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Synthesis of N-Alkylated Chitosan and its Aggregation Behavior

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***Abstract*—A series of N-alkylated chitosan (NACS) with various alkyl chain length and degree of substitution (DS) was prepared by a reductive amination method. The synthesis and structural characteristics of NACS was determined by FTIR, NMR and elemental analysis (EA). Fluorescence spectroscopy was used to analyze the aggregation behavior of the hydrophobic alkyl chain in aqueous solution and determine critical aggregation concentration (CAC). The results show that N-dodecyl chitosan and N-Octadecyl chitosan with a DS of 30%, 20%, 10% were successfully prepared. The CAC of NACS was smaller than that of chitosan (CS), and the CAC decreased with a larger DS and a longer alkyl chain.**

Keywords-N-alkylated chitosan; aggregation behavior; CAC

I. INTRODUCTION

As a cationic polysaccharide, chitosan (CS) is widely used in various fields such as tissue engineering and drug delivery because of its excellent biocompatibility, well degradability, antimicrobial activity, wound healing property and low cost. [1-3]There are a large number of amino and hydroxyl groups on CS chain, which are beneficial to modification of CS. To expand the application fields of CS, various CS derivatives were synthesized by grafting different functional groups such as carboxymethyl, aldehyde and alkane. Among many of its derivatives, hydrophobic modification of CS is a promising one, since they are capable of self-assembling to form micellar nanoparticles with a hydrophobic core in water and therefore it can be used as a hydrophobic drug carrier such as Taxol to improve the stability of drugs and facilitate the delivery and release. [4-6] Nowadays, there are many researches based on the hydrophobic alkyl modification of CS biomaterials, which can be synthesized by grafting alkyl onto CS backbone by a reductive method [7-9]. However, the effect of alkyl chain length and degree of substitution (DS) on the ability of self-assembling to form micelles is unclear. Therefore, it is a burning question to study the aggregation behavior of alkyl modification of CS.

In this study, we prepared a series of N-alkylated chitosan (NACS) with various alkyl chain length (C12, C18) and DS (30%, 20%, 10%) by a reductive amination method. The effects of DS and alkyl chain length on the aggregation behavior of NACS in aqueous solution were investigated.

II. MATERIALS AND METHODS

A. Materials

CS of molecular weight 560kDa was purchased from Shandong Aokang Biotechnology Co., Ltd (Shandong, China); acetic acid and pyrene were purchased from Tianjin Brett Biotechnology Co., Ltd. (Tianjin, China); dodecyl aldehyde, and octadecyl aldehyde were purchased from Sigma (St. Louis, MO, USA); deuterium (D_2O) and deuterated hydrochloride (DCl) from Tianjin Weikai Bioeng Ltd. (Tianjin, China).

B. Preparation of N-Alkylated Chitosan

NACS was prepared according the method reported by Dowling et al [10]. Chitosan 2 g was dissolved in 200 mL of 1% acetate solution. After completely dissolved, a certain amount of dodecyl aldehyde or octadecyl aldehyde was added to adjust the pH to 5.1. After stirring at room temperature for 4h, sodium borohydride (3 mol per chitosan monomer) was added, with an excess stirring for 12 h. The precipitate was obtained by adjusting the pH to10 using a NaOH solution, then washed with ethanol to remove the residuary aldehyde. Finally, the product NACS was obtained after freeze-drying.

C. FTIR

FTIR spectra of CS and NACS were recorded on Tensor 37 spectrometer (Thorlabs, Newton, NJ, USA) by KBr tableting method. And then the chemical structures of different samples were analyzed.

D. NMR

1H NMR spectra of samples were recorded on Bruker spectrometer (AV-500, Biospin, Billerica, MA, USA). The

deacetylation degree (DD) of CS and the structure of NACS were determined. DCl/D_2O (1:9) was used as a solvent for CS and NACS.

E. Element Analysis

An elemental analyser (Vario EL/microcube, Elementar, Langenselbold, Germany) was used to determine the content of C and N. And the DS of NACS was evaluated according to formula (1).

$$\frac{[8(1-x)+6x+n\times DS]\times 12}{14}=\frac{C}{N} \quad (1)$$

In which,
x --- DD of CS
C, N --- C, N element percentage
n --- The number of C atoms in alkyl aldehyde: n=12, 18

