

Preparation and physicochemical characteristics of self-assembled nanoparticles of deoxycholic acid modified-carboxymethyl curdlan conjugates

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Abstract

Various deoxycholic acid (DOCA)-modified-carboxymethylated (CM)-curdlan (DCMC) were synthesized and characterized by FTIR, ¹H NMR and XRD. The degree of DOCA substitution (DS), as spectrophotometrically determined, was 2.1, 3.2, 4.1, or 6.3 DOCA groups per hundred sugar residues of CM-curdlan. The physicochemical properties of the self-assembled nanoparticles in aqueous media were investigated using ¹H NMR, dynamic light scattering, zeta potential, transmission electron microscopy (TEM) and fluorescence spectroscopy. DCMC conjugates provided monodispersed self-assembled nanoparticles in water, with mean diameter decreasing from 192 to 347 nm with DOCA DS increasing. Moreover, the mean diameter also increased with decreasing pH in PBS. Zeta potential of DCMC self-assembled nanoparticles exhibited near −60 mV in distilled water and −26 to −36 mV in PBS, indicating these nanoparticles were covered with negatively charged CM-curdlan shells. The critical aggregation concentration (cac) of the DCMC were dependent on the degree of substitution (DS) of DOCA and were slightly lower in PBS than in distilled water. The TEM images demonstrated that these self-assembled nanoparticles were of spherical shape.

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1. Introduction

Recently, polymeric amphiphiles have received increasing attention because of their potential biotechnological and pharmaceutical application (Gref et al., 1994; Kataoka, Harada, & Nagasaki, 2001). In the aqueous media, polymeric amphiphiles spontaneously form micelles or

micelle like aggregates via undergoing intra or intermolecular associations between hydrophobic moieties, primarily to minimize interfacial free energy (Mortensen, 2001; Nagasaki, Yasugi, Yamamoto, Harada, & Kataoka, 2001). These polymeric micelles or micelle like aggregates have been recognized as a promising drug carrier, because their hydrophobic domain, surrounded by hydrophilic outer shell, can serve as a preservative for various hydrophobic drugs (Kataoka et al., 2001; Nakanishi et al., 2001). Therefore, many efforts have been made for the past decades to develop some novel polymeric amphiphiles such as amphiphilic block copolymers (Gref et al., 1994;

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Kabanov et al., 1995; Lee et al., 1999; Nakanishi et al., 2001; Poppe, Willner, Allgaier, Stellbrink, & Richter, 1997) and hydrophobically modified water-soluble polymers (Huh et al., 2000; Kim et al., 2000) which can self-assemble to form compact micellar structure in the aqueous media. In recent years, natural polysaccharides, such as chitosan, dextran and pullulan, have been hydrophobically modified with the long alkyl chains or cholesteryl groups, to form micelles or nanoparticles for being used as the controlled drug delivery systems or gene carriers (Miwa et al., 1998; Zhang, Ping, & Zhang, 2004; Jiang, Quan, Liao, & Wang, 2006; Lee, Kim, Kwon, & Jeong, 2000; Yuan, Li, & Yuan, 2006; Yuan, Li, Zhu, & Woo, 2006; Nichifor, Lopes, Carpov, & Melo, 1999; Na & Bae, 2002; Kim, Gihm, & Park, 2001; Lee, Kwon, Kim, Jo, & Jeong, 1998).

In this study, we used DOCA modified CM-curdlan conjugates to prepare self-assembled hydrogel nanoparticles. Curdlan, one of the microbial polysaccharides, is a naturally occurring linear (triple helix) polysaccharide composed of 1,3- β -linked D-glucose units produced by a strain of *Alcaligenes faecalis* (Fig. 1a) and mainly used as a food additive now. As reported previously (Greinacher, Alban, Dummel, Franz, & Muellereckhardt, 1995; Katsuraya et al., 1994; Yoshida et al., 1994), curdlan has a potential inhibitory effect against AIDS virus infection and blood anti-coagulant activity, as well as low toxicity in vitro and in vivo. Carboxymethylated curdlan (CM-curdlan), as a negatively charged curdlan derivative, is known to have anti-tumor activity (Saito, Yoshioka, & Uehara, 1991; Sasaki, Abiko, Nitta, Takasuka, & Sugino, 1979; Sasaki, Abiko, Sugino, & Nitta, 1978). Although the mechanism of its anti-tumor action is not clearly understood, CM-curdlan may not act on tumor cells directly. Ohya et al. reported the immunological enhancement activity of the muramyl dipeptide analogue (GADP)/CM-curdlan conjugate (Ohya, Nishimoto, Murata, & Ouchi, 1994) and suggested that the enhancement was due not only to giving polymeric character to GADP but also to the hybridization of GADP with the immunologically active polysaccharide, curdlan. Due to its anti-tumor activity,

CM-curdlan may act as a new anti-tumor drug delivery carrier.

