1×10^{-2} mg/mL,[11] and the value for the reductive derivative of 3-*O*-dodecyl-D-glucose coupled with chitosan was 0.1 mg/mL in neutral aqueous media.[2] The lower CAC values of the derivatives indicate that a small amount of the chitosan derivatives can form self-aggregates and maintain the stability in dilute condition. It can be noticed that the CAC values decrease with increasing DS (Table 2); the reason for this phenomenon can be attributed to the increase of hydrophobicity by introduction of a large amount of hydrophobic groups, which agrees with the idea that increasing the level of hydrophobic attachment will result in the high stability of the polymer micelles proposed by Li and Kwon.[15]

(25) Whihelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J. L.; Riess, G.; Croucher, M. D. Poly(styrene-ethylene oxide) block copolymer micelle formation in water: A fluorescence probe study. *Macromolecules* **1991**, *24*, 1033−1040.

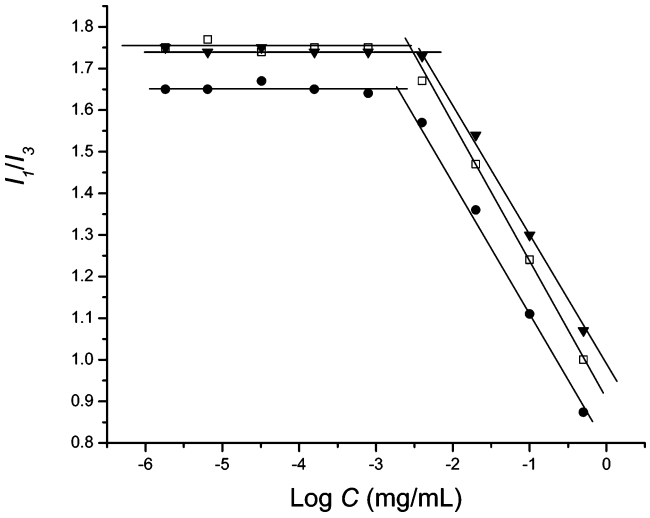


Figure 5. Change of intensity ratio (I_{372}/I_{383}) for pyrene in water with various concentrations of PLCS: (▼) DS5.6; (□) DS9.4; (●) DS14.2.

Table 2. Effects of DS on the Properties of PLCS

sample	CAC[a] × 10^3 (mg/mL)	*d* [b] (nm)	polydispersity[c]	morphology[d]
DS1.2	37.2	608	1.821	irregular
DS5.6	3.7	158	0.219	near spherical
DS9.4	2.8	149	0.241	near spherical
DS14.2	2.0	140	0.263	near spherical

[a] CAC determined by fluorescence spectroscopy. [b] Mean diameter measured by DLS. [c] Polydispersity determined by DLS. [d] Morphology measured by TEM.

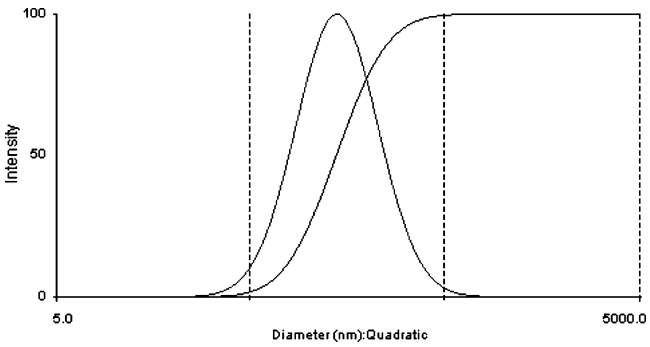


Figure 6. Size of PLCS (DS14.2) mean diameter in pure water.

