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Amphiphilic Chitosan Derivatives-Based Liposomes: Synthesis, Development, and Properties as a Carrier for Sustained Release of Salidroside

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ABSTRACT: A novel amphiphilic chitosan derivative of *N*,*N*-dimethylhexadecyl carboxymethyl chitosan (DCMCs) was synthesized. The structure of DCMCs was confirmed via FT-IR and ^{1}H NMR, and the critical micelle concentration (CMC) was investigated by fluorescence spectroscopy. The results indicated that DCMCs has hydrophilic carboxyl and hydrophobic methylene groups and the CMC value was 23.00 mg·L^{-1}. The polymeric liposomes (DCMCs/cholesterol liposomes, DC-Ls) were developed, and its properties were evaluated. The DC-Ls exhibited multilamellar spheres with positive charge (+73.30 mV), narrow size distribution (PDI = 0.277), and good crystal properties. Salidroside was first to encapsulate into DC-Ls. Compared with traditional liposomes (phosphatidylcholine/cholesterol liposome, PC-Ls), DC-Ls showed higher encapsulation efficiency (82.46%) and slower sustained release rate. The in vitro salidroside release from DC-Ls was governed by two distinct stages (i.e., burst release and sustained release) and was dependent on the pH of the release medium. The case II transport and case I Fichian diffusion were the main release mechanisms for the entire release procedure. These results indicated that DC-Ls may be a potential carrier system for the production of functional foods that contain salidroside or other bioactive food ingredients.

KEYWORDS: *amphiphilic chitosan, liposomes, salidroside, sustained release, release mechanism*

INTRODUCTION

Because of a lipophilic and a hydrophilic group on the same molecules, polar lipids can be self-assembled and form self-organized traditional liposomes after interaction with water.[1] As an active agent's carrier, traditional liposomes have advantages, including biocompatible and biodegradable properties and targeting and protection of entrapped agents.[2] In addition, traditional liposomes are capable of entrapping both hydrophilic and hydrophobic agents.[3] Recently, traditional liposomes have been widely applied in pharmaceutical,[4] cosmetic,[5] nutrition, and food industries.[1,6,7] However, traditional liposomes exhibit notable defects on short circulation half-life in vitro, instability of the lipid, and a few certain chemical groups on the liposome surface. Hence, many efforts have been exerted to design sufficiently stable polymeric liposomes, which may be a promising carrier with good physical and thermal stability, excellent solubility, and high encapsulation efficiency.[8,9] For promoting the application of polymeric liposomes in the food industry, choosing the optimal food-grade biopolymers is a critical factor.

Chitosan (CS) is a natural polymer composed of randomly distributed β-(1−4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit).[10] CS has been widely used in several areas such as biomedical, pharmaceutical, and biotechnological fields as well as in the food industry because of its good properties such as nontoxicity, good biodegradability, and biocompatibility.[11−13] Among various food biopolymers for encapsulation and delivery systems, CS is one of the most popular polymers and has also been extensively investigated for its potential using in delivery of various nutrients.[14] However, CS is only soluble in aqueous acidic solutions below pH 6.5 but insoluble in neutral pH and physiological environments. Therefore, different chemical agents have been used to improve the CS solubility, and the formation of micelles has been confirmed. However, only a small section of the hydrophobic segment can conjugate to the CS backbone (substitution degree, DS < 10%) because of the limited solubility property.[15] Hence, the loading capacities of the micelles for hydrophobic bioactive agents are very low. Thus, CS derivatives were synthesized using the soluble CS (such as carboxymethyl chitosan, CMCs) as a necessary backbone.

Among these CS derivatives, amphiphilic CS derivatives with hydrophilic and lipophilic blocks have received extensive study.[15−17] The characteristics of amphiphilic CS derivatives are quite similar to that of polar lipids and surfactant complexes, which can form micelles via intra- or intermolecular association between hydrophobic moieties, and this process primarily minimizes interfacial free energy in contact with aqueous solvents.[13] Evidence has shown that amphiphilic CS derivatives have been applied to develop liposomes for drugs and genes delivery[8,9] because such polymeric liposomes exhibit higher stability in vivo/vitro and encapsulation efficiency.

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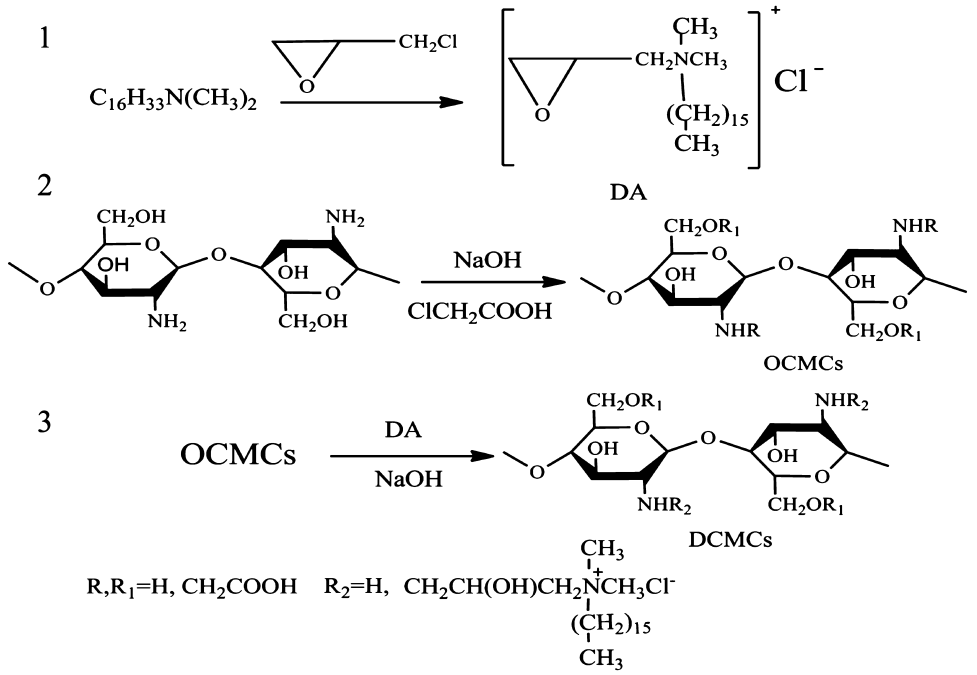