In this study, we prepared a novel kind of the negatively charged nanoparticles to develop a new drug delivery system by hydrophobical modification of CM-curdlan with DOCA. DOCA is a main component of bile acid and has the amphiphilic property due to the presence of hydrophilic moieties such as the carboxyl group at the end of the branched side chain of carbon atoms and the hydroxyl groups at both 3 α and 12 α positions, and the hydrophobic cyclopentenophenanthrene nucleus in its molecule (Fig. 1b). Therefore, DOCA can form micelles in water which plays an important role in the emulsification, solubilization, and absorption of cholesterol, fat, and lipophilic vitamins in the body (Enhsen, Kramer, & Wess, 1998). Thus, it was expected that the introduction of DOCA moieties into the CM-curdlan molecule would induce association to form self-assembled nanoparticles. Herein, we investigated the effect of DOCA attached to CM-curdlan on the formation and physicochemical characteristics of self-assembled nanoparticles. The detailed synthesis scheme of CM-curdlan/DOCA conjugates in this study is shown in Fig. 2.

2. Experimental

2.1. Materials

Curdlan (M_w 81,000) was purchased from Wako Co. (Japan). DOCA and 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) were purchased from Sigma Co. (St. Louis, MO, USA). *N*-Hydroxyl succinimide (NHS) and pyrene were purchased from Aldrich Co. (Milwaukee, WI). All other chemical reagents were of analytical grade and obtained from commercial sources.

2.2. Carboxymethylation of curdlan (CMC)

Curdlan was carboxymethylated by the method described by Na, Park, Kim, and Bae (2000), Yang, Zhang, Yimei Yin, and Nishinari (2006), and Zhang, Cheung, Zhang, Chiu, and Ooi (2004). Briefly, a suspension of 3 g of curdlan in 80 mL of isopropyl alcohol was stirred at room temperature for 30 min. Then, 8 mL of a 30% sodium hydroxide solution was added in 1 mL portions at 15 min intervals by stirring at room temperature for 90 min. Then, 3.6 g monochloroacetic acid was added over three separate intervals of 10 min each and the mixture was stirred at 55 °C for 5 h. The suspended product was recovered by filtration and washed successively with methanol–acetic acid (7:3 v/v), methanol–water (4:1 v/v), methanol and acetone, respectively. The final precipitate was dissolved in water, and dialyzed against distilled water at 4 °C for 3 days. The retentate was frozen and lyophilized to give the product. CM-curdlan was identified using Fourier transform infrared (FT-IR) spectrophotometer (FTS 3000, BIO-RAD, USA) ^1H NMR and ^{13}C -NMR (D_2O) (AV600,

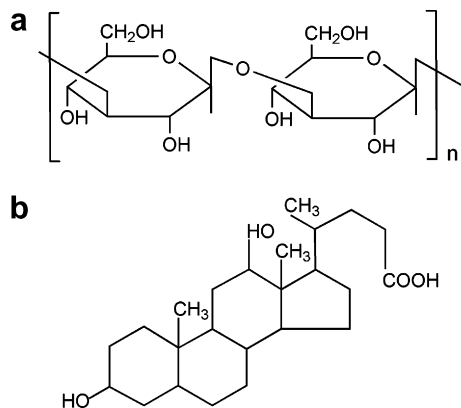


Fig. 1. Chemical structures of a repeat unit of curdlan (a) and deoxycholic acid (b).

