Preparation and Characteristic of Lactose-Oleoylchitosan and the Application of Its Self-Aggregates as Drug Delivery System

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ABSTRACT: Amphiphilic polymers lactose-oleoylchitosan (Lac-OCH) with different degree of substitution (DS) of lactose were prepared. The chemical structure of the new chitosan derivative was tested and verified. The rheological features including solubility and viscosity of Lac-OCH were investigated. The introduction of hydrophilic group lactose could improve the solubility of the polymer and Lac-OCH was soluble in acetic acid solution under pH 7.0. The viscosity of Lac-OCH decreased a little along with the increasing of DS of lactose. Lac-OCH with high DS, middle DS, and low DS of lactose possessed small critical aggregation concentration value, and the critical aggregation concentration value rised along with the increasing of DS of lactose. However, the affect was not obvious. In brief, the CAC values were 0.0325, 0.0340, and 0.0344 mg/mL corresponding to the samples of low DS, middle DS, and high DS. Lac-OCH, obtained by hydrophilic modified using lactose, could also form self-assembled nanoparticles by oil/water (O/W) emulsification method comparing with OCH. The Lac-OCH nanoparticles showed dense, axiolitic texture, and the average diameter was approximate 200 nm. The sustained-release characteristics of Lac-OCH nanoparticles were studied using Doxorubicin as model drug. The results revealed the promising potential of amphiphilic Lac-OCH as drug carrier. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3359–3367, 2011

Key words: amphiphilic polymers; lactose-oleoylchitosan (Lac-OCH); self-assembly; nanoparticles; drug delivery system

INTRODUCTION

Polymeric amphiphiles with both hydrophobic and hydrophilic segments have been recognized as one of the most promising of antitumor drug deliveries owing to their self-assembled capability. The self-assembled micelles in aqueous solution possessed the core-shell architecture that composed of a hydrophobic inner core serving as storage tank for drugs and a hydrophilic outer shell which provides a stabilizing interface between the micelle core and the aqueous environment.[1] These self-assembled nanoparticles, used as drug delivery system, could protect the activity of the drugs, reduce the unwanted toxic side effects, and enhance the therapeutic index of the drugs. Recently, various materials have been exploited for amphipathic polymers.[2,3] Among them, chitosan has attracted significant attention as biodegradable polysaccharide.

Chitosan, a native cationic polymer consisting of D-glucosamine and *N*-acetyl-D-glucosamine[4,5] linked by β-(1→4) glycosidic bond,[6–8] is harvested from the exoskeleton of crustaceans and insects as chitin and goes through alkaline deacetylation.[9] Chitosan is known to be a favorable pharmaceutical material because of its excellent biocompatibility, biodegradability, low immunogenicity, and biological activities. Apart from this, the positively charged amine groups of chitosan would be expected to adhere to the negatively charged cell surface,[10–13] facilitating the penetration of chitosan across the cell membrane. Additionally, the reactive amine groups located on the backbone of chitosan allow chemical modifications to control its physical properties.

There have been numerous reports on amphiphilic chitosan derivatives and their utilization as delivery carriers.[14–17] Hydrophobic chitosan derivatives modified by long chain fatty acyl have shown out huge potential application in drug delivery systems and have been investigated due to their strong property of forming self-assembled nanoparticles, for example, cholesterol-modified *O*-carboxymethyl chitosan,[17] linoleic-acid modified carboxymethyl-chitosan,[18] and stearic acid-grafted chitosan oligosaccharide.[1] Oleoylchitosan (OCH),[19] which we formerly prepared, had the similar potential capability of self-aggregation behaviors in aqueous solution. Furthermore, the oleoyl chain, possessing the lipophilic character, had the favor to contact with

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phospholipids bilayer of membrane and was likely to enter cell.[20] Now to exploit a novel liver target drug carrier, OCH was modified by lactose. It was well known that the hepatic targeted drug delivery systems mainly included passive targeting delivery system and active targeting delivery system. Liposome, nanocapsules, emulsion, and cholate were the common materials for drug carriers belonging to the former, the latter mainly depended on the hepatic asialoglycoprotein receptor (ASGP-R) or the mannose receptor (MR) such as lactose modified chitosan,[7,21,22] galactosylated chitosan.[23,24] Consequently, Lac-OCH would not only own the potential passive targeting quality as nanoparticles profiting from the long chain fatty acyl based on the permeability and retention effect (EPR), but also the active targeting quality due to the galactose from lactose which could be identified by ASGP-R located on the liver parenchymal cells.[21,25]

In this article, chitosan was first hydrophobically modified by oleoylchloride, which provided a hydrophobic function, then Lac-OCH was synthesized by reacting OCH with the hydrophilic radical lactose through the reaction between the aldehyde group from the lactose and the amine group belonging to OCH. The physicochemical characteristics and its potential applications in drug delivery system were investigated. Lac-OCH, obtained by hydrophilic modified using lactose, could also form self-assembled nanoparticles by O/W emulsification method comparing with OCH. This study was aimed to develop a novel chitosan derivative in the basis of OCH, which hoped to be applied as hepatic target drug carrier.