Figure 1. Schematic illustration of the synthesis process of DCMCs: (1) DA was synthesized using epoxychloropane and *N,N*-dimethylhexadecylamine; (2) OCMCs were synthesized using CS and monochloroacetic acid in an alkali environment; (3) DCMCs were then synthesized using OCMCs and DA in 2-propanol aqueous solution in an alkali environment.

However, to the best of our knowledge, the application of amphiphilic CS derivatives liposome in the food industry is rarely reported.

Salidroside has been widely used as a functional food for the various pharmacological actions such as antiaging, antioxidative, anticancer, and antidepressant activities.[18−20] Salidroside was widely applied in functional food industries in oral solution, powder, and capsule forms. The application of these forms has been significantly restricted because of the fluctuation of its concentration in blood, very high intake frequency, and poor oral bioavailability.[21,22] To overcome these drawbacks, traditional liposomes (phosphatidylcholine/cholesterol liposomes, PC-Ls) were developed to encapsulate salidroside.[21,22] However, PC-Ls suffer from various disadvantages such as instability in vivo/vitro environments and leaking of entrapped salidroside. On the basis of the advantages of amphiphilic CS derivatives, formulating a polymeric liposome by using amphiphilic CS derivatives for salidroside encapsulation may be an effective approach to overcome the above-mentioned problems.

In this study, the amphiphilic CS derivative (DCMCs) was synthesized. DCMCs were then used to develop liposomes with cholesterol (DC-Ls). As a functional food agent, salidroside was encapsulated first into DC-Ls. The physicochemical properties of DCMCs and DC-Ls were studied. Meanwhile, the encapsulation capacity and in vitro release properties of DC-Ls were also investigated.

EXPERIMENTAL PROCEDURES

Reagents and Materials. Salidroside (with 98.00% purity) was purchased from Dingsheng Pharmaceutical Technology Co., Ltd. (Hunan, China). Chitosan (CS) (300 KDa molecular weight, 90% deacetylation degree) was obtained from Lanji Technology Co., Ltd. (Shanghai, China). Epoxychloropane was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). *N,N*-Dimethylhexadecylamine was purchased from Aoke Industrial Co., Ltd. (Shanghai, China). Cholesterol (Chol) and pyrene (with 99.00% purity) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). All other chemical agents used were of analytical grade.

Preparation of DCMCs. Figure 1 illustrates the synthesis procedure of DCMCs. Epoxychloropane (10 mg) and *N,N*-dimethylhexadecylamine (4 mg) were added into a flask and were reacted at 60 °C for 2 h with stirring. The residual epoxychloropane was then removed via reduced pressure distillation. The hexadecyl dimethylammonium chloride (DA) production was recrystallized using acetone and was washed with ether. CS (5 g) was subjected to swelling by using NaOH solutions (75 mL, 50 wt %) for 24 h in a flask. Monochloroacetic acid (5 g, 53 mmol) was dissolved in 25 mL of 2-propanol and then added dropwise into the flask containing CS for reaction of 24 h at room temperature. The pH of the reacted systems was adjusted to 7 using HCl (2.5 M). The product of *O*-carboxymethyl chitosan (OCMCs) was filtered and washed with ethanol. The production was then dialyzed for 3 days and was subsequently freeze-dried. OCMCs (5 g) were dissolved in an 2-propanol aqueous solution (100 mL, 2-propanol/deionized water = 1/3, V/V). In addition, DA was added slowly. The mixture was trickled with 10 mL of NaOH solution (42%, w/w) and reacted at 50 °C for 24 h with stirring. Finally, the solution was dialyzed for 3 days and lyophilized and the DCMCs were then obtained.

Structure Characterizations of DCMCs. The structure of DCMCs was determined via FT-IR and ^{1}H NMR. The FT-IR spectra of CS, OCMCs, and DCMCs were obtained using KBr pellets on a spectrophotometer (Nicolet 5700, Thermo Electron Corporation, Waltham, MA, USA) in the region of 400−4000 cm^{-1}. The ^{1}H NMR spectra of DCMCs were obtained using a spectrometer operated at 500 MHz (Bruker DPX300, Bruker Corporation, Karlsruhe, Germany) with D_2O as the solvent.

Substitution Degree (SD) of OCMCs. The SD of OCMCs was measured via the conductometric method.[23] OCMCs (0.1 g) was dissolved in an HCl solution (100 mL, 0.05 M) with mild stirring. The pH was adjusted to 2.0−2.2 by adding NaOH solution (0.1 M). The OCMCs solution was then titrated with NaOH solution (0.1 M) to pH 11.0−12.0. The typical titration curve was calculated, and the SD of OCMCs was measured based on the following equation:

$$SD = (V_2 - V_1) \times DD/(V_3 - V_2) \quad (1)$$

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where SD is the OCMCs substitution degree and DD is the deacetylation degree of original CS. V_1, V_2, and V_3 are the consumed volume of NaOH in different linear sections of the titration curve.