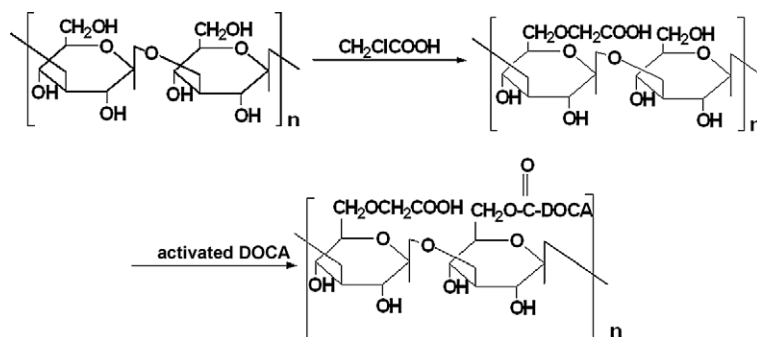


Fig. 2. Synthesis schemes of CM-curdlan/DOCA conjugate.

Switzerland, 600 MHz). The degree of substitution (DS) of the CMC sample was 0.49 units of carboxymethyl group per one glucose determined by conductometric back titration in accordance with a previous report (Capitani, Porro, & Segre, 2000; Chen & Park, 2003; Ge & Luo, 2005) and by ^1H NMR.

2.3. Synthesis of CM-curdlan/DOCA conjugates (DCMCs)

The swollen CM-curdlan power (0.5 g) was dispersed in 40 mL dried DMSO by sonication in ultrasonic bath. The carboxyl groups of DOCA in dried DMSO were activated by addition EDC (1.2 equiv DOCA) and NHS (0.5 equiv EDC). Different amounts of activated DOCA (0.20–0.50 mol/mol sugar residues of CM-curdlan) were added into the dried DMSO solution containing CM-curdlan. The mixture was reacted by stirring for 48 h at 45 °C. Then, the reactant mixture was poured into acetone, and the precipitate was collected by filtration and washed with acetone and methanol, respectively. The product was dispersed in distilled water and exhaustively dialyzed against distilled water for three day using a dialysis tube (molecular cut-off 8000–12,000), and the distilled water was exchanged at intervals of 6 h. Then, the sample was lyophilized to obtain DCMC conjugates.

2.4. Characteristics of DCMCs

The chemical structure of DCMCs was confirmed using FT-IR spectrophotometer (KBr pellets) and ^1H NMR (D_2O or $\text{D}_2\text{O}/\text{DMSO}-d_6$ (3:1 v/v), 600 MHz).

X-ray diffraction spectrometry was obtained using an XD-3A powder diffraction meter (Rigaku D/Max 2500 PC, Japan) with $\text{CuK}\alpha$ radiation in range of 5°–80° (2 θ) at 40 kV and 150 mA.

2.5. Determination of DOCA degree of substitution

The amount of DOCA covalently bound to CM-curdlan was spectrophotometrically determined according to the procedure described by Nichifor and Carпов (1999), and Yuan et al. (2006). Briefly, the polymer sample (4–10 mg) was accurately weighed and dissolved in 0.5 mL DMSO.

The mixture of 0.5 mL acetic acid solution (60%) and 9.0 mL water/sulfuric acid (65/50 v/v) was subsequently added to the solution. The sample solution was homogenized, heated at 70 °C for 30 min, and then cooled to room temperature. The UV (Sunnyvale, CA, USA) absorbance of sample solution at 380 nm was measured against a blank sample containing the same components as the sample but without the polymer. Individual calibration curves of absorbance versus DOCA concentrations were made. The degree of substitution (DS, mol%, expressed as mole DOCA/100 sugar residues of CM-curdlan) was calculated according to the following formula

$$\text{DS} = (c/M_{\text{DOCA}})/((m - c)/M_{\text{CMC}})$$

where c is the content of the DOCA determined from the corresponding calibration curve; m is the amount of the modified polymers used in experiment, M_{DOCA} is the molecular weight of the DOCA residue, and M_{CMC} is the molecular weight of anhydroglucose units of CMC.

2.6. Preparation of self-assembled nanoparticles

The DCMCs were suspended in distilled water or various pH phosphate-buffered saline (PBS) solution (identical ionic strength) under gentle shaking at 37 °C for 12 h, followed by sonication three times using a probe-type sonifier (Automatic Ultrasonic Processor UH-500A, China) at 70 W for 2 min each, in which the pulse was turned off for 2 s with the interval of 5 s to protect the solution from heat build-up during sonication. Then, the solution of self-assembled nanoparticles was passed through membrane filter (pore size: 0.45 μm , Millipore) and stored at 4 °C refrigerator.

