Preparation and Rheological Behaviors of 6-Carboxy-Chitosan

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ABSTRACT: The hydroxymethyl groups of 2-amino-2-deoxy-D-glucose units in chitosan were selectively oxidized to carboxyl groups with NO_2 gas to form 6-carboxy-chitosan in aqueous 0.5 mol/L acetic acid. In optimal conditions, the 52.5% hydroxymethyl groups in chitosan could be converted into carboxyl groups. When the pH value of the solution is lower than 4.0 or more than 5.4, 6-carboxy-chitosan has common characteristics of a polycation or polyanion electrolyte, respectively, and when the pH value of the solution is 4.0—5.4, 6-carboxy-chitosan has common characteristics of an amphoteric polyelectrolyte. 6-Carboxy-chitosan has same macromolecular backbones as chitosan, and its isoelectric point (pI) value is 4.9. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 1126–1130, 2004

Key words: chitosan; polysaccharides; synthesis; 6-carboxy-chitosan; rheology

INTRODUCTION

Polyampholytes or amphoteric polyelectrolytes, containing both anionic and cationic groups along the macromolecular backbone, have a similar structure as biopolymer (protein, nucleic acid, etc.)[1] and superior biocompatibility, which has attracted much attention in the biomedical, pharmacological, agricultural, and biotechnological fields.[2–5] Chitosan is a biopolymer consisting of $\beta(1\rightarrow4)$-2-amino-2-deoxy-D-glucose repeat units and is normally obtained by alkaline deacetylation of chitin, which is the second most abundant polysaccharide in nature. Having common characteristics of a polycation electrolyte and good biocompatibility, chitosan can selectively adsorb some other biopolymers and is widely used in enzyme engineering, biomedicine, and pharmacology, etc. However chitosan can easily adsorb erythrocytes and thrombocytes that carried negative charges on its surface to form thrombii or cause red blood cell lysis[3] because of the electrostatic attraction between chitosan and erythrocytes or thrombocytes, which may cause inconvenience when it is used as a medical material, therefore chitosan is usually modified by grafting anionic groups, such as sulfo groups or heparin etc., to improve its blood compatibility. Hirano et al.[6] have synthesized polysulphate chitosan derivatives and determined its antithrombin activity. Chandy and Rao[7] have made chitosan and heparin crosslinked with glutaraldehyde and Liu et al.[8] have prepared a blend microcapsule containing chitosan and algin for increasing the biocompatibility of chitosan. However, so far there are no papers reporting the preparation and behaviors of 6-carboxylic chitosan to the best of our knowledge.

In this paper, 6-carboxy-chitosan was synthesized by selectively oxidizing the $-CH_2OH$ groups in chitosan to –COOH groups with NO_2 gas (Scheme 1) and its structure and rheological behaviors were studied.

EXPERIMENTAL

Materials

The preparation of chitin was conducted following a similar procedure described by Zhou et al.[9] using mantis shrimp shell, and chitosan with high degree of N-deacetylated (DD = 90%) was prepared by repeated N-deacetylation of chitin with 50% w/v NaOH at 100°C under nitrogen. Acetic acid, nitric acid, copper, and other reagents were purchased from Peking Chemical Co., China, and were used without further purification.

Preparation of 6-carboxy-chitosan

Chitosan (5.0 g) was thoroughly swollen in 50 mL of aqueous 0.5 mol/L acetic acid to form a semisolid gel. With constant stirring, NO_2 gas, prepared by the reaction of copper with concentrated nitric acid, was added into the above sample under room tempera-

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

ture, and the viscosity of the mixture decreased rapidly. When the pH value of the system reached 3.5, the addition of NO_2 was stopped, the NO_2 remaining in the solution was removed under vacuum, and the 6-carboxy-chitosan began to precipitate after adding acetone. Purified by washing with acetone three times and dried at room temperature, 6-carboxy-chitosan (4.5 g, degree of oxidation 52.5%) was obtained.