Size and Morphology. The sizes of micelles were determined by DLS. Figure 6 shows size distributions of DS14.2. The sizes of self-aggregates decreased as the DS increased (Table 2), which indicated that more hydrophobic substitution resulted in more compact and stable micelles. This phenomenon was consistent with the results reported by Kwon et al.[10] and Li et al.,[11] that the increase of DS may enhance the chances of hydrophobic interactions among hydrophobic pendant groups, resulting in the formation of more compact hydrophobic cores. TEM images depicted that

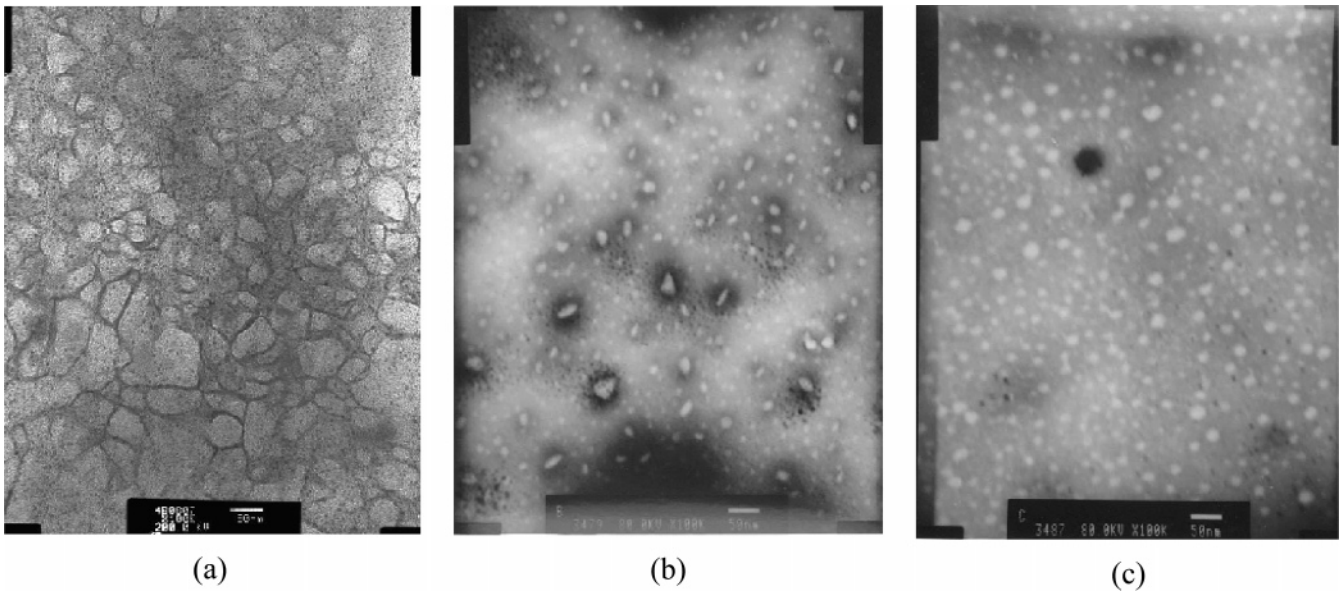


Figure 7. TEM of PLCS: (a) DS1.2; (b) IBU encapsulated DS1.2 (LC = 5.7%); (c) DS9.4.

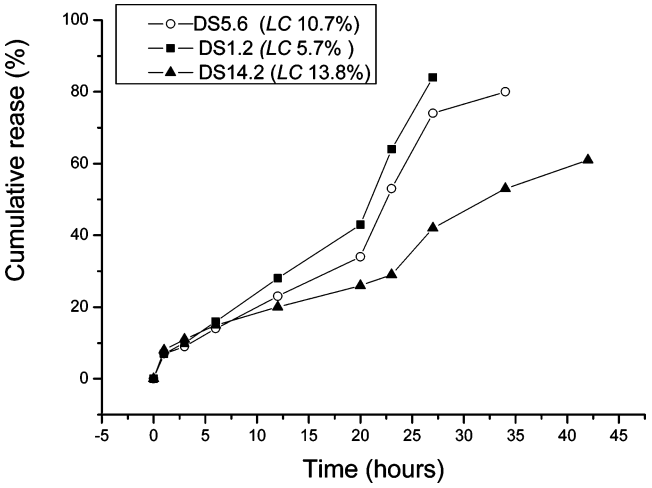


Figure 8. In vitro drug release profiles for PLCS with various DS in phosphate buffer (pH 7.4; 37 °C).