MATERIALS AND METHODS

Materials

Chitosan (87% deacetylation degree, M_w = 35 kDa) was supplied by Haili Biological Products Co. Ltd. (China). Oleic acid was purchased from Tianjin Chemical Reagent Factory (China). Lactose and sodium tripolyphosphate (TPP) were obtained from Tianjin Beifang Tianyi Chemical Reagent Factory (China). Potassium borohydride (KBH_4) was purchased from Tianjin JinKe Chemical Industry Research Institute (China). Pyrene was purchased from Sigma Chemicals. Doxorubicin was obtained from Zhejiang Haizheng Co. Ltd. (China). All other chemicals used in this study were of analytical grade.

Synthesis of Lac-OCH

Lac-OCH conjugate was synthesized via two reaction steps: (1) the preparation of OCH and (2) conjugation of lactose and OCH.

OCH was prepared by reacting chitosan with oleoylchloride according to the method proposed by Zong et al.[26]

Lac-OCH was synthesized by reductive amination of OCH and lactose using KBH_4 according to Yalpani et al.[27] Briefly, 0.3 g OCH and 2.0 g lactose were dissolved in 20 mL solutions of methanol and 3% (v/v) acetic acid mixture (1 : 2) (pH 4.5), into which 0.3 g KBH_4 was added gradually after 4 h. The solution were stirred at room temperature for 3 days, and the resultant product was gained by freeze-dried after exhaustively dialyzed against triply distilled water for 5 days in a dialysis bag (molecular weight cut off limit = 8000–14,000). Additionally, Lac-OCH with different DS of lactose were prepared by controlling the feed ratio of OCH to lactose.

FTIR and ^{1}H-NMR study of Lac-OCH

FTIR spectroscopy and ^{1}H-NMR spectroscopy were adopted to confirm the particular chemical structure of Lac-OCH.

The FTIR spectrum of Lac-OCH was recorded by Nicolet 380 Fourier Transform Infrared Spectrometer (Thermo Electron Corporation) at room temperature. Lac-OCH was mixed with KBr and pressed to plate for measurement. Data analysis was carried out using Omnic 8.0 (Windows xp).

The ^{1}H-NMR spectrum of Lac-OCH was measured using digital superconducting magnetic resonance spectrometer (600 MHz, Bruker, Switzerland). The sample was dissolved in D_2O to gain a concentration of 30 mg/mL.

The solubility of Lac-OCH

The solubility of Lac-OCH was evaluated from the turbidity based on the method of Sashiwa and Shigemasa.[28] The sample was dissolved in 0.5% hydrochloric acid solution. The transmittance of the solution at different pH which was adjusted by 10% NaOH was determined by UV spectrophotometer (T6, Beijing Purkinje General Instrument Co. Ltd.) at 600 nm using quartz cell with optical path length of 1 cm.

The viscosity of Lac-OCH

The viscosity of Lac-OCH was assayed referring to Zhao et al.[29]: Lac-OCH was dissolved in 0.5% acetic acid solution (pH 1.7). A 15 mL pure solvent or Lac-OCH solution at different concentrations were moved into the Ubbelohde viscometer which was vertically fixed in the thermostatic water bath at 25°C. The lowering time of the sample was

accurately recorded using a second chronograph. Each measurement was repeated three times.

Relative viscosity (η_r) is the ratio of the viscosity of the Lac-OCH solution to that of the pure solvent ($\eta_r = t/t_0$, where t and t_0 are the lowering times of the Lac-OCH solution and the pure solvent, respectively).

Measurement of CAC for Lac-OCH

The CAC was investigated using pyrene as fluorescence probe with the purpose of proving its potential self-assembly property.[30] In brief, pyrene, dissolved in methanol, was added into the test tubes and evaporated to remove the organic solvent; then, 5 mL Lac-OCH solution at different concentrations varied from 0.0001 to 2 mg/mL were added into test tubes. The final concentration of pyrene in each sample solution was $2 \times 10^{-6}M$. The pyrene and Lac-OCH mixtures were equilibrated at 65°C water bath for 3 h and then at 37°C with gentle shaking overnight before detection by fluorescence spectrophotometer (HITACHI F-4600, Hitachi High-Technologies Corp., Tokyo, Japan). All samples were excited at 343 nm, and the emission spectra were recorded in the range of 360–500 nm, with excitation and emission slit openings were both 2.5 nm, separately.