Critical Micelle Concentration (CMC) of DCMCs. The CMC of DCMCs was investigated via fluorescence spectroscopy by using pyrene as a hydrophobic probe. A known volume of pyrene in acetone (6.0×10^{-6} M, 1 mL) was added to each of a series of 10 mL vials. Then 10 mL of various concentration of DCMCs (1.0×10^{-4}, 1.0×10^{-3}, 2.0×10^{-3}, 5.0×10^{-2}, 1.0×10^{-1}, and 1.0 mg·mL^{-1}) were added into each vial after acetone evaporation was complete. The fluorescence emission spectra were obtained using a fluorometer (F-4500, Hitachi, Tokyo, Japan) at an excitation wavelength of 339 nm, and the emission wavelength was 360−t450 nm for excitation spectra.

Preparation of DC-Ls. The DC-Ls were developed via thin-layer evaporation method.[9] DCMCs and cholesterol (Chol) (weight ratio 1/0.81, total lipids 90 mg) were dissolved in ethanol (15 mL) in a round-bottomed flask. The mixture was dried under reduced pressure by using an Eyela rotary evaporator (model N-1000; Eyela, Tokyo, Japan) at 40 °C under condition of N_2 environment to form a thin lipid film. The trace solvent was then removed by holding the lipid film at high vacuum overnight. The thin lipid film was hydrated by using deionized water (15 mL, with salidroside of 0.5 mg·mL^{-1}) and was then ultrasonicated using an ultrasonic processor (KQ-600B, Kun Shan Ultrasonic Instruments Co., Ltd., Jiangshu, China) at 25 °C for 30 min at 45 W to produce multilamellar liposomes (DC-Ls). For comparison, traditional PC-Ls were also prepared via the same procedures and conditions except egg yolk phosphatidylcholine (PC) replaced the DCMCs.

For verifying that DC-Ls have the capacity of forming multilamellar film structure, 25 mg of OA-Fe_3O_4 was also dissolved in ethanol (15 mL) with DCMCs and Chol, and other procedures were the same as that in the preparation of DC-Ls. The physical mixtures of DCMCs/Chol were prepared as follows: DCMCs/Chol (weight ratio 1/0.81) was mixed with chloroform, and the chloroform was then evaporated to obtain the dried physical mixtures of DCMCs/Chol.

Physicochemical Properties of DC-Ls. Transmission electron microscopy (TEM) images were obtained using JEOL (JEM-2010HR, Hitachi, Tokyo, Japan) with an operating voltage of 200 kV. X-ray diffraction (XRD) patterns were recorded using an XRD analyzer (D/max 2400, Rigaku Corporation, Tokyo, Japan) with a scattering angle range of 3−45°. DSC was performed using DSC equipment (Perkin-Elmer, Waltham, MA, USA) with a heating rate of 10 °C·min^{-1}. The average particle size was determined by a dynamic light scattering Zetasizer (Nano ZS90, Malvern Instruments, Worcester, UK). The ζ potential was measured using the Nano ZS90 zetasizer (DTS1060, Malvern Instruments) at 25 °C. The vibrating specimen magnetometer (VSM) (model 7407, Lakeshore, Westerville, OH, USA) was used to examine the magnetic properties of the DC-Ls.

Encapsulation Efficiency (EE) of DC-Ls. The Sephadex G_{25} column method was used to determine the salidroside EE in DC-Ls. A predetermined aliquot of salidroside loaded DC-Ls were first eluted through the Sephadex G_{25} column to remove free salidroside. The DC-Ls was dissolved in chloroform to destroy the liposomes structure. The trapped salidroside was extracted with deionized water and was then determined with high performance liquid chromatography (HPLC) (1100 series, Agilent Technologies Co., Ltd., Palo Alto, CA, USA) at a wavelength of 480 nm. The analytical column was a C_{18} column (5 μm, 4.6 mm × 150 mm, Waters Company, Milford, MA, USA). The mobile phase was a mixture of methanol/water (20:80, v/v) at a flow rate of 1.0 mL min^{-1}, and the injection volume was 20 μL at 25 °C. The EE of DC-Ls was determined using the equation below. EE test were performed in triplicate with data reported as the mean values.

$$\mathrm{EE}(\%) = (\text{salidroside loaded in DC-Ls/total salidroside added}) \times 100 \quad (2)$$

In Vitro Release of DC-Ls. First, 5 mL of salidroside loaded DC-Ls suspension were placed in dialysis bags and were suspended in 30 mL of simulated gastric fluid (SGF, pH 1.2), phosphate buffer saline (PBS, pH 7.0), and simulated intestinal fluid (SIF, pH 7.4), respectively. The release media was incubated at 37 ± 0.1 °C with stirring. At specified time intervals, 1 mL of the sample was taken and the same volume of fresh media was added to maintain a constant volume. The amount of salidroside release from DC-Ls was estimated via HPLC. All release tests were performed in triplicate, and the mean values were reported. The ingredients of simulated fluids were as follows:[24]

SGF (pH 1.2): NaCl (0.2 g), HCl (7 mL), and pepsin (3.2 g), pH was adjusted by NaOH to 1.2 ± 0.5.

SIF (pH 7.4): KH_2PO_4 (6.8 g), 0.2 N NaOH (190 mL), and pancreatin (10.0 g).

PBS (pH 7.0): 0.2 M KH_2PO_4 (250 mL), 0.2 M NaOH (145.5 mL), and H_2O (604.5 mL).