the shapes of self-aggregates with higher DS (5.6−14.2%) were approximately spherical, however, the nanoparticle based DS1.2 had irregular morphological features as shown in Figure 7a. An interesting phenomenon was that micelles of DS1.2 contracted obviously after encapsulating IBU though their morphologies were still irregular (Figure 7b). The nanoparticle sizes observed by TEM were all smaller than 50 nm (except DS1.2), and the values were smaller than the data determined by DLS obviously.

Release of IBU from PLCS Micelles. All samples showed a good drug control capacity; the release rate depended on the hydrophobic DS of the PLCS, pH, and temperature of the medium. As shown in Figure 8, the release of IBU was characterized with four phases in PBS medium at 37 °C. The first phase corresponded to an initial burst release for the first hour in all PLCS with different DS, followed by a near zero-order release for the second period of 20 h. During the first hour, as the drug concentration in medium was zero, a little IBU carried in the micelle corona quickly diffused into the medium. After 20 h (for DS1.2 and DS5.6) or 23 h (for DS14.2) of an almost constant release, a burst release occurred again. One possible explanation for these phenomena is that some significant dissociation of the micelles has happened and the structures of micelles become loose. The rapid release sustained 7 h (for DS5.6) and then slowed down. The rest of the loaded IBU was released in the final phase, which revealed that the micelle structure did not collapse. The reason was that the release through diffusion slowed down along with the increase of IBU concentration in the medium. On that basis, we can conclude that the mechanism of drug release in the first period (1−20 h) was mainly controlled by diffusion through the solid micelles. In addition, some interactions between IBU and PLCS probably existed, such as the hydrophobic attraction between IBU and palmitoyl groups and hydrogen bond interaction between the carbonyl group of IBU and the amino group and hydroxyl group of PLCS; all of these interactions could retard the drug release. It could be noticed that the release depended on hydrophobic DS markedly. Although the release of DS14.2 in the initial period was faster than that of DS5.6 and DS1.2 due to a higher loading capacity (LC), the release in the latter period was much slower than that of DS5.6 and DS1.2. Compared with DS5.6, the burst release time of DS14.2 came 3 h later, which might strongly suggested that the structure of PLCS with high DS was more compact and dissociated more slowly. Moreover, the more hydrophobic drug content in DS14.2 might reduce the level of hydration of matrix, which would result in slower release of drug too. The pH of the medium significantly affected the release rate; for instance, the drug release of DS5.6 in dilute acidic solution (pH 3.5) had a faster rate as shown in Figure 9. The reason can be explained by the same explanation of Lee's previous work,[11] which indicated that the CAC value of micelles based on hydrophobically

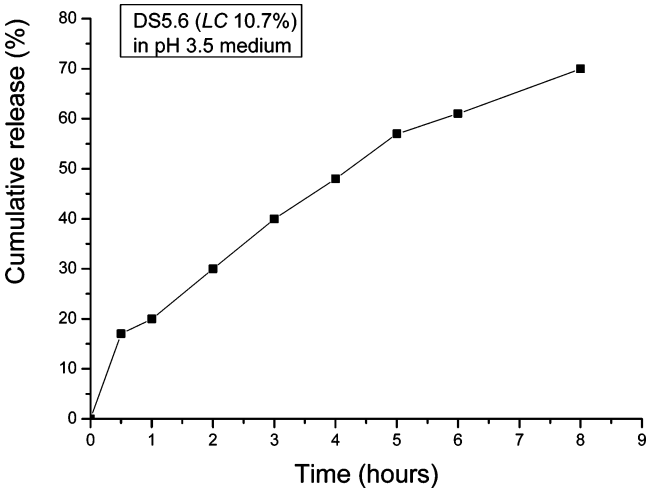


Figure 9. IBU release profile of DS5.6 (LC 10.7%) in dilute hydrochloride acid (pH 3.5) at 37 °C.