Preparation and characterization of Lac-OCH nanoparticles

Lac-OCH self-assembled nanoparticles were prepared by O/W emulsification method referring to Chen.[31] In general, 24 mg Lac-OCH was suspended in 12 mL 0.5% acetic acid solution, then 0.36 mL methylene chloride was added to the solution followed by sonication in ice-bath with a probe-type sonicator (BILON92-II L, Shanghai Bilon Instruments Co. LTD., Shanghai, China) setting power output 40 W. This procedure continued for 20 min (active every 2 s for 5 s duration) to obtain clear solution. The nanoparticles suspension was under vacuum for 2 h to remove the residual organic solvent. TPP in water solution was added into the suspension under stirring, conducing to the formation of nanoparticles. The DOX-loaded nanoparticles were formed similarly to blank nanoparticles described above, except that DOX was added to the solution. The final blank nanoparticles suspension and DOX-loaded Lac-OCH nanoparticles suspension were stored in dark for later detection. It was worth noting that all procedures were carried out in the absence of light to avoid photodegradation of DOX.

The nanoparticle morphology was examined by TEM (JEM-100SX, Japan). Samples were placed onto copper grid covered with nitrocellulose. They were dried at room temperature, and then were observed using TEM after negative staining with the aqueous solution of sodium phosphotungstate.

Evaluation of DOX encapsulation and DOX release

The encapsulation efficiency was calculated based on the percent ratio of the quantity of DOX entrapped into the nanoparticles to the whole quantity added. The method was as follows: the DOX-loaded Lac-OCH nanoparticles suspension was centrifuged at 14,000 × g for 30 min. Supernatant DOX concentration was calculated by fluorimetry with excitation and emission wavelength at 498 and 593 nm, excitation and emission slit openings 10 and 2.5 nm, respectively. All measurements were performed in triplicate.[11] Encapsulation efficiency was calculated as follows:

$$\text{DOX encapsulation efficiency} = \frac{\text{total DOX} - \text{free DOX}}{\text{total DOX}} \quad (1)$$

To study the drug release behavior of Lac-OCH nanoparticles, 3 mL DOX-loaded Lac-OCH nanoparticles suspension was placed in dialysis bag (MWCO 14,000) submerged in 40 mL acetate buffer of 0.1M. The system was holded at 37°C with continuous shaking at 60 rpm in absence of light for several days. Three milliliter of sample medium was taken out from the system at scheduled time intervals and replaced by the equivalent quantity of fresh acetate buffer to maintain the original volume. The concentration of the released DOX was determined by fluorimetry as described above. The DOX release experiments were performed in triplicate.

Statistical analysis

The data were showed as mean ± standard deviation. Difference between groups was evaluated using independent samples T-test. P value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Synthesis of Lac-OCH

Lac-OCH was synthesized according to the procedure exhibited in Figure 1. Lactose is a disaccharide consisting of galactose and glucose. Amine groups of OCH were reacted with aldehyde group of glucose from lactose; then, the Schiff's base already formed were reduced by KBH_4.[32] Therefore, the resultant product Lac-OCH possessed two new groups which were oleoyl chain and lactose compared with chitosan. The former supplied hydrophobic function, and the latter retained its effective structure which

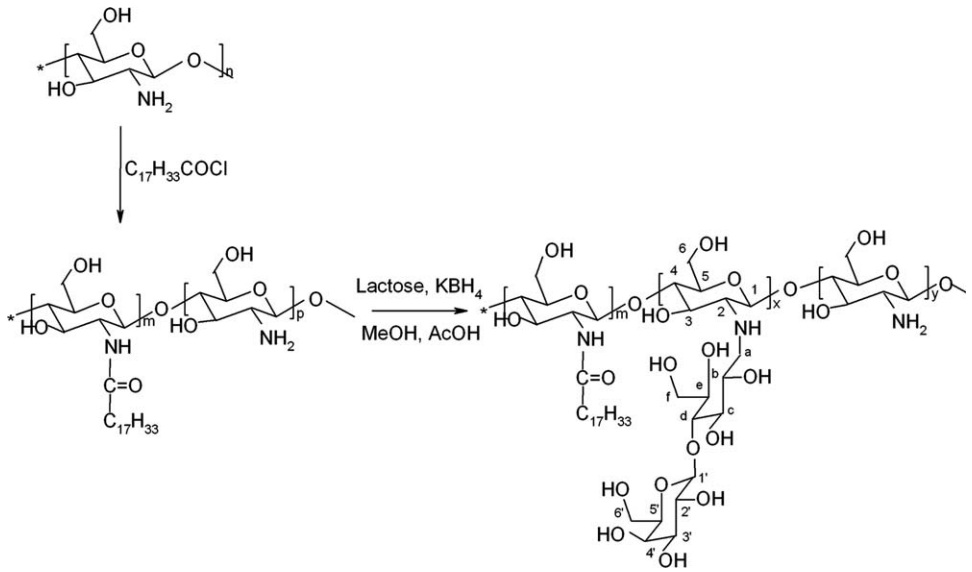

Figure 1 Synthetic procedure of Lac-OCH.

could be recognized by the ASGP-R and could be internalized by liver parenchymal cells.[33,34] Additionally, Lac-OCH with different DS of lactose were prepared and the substitution degree was calculated by elemental analysis. The DS of oleoyl group was 1.7%. The DS of lactose were 1.3% (low DS), 7.8% (middle DS), and 15.3% (high DS), respectively.

