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Amphipathicity and self-assembly behavior of amphiphilic alginate esters

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ABSTRACT

Amphiphilic alginate esters (Alg-C_n) with different degree of substitution (DS) and hydrophobic alkyl length were synthesized by the reaction between partially protonated sodium alginate and aliphatic alcohols (octanol, dodecanol or hexadecanol) and characterized by conventional methods. The critical micelles concentration (CMC) of Alg-C_n was determined by measuring the fluorescence intensity of pyrene as a fluorescent probe, conductance and surface tension (SFT). Formation and characteristics of the self-assembly micelles of Alg-C_n were studied by transmission electron microscopy (TEM) and Zetasizer Nano Series method. The results indicate that CMC value and the self-assembled micelle size decreased with the increasing of the hydrophobic alkyl chain length, as the DS of Alg-C_n is similar.

1. Introduction

In recent decades, increasing attention has been shifted to designing self-assembly of amphiphilic copolymers or hydrophobically modified polymers which are degradable, tissue-compatible and non-toxic. Amphiphilic copolymers consisting of hydrophilic and hydrophobic segments can form micelle structures with the hydrophobic inner core and the hydrophilic outer shell in aqueous media (Wu, Zheng, & Yang, 2005).

Sodium alginate is a linear anion natural polysaccharide, composing of 1,4-linked residues of β-D-mannuronic (M) acid and α-L-guluronic acid (G) (Atkins, Nieduszynski, Mackie, Parker, & Smolko, 1973; Haug, Larsen, & Smidsrod, 1967), which is applied in food (Júnior et al., 2009), textile industry (Musa et al., 1999), cosmetics industry and pharmaceutical field (Liu, Wang, Gao, Liu, & Tong, 2008). Being strong hydrophilic, sodium alginate has some defects in the application of hydrophobic drug. For example, the capacity is not big and it is easy to burst release as control release material (Yao, Li, Ma, & Ni, 2009), and the adhesion force is not big as mucosal adhesion agent (Zhang, Song, Liang, & Ji, 2009), etc. Therefore, much attention has been paid to modified sodium alginate in order to obtain broader applications of alginate derivatives (Coleman et al., 2011; Omagari, Kaneko, & Kadokawa, 2010; Schutz, Juillerat-Jeanneret, Käuper, & Wandrey, 2011).

Today, increasing scholars research on amphiphilic macromolecules so as to have a deeper understanding of self-assembly behavior of macromolecules (Li et al., 2008; Yuan, Li, Gu, Cao, & Ren, 2011). However, very little result has been reported about the synthesis and the self-assembly of amphiphilic alginate graft aliphatic alcohols. To combine the advantages of synthetic and maintain the favorite properties of natural polymers such as biodegradation and bioactivity, in this paper, we adopt esterification which is often used as a simple method whereby alkyl groups are attached to molecules. Alginate esters (Alg-C_n) with different degree of substitution (DS) and hydrophobic alkyl chain were prepared by the reaction between sodium alginate (SA) and aliphatic alcohols (octanol, dodecanol or hexadecanol). Furthermore, in this study we investigated the chemical structure, self-assembly behavior and the micelle morphology of alginate esters. The study may be used to assess the possibility of the amphiphilic Alg-C_n as potential materials for biomedical applications.

2. Materials and methods

2.1. Materials

Sodium alginate (SA, $\bar{M}_\eta$~430 kDa, M/G = 0.18), octanol, dodecanol, hexadecanol, *p*-toluenesulfonic acid (PTSA), formamide (FA), dimethyl formamide (DMF), 4-(N,N-dimethylamino) pyridine (DMAP) and absolute ethanol were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Sangon Biotech Co. Ltd. (Shanghai, China). Distilled water was used for the preparation of all solutions. Water-free PTSA was prepared by drying under vacuum at 120 °C for 30 min and then was stored in dried DMF. Pyrene (99%) was purchased from Aldrich and recrystallized from absolute ethanol.

