Preparation and Characterization of 6-Carboxychitosan

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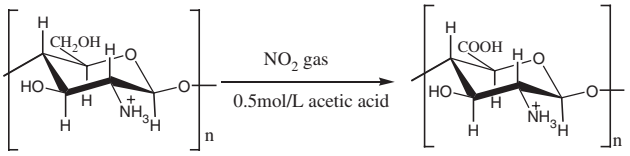
The hydroxymethyl group of 2-amino-2-deoxy-D-glucose unit in chitosan was selectively oxidized to carboxyl group with NO_2 to form amphoteric chitosan by one step so as to give chitosan a new bioactivity. The structure and properties of amphoteric chitosan are discussed.

Polyampholytes or amphoteric polyelectrolytes, containing both anionic and cationic groups along the macromolecular backbone, have similar structure with biopolymer (protein, nucleic acid, etc.) and superior biocompatibility, which has attracted much attention in biomedical, pharmacological, agricultural, and biotechnological fields.[1–4] Chitosan, being a polycation electrolyte and consisting of β (1 → 4)-2-amino-2-deoxy-D-glucose repeat units, is widely used in enzyme engineering, biomedicine, and pharmacology, etc. Because of the large quantity of cationic groups ($-NH_3^+$) in the molecule, chitosan can easily absorb erythrocytes and thrombocytes that carry negative charges to form thrombus or to cause red blood cell lysis,[2] so it is usually grafted anionic groups, such as sulfo or heparin groups etc, in order to improve its blood compatibility.[5–7]

In this work, the hydroxymethyl group of 2-amino-2-deoxy-D-glucose unit in chitosan was selectively oxidized to carboxyl group as follows (Scheme 1).



Scheme 1. A schematic illustration of the oxidization chitosan with NO_2.

7.0 g chitosan, with 85% deacetylation, prepared by repeated N-deacetylation of chitin obtained from mantis shrimp shell with 50% w/v NaOH at 100 °C under nitrogen, was thoroughly swollen in 93.0 mL aqueous 0.5 mol/L acetic acid to form a semisolid gel. With constant stirring, NO_2 gas was added into the above sample under a room temperature of 25 °C. When pH value of the system reached 3.5, the adding NO_2 was stopped, the NO_2 remained in the solution was removed under vacuum, and the amphoteric chitosan begun to precipitate after adding acetone. Purified by washing with acetone five times and dried in air at a room temperature of 25 °C, about 6.0 g amphoteric chitosan was obtained.

As shown in Figure 1, the FTIR spectrum reveals that the obtained amphoteric chitosan (Figure 1. b) has a new carbonyl ν(C=O) at 1709.5 cm^{-1} comparing with chitosan (Figure 1. a). Since –COOH groups in the molecule exhibit ionization equilibrium in aqueous solution, some of –COOH groups are converted into $-COO^-$ groups and the new $\nu_{as}(COO^-)$ at 1566.1 cm^{-1} and $\nu_s(COO^-)$ at 1409.9 cm^{-1} were observed too. Because the sample was prepared in an acidic medium, and carbonyl oxygen of acetylamino group in chitosan was capable of protonation ($^+H\cdots O{=}C\text{-}CH_3$) with H^+, the spectrum shows a shift in ν(C=O) of acetylamino groups from 1657.1 cm^{-1} in chitosan to 1623.8 cm^{-1} in amphoteric chitosan. For examining the existence of –COOH in the molecule, a sample of amphoteric chitosan, in which all of –COOH groups were converted into $-COO^-$ groups by dissolving amphoteric chitosan in 0.1 mol/L NaOH solution and precipitated with acetone, was tested with an FTIR spectrometer. The spectrum of sample (Figure 1. c) shows the carbonyl ν(C=O) disappeared, and the two strong vibrations, $\nu_{as}(COO^-)$ at 1571.4 cm^{-1} and $\nu_s(COO^-)$ at 1404.1 cm^{-1}, were observed. These results can tell us that no aldehyde and ketone groups existed in amphoteric chitosan molecule and the hydroxymethyl group of 2-amino-2-deoxy-D-glucose units in chitosan was selectively oxidized to carboxyl group by NO_2 successfully. Compared (a), (b), and (c), the characteristic vibrations of pyranpolyose combined with β (1 → 4) glycoside bond at 895.2 cm^{-1} and 1151.4 cm^{-1} show no shift, which indicated that the amphoteric chitosan has the same macromolecular backbone as chitosan.