The FTIR spectroscopy and ^{1}H-NMR spectroscopy

The infrared spectra of chitosan, OCH and Lac-OCH are shown in Figure 2. In the FTIR of chitosan, distinctive absorption bands appear around at 3429 cm^{-1} (OH, NH_2), 2917 cm^{-1} (δ CH_2), 2872 cm^{-1} (δ CH_2), and 1642 cm^{-1} (ν C = O). Compared with chitosan, the spectrum of OCH exhibited some alterations: the peaks at 2920 cm^{-1} (δ CH_2) and 2850 cm^{-1} (δ CH_2) were stronger and sharper, the prominent band at 1658 cm^{-1} (ν C = O) was observed, the peak at 1543 cm^{-1} (δ *N*-H of amide II) was clear after oleoyl substitution. These results confirmed that chitosan was substituted by oleoyl.[35] Compared with the FTIR spectrum of OCH, the spectrum of Lac-OCH exhibited tiny alterations: the peak at 1380 cm^{-1} (δ CH_2) had a relatively little intensive trend after OCH modified by lactose.

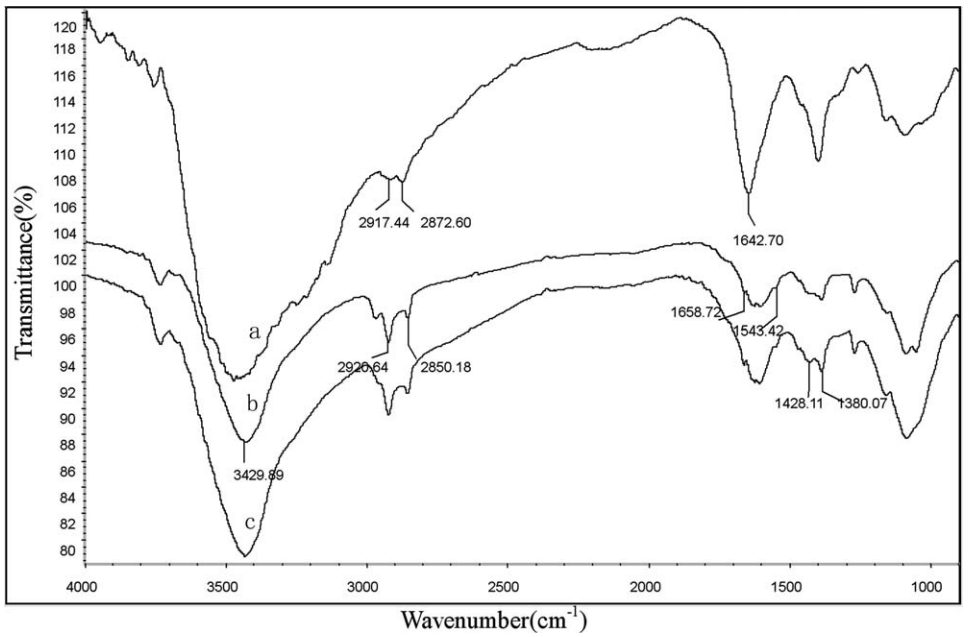

Figure 2 FTIR of chitosan, OCH and Lac-OCH. (a) chitosan; (b) OCH; (c) Lac-OCH.

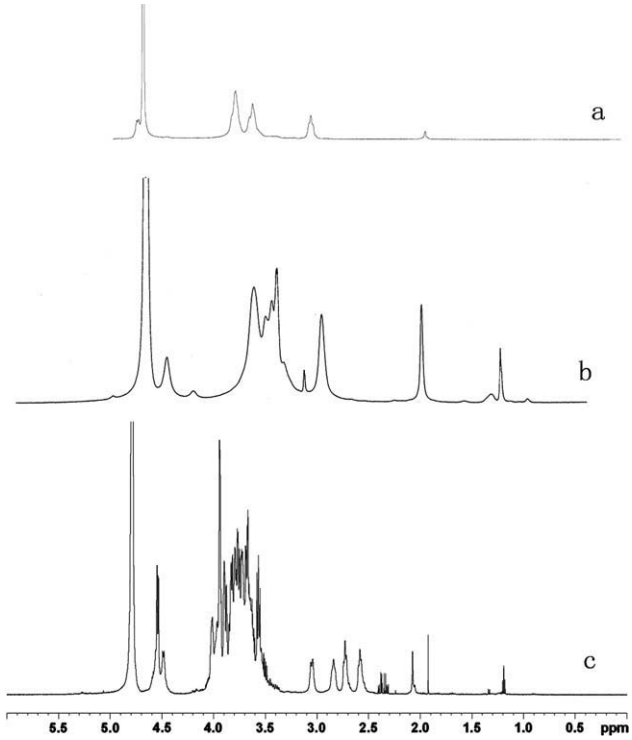

Figure 3 ^{1}H-NMR spectra of (a) chitosan; (b) OCH; (c) Lac-OCH.