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2.2. Synthesis and purification of Alg-C$_n$

Synthesis was carried out according to a previously reported procedure (Yang and He, 2012; Yang, Jiang, He, & Xia, 2012). The FA/DMF (10/9, v/v) containing some PTSA by stirring at 55 °C for 30 min was used to make partially protonated sodium alginate. Subsequently, the esterification reaction between the carboxylic acid groups of protonated sodium alginate and the hydroxyl of aliphatic alcohol (octanol, dodecanol or hexadecanol) was carried out at 45 °C for 30 h, then adding of DMAP, some of the EDC and aliphatic alcohol. Some absolute ethanol was poured into the reaction mixture, and the precipitated product was separated by centrifugation, thoroughly washed with absolute ethanol, then dissolved in the distilled water and neutralized by adding 1.5 wt% Na_2CO_3 solution. The solution was dialyzed against the distilled water for 4 days and lyophilized to get the pure product.

2.3. Characterization of Alg-C$_n$

The chemical structure of Alg-C$_n$ was determined by Fourier transform infrared spectroscopy (FTIR, Tensor 27, Germany), proton nuclearmagnetic resonance (^{1}H NMR, Bruker AVANCE 600, Germany) and thermogravimetric analysis (TG, STA409PC, Germany). The DS ($N_{alkyl}/N_{hexuronic}$) of Alg-C$_n$ was determined by gas chromatography (Agilent GC6890N, USA). All tests were carried out according to previous reports (Yang and He, 2012).

2.4. Measurement of critical micelle concentration (CMC)

The self-aggregation property of Alg-C$_n$ and its CMC was determined using fluorescence spectroscopy with pyrene as a fluorescent probe (Ren, Gao, Lu, Liu, & Tong, 2006) and surface tension as well as conductivity.

Fluorescence emission spectra of pyrene probe were recorded using a fluorescence spectrophotometer (Hitachi F4500, Japan) at 25 ± 0.1 °C. The probe was excited at 335 nm, and the emission spectra were obtained in the range of 350–550 nm. The slit width for excitation and emission was 5.0 and 2.5 nm, respectively.

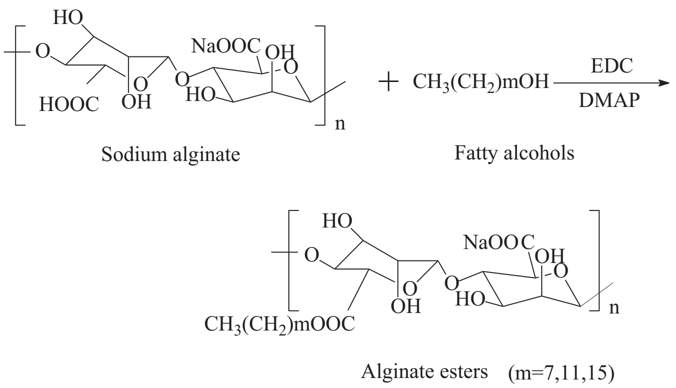
The surface tension (SFT) measurements of Alg-C$_n$ solutions with various concentrations were carried out on a tensionmeter with plate method of Wilhelmy (DCAT11, Germany) at 25.0 ± 0.1 °C. All the solutions were kept at room temperature for 1 day before surface tension measurement.

The conductivity of Alg-C$_n$ solutions was measured by a digital conductivity meter (DDS-11A, China) at 25.0 ± 0.1 °C. At least a set of 10 specific conductance readings for 10 different concentrations of sample was noted in order to get the CMC values for each system.

2.5. Determination of micelle

The sizes of polymeric micelle were measured by Zetasizer Nano Series laser particle size analyzer (Malvern ZEN3690, UK) at 25.0 ± 0.5 °C. For size measurement, 5 mL micelles were diluted with 10 mL distilled water and the experiment for each solution was repeated at least three times.