2.7. Transmission electron microscopy (TEM) observation

To observe the morphology of DCMC self-assembled nanoparticles, the sample solution (1 mg/mL) was placed on a carbon-coated copper grid. The grid was held horizontally for 20 s and then at 45° to allow excess fluid to drain for 10 s. The grid was returned to the horizontal position. Then, the grids were air-dried and examined using a

transmission electron microscope (Tecnai G² 20S-Twin, USA) at an acceleratin voltage of 80 kv.

2.8. Measurements of dynamic light scattering (DLS) and zeta potential

The average particle size and size distribution of the self-assembled nanoparticles were measured by dynamic laser light scattering with a digital autocorrelator (Brookhaven BI-90 Plus, USA) at a scattering angle of 90°, a wavelength of 658 nm and a temperature of 25 ± 0.1 °C. DLS measurements were performed over a time period sufficient to reach equilibrium, with no evidence of change in size with time.

The zeta potential of nanoparticles was measured using a zeta potentiometer (Zetasizer 3000HS, Malvern Instruments Ltd, Malvern UK) operated at 11.4 V/cm, 13.0 mA and 25 °C. The sample concentration was maintained at 2.0 mg/mL in water or in PBS solution.

2.9. Measurement of fluorescence spectroscopy

The self-aggregate property of DCMC conjugates and their critical aggregation concentration (cac) were estimated by the probe fluorescence technique (Ananthapadmanabhan, Goddard, Turro, & Kuo, 1985) in which pyrene was used as a hydrophobic probe. Briefly, a known amount of pyrene solutions (3×10^{-4}) in acetone were added to a series of vials, and followed evaporation to remove the acetone. Then, various concentrations of DCMC conjugate suspension were added to each vial and sonicated in an ultrasonic bath (AS5150A, China) for 2 h to equilibrate the pyrene and the nanoparticals. The fininal concentration of pyrene was 6.0×10^{-7} mol/L. The emission spectra of pyrene were recorded in the range of 350–500 nm using a fluorescence spectrophotometer (Shimadzu RF-4500, Japan) at the excitation wavelength (λ_{ex}) of 336 nm and an integration time of 4 s/nm. For the intensity ratio measurement of the first and the third highest energy bands in the emission spectra of pyrene, the slit opening for excitation and emission was set at 5 and 1 nm, respectively.

3. Results and discussion

3.1. Synthesis and characterization of DCMCs

Curdlan is insoluble in water, which limits its biological applications. Carboxymethyl substitution is considered as a method to improve the functional properties for many polysaccharides. In the case of curdlan, its carboxymethylated derivative has good water solubility as well as good bioactivity. Therefore CM-curdlan was firstly synthesized, and then DOCA was covalently attached to CM-curdlan in the presence of EDC and NHS, thus producing a novel kind of polymeric amphiphiles. Fig. 3 shows the IR spectra of curdlan, CM-curdlan and DCMC. The peak at 1640 cm^{-1} in the spectrum of native curdlan was attributed

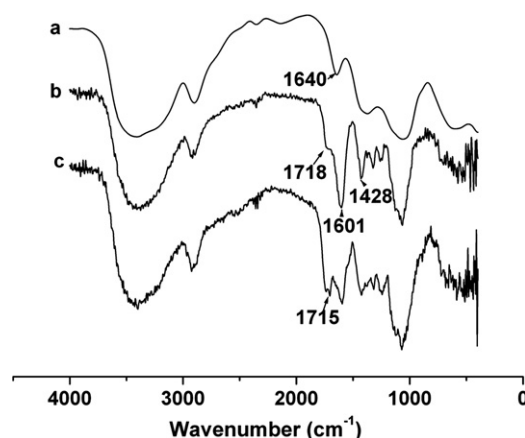


Fig. 3. FT-IR spectra of (a) curdlan, (b) CM-curdlan, (c) DCMC.

to the existence of water, which seems to be unable to be completely eliminated from the sample (Yang et al., 2006). Compared with natived curdlan (Fig. 3a), in CM-curdlan spectrum (Fig. 3b), the absorption band at 1718 cm^{-1} was the carboxyl characteristic peaks in protonated form (Sandula, Kogan, Kacurakova, & Machova, 1999), absorption band at 1601 cm^{-1} was due to the asymmetrical COO^- stretching vibration, whereas the band at 1428 cm^{-1} was due to the symmetrical COO^- stretching vibration in salt form (Sandula et al., 1999; Yang et al., 2006). Compared with CM-curdlan, the absorption band at 1715 cm^{-1} was evidently increased in DCMC conjugate spectrum (Fig. 3c). This indicated DOCA was conjugated to CMC by carboxyl ester. Furthermore, the presence of DOCA in CM-curdlan was evaluated by the characteristic peaks of DOCA appearing at 0.6–2.5 ppm in the ^1H NMR spectra (Kim et al., 2005).