^{1}H-NMR spectra were used to confirm the structure of Lac-OCH. Figure 3 showed the ^{1}H-NMR spectra of chitosan, OCH, and Lac-OCH.

The characteristic peaks of chitosan were as follows: ^{1}H-NMR (D_2O/CD_3COOD) $\delta = 1.9$ ($NHCOCH_3$), $\delta = 2.95$ (H2), $\delta = 3.6$–3.8 (H3, H4, H5, H6), $\delta = 4.7$(H1).[36] The proton assignment of OCH: $\delta = 1.2$ (CH_3 of oleyl), $\delta = 1.9$ ($NHCOCH_3$), $\delta = 2.95$ (H2), $\delta = 3.15$ (CH_2 of oleyl), $\delta = 3.5$–3.8 (H3, H4, H5, H6), $\delta = 4.8$ (H1). The new peak at 1.2 was mainly due to characteristic methyl protons of the reacted chitosan with olenic acid.[19] The ^{1}H-NMR spectrum of Lac-OCH showed new signals at $\delta =$ 2.6–2.8, it was assigned to Ha. The other protons of Lac-OCH were assigned to $\delta = 4.6$–4.8 (H1), $\delta = 3.0$ (H2), $\delta = 3.5$–4.0 (H3, H4, H5, H6, H2′, H3′, H4′, H5′, H6′, Hb, Hc, Hd, He, Hf), $\delta = 2.0$ ($NHCOCH_3$), $\delta = 1.2$ (CH_3 of oleyl).

The solubility of Lac-OCH

The solubility curves of Lac-OCH were displayed in Figure 4. Lac-OCH with different DS had similar solubility trend. The samples were soluble in acetic acid solution under pH 7.0, whereas they were insoluble when pH > 7. It was worth mentioning that the solubility had improved along with the increasing of DS of lactose. To put it more specifically, samples of high DS and middle DS rised about 10 and 9 unit in transmittance compared with the low DS, respectively.

The results could be explained as follows. The remaining primary amino groups of Lac-OCH after oleoyl substitution and lactose substitution could still combine with hydrogen proton in the acetic acid solution under pH 7,[28] converting Lac-OCH high polymer to positively charged polyelectrolytes which could be soluble. This role were not allowed to be ignored. On the other hand, after the hydrophilic group lactose was introduced into OCH, it also improved the solubility of the polymer just as Donati have reported that the reductive amination reaction between the aldehyde group of lactose and the amino group of the glucosamine residues of chitosan obtained a highly soluble engineered polysaccharide (chitlac).[22] In summary, the protonation of amino and the introduced lactose into the polymer could be the primary causes.

The viscosity of Lac-OCH

Figure 5 showed the viscosity details of Lac-OCH. The contribution of lactose was that it could reduce viscosity of the polymer. With increasing of DS of lactose, a degressive trend about the viscosity was disclosed.

Compared with chitosan, the main influence factors about the viscosity of Lac-OCH were the two new groups oleoyl and lactose. Being a long hydrophobic chain, oleoyl chain played the role of junction zones, which linked multiple polymer molecules together in intermolecular aggregate.[19] Consequentially, this would lead to increase of viscosity. On the other hand, the introduction of lactose, a highly hydrophilic and flexible arms, eventually led to decrease of the viscosity of the polymer.[22] Put concretely, when the polymer dissolved in solution, the hydrophobic chain tended to be self-associated into

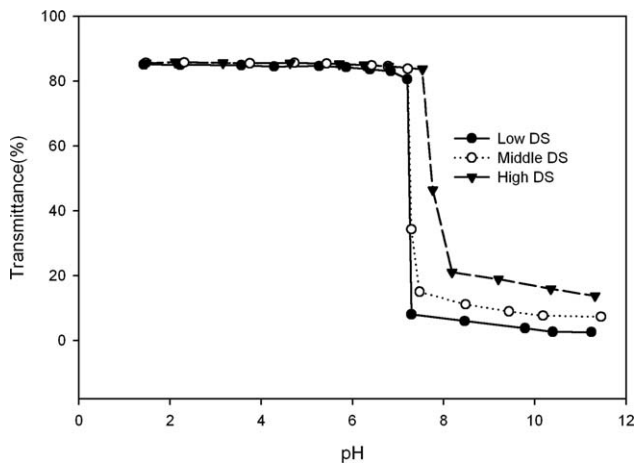

Figure 4 Solubility of Lac-OCH with different DS based on pH.