The morphology of polymeric micelle was observed using transmission electron microscope (TEM, Tecnai 12, Holland). Samples were placed onto copper grill covered with nitrocellulose. They were dried at room temperature, and then were examined using a TEM by negative staining with an aqueous solution of sodium phosphotungstate.



Scheme 1. The synthesis principle of alginate esters.

3. Results and discussion

3.1. Preparation and characterization of Alg-C$_n$

Alginate has an abundance of free hydroxyl and carboxyl groups distributed along the polymer chain backbone, and it, therefore, unlike neutral polysaccharides has two types of functional groups that can be modified to alter the characteristics in comparison to the parent compounds (Yang, Ren, & Xie, 2011; Yang, Xie, & He, 2011). Esterification often used as a simple method whereby alkyl groups are attached to molecules. This method was successfully used by researchers to modify SA, increasing its hydrophobic nature by the addition of alkyl groups to the backbone of the native alginate (Leonard, Rastello De Boisseson, Hubert, Dalencon, & Dellacherie, 2004). The synthesis principle of Alg-C$_n$ is shown in Scheme 1. In this process EDC as the coupling agent was used to modify SA which only has hydrophilicity to form ester linkages between the carboxylate moieties on the alginate polymer and aliphatic alcohols (octanol, dodecanol or hexadecanol). The reaction products are denoted as xAlg-C$_n$, where x, n represent the DS and the length of alkyl chain, respectively. In this paper the samples involved are 9.87% Alg-C$_8$, 10.23% Alg-C$_{12}$ and 10.15% Alg-C$_{16}$, respectively.

The FTIR spectra of SA and 10.23% Alg-C$_{12}$ can be seen from previous articles (Yang and He, 2012). For the spectrum of SA, it is being observed that a broad peak at 3450 cm^{-1} is due to the stretching vibrations of O—H, and a small peak at 2930 cm^{-1} is attributed to the C—H stretching vibrations of methylene groups. The bands at 1090 and 1030 cm^{-1} are assigned to C—O—C stretching vibrations of the saccharide structure (Kanti, Srigowri, Madhuri, Smitha, & Sridhar, 2004). Two strong peaks at 1609 and 1417 cm^{-1} are assigned to asymmetric and symmetric stretching vibrations of carboxyl groups. Comparing that of SA, the spectrum of Alg-C$_{12}$ contains the characteristic hydroxyl and carboxyl bands, but also features additional peaks. The peak of 2855 cm^{-1} is assigned to methylene groups of dodecyl, and the peaks of 1730 and 1250 cm^{-1} are attributed to the C═O and C—O component of an ester bond respectively. The appearance of the peaks suggests that dodecyl groups successfully grafted onto alginate. Compared with 10.23% Alg-C$_{12}$, 9.87% Alg-C$_8$ and 10.15% Alg-C$_{16}$ also have similar features additional peaks, which indicate that aliphatic alcohols successfully grafted into alginate.

^{1}H NMR was investigated to further confirm the chemical structure of the graft copolymers. The ^{1}H NMR spectra of SA and 10.23% Alg-C$_{12}$ have been shown in previous literature (Yang and He, 2012). The H of SA proton peaks appeared at 3.6–5.1 ppm, in which the anomeric protons are observed in the region 4.5–5.1 ppm. In contrast with the spectrum of SA, additional peaks in spectrum of Alg-C$_{12}$ are observed from 0.9 to 3.0 ppm, which were assigned to the methyl and methylene protons of lauryl chain respectively