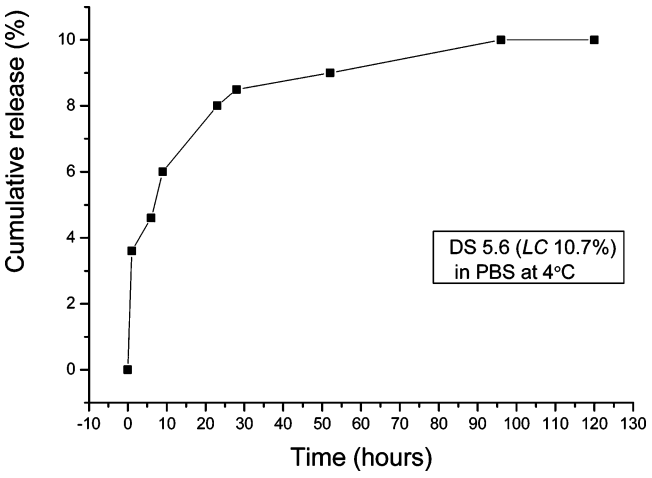


Figure 10. IBU release profile of DS5.6 (LC 10.7%) in phosphate buffer (pH 7.4) at 4 °C.

modified chitosan increased obviously in acidic medium, the reason being that, at low pH, amino groups of chitosan are well-protonated and inter- or intramolecular electrostatic increasing repulsions counteract the hydrophobic interactions, resulting in loose and large self-aggregates. However, the PLCS still tended to occur as self-aggregates (micelles) even in dilute acidic solution, just as Esquenet et al.[26] and Liu et al.[8] had demonstrated that self-aggregates of N-alkylated chitosan existed in 0.1−0.3 M CH_3COOH. The reason is that these water-soluble polymers bearing a small amount of highly hydrophobic groups show a strong tendency of associations.[26] On the other hand, the hydrogen bonds involving C(2)NH···O═C(IBU) and C(6)OH···O═C(IBU) were weakened greatly in acidic medium, so the release became much quicker. In addition, the release rate is affected greatly by medium temperature. Figure 10 shows that IBU-loaded PLCS released very slowly at 4 °C. From 1 to 96 h, the IBU released only reached 10% of the total amount loaded. However, the release nearly stopped from 96 h to day 13 (the data are not shown in Figure 10), and the release kept at a slow rate till day 24 when the released IBU came up to 30% of the total amount. The probable reason could be that the micelles became more stable at low temperature and the diffusion of IBU decreased in cold medium; in addition, the hydrogen bonds between IBU and PLCS became more stable at low temperature; all of these slowed the drug release.

Discussion

It is well-known that chitosan is insoluble due to its crystalline structure, and the crystalline structure can be destroyed by the introduction of hydrophilic groups or hydrophobic substitutes. Using palmitoyl groups as hydrophobic pendant chains, Uchegbu et al.[27] synthesized chitosan-based polysoap (quaternary ammonium palmitoyl glycol chitosan), and the polysoap could aggregate in aqueous solution and form either a necklace of intrachain spherical micelles or larger cyclindrical micelles. In fact, there are some ingenerate similarities among those chitosan-based polysoaps,[27] polymeric surfactants,[2,28,29] hydrogels,[30,31] vesicles,[32,33] and micelles.[6−13] Although micelles have definite CAC and polysoaps et al. appear to lack definite CAC,[27,34] they all show self-aggregate behavior for example, Martin et al. declared that physically cross-linked chitosan-based gels can be formed by exploiting the hydrophobic attraction.[31] Perhaps chitosan-based polysoaps et al. can be regarded as one kind of special micelles with indefinite CAC and very big sizes of self-aggregates. Now that many researchers[27,35−37]

(26) Esquenet, C.; Buhler, E. Phase behavior of associating polyelectrolyte polysaccharides. 1. Aggregation process in dilute solution. *Macromolecules* **2001**, *34*, 5287−5294.