Determination of the degree of oxidation (DO) in 6-carboxy-chitosan

About 0.2 g 6-carboxy-chitosan sample was dissolved in 15.0 mL of water, and 5.0 mL of 20% (v/v) formaldehyde was carefully added in the solution to mask $-NH_2$ groups in 6-carboxy-chitosan. The mixture was titrated with 0.05 mol/L NaOH standard solution using phenolphthalein as indicator. The amount (m) of aminoglucose units in which the $-CH_2OH$ groups was oxidized into –COOH groups in the sample was determined from the equation

$$m = \frac{c_1 v_1}{1{,}000}\ (\text{mol})$$

where c_1 and v_1 are concentration (mol/L) and volume (mL) of NaOH standard solution, respectively. The *DO* of 6-carboxy-chitosan, which is the percentage of the $-CH_2OH$ groups that were oxidized into –COOH groups in chitosan, was given by

$$DO = \frac{m}{m + \dfrac{M - 175m}{161}} \times 100$$

where c_1 and v_1 are concentration (mol/L) and volume where M is the weight (g) of the sample and 175 and 161 are the M_w of the aminoglucuronic acid unit and D-glucosamine unit in 6-carboxy-chitosan molecules, respectively.

FTIR spectroscopy

A FTIR-8900 (Shimadzu Corp., Japan) infrared spectrophotometer was used to identify the chitosan and 6-carboxy-chitosan. All samples were prepared for analysis using a KBr pellet. Pellets were prepared using a 50 : 1 weight ratio of KBr to sample. All spectra were acquired at room temperature.

Morphology and thermal stability

The morphology of chitosan and 6-carboxy-chitosan samples were evaluated by scanning electron microscopy using a KYKY-2800 (China) instrument and the thermal stabilities were examined with a DSC-7 differential scanning calorimeter (Perkin—Elmer, USA) at a heating rate of 20°C/min under nitrogen.

Rheological behaviors of 6-carboxy-chitosan

6-Carboxy-chitosan (1.0 g) was dissolved in 100 mL of distilled water and 0.5 g chitosan was dissolved in 100 mL of 0.1 mol/L acetic acid, respectively. The relative viscosities (η_{red}) of the samples with different concentrations were determined using a standard Ubbelohde viscometer at 30 ± 0.1°C, and the intrinsic viscosity ($[\eta]$) of the sample was defined as follows:

$$[\eta] = (\eta_{red})C \to 0$$

which is obtained by extrapolating the reduced viscosity versus concentration data to zero, and the intercept on the abscissa is the intrinsic viscosity. The influences of the acidity (adjusted with HCl or NaOH) and salt addition on intrinsic viscosity of the sample were examined.

RESULTS

Characterization of 6-carboxy-chitosan

The FTIR spectra were recorded with a SHIMADZU FTIR-8900 infrared spectrophotometer (Fig. 1). The spectrum of chitosan [Fig. 1(a)] shows one amideamine band at 1,657.1 cm^{-1} as well as $\delta(CH_3)$ at 1,380.9 cm^{-1}. This evidence indicated that the chitosan wasn't deacetylated by 100%. Compared with chitosan, the FTIR spectrum of 6-carboxy-chitosan [Fig. 1(b)] shows a new carbonyl ν(C=O) at 1,709.5 cm^{-1}. Since a part of the –COOH and $-NH_2$ groups in the sample changed into $-COO^-$ and $-NH_3^+$ groups, the new $\nu_{as}(COO^-)$ at 1,566.1 cm^{-1} and $\nu_s(COO^-)$ at 1,409.9 cm^{-1} were observed also. For examining the existence of –COOH in the molecules, a sample of 6-carboxy-chitosan, in which all of –COOH groups were converted into $-COO^-$ groups by dissolving 6-carboxy-chitosan in aqueous 0.1 mol/L NaOH solution and precipitating with acetone, was tested with the FTIR spectrometer. The spectrum of the sodium salt of 6-carboxy-chitosan [Fig. 1(c)] shows the carbonyl ν(C = O) at 1,709.5 cm^{-1} disappeared, and the two strong vibrations, $\nu_{as}(COO^-)$ at 1,571.4 cm^{-1} and $\nu_s(COO^-)$ at 1,404.9 cm^{-1}, were observed. These results indicate that no alde-