Release Mechanism of DC-Ls. The first-order, Higuchi, and Peppas models were used to fit the release data of salidroside from DC-Ls and were then used to investigate potential release mechanisms.

$$\text{first-order model: } \ln(1 - M_t/M_\infty) = -k_t \quad (3)$$

$$\text{Higuchi model: } M_t/M_\infty = k_t^{1/2} \quad (4)$$

$$\text{Peppas model: } \ln(M_t/M_\infty) = n \ln t + \ln k \quad (5)$$

where M_t/M_∞ is the fractional active agents release at time t, k is a constant incorporating the properties, and n gives an indication of the release mechanism. The correlation coefficient (R^2) is the linear relationship between salidroside release and time.

RESULTS AND DISCUSSION

Synthesis and Structural Characterization of DCMCs. A novel amphiphilically modified CS molecule with hydrophilic carboxyl and long hydrophobic methylene chains were synthesized. Detailed schemes for the DCMCs preparation are shown in Figure 1. FT-IR and ^{1}H NMR were performed to confirm the structural properties of DCMCs. The FT-IR spectra of CS, OCMCs, and DCMCs are shown in Figure 2A. In the FT-IR spectra of CS, the stretching vibrations at 3444.62 and 2927.73 cm^{-1} were attributed to the −OH and C−H groups, respectively. Meanwhile, the stretching vibration of C−O was found at 1092.96 cm^{-1}.[25] In the FT-IR spectra of OCMCs, the vibration absorption peaks were observed at 1575.94 and 1419.22 cm^{-1}, which indicates that carboxylate groups were successfully conjugated into CS.[26] In the FT-IR spectra of DCMCs, the characteristic stretching vibration absorption of long methylene chain was found at 2921.31 and 2853.49 cm^{-1}. Additionally, the appearance of new stretching vibration absorption at 721.55 cm^{-1} corresponds to the methylene planar rocking vibration. These results showed that the quaternary ammonium salt group was grafted into DCMCs. The DCMCs structure was further confirmed by the ^{1}H NMR spectra. In the ^{1}H NMR spectra of DCMCs (Figure 2B), the signal at δ 4.78, 1.72, 3.67, 3.65, 3.60, and 3.37 ppm were attributed to H-1, H-2, H-3, H-4, H-5, and H-6 of the D-glucosamine unit of DCMCs, respectively. The new signals at δ 0.91, 1.32−1.40, 3.25, 3.27, and 3.90 ppm were attributed to H-e, H-d, H-b, H-a, and H-c, which correspond to the proton assignment of long-chain alkyl of quaternary ammonium salt. The results of FT-IR and ^{1}H NMR confirmed that DCMCs were successfully synthesized, which was consistent with the previous report of Liang at el.[27]

Substitution Degree (SD) of OCMCs. A typical conductometric titration curve of OCMCs that consists of four linear branches is shown Figure 3. The excess HCl was neutralized using the volume of NaOH ($0-V_1$). (V_1-V_2) corresponds to the volume of NaOH that reacted with the

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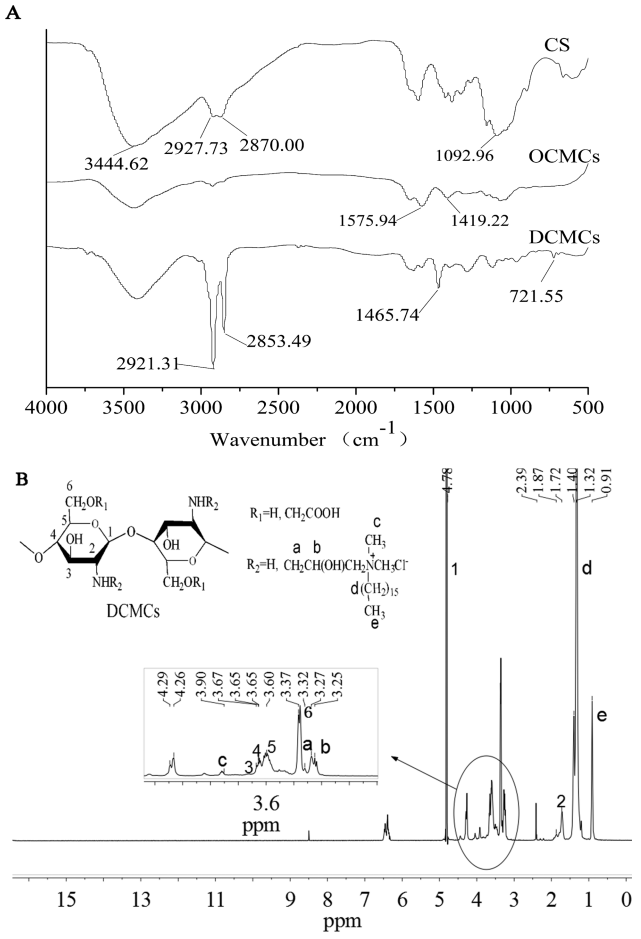

Figure 2. FT-IR spectra (A) of CS, OCMCs and DCMCs, and ^{1}H NMR spectra (B) of DCMCs.