(27) Uchegbu, I. F.; Sadiq, L.; Arastoo, M.; Gray, A. I.; Wang, W.; Waigh, R. D.; Schätzleinä, A. G. Quaternary ammonium palmitoyl glycol chitosan-a mew polysoap for drug delivery. *Int. J. Pharm.* **2001**, *224*, 185−189.

(28) Ramos, V. M.; Rodríguez, N. M.; Rodríguez, M. S.; Heras, A.; Agulló, E. Modified chitosan carrying phosphonic and alkyl groups. *Carbohydr. Polym.* **2003**, *51*, 425−429.

(29) Yoshioka, H.; Nonaka, K.; Fukuda, K.; Kazama, S. Chitosan derive polymer-surfactants and their micellar properties. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1901−1904.

(30) Noble, L.; Gray, A. I.; Sadiq, L.; Uchegbu, I. F. A non-covalently cross-linked chitosan based hydrogel. *Int. J. Pharm.* **1999**, *192*, 173−182.

(31) Martin, Lee.; Wilson, C. G.; Koosha, F.; Tetley, L.; Gray, A. I.; Senel, S.; Uchegbu, I. F. The release of model macromolecules may be controlled by the hydrophobicity of palmitoyl glycol chitosan hydrogels. *J. Controlled Release* **2002**, *80*, 87−100.

(32) Mcphail, D.; Tetley, L.; Dufes, C.; Uchegbu, I. F. Liposomes encapsulating polymeric chitosan based vesicles−a vesicle in vesicle system for drug delivery. *Int. J. Pharm.* **2000**, *200*, 73−86.

(33) Wang, W.; Mcconaghy, A. M.; Tetley, L.; Uchegbu, I. F. Controls on polymer molecular weight may be used to control the size of palimitoyl glycol chitosan polymeric vesicles. *Langmuir* **2001**, *17*, 631−636.

(34) Anton, P.; Laschewsky, A. Zwitterionic polysoaps with reduced density of surfactant side groups. *Macromol. Chem. Phys.* **1993**, *194*, 601−624.

have realized that self-aggregate behavior can be promoted by increase of hydrophobic DS and increase of hydrophobic group chain length, to obtain stable chitosan-based micelles, why not modify chitosan only by graft of hydrophobic groups? Because the reverse effects (introduction of hydrophilic will counteract the hydrophobic self-assembling ability) should be taken into account. Since previous reports have shown that water-soluble hydrophobically acylated chitosan can be prepared by chitosan grafting organic acid in the presence of EDC[11−13] or by chitosan reacting with acid anhydride,[38] in this paper, the PLCS were synthesized following the method of Hirano with modification in view of EDC not owning the properties of wide availability and low cost. The PLCS micelles based on only hydrophobic modified chitosan show high hydrophobic-drug loading capacity due to their compact hydrophobic cores; moreover, the PLCS micelles can sustain model drug IBU release for at least 40 h, while chitosan-based hydrogels sustained less than 5 h.[30,32]

Since PLCS can be dissolved in water easily, but cannot be dissolved in any organic solvent except DMSO, a distinctive drug encapsulating method fitting PLCS micelles was carried out. In fact, this drug encapsulating method is very interesting; for example, as the hydrophobic cores of PLCS micelles can capture small-sized hydrophobic solids, so some water-insoluble and organic solvent almost-insoluble drugs such as the anticancer drug camptothecin can be solubilized by these micelles, some similar work having been done successfully.[39] The drug loading capacity was determined by indirect measurement, as the PLCS micelles cannot be destroyed utterly by any organic solvent. It was difficult to measure the total loading drug through the dissociation of the micelles, so the test method was different from the general method appropriate for those block copolymer micelles. Sometimes, this indirect measurement is more simple and convenient than direct measurement especially when the loaded drug cannot release utterly by destroying the micelles.