F. The Aggregation Behavior of NACS

Fluorescence spectroscopy studies were determined using a Varian Cary Eclipse Fluorescence Spectrophotometer. Pyrene was employed as a hydrophobic probe to study the formation of hydrophobic domains in aqueous solution and to determine the critical aggregation concentration (CAC), which is an important parameter that measures the stability of the self-assembly polymer. The ratio of fluorescence intensity of pyrene monomer emission spectra at 374nm to 385nm (I_{374}/I_{385}) is defined as hydrophobic factor to analyze the variation of micro-polarity in aqueous solution. In this study, pyrene (at concentration of 6.0×10^{-7}mol/L) was used as fluorescence probe, the I_{374}/I_{385} value of a series of NACS acetic acid solution of different concentration gradient was determined. The excitation wavelength was 339 nm, while spectra was recorded in the interval 350-400 nm at an integration time of 1.0 s. [11]

III. Results And Discussion

A. FTIR

The FTIR spectra of C12a (Amino/Aldehyde = 1:0.8) and C18a (Amino/Aldehyde = 1:1.2) were shown in Figure 1, and the results were consistent with the literature [12]. Compared with CS, it was clear that the band intensity of the stretching vibration band of CH_3 (2900-3000cm^{-1}) and CH_2 (2800-2900cm^{-1}) were enhanced, the bending vibration absorption peak of alkyl chain (1440cm^{-1}) and the backbone vibration peak of CH_2 (720cm^{-1}) appeared while the bending vibration band of NH (1560cm^{-1}) were enhanced, which indicated that the dodecyl and octadecyl were successfully grafted onto the amino of CS chain respectively.

B. NMR

The DD of CS was 87% by determining the integral of CH3, H3, H4, H5, and H6, according to the equation reported previously [13]. As the description in Figure 2, the positions of the peaks in the spectra are basically consistent with the report in the literature [14]. For the spectra of CS, the distribution of peaks is as follow: 1.8(CH_3), 4.3(H1), 2.9(H2), 3.3-3.9(H3, H4, H5, H6). For the spectra of C12a, the distribution of peaks is as follow: 2.9-3.0(-NH-CH_2-$(CH_2)_{10}$-CH_3), 1.0-2.0(-NH-CH_2-$(CH_2)_{10}$-CH_3), 0.6(-NH-CH_2-$(CH_2)_{10}$-CH_3). For the spectra of C18a, the peaks in the range of 2.9-3.0 and 1.7-2.0 ppm indicated the methylene hydrogen of octadecyl groups grafted onto the CS backbone successfully.

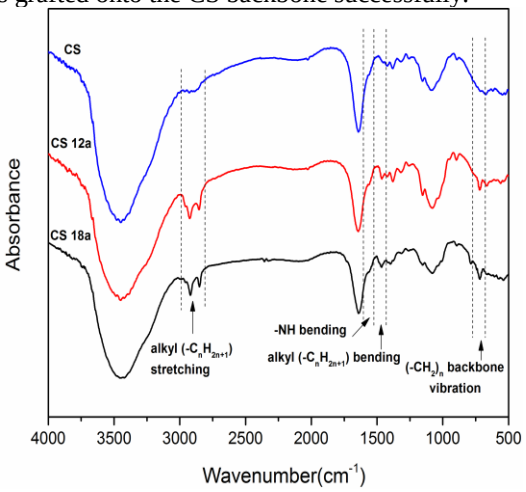

Figure 1. FTIR spectrum of CS, C12a and C18a.

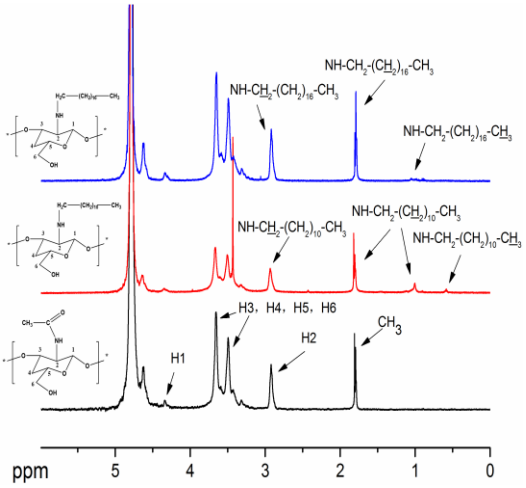

Figure 2. 1H NMR spectra of CS, C12a and C18a.

C. Element Analysis

The content of C and N and the DS of NACS was shown in Table 1. With the ratio of amino/aldehyde increased, the content of N in the NACS increased while the content of C decreased and the DS decreased. The DS of NACS was about 30%, 20%, 10% by changing the ratio of amino to aldehyde group.