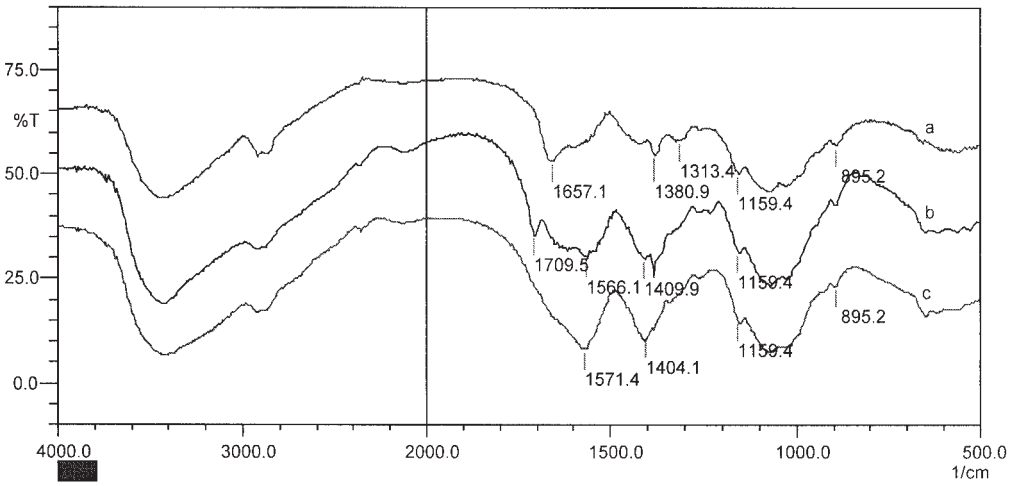

Figure 1 FTIR spectra: (a) chitosan, (b) 6-carboxy-chitosan, and (c) the sodium salt of 6-carboxy-chitosan.

hyde and ketone groups existed in 6-carboxy-chitosan molecules, and the hydroxymethyl groups of 2-amino-2-deoxy-D-glucose units in chitosan were selectively oxidized to carboxyl groups by NO_2 gas successfully. Comparing Figures 1(a), (b), and (c), the characteristic vibrations of pyranpolyose combined with $\beta(1\rightarrow4)$ glycoside bond at 895.2 cm^{-1} and 1,159.4 cm^{-1} have no shift, which indicates that the 6-carboxy-chitosan has the same macromolecular backbone as chitosan.

Figure 2 shows the SEM pictures of chitosan precipitated from aqueous 0.5 mol/L acetic acid solution with aqueous NaOH solution and 6-carboxy-chitosan. There is spherulite [Fig. 2(b)] and no fiber structure like chitosan [Fig. 2(a)] in the 6-carboxy-chitosan sample. The reason probably is that the electrostatic repulsion between $-NH_3^+$ groups along the macromolecular backbone of chitosan made the molecule have a stretch conformation in solution; the molecules of chitosan could maintain the conformations to form fiber structure by parallel orientation when they were precipitated from aqueous solution. As for 6-carboxy-chitosan, having $-NH_3^+$ and $-COO^-$ groups along the macromolecular backbone, the electrostatic attraction between them made the molecule of 6-carboxy-chitosan have a fold conformation in solution so that the molecules couldn't form a fiber structure by parallel orientation as chitosan when they were precipitated from aqueous solution.

The DSC analysis was consistent with the TEM observation. The DSC curve (Fig. 3) showed the hydrogen bond absorption peak of 6-carboxy-chitosan at a lower temperature (100°C) than that of chitosan (105°C), which indicated that the intermolecular hydrogen bond of 6-carboxy-chitosan was weaker than that of chitosan because of its fold conformation caused by the attraction between $-NH_3^+$ and $-COO^-$ groups along the macromolecular backbone.

The absorption peak at 194°C indicated that 6-carboxy-chitosan has a decarboxylation reaction and chitosan doesn't under the same condition, indicating 6-carboxy-chitosan has lower thermal stability than chitosan.

Rheological behavior of 6-carboxy-chitosan

Influence of ph on [η] of chitosan and 6-carboxy-chitosan

Because of the $-NH_2$ groups changing into $-NH_3^+$ groups in 0.1 mol/L acetic acid, chitosan has the common characteristics of a polycation electrolyte. The molecule of chitosan has a stretch conformation because of electrostatic repulsion between $-NH_3^+$

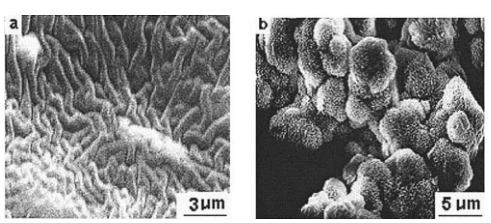

Figure 2 The SEM pictures of (a) chitosan and (b) 6-carboxy-chitosan.