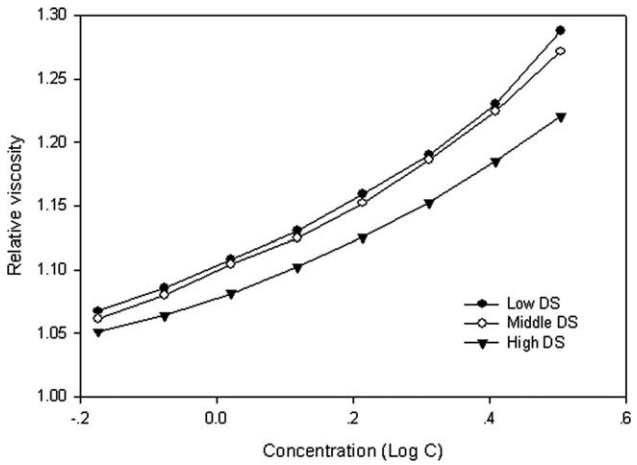

Figure 5 Viscosity of Lac-OCH with different DS.

a hydrophobic core, while the hydrophilic lactose would play the part of surrounding the core.[37] This could help to reduce the contact between hydrophobic chain and water environment, as a result it would reduce the viscosity of the polymer. In our research, samples of the same content of oleoyl, but with different DS of lactose were designed, and the influence of lactose on viscosity was investigated.

Self-assembly behavior of Lac-OCH

The aggregation behavior of Lac-OCH in solution was monitored by fluorometry in the presence of pyrene as fluorescent probe. As hydrophobic molecule, pyrene is apt to concentrate in the hydrophobic domains of amphipathic molecules.[8,38] The intensity ratio of the third peak (at 384 nm) and the first peak (at 373 nm) I_3/I_1 is regularly utilized to study the change in environmental polarity based upon the association of amphipathic molecules in solutions.[39,40] As soon as the micellization of Lac-OCH, the hydrophobic microdomain attributed to oleoyl chain will be occupied by pyrene,[1] causing the increasing of the intensity ratio I_3/I_1. Hence, the CAC of Lac-OCH could be determined by this way.

Figure 6 showed the fluorescence spectra of pyrene in Lac-OCH solution in 0.5% acetic acid. Figure 7 showed the plots of I_3/I_1 versus log C of Lac-OCH. The CAC value of Lac-OCH was 0.0340 mg/mL. This phenomenon suggested the intermolecular hydrophobic interactions between *N*-acetyl-D-glucosamine (GlcNAc) groups,[41] as well as the intramolecular and intermolecular hydrophobic interactions between oleoyl chain of Lac-OCH.

The CAC of different DS of Lac-OCH were also discussed, as showed in Table I. It was just as conjectured, the sample of high DS had relative larger CAC value, and the sample of low DS possessed relative smaller CAC value. To put it simply, it had

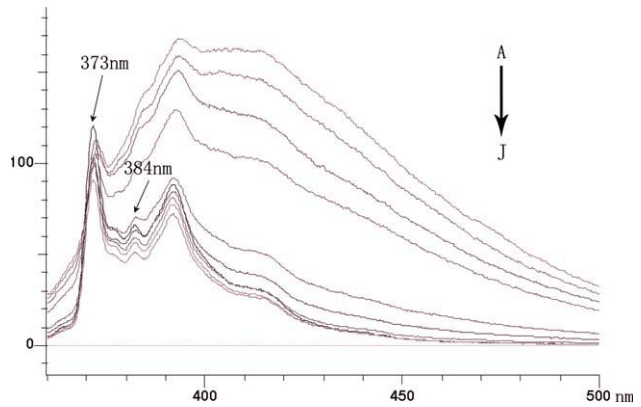

Figure 6 Fluorescence spectra of pyrene ($2 \times 10^{-6}M$) in acetic acid solution in the presence of increasing concentrations of Lac-OCH. (A) 2.0, (B) 1.5, (C) 1.0, (D) 0.5, (E) 0.25, (F) 0.1, (G) 0.025, (H) 0.01, (I) 0.001, (J) 0.0001 mg/mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

been found that the CAC value would decreased along with the increasing ratio of hydrophobic group.[42,43] The ratio of hydrophobic group to hydrophilic group would change after lactose was introduced into chitosan derivatives, the decrease of hydrophobic group proportion led to the increasing CAC value.

Morphology of nanoparticles

TEM image of Lac-OCH (middle DS of lactose) nanoparticles was showed in Figure 8. The Lac-OCH nanoparticles showed dense, axiolitic texture, and the average size of the dried particles was about 200 nm. We could suggest that Lac-OCH could form nanoparticles. Additionally, we could adjust the ratio of oleoyl chain to the lactose groups to get an

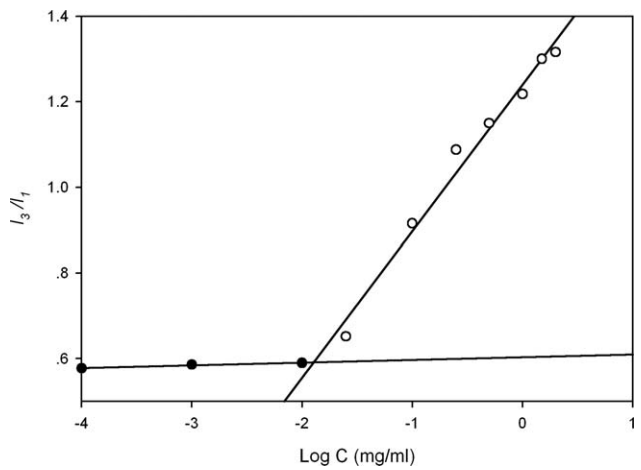

Figure 7 Fluorescence intensity ratio variation of I_3/I_1 for pyrene emission against the concentration of Lac-OCH. CAC = 0.0340 mg/mL.