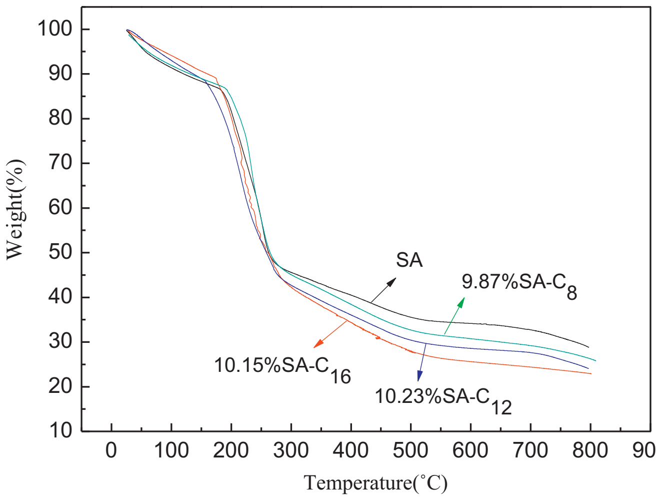


Fig. 1. The TG analysis curves of SA and Alg-C_n.

(Yang, Ren, et al., 2011; Yang, Xie, et al., 2011). At same time, compared with 10.23% Alg-C_{12}, 9.87% Alg-C_8 and 10.15% Alg-C_{16} also have similar characteristics.

Fig. 1 shows the TG analysis curves of SA and Alg-C_n. As we know, the first stage region belongs to the loss of combined water of SA in the 40–160 °C (Laurienzo, Malinconico, Motta, & Vicinanza, 2005), and in the second stage from 220 to 280 °C, the weight loss of SA should be the carboxyl take off CO_2 and neighboring hydroxyl dehydration. The weightlessness of 9.87% Alg-C_8 in the second stage was obviously higher than the SA because in this period the side chain heat cracked into small molecules increased the weight. This phenomenon is also found in the 10.23% Alg-C_{12} and 10.15% Alg-C_{16} which further supports the presence of ester bond formation between aliphatic alcohol and SA.

3.2. Self-aggregation behavior of Alg-C_n

In aqueous media the aggregation behavior of Alg-C_n was monitored by the fluorescence probe technique in the presence of pyrene as fluorescent probe. Pyrene shows a small fluorescence intensity in a polar environment owing to poorly soluble and self-quenching, but it strongly emits radiation when micelles or other hydrophobic microdomains are formed in an aqueous solution, as it preferably close to the microdomains (Amiji, 1995; Gao et al., 2008). Pyrene has five peaks in the emission spectra, and the emission intensity ratio of the first peak (I_1 = 373 nm) and the third peak (I_3 = 383 nm) I_1/I_3 is sensitive to the polarity of the microenvironment. Thus the change in peak I_1/I_3 ratio of pyrene monomer fluorescence could be used to examine the aggregation behavior of surfactants or polymers in aqueous solution (Sui, Song, Chen, & Xu, 2005). The critical micelle concentration (CMC), which can be determined from the change in the intensity ratio (I_1/I_3) of the pyrene in the presence of polymeric amphiphiles, is the lowest concentration of self-aggregation formation by intramolecular or intermolecular association.

Table 1
Three methods for measuring CMC values.

Sample	CMC ($\times 10^{-2}$ mg/mL)		
	Fluorescence	Conductivity	SFT
9.87% Alg-C_8	0.34	0.38	0.33
10.23% Alg-C_{12}	0.29	0.27	0.24
10.15% Alg-C_{16}	0.17	0.17	0.16

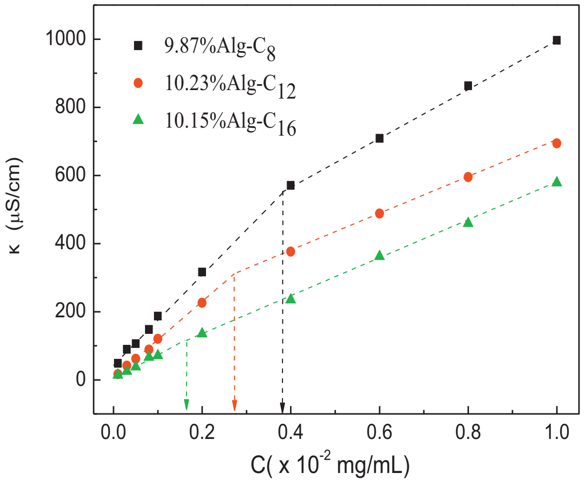


Fig. 3. The relationship of conductivity with concentration of Alg-C_n.