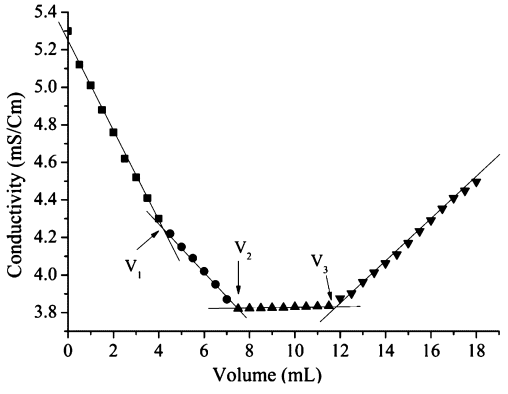

Figure 3. Conductimetric titration curve of OCMCs.

carboxymethyl groups of OCMCs. (V_2−V_3) represents the volume added to react with NH_3^+ of CS. The final volume (from V_3 to the end) indicated the excess amount of NaOH in the solution. The SD of OCMCs was determined according to eq 1, and the SD value was 70.00%. The results obtained in the current study was higher than that of the previous study.[23]

Critical Micelle Concentration (CMC) of DCMCs. For evaluation of the aggregation properties of DCMCs, the probe fluorescence technique was applied to determine the CMC by using pyrene as the fluorescence probe. Pyrene has a strong hydrophobic character with a very low solubility in water and tends to solubilize into the hydrophobic region of the micelles, thereby resulting in the fluorescence emission changes. Figure 4A shows the fluorescence emission spectra of pyrene in

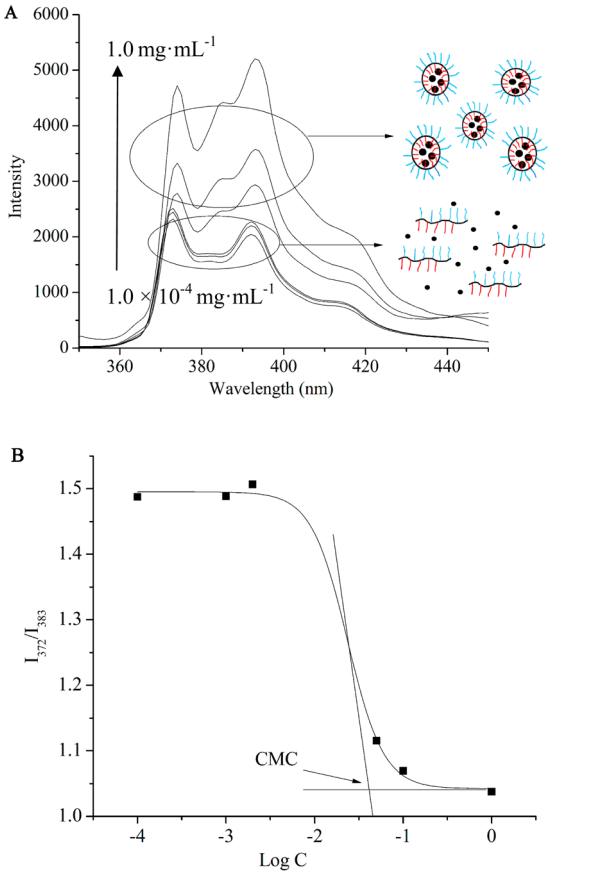

Figure 4. Fluorescence spectra of pyrene in different DCMCs concentrations (1.0×10^{-4}, 1.0×10^{-3}, 2.0×10^{-3}, 5.0×10^{-2}, 1.0×10^{-1}, and 1.0 mg·mL^{-1}) (A), and intensity ratio (I_{372}/I_{383}) versus the DCMCs concentration (B).

DCMCs solution with concentrations varying from 1.0×10^{-4} to 1.0 mg·mL^{-1}. The results indicated that the fluorescence intensity increased with increasing the concentration of DCMCs. The pyrene solubility in water is extremely low, and its dissolved fraction produces negligible fluorescence. When the concentration is above CMC, the hydrophobic phase (micelle core) was formed, which solubilizes pyrene and results in increased solution fluorescence. Plots of pyrene I_1 (372 nm /I_3 (383 nm) ratio versus logarithm of DCMCs concentration are shown in Figure. 4B. The scatters are fitted by using a Boltzmann function, which is expressed as following:

$$y = 1.29 + 0.52/[1 + e^{(x+1.88)/\Delta x}] \quad (6)$$

where y and x is the pyrene I_1/I_3 ratio and the DCMCs concentration logarithm, respectively. A common tangent can be obtained by determining the midpoint, which is obtained by using the second derivative of y ($y'' = 0$), and the CMC was defined as the crossover point of common tangent and flat tangent.[28] The CMC value of DCMCs was determined as approximately 23.00 mg·L^{-1}, which was lower than other similar CMCs-based micelle systems such as deoxycholic acid-*O*-carboxymethylated chitosan−folic acid conjugates and acylated CMCs.[29,30] These results indicated that DCMCs are capable of forming self-organized liposomes easily and are also

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capable of maintaining their integrity even upon strong dilution.

Formulation of DC-Ls. Figure 5 illustrats the preparation processes of DC-Ls via the thin-layer evaporation method.

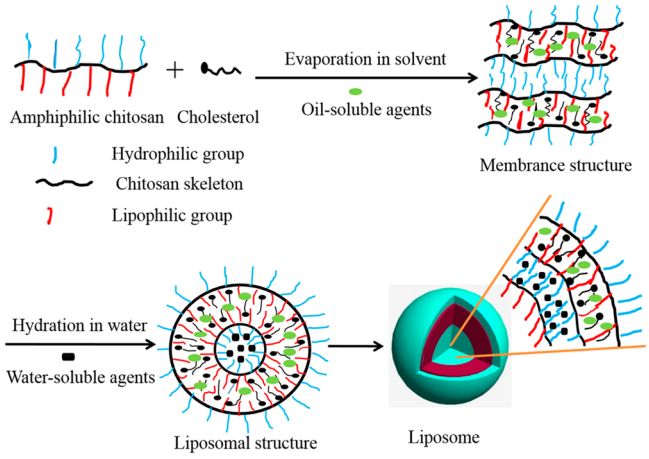

Figure 5. Schematic illustration of the formation process of DC-Ls. DCMCs and Chol (or with poorly water-soluble agents) were dissolved in an organic solvent, which was then completely removed, and the thin film was obtained. The thin film was hydrated using deionized water (or with water-soluble agents) and sonicated to obtain the liposomes. As a soluble agent, salidroside was dissolved in deionized water and used to hydrate the thin film and thus obtained the salidroside-load DC-Ls.

