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.

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\textsubscript{2}O) 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 residiary 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

\textsuperscript{1}H 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$_2$O (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).

$$\frac{[8(1-x)+6x+nxDS]}{14} = \frac{C}{N}$$

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 6.0x10$^{-7}$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 CH$_3$, H3, H4, H5, and H6, according to the equation reported previously [13]. As described 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: 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.

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 C12 and 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.

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.

ACKNOWLEDGEMENT

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

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

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>C12a</th>
<th>C12b</th>
<th>C12c</th>
<th>C18a</th>
<th>C18b</th>
<th>C18c</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAC</td>
<td>0.291</td>
<td>0.335</td>
<td>0.442</td>
<td>0.206</td>
<td>0.317</td>
<td>0.361</td>
<td>0.504</td>
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REFERENCE