It is interesting that the release is accelerated greatly by low pH or high temperature. The acid sensitivity of PLCS micelles can be explained by PLCS's being cationic polyelectrolytes and their self-aggregation's being affected by pH significantly. In acidic solution, amino groups of PLCS are well-protonated and the electrostatic repulsion increases obviously, which counteracts the hydrophobic aggregation and results in loose or lax micelles with big sizes and great CAC values, so the drug sustaining ability decreases obviously. However, this accelerating drug release in a lower pH environment is an advantage in the view of tumor targeting, because it may allow more drug release at the solid tumor where local pH is reported to be 1 order lower than that of normal tissue.[40] In the field of cancer drug delivery, the thermoresponsive property of PLCS micelles is also an advantage. It can be expected that the drug release can be controlled well by controlling the temperature of the target site, because the solid tumor has higher temperature than normal tissue generally. Another advantage of the PLCS micelles is that they have an initial little burst release, so the released drug can quickly reach an effective drug concentration. Although the second burst release is a disadvantage, the release time can continue for 40 h; such a prolonged period should be satisfying for some long-circulation desires. The PLCS micelles will be further used to deliver bioactive macromolecules such as protein and DNA; and some initial works for carrying of DNA revealed many attractive features.

Conclusion

Water-soluble *N*-palmitoyl chitosan (PLCS) was synthesized by swollen chitosan coupling with palmitic anhydride in dimethyl sulfoxide (DMSO), which formed novel polymer micelles after being dissolved in pure water. Ibuprofen (IBU) as hydrophobic model drug was encapsulated by the PLCS micelles. IR, ^{1}H NMR, and TEM have demonstrated that some reactions exist between the IBU molecule and polymer molecule in IBU-loaded PLCS. The study of drug release in vitro shows that the release slows down with increase of DS of PLCS and the release is accelerated significantly by the decrease of pH and the rise of temperature.

Acknowledgment. Financial support (30270393) from the National Science Foundation of China is gratefully acknowledged.

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(35) Yang, Y. J.; Engberts, J. Fluorescence spectroscopic study of the formation of hydrophobic microdomains in aqueous-solutions of poly(alkylmethyldiallylammonium bromides). *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 384−386.

(36) Wang, G. J.; Engberts, J. Fluorescence spectroscopic study of the aggregation behaviour of non-cross-linked and cross-linked poly-(alkylmethyldiallylammonium bromides) having decyl, octyl, and hexyl side chains in aqueous solution. *Langmuir* **1996**, *12*, 652−656.

(37) Mccormick, C. L.; Chang, Y. Water-soluble copolymers 58. Associative interactions and photophysical behaviour of amphiphilic terpolymers prepared by modification of maleic-anhydride ethyl vinyl ether copolymers. *Macromolecules* **1994**, *27*, 2151−2158.

(38) Hirano, S.; Yamaguchi, Y.; Kamiya, M. Water-soluble *N*-(*n*-fatty acyl) chitosans. *Macromol. Biosci.* **2003**, *3*, 629−631.

(39) Jiang, G. B. Nanosize $CaCO_3$ being encapsulated by chitosan-based micelles and being solubilized in water. Unpublished results.

(40) Hoes, C. J. T.; Boon, P. J.; Kaspersen, F.; Feijen, J. Design of soluble conjugates of biodegradable polymeric carries and adriamycin. *Makromol. Chem., Macromol. Symp.* **1993**, *70*, 119−136.