3.2. Physical properties of curdlan derivatives

X-ray diffraction diagrams of curdlan and its derivative are shown in Fig. 4. Native curdlan had diffraction at $2\theta = 10^\circ$ and sharp peaks at $2\theta = 20^\circ$ which was assigned

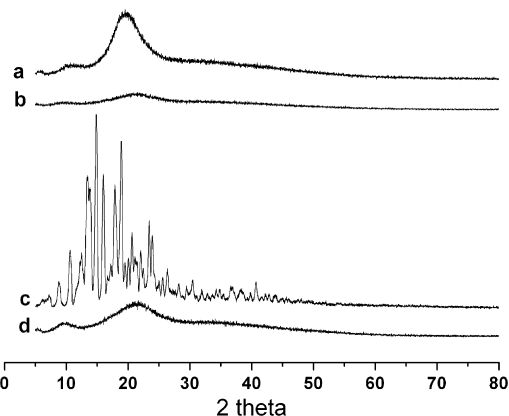


Fig. 4. WAXD patterns of (a) curdlan, (b) CM-curdlan, (c) DOCA, (d) DCMC.

to the crystal form. The peak at $2\theta = 20^\circ$ of CM-curdlan was hardly appreciable. DOCA had eight typical crystal peaks at 2θ of 10.6, 13.4, 13.9, 14.8, 16.0, 17.9, 18.9, and 23.4° and numerous small peaks between 25 and 40° . However the DCMC showed broad diffraction peaks at around $2\theta = 10^\circ$ and $2\theta = 23^\circ$. These results indicated the crystal-line structure of curdlan had been disrupted after chemically modification. These results was similar to chemical modified chitosan (Xu et al., 2007).

3.3. Self-aggregation of DCMCs

The amphiphilic nature of the DCMC conjugates, composed of hydrophilic CM-curdlan and hydrophobic DOCA, was characterized using ^1H NMR in D_2O and $\text{D}_2\text{O}/\text{DMSO-}d_6$ (3:1 v/v; Fig. 5). All the characteristic peaks appearing from both CM-curdlan and DOCA were clearly observed in $\text{D}_2\text{O}/\text{DMSO-}d_6$ (3:1 v/v), whereas the characteristic peaks of DOCA, including 18- CH_3 (0.67 ppm), 19- CH_3 (0.90 ppm), 21- CH_3 (0.99 ppm) and methylene–methine envelope (1–2.5 ppm), significantly decreased in D_2O . It indicated that the molecular motion of hydrophobic DOCA moieties in DCMC molecule was limited due to the hydrophobic aggregation in D_2O , thus causing proton signal shielding or peak broadening of DOCA. This trend of ^1H NMR spectra is consistent with other polymeric amphiphiles that form micelles in the aqueous phase (Kim et al., 2005, 2000; Kwon, Park, Chung, Kwon, & Jeong, 2003; Park et al., 2004; Yuan et al., 2006).

The sizes and size distributions of self-assembled nanoparticles of DCMC conjugates in the aqueous media with different DS were measured by DLS. As shown in Table 1, the mean sizes of self-assembled nanoparticles were in the range of 192–347 nm, which depending on the DS value. The size of self-assembled nanoparticles decreased as the DS increasing, indicating formation of more compact hydrophobic cores. Fig. 6 shows the size of self-assembled nanoparticles of DCMC4.1 was 290 nm with a unimodal size distribution. In our study, the size of self-assembled nanoparticles was scarcely affected by the concentration of DCMCs from 0.5 to 2 mg/mL, which implied that the interaction among self-assembled nanoparticles

Table 1

Characterization of CM-curdlan-deoxycholic acid conjugates in distilled water

Sample	DS ^a	d^b (nm)	Variance ^b	ξ^c (mV)	Cac ^d (mg/mL)
DCMC2.1	2.1	347	0.151	−60.1	0.052
DCMC3.2	3.2	326	0.133	−60.4	0.043
DCMC4.1	4.1	290	0.307	−61.0	0.023
DCMC6.3	6.3	192	0.268	−54.8	0.014

^a Degree of substitution of deoxycholic acid, determined by UV-spectroscopy.