TABLE I
The CAC and Encapsulation Efficiency of Lac-OCH with Different DS

Sample	Molar ratio of lactose to amino-group of chitosan	DS of lactose (%)	CAC value (mg/mL)	*Encapsulation efficiency (%)*
Low DS	1	1.3	0.0325 ± 0.00010	54.81 ± 0.16
Middle DS	3	7.8	0.0340 ± 0.00015	47.97 ± 0.70
High DS	6	15.3	0.0344 ± 0.00008	47.98 ± 0.86

ideal proportion which could form appropriate nanoparticles depending on the previous work.[19]

Release behavior of DOX from the nanoparticles

The encapsulation efficiency of Lac-OCH nanoparticles was also showed in Table I. As it illustrated, all Lac-OCH with different DS of lactose achieved approximate encapsulation efficiency of 50%; however, with the increasing of DS of lactose, there was a slight downtrend about the DOX encapsulation efficiency. This result may be explained as follows: the accession of lactose weakened the hydrophobic effect and when the quantity of lactose reached a certain level, the effect would not increase.

Release researches were performed in acetate buffer (pH 3.8 and 5.0). This medium was chosen because DOX is maximally stable at pH 3–5. Moreover, at higher pH there might be problems of fluorescence quenching or interference for the quantification of released DOX.[11]

Figure 9 showed the drug release result of Lac-OCH depending on different DS. All of the release patterns displayed an initial burst for 3 h ahead, then sustained release following up to 72 h, and the cumulative release of DOX in acetate buffer were 50.27%, 48.54%, and 45.38% corresponding to the sample of high DS, middle DS, and low DS, respectively. The burst effect was probable owing to the following reason: part of the drug was adsorbed onto the surface of nanoparticles or loosely encapsulated in the core. However, the nanoparticles might act as barrier against the release of DOX located in the hydrophobic core after 3 h. Since the tight structure of nanoparticles forming by amphipathic molecule, all the results revealed common feature. There was quite a bit of drug remaining in the core of nanoparticles, and the complete release of them needed further depolymerization or degradation of the Lac-OCH. In addition, the *in vivo* situation of Lac-OCH nanoparticles required further research.

The release case of DOX from Lac-OCH nanoparticles in acetate buffer at pH 3.8 and 5.0 were investigated and presented in Figure 10. The cumulative releasing of DOX from Lac-OCH nanoparticles in the buffer of pH 3.8 was quicker than that in pH 5.0 at the same time. The result was consistent with the result of Ye et al.[1] The faster drug release rate in lower pH medium could be contributed to two factors: the first is the looser nanoparticles structure, which caused by the stronger protonation of amino groups of Lac-OCH; the other is the higher solubility of DOX in lower pH.

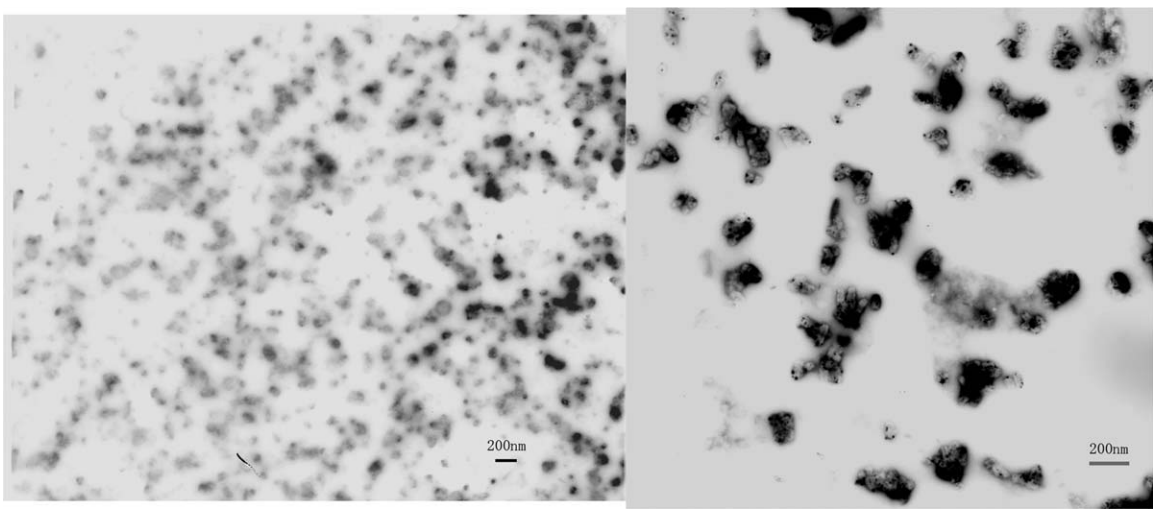

Figure 8 Transmission electron micrograph of Lac-OCH nanoparticles.