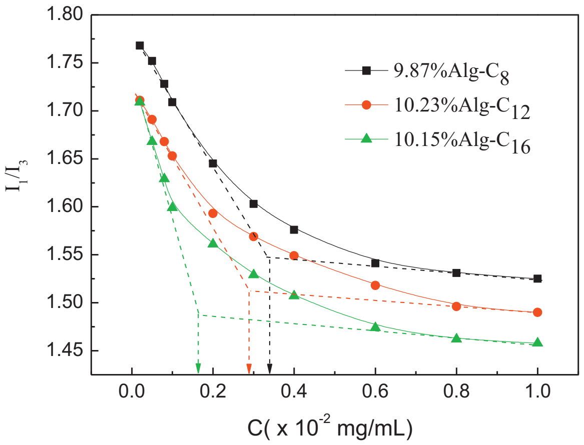


Fig. 2. The relationship of pyrene fluorescence intensity ratios (I_1/I_3) with concentration of Alg-C_n.

Fig. 2 shows the changes of the I_1/I_3 values as a function of Alg-C_n concentrations which proved that Alg-C_n have happened self-aggregation behavior in aqueous solution. It can be seen from Fig. 2 that the inflection point of each curve corresponds to the CMC value respectively and the I_1/I_3 values of Alg-C_n declined with the increasing of the concentration. As shown in Table 1, when the

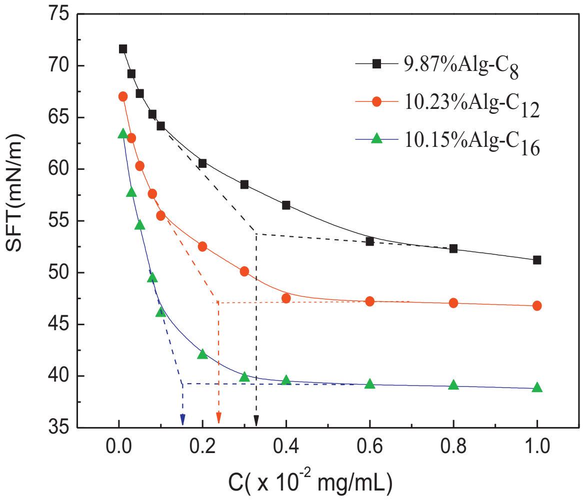


Fig. 4. The relationship of SFT with concentration of Alg-C_n.

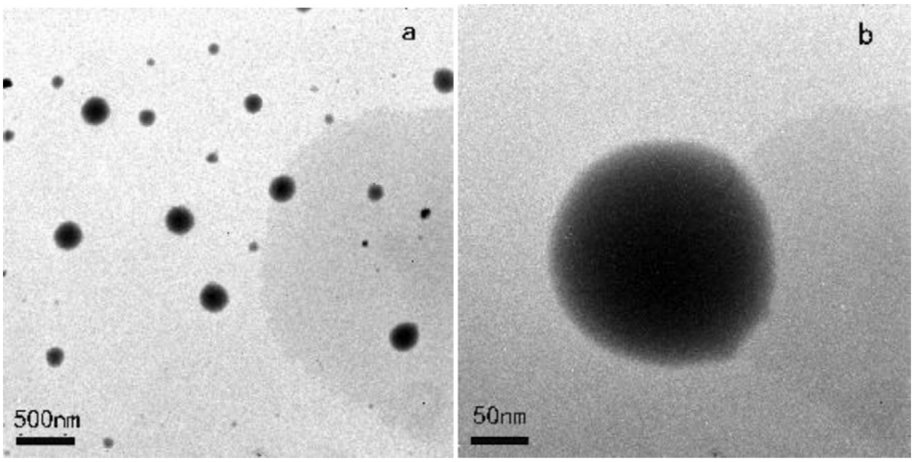


Fig. 5. TEM images of 9.87% Alg-C_8 self-assembled micelle.