^b Mean diameter and variance in distilled water determined by dynamic light scattering ($\theta = 90^\circ$).

^c The ξ potential of DCMC conjugated in distilled water at 2 mg/mL.

^d Critical aggregation concentration in distilled water determined from pyrene emission spectra.

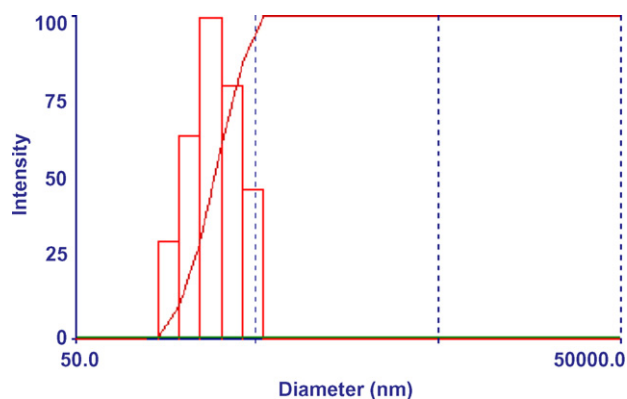


Fig. 6. Histograms of the distribution of the self-aggregated nanoparticles (DCMC4.1).

was negligible. Moreover, as shown in Table 2, the partial size of DCMC4.1 increased with the decrease of pH value of PBS. We believed this was because that the surface carboxyl groups were unionized in low pH, therefore the electrostatic repulsion reduced between particles, leading to the aggregation of nanoparticles.

The zeta potentials of DCMC self-assembled nanoparticles were very negative in distilled water, which indicated that negatively charged carboxyl groups of CM-curdlan covered nanoparticles. It deduced that these nanoparticles were electrostatically stabilized by the negatively charged CM-curdlan shells, which providing a long term stability

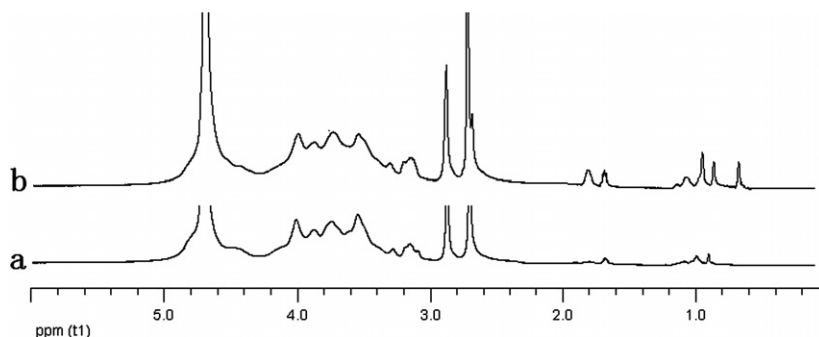


Fig. 5. ^1H NMR spectra of DCMC4.1 in (a) D_2O and (b) $\text{D}_2\text{O}/\text{DMSO-}d_6$ (3:1 v/v).

Table 2
Characterization of CM-curdlan-deoxycholic acid conjugates^a in PBS of different pH

pH	d^b (nm)	Variance ^b	ζ^c (mV)	Cac ^d
5.0	315	0.086	−25.9	0.011
6.5	299	0.005	−32.2	0.015
7.4	241	0.005	−36.1	0.017

^a [DCMC4.1] = 2mg/mL. $T = 25^\circ\text{C}$.

^b Mean diameter and variance determined in PBS by dynamic light scattering ($\theta = 90^\circ$).

^c The ζ potential of DCMC conjugated in PBS.

^d Critical aggregation concentration in PBS determined from pyrene emission spectra.

in the aqueous phase. However, the absolute value of zeta potential of self-assembled nanoparticles decreased in PBS (Table 2). It may be considered that electrolytes in PBS screen the negative charges in the CM-curdlan backbone. Furthermore, the absolute value of zeta potential of DCMC self-assembled nanoparticles decreased with the pH value decreasing, which resulting the aggregation of nanoparticles.