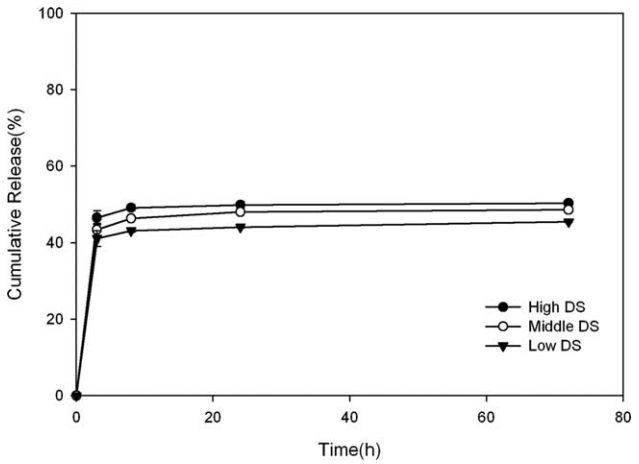

Figure 9 *In vitro* DOX release profile of different DS of Lac-OCH.

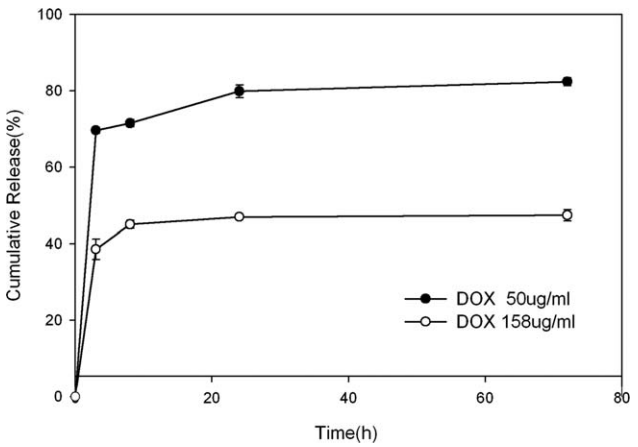

Figure 11 *In vitro* DOX release profile of Lac-OCH nanoparticles incorporating different amount of DOX.

Releasing behavior of DOX from Lac-OCH based on the drug loading content was showed in Figure 11. This result indicated the higher the drug content, the slower the drug releasing. Lac-OCH nanoparticles loading less DOX (50 μg/mL) showed burst release of 69.63% at 3 h, followed by an additional release of 12.75% over the next 3 days, whereas, the sample incorporating more DOX (158 μg/mL) showed release of 38.57% at 3 h, followed by an additional release of 8.82% over the next 3 days. This awoke us that suitable drug delivery system could be designed by means of controlling the drug content.

CONCLUSION

In this study, the novel material Lac-OCH was synthetized, the relevant physicochemical property and the possibility of its behavior as drug delivery system were discussed. The structure of Lac-OCH was proved by FTIR and ^{1}H-NMR. Lac-OCH was soluble in acetic acid solution under pH 7.0. The viscosity of Lac-OCH decreased a little along with the increasing of DS of lactose. Lac-OCH with middle DS of lactose possessed a CAC value of 0.0340 mg/mL. Lac-OCH could form self-assembled micelles in aqueous solution benefiting from the hydrophobic oleoyl group, what's more, the content of lactose in Lac-OCH had little effect on CAC value. The Lac-OCH nanoparticles were prepared by means of O/W emulsification method and showed dense, axiolitic texture. DOX was physically entrapped into the self-assembled Lac-OCH nanoparticles by sonication. These results revealed the promising potential of amphiphilic Lac-OCH as sustained-release carrier. In addition, the controlled-release behavior of Lac-OCH *in vivo* and its targeted function should be further studied.

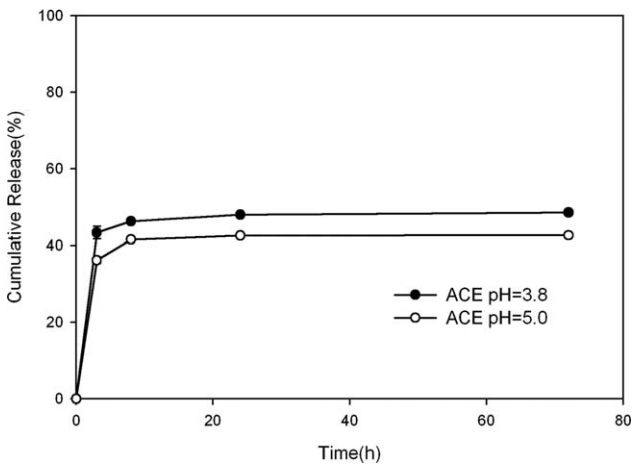

Figure 10 *In vitro* DOX release profile of Lac-OCH nanoparticles using dissolution medium with different pH.

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