DCMCs and Chol were dissolved in organic solvents, and a thin film was obtained after removing the solvent via evaporation. The DC-Ls solution was obtained after hydration of thin film by adding an aqueous phase. The cross section of DC-Ls (Figure 5) showed that the hydrophilic heads of the DCMCs are oriented toward the water compartment, whereas the lipophilic tails are directed toward the center of the vesicle. Thus, the hydrophilic and lipophilic bioactive agents can be loaded into the water compartment and lipid section of DC-Ls, respectively.

Characterization of DC-Ls. The morphology and bilayer structure of DC-Ls were observed by TEM (Figure 6). The results indicated that DC-Ls exhibit a nearly spherical bubble with slight aggregation (Figure 6A,B). For verifying OA-Fe_3O_4 incorporated into DC-Ls, VSM was employed to study the magnetic properties of the DC-Ls@OA-Fe_3O_4. From the magnetic hysteresis loop of OA-Fe_3O_4 and DC-Ls@OA-Fe_3O_4 (Figure 7), it was found that the saturation magnetization of OA-Fe_3O_4 and DC-Ls@OA-Fe_3O_4 was 66.45 and 23.45 em$\mu\cdot g^{-1}$, respectively. These results (Figures 6C and 7) indicated that OA-Fe_3O_4 was encapsulated into DC-Ls with properties of core (OA-Fe_3O_4) and shell (DCMCs films) structure. Meanwhile, the multilamellar film structure was also found on the surface of OA-Fe_3O_4 nanoparticles (Figure 6D).

The particle size and ζ potential of DC-Ls were determined and are shown in Figure 8. The average size of DC-Ls was 32.21 nm with a narrow distribution (PDI = 0.277 < 0.5), and the ζ potential was approximately +73.30 mV. A previous study reported that the average size, PDI and ζ potential of the traditional liposomes (PC-Ls) were 604.00 nm, 0.36, and +7.50 mV,[21] respectively. These results indicated that DC-Ls has better properties than PC-Ls. Evidence has shown that nanocarriers with a small particle size (<200 nm) and positive

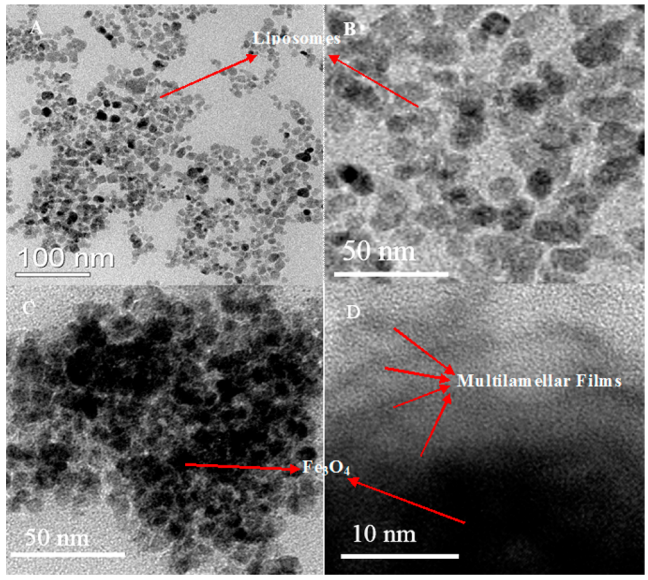

Figure 6. TEM images of DC-Ls and DC-Ls@OA-Fe_3O_4. DC-Ls exhibit a nearly spherical bubble shape with slight aggregation (A,B), DC-Ls@OA-Fe_3O_4 exhibit property of core (OA-Fe_3O_4) and shell (DMCMs films) structure (C,D), and multilamellar films can be seen on the surface of OA-Fe_3O_4 (D).

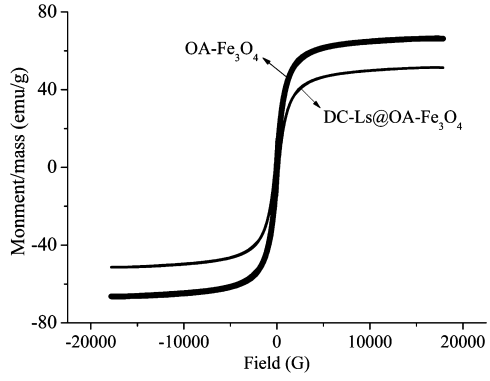

Figure 7. Magnetic hysteresis loop of OA-Fe_3O_4 and DC-Ls@OA-Fe_3O_4.

charge were not captured by the reticuloendothelial cell systems but were easily absorbed by the negatively charge cellular membrane.[31] Consequently, the bioavailability of bioactive agents can be improved after encapsulation into DC-Ls.

The XRD of DCMCs, Chol, DC-Ls, and DCMCs/Chol physical mixture were investigated, and the results are shown in Figure 9A. CS has the property of the crystal form II at 2θ = 20.5°. However, the crystal peaks of DCMCs at 2θ were sharply changed with shifting from 20.5° to 21.6°, and a new peak at 2θ of 5° appeared. These results indicated that DCMCs molecules have good crystal properties with a billayered lamellar structure.[32] The DCMCs peak of 21.6° and all Chol peaks disappeared for DCMCs/Chol physical mixture, which indicated that the interaction (i.e., hydrogen bonding and hydrophobic interactions) between DCMCs and Chol was occurred.[9] However, the peak at 20.9° appeared for DC-Ls after hydration, which suggested that DC-Ls can form lamellar-like structures as lipid.