As shown in Fig. 7, the morphology of DCMC self-assembled nanoparticles observed by TEM was spherical shape, and their diameter appeared a little difference from the results determined by DLS measurement. This was mainly due to the process involved in the preparation of samples. In the case of the TEM method, TEM images depicts the size at the dried state of the sample, whereas DLS method involves the measurement of size in the hydrated state. In other words, the size determined by TEM is an actual diameter (dry state) of the nanoparticles, whereas the size measured by the laser light scattering method is a hydrodynamic diameter (hydrated state), and therefore the nanoparticles will have a larger hydrody-

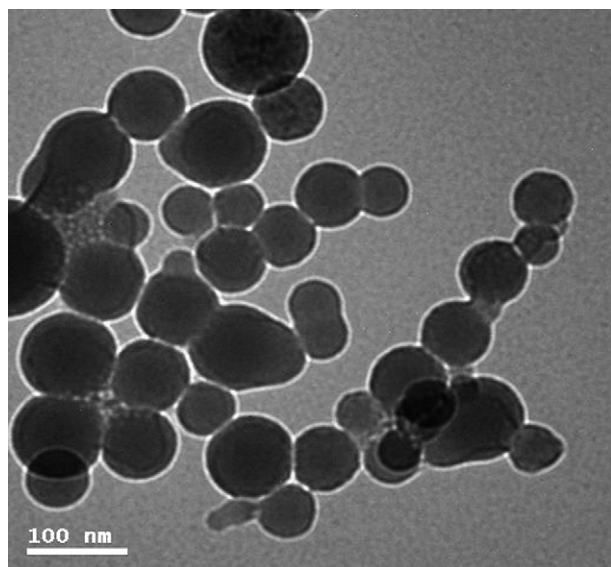


Fig. 7. TEM images of self-aggregated of DCMC4.1 conjugate (1 mg/mL).

namic volume due to solvent effect in the hydrated state; hence, the size measured by DLS method was larger than the size observed by TEM method. A similar finding has been reported by other authors (Jiang et al., 2006; Liu, Desai, Chen, & Park, 2005; Prabha, Zhou, Panyam, & Labhasetwar, 2002).

3.4. Critical aggregation concentration of self-assembled nanoparticles

The aggregation behavior of DCMC conjugates in the aqueous media was monitored by the probe fluorescence technique in which pyrene was used as a fluorescence probe. Pyrene shows only a small fluorescence intensity in a polar environment (water) due to its poor solubility and self-quenching, but if micelles or other hydrophobic microdomains are formed in an aqueous solution, the pyrene preferably lies close to (or inside) these microdomains and strongly emits (Amiji, 1995; Ananthapadmanabhan et al., 1985). Fig. 8 shows the fluorescence emission spectra of pyrene in DCMC4.1 self-assembled nanoparticles incorporated into various concentrations of in distilled water after sonication. When pyrene coexists with self-aggregates in the aqueous media, the total emission intensity increases with the increase of DCMC concentration, especially the intensity of the third highest vibrational band at 383 nm (I_3) starts to drastically increase at a certain concentration of polymeric amphiphiles. The critical aggregation concentration (cac), defined as the threshold concentration of self-aggregation of polymeric amphiphiles by intra- and/or intermolecular association, can be determined by measuring the intensity ratio (I_1/I_3) of the first and the third highest energy bands in the emission spectra of pyrene. Fig. 9 illustrates the changes of the I_1/I_3 values as a function of DCMC4.1 concentration. At low concentration of polymeric amphiphiles, the I_1/I_3 values are close to the value (Dong & Winnik, 1984) (1.87) for pyrene in water, and followed by a linear decrease with the addition of polymeric amphiphiles. Therefore, cac could be determined by the