D. The Aggregation Behavior of NACS

Polarity parameter I_{374}/I_{385} of pyrene in aqueous solutions for C12 and C18 with various DS was shown in Figure 3 and Figure 4. As the concentration of NACS increased, the curve of I_{374}/I_{385} took a turn and the value of the turning point was CAC. The CAC of C12 and C18 with various DS was shown in Table 2. Compared with CS, the CAC of NACS decreased

obviously. For the C12and C18 the CAC decreased with a larger SD and a longer alkyl chain. With the increase of SD and the hydrophobic chain length, the hydrophobic interaction force enhanced while NACS tend to form a core-shell structure in polarity solution. So the pyrene entered the hydrophobic core easily and the therefore fluorescence spectrum of pyrene was increased.

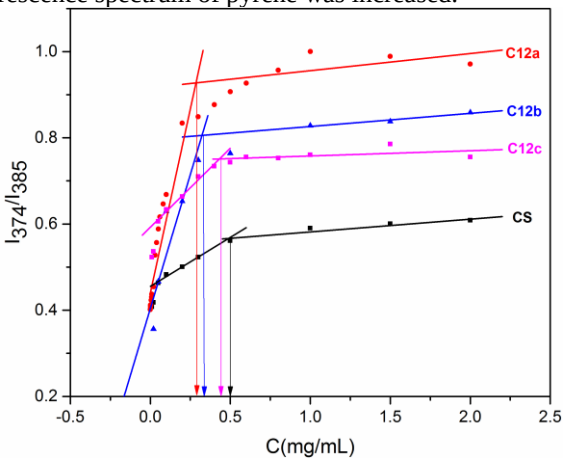

Figure 3. Variation of the I374/I385 with concentration of CS, C12a, C12b, C12c.

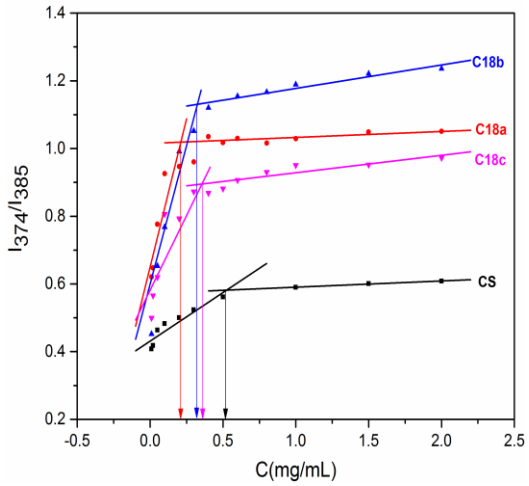

Figure 4. Variation of the I374/I385 with concentration of CS, C18a, C18b, C18c.

TABLE I THE CONTENT OF C AND N AND THE DS OF NACS

Sample	Amino/Aldehyde (mol/mol)	C (%)	N (%)	SD (%)
C12a	1:0.8	49.71±1.59	5.69±0.28	32.69±0.86
C12b	1:0.5	44.27±2.18	6.14±0.33	17.99±0.75
C12c	1:0.3	41.26±1.12	6.71±0.40	7.57±0.37
C18a	1:1.2	51.48±2.01	5.13±0.37	30.27±0.79
C18b	1:0.6	47.64±1.77	5.73±0.29	19.15±0.63
C18c	1:0.3	41.90±1.89	5.97±0.42	10.67±0.57

TABLE II CAC (MG/ML) OF NACS AND CS

Sample	C12a	C12b	C12c	C18a	C18b	C18c	CS
CAC	0.291	0.335	0.442	0.206	0.317	0.361	0.504

IV. CONCLUSION

A series of NACS with various alkyl chain length (C12, C18) and DS (30%, 20%, 10%) was prepared. The results of FTIR and ^{1}H NMR indicated that the dodecyl and octadecyl groups were successfully grafted onto the amino group of CS respectively. CAC was obtained by the fluorescence spectra in concentrated solution of all the sample which indicated that the aggregation behavior of NACS was affected by its alkyl chain length and the DS. CAC of NACS was higher than that of CS, as well as decreased with a larger SD and a longer alkyl chain. So the alkyl chain can promote the formation of local hydrophobic environments. And with the increasing of alkyl chain length and the DS, the intra- and inter-hydrophobic interaction enhanced.

The CAC study of amphiphilic polymer NACS provided a theoretical basis for its use as a carrier of hydrophobic drugs. [15, 16] The amphiphilic polymer can self-assemble into micelles in selective solvents. The hydrophilic chains of NACS can form a hydrophilic shell and hydrophobic alkyl chains can form a hydrophobic core. Hydrophobic drugs can be loaded into the micellar core through the physical synergy. In addition, the ability of self-aggregation to form micelles and the adsorption capacity of hydrophobic drugs can be controlled by adjusting the DS of alkyl chain and the chain length. [17-20] Therefore, NACS can be used as a carrier of hydrophobic drugs to improve the stability of drugs and facilitate the delivery and release of hydrophobic drugs.

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