DS of Alg-C_n was similar, CMC value decreased with the increasing of the hydrophobic alkyl chain length. Furthermore, this indicates that the longer hydrophobic alkyl chain of Alg-C_n, the more easy to assemble, and the weaker polarity of formation of hydrophobic microdomains.

As shown in Fig. 3, the conductivity curves of Alg-C_n solution change with the concentration and the inflection point of each curve also corresponds to the CMC value. From the figure we can see that when the DS of Alg-C_n were similar, the CMC value decreased with the increasing of the hydrophobic alkyl chain length, which also can be seen in Table 1.

The relationship of SFT with concentration of Alg-C_n is shown in Fig. 4. It can be noticed that when the DS of Alg-C_n was similar, SFT value decreased with the increasing of the hydrophobic alkyl chain length at the same concentration. The longer the hydrophobic alkyl chain, the stronger reduce the SFT, this is probably because with the hydrophobic alkyl chain length increased, and the density of the hydrophobic groups in solution surface increased as well, lead to the SFT of solution drop even lower. The data from Table 1 shows the CMC value obtained by SFT, electrical conductivity and fluorescence are similar.

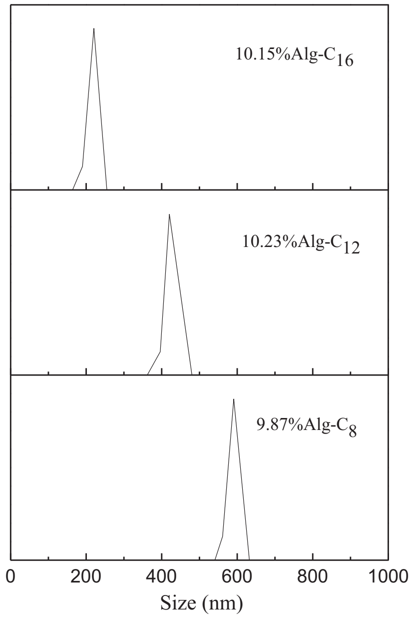


Fig. 6. The micelle size of Alg-C_n.

3.3. Micelle of Alg-C_n

As shown in Fig. 5, the micelle morphology of 9.87% Alg-C_8 observed by TEM was almost spherical shape, a similar micelle morphology appearing in other samples, which proved that alginate esters could form self-aggregates in the aqueous media.

The self-assembled micelle size of Alg-C_n was determined by Zetasizer Nano Series method which has been shown in Fig. 6. When the DS of Alg-C_n was similar, the size of aggregates decreased as the long hydrophobic chain increased, which indicated that with the growth of the hydrophobic carbon of alginate ester, hydrophobic interaction became stronger and combined more closely.

4. Conclusion

Through grafting aliphatic alcohols (octanol, dodecanol or hexadecanol) onto water soluble SA, a novel amphiphilic alginate esters (Alg-C_8, Alg-C_{12} or Alg-C_{16}) graft copolymers have been successfully synthesized. Alginate esters can self-assembly of regular sphere morphology in water. When the DS of Alg-C_n is similar, CMC value and the self-assembled micelle size decreased with the increasing of the hydrophobic alkyl chain length. Those indicate that the longer hydrophobic alkyl chain of alginate esters, the stronger of hydrophobic interaction, and the more easy to assemble. Moreover, this novel amphiphilic alginate derivatives system is expected to be used as protein drugs and a carrier of hydrophobic drugs, and the further investigations are underway.

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