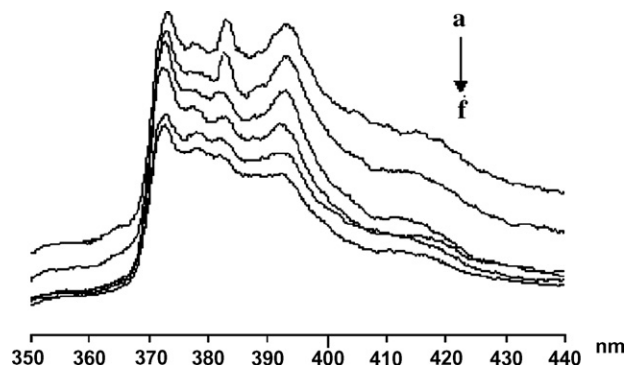


Fig. 8. Effect of polymer concentration on the fluorescence emission spectra of pyrene ($6.0 \times 10^{-7}\text{M}$) in distilled water in the presence of DCMC4.1 at 25°C : [DCMC4.1] = (a) 1 mg/mL, (b) 0.5, (c) 0.25, (d) 0.1, (e) 0.01, (f) 0.001. The excitation wavelength was 336 nm, and the spectra were accumulated with an integration time of 4 s/1 nm.

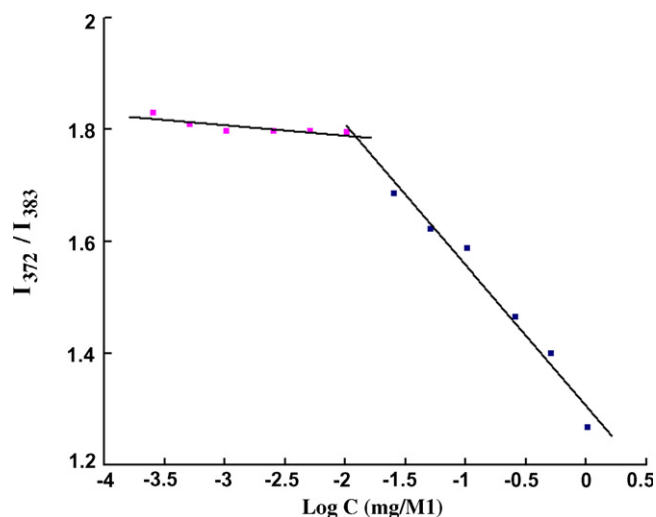


Fig. 9. Intensity ratio (I_1/I_3) from emission spectra vs. log C of DCMC4.1 self-aggregates.

interception of two straight lines. The cac values of DCMCs (Table 1) are lower than the critical micelle concentration (cmc) of some low-molecular-weight surfactants, e.g., 2.3 mg/mL for sodium dodecyl sulfate (SDS) in water (Rahman & Brown, 2003) and 1.0 mg/mL for deoxycholic acid in water (Kratohvil, Hsu, & Kwok, 1986), which implied that DCMCs were a novel kind of polymeric amphiphiles and could form the stable self-assembled nanoparticles at a dilute condition. The cac values of the DCMC conjugates decreased with the increase in the content of hydrophobic deoxycholic acid because of enhanced hydrophobicity (Table 1). The cac of DCMC4.1 was lower in PBS than in distilled water, moreover the cac slightly decreased with the pH value decreasing. The other DCMCs had the same phenomenon. Unlike nonionic amphiphiles, the aggregation behavior of ionic amphiphiles in water is influenced by electrolytes. There are some electrolytes in PBS which screen the negative charges in the CM-curdlan backbone leading to relative increasing of hydrophobic interaction, therefore DCMCs easily aggregated in PBS than in distilled water. Moreover, as mentioned above, ionization extent of carboxyl decreased with pH decreasing, electrostatic repulsion reduced between intra and/or intermolecular leading to cac slightly decreasing in low pH.

4. Conclusion

Deoxycholic acid modified CM-curdlan (DCMC) conjugates, a novel kind of polymer amphiphiles, were synthesized and characterized by FTIR, ^1H NMR and XRD. DCMC self-assembled nanoparticles were prepared by the probe sonication method and their physicochemical properties in the aqueous media were investigated. The mean diameters of DCMC self-assembled nanoparticles were in the range of 192–347 nm with a unimodal size distribution. The zeta potentials of DCMC self-assembled

nanoparticles were very negative in distilled water, however the absolute value of zeta potential of self-aggregates decreased in PBS. The mean size and zeta potential changed with the pH value. The TEM images of self-aggregates showed a spherical shape. The critical aggregation concentration of DCMC conjugates depended on the DS of deoxycholic acid over a range of 0.014–0.052 mg/m and lowered in PBS. This novel nanoparticle system may be useful in the pharmaceutical and biomedical fields, especially being used as the delivery of anti-tumor drugs, and the further investigations are in progress.

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