The lipid physical state can change from an ordered gel phase to a disordered liquid-crystalline phase when temperature is

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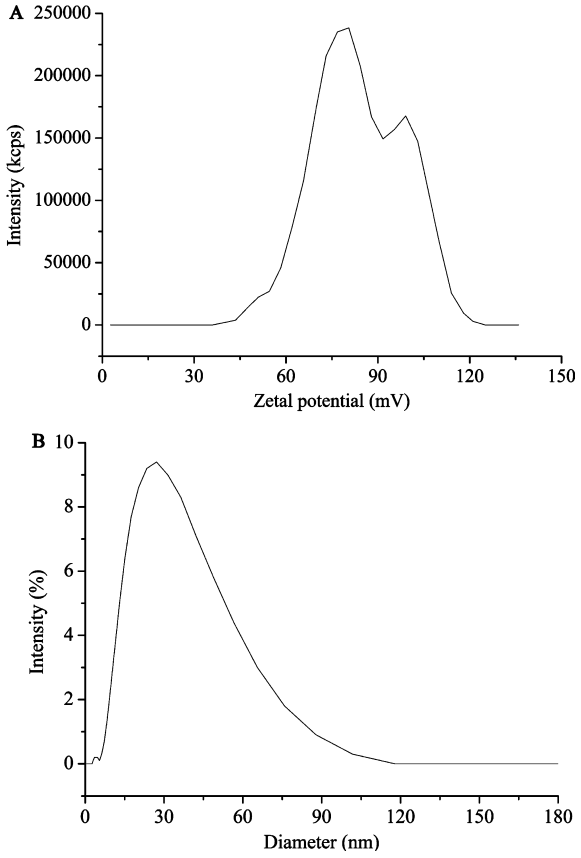

Figure 8. Size distribution (A) and ζ potential (B) of DC-Ls.

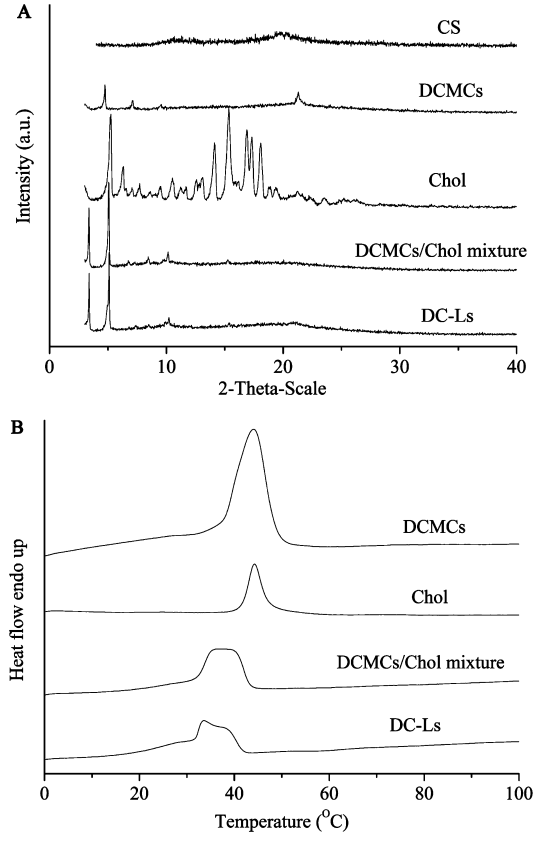

Figure 9. XRD (A) and DSC (B) spectra of DCMCs, Chol, DCMCs/Chol physical mixture, and DC-Ls.

changed. This change was defined as phase transition temperature (T_m). The DSC of DCMCs, DC-Ls, Chol, and DCMCs/Chol physical mixture was investigated and is shown in Figure 9B. The T_m values of Chol and DCMCs were 44.43 and 44.29 °C, respectively. However, the T_m of DCMCs/Chol physical mixture and DC-Ls decreased and the values were 37.50 and 33.61 °C, respectively. The results indicated that the structure of DCMCs was rearranged and interacted with Chol. The Chol interaction with DCMCs would increase the rigidity of the "fluid state" liposomal bilayers of DC-Ls, which results in good thermal stabilization.[33] The XRD and DSC results suggested that DC-Ls have the same physiochemical properties as that of PC-Ls.

Encapsulation Efficiency (EE) of DC-Ls. Compared with DC-Ls, PC-Ls of salidroside loading was also developed and the EE was only about 54.72%. Previous studies have also reported that the EE of salidroside loading in PC-Ls was lower than 60.00%.[21,22] Fortunately, the EE of salidroside in DC-Ls increased to 82.46%. The high EE (82.46%) may be attributed to the multilamellar structure of DC-Ls, which favored the formation of a high inner volume.[15] EE depends on the inner volume of liposomes for water-soluble compounds, thus, a large inner volume of DC-Ls results in a high EE.