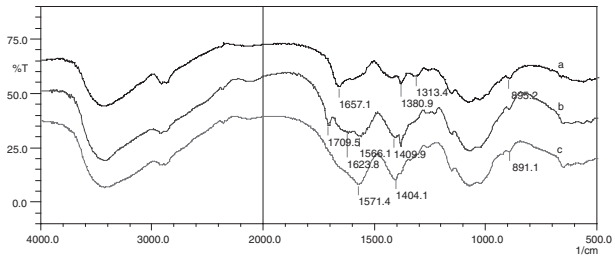


Figure 1. The FTIR spectra of chitosan (a), amphoteric chitosan (b), and sodium salt of amphoteric chitosan (c).

1.0 g amphoteric chitosan was dissolved in 100.0 mL of distilled water and 0.5 g chitosan was dissolved in 100.0 mL of 0.1 mol/L acetic acid respectively. The relative viscosities (η_{rel}) of the samples with different concentrations were determined using a standard Ubbelohde's viscometer under 30 ± 0.1 °C, respectively, and the intrinsic viscosity ([η]) of the sample was obtained by extrapolating the reduced viscosity vs concentration data to zero. The results (Figure 2) indicate that chitosan has much higher intrinsic viscosity than amphoteric chitosan. The reason is that the electrostatic repulsion between $-NH_3^+$ groups along the macromolecular backbone made the molecule of chitosan stretched. As to amphoteric chitosan, both its –COOH and $-NH_2$ groups being in the ionization equilibriums in aqueous solution are converted partially into $-COO^-$ and $-NH_3^+$ groups along the macromolecular backbone. The electrostatic attraction between them made the mole-

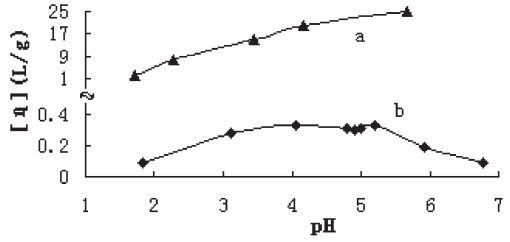


Figure 2. The effects of pH on [η] of chitosan (a) and amphoteric chitosan (b).

cule fold and resulted in amphoteric chitosan having different rheological properties from chitosan. When pH<4.0 or pH>5.4, amphoteric chitosan, as chitosan, has common characteristics of a polyelectrolyte that the high ionic strength of the solution made its intrinsic viscosity decreased. When pH=4.0–5.4, amphoteric chitosan has common characteristics of amphoteric polyelectrolytes[8] that the high ionic strength of the solution made its intrinsic viscosity raised, and its isoelectric point (pI) value is 4.9.

Figure 3 shows that there is spherulite (Figure 3. b) and no fiber structure like chitosan (Figure 3. a) in amphoteric chitosan sample. The reason probably is that the electrostatic attraction between $-NH_3^+$ and $-COO^-$ groups along the macromolecular backbone made the molecule of amphoteric chitosan fold so that

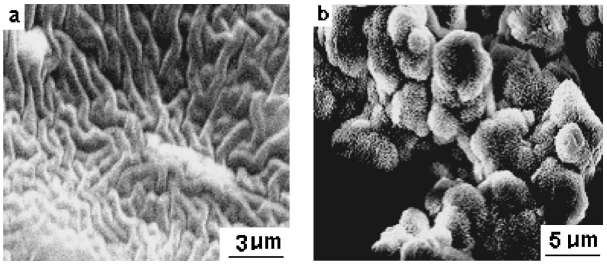


Figure 3. The SEM pictures of chitosan (a) and amphoteric chitosan (b) sample.

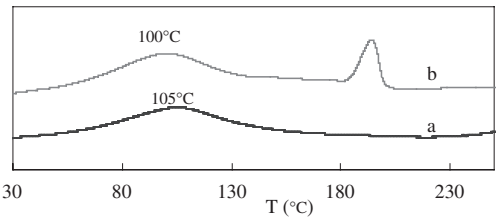


Figure 4. The DSC curves of chitosan (a) and amphoteric chitosan (b) at a heating rate of 20 °C/min.

it couldn't form fiber structure as chitosan.

The DSC curve (Figure 4) shows that amphoteric chitosan has an endothermal peak at lower temperature (100 °C) than that of chitosan (105 °C), which indicates that the intermolecular hydrogen bond of amphoteric chitosan is weaker than chitosan because of its fold molecule. The endothermal peak at 194 °C indicates that the amphoteric chitosan take place decarboxylation, whereas chitosan does not under the same condition.

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