Release of Salidroside from DC-Ls. The release of salidroside from salidroside solution (control sample), PC-Ls, and DC-Ls was investigated in PBS solutions (pH 7.0) (Figure 10A). The release of salidroside from the control solution occurred rapidly with a release rate of 86.44% after 6 h. As expected, the salidroside release from liposomes was slower than that in the control solution. The release rates were only about 38.38% and 30.77% for PC-Ls and DC-Ls after 6 h,

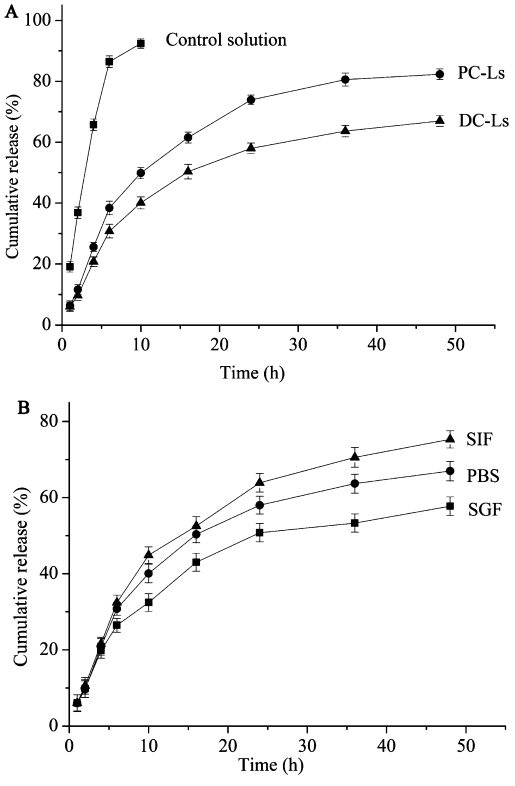

Figure 10. In vitro release of salidroside from control solution, PC-Ls, and DC-Ls in PBS solutions (A), and from DC-Ls in SGF, PBS, and SIF solutions (B), respectively. Data represent the mean ± SD (n = 3).

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Table 1. Correlation Coefficients (R^2) and Release Exponent (n) of Different Models in SGF, PBS, and SIF Solution, Respectively[a]

		SGF solution		PBS solution		SIF solution	
model		stage 1	stage 2	stage 1	stage 2	stage 1	stage 2
First model	R^2	0.939	0.897	0.975	0.941	0.993	0.943
Higuchi model	R^2	0977	0908	0.984	0.951	0.989	0.933
Peppas model	R^2	0.983	0.948	0.987	0.979	0.995	0.964
	n	0.767	0.252	0.870	0.259	0.901	0.322

[a]Stage 1 and 2 is the burst (0−10 h) and sustained release (10−48 h) process, respectively.

respectively, and more interestingly, the release rate was 66.98% and 82.33% for DC-Ls and PC-Ls after 50 h, respectively. These results indicated that DC-Ls had a relatively longer release time than PC-Ls, which may be due to the high molar mass of DCMCs. Thus, DC-Ls can be used for sustained release of salidroside.

To further investigate the effect of gastrointestinal environment on salidroside release from DC-Ls, the solutions of SGF (pH 1.2), PBS (pH 7.0), and SIF (pH 7.4) were used as release media (Figure 10B). The results suggested that the release rate of salidroside from DC-Ls decreased with decreasing pH value. In the SGF solution, the H-bond between −COOH and −OH caused the aggregation of salidroside in the inner portion of DC-Ls. Moreover, the repulsive force between H^+ and the positive charge of liposomal surface made DC-Ls more stable. Consequently, the release rate of salidroside from DC-Ls was lower in the SGF solution. On the other hand, the positive charge on the surface of DC-Ls was partially neutralized with OH^- in the SIF solution, which leads to the destruction of DC-Ls and an improvement in the release rate of salidroside.

Release Mechanism of DC-Ls. Figure 10B shows that two stages were observed for the release of salidroside from DC-Ls. The first stage of release was initially rapid and was considered the burst release, which may be due to the unbound excess of salidroside on the DC-Ls surfaces. The second stage was a sustained release process. The burst release of salidroside may help to reach the effective concentration rapidly in plasma, whereas the sustained release would maintain the effective concentration in plasma for a long time. Thus, the bioavailability and biological half-life time of salidroside can be improved after loading into DC-Ls.

The amphiphilic CS derivatives have been used to develop liposome for drugs and genes delivery. However, the release mechanism is rarely reported. Thus, different kinetic models of first-order, Higuchi, and Peppas were used to fit the two release stages for further investigation of the probable DC-Ls release mechanism, and the results are shown in Table 1. It can be seen that the R^2 of Peppas model was highest among models. This finding indicated that Peppas is the most suitable model for describing the release kinetic of salidroside from DC-Ls. The value n of the Peppas model is characteristic for the release mechanism such as $n \leq 0.43$ for Case I Fichian diffusion, $0.43 < n < 0.85$ for anomalous behavior or non-Fickian transport, and $n \geq 0.85$ for case II transport.[34] The n values of DC-Ls were determined and are shown in Table 1. The results suggested that case II transport was the main release mechanism for stage 1 and case I Fichian diffusion for stage 2.

In conclusion, amphiphilic CS derivatives of DCMCs were successfully synthesized and the structure was confirmed via FT-IR and ^{1}H NMR. DCMCs have a self-assembling property with low CMC as the traditional lipid. DCMCs were used to develop liposomes with Chol (DC-Ls), which exhibits multilamellar structure with positive charge and narrow size distribution. Compared with traditional liposomes, DC-Ls have higher thermal stabilization and encapsulation efficiency. The release of salidroside from DC-Ls was initially burst release, followed by sustained release. Furthermore, the case II transport and case I Fichian diffusion were the main release mechanisms for the whole release process. In conclusion, DC-Ls may be a promising carrier system for the production of functional foods that contain salidroside or other bioactive food ingredients. However, further in vivo investigation is required to demonstrate the efficacy and safety of DC-Ls.

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Notes

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