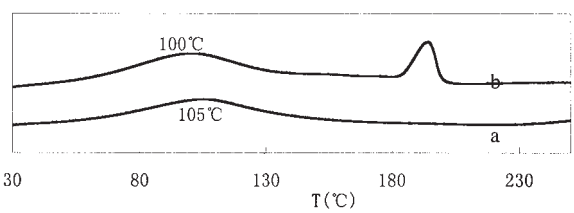

Figure 3 The DSC curves of (a) chitosan and (b) 6-carboxy-chitosan at a heating rate of 20°C/min.

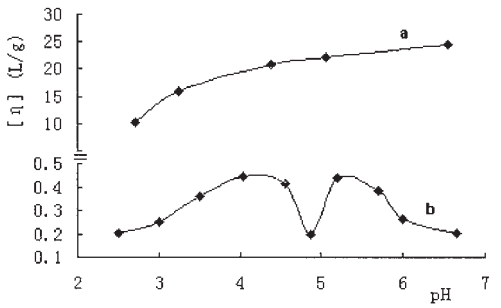

Figure 4 The effects of pH on [η] of (a) chitosan (DD = 90%) and (b) amphoteric chitosan (DD = 90%, OD = 52.5).

groups along the macromolecular backbone in solution and has high intrinsic viscosity. With decreasing pH, the ionic strength of the solution increased, and the electrostatic repulsion between $-NH_3^+$ groups along the macromolecular backbone was weakened because the excess negative ions in the solution screened or neutralized some of the positive charges on the polymer molecule, and thus the conformation of chitosan molecule became folded, which resulted in the intrinsic viscosity of chitosan decreasing [Fig. 4(a)]. This result is in agreement with Guo et al.'s conclusion.[10]

As for 6-carboxy-chitosan, the ionization equilibriums of both –COOH and $-NH_2$ groups in aqueous solution made the same molecule of 6-carboxy-chitosan containing $-COO^-$ and $-NH_3^+$ groups along the macromolecular backbone and having electrostatic attraction between them. In addition, the 6-carboxyl groups could easily form intramolecular hydrogen bonds, which was confirmed by the observation that the ν(C=O) of carbonyl groups in 6-carboxy-chitosan at 1,709.5 cm^{-1} was lower than that of the normal α-alkoxy-substituted carboxylic acid at about 1,730 cm^{-1}. Both the reasons above made 6-carboxy-chitosan molecule have a fold conformation so that its intrinsic viscosity was much lower than that of chitosan [Fig. 4(b)]. When the pH value of the solution is lower than 4.0 or more than 5.4, 6-carboxy-chitosan has common characteristics of a polycation or polyanion electrolyte, respectively, that is, the high ionic strength of the solution made its intrinsic viscosity decrease. When pH is 4.0–5.4, the changes on acidity of the solution may cause the ratio of negative charges to positive charges to change along the macromolecular backbone and give rise to change on the conformation of polymer molecule, so the pH value of the solution has a significant influence on [η] of 6-carboxy-chitosan and its isoelectric point (pI) value is 4.9. This is consistent with the facts that the amino group in chitosan has a $pK_b$of about 7–7.8[11] and the carboxyl group in α-alkoxy-substituted carboxylic acid has a pK_a of about 4.0. All of the above phenomena are in good agreement with the FTIR spectrum analysis.

Effects of salt addition on [η] for 6-carboxy-chitosan

The effects of salt addition on [η] for 6-carboxy-chitosan in different pH solutions are depicted in Figure 5. When the pH value of the solution is far away from the pI of 6-carboxy-chitosan, the general behavior for 6-carboxy-chitosan is that the value of [η] decreases with increasing concentrations of salts added [Figs. 5(a) and (c)] because the salt addition may cause electrostatic screening and neutralization of some of the charges on the macromolecular backbone and result in the polymer molecules shrinking. We may note that, at the same concentration, the influence of salt addition on the value of [η] of the sample decrease in the order $CaCl_2 > Na_2SO_4 > NaCl$, when the pH value of the aqueous 6-carboxy-chitosan solutions is 1.94 [Fig. 5(a)]. The reason is that, when the pH value of the solution is much lower than the pI value of the polymer, 6-carboxy-chitosan has common characteristics of a polycation electrolyte. At same salt concentration, the anion concentration in aqueous $CaCl_2$ solution is double that in Na_2SO_4 or NaCl solution and the same as that in aqueous Na_2SO_4 and NaCl solution, so the ability of the anions to screen the positive charges on the polymer molecule in aqueous solution decreases in the order $CaCl_2 > Na_2SO_4 > NaCl$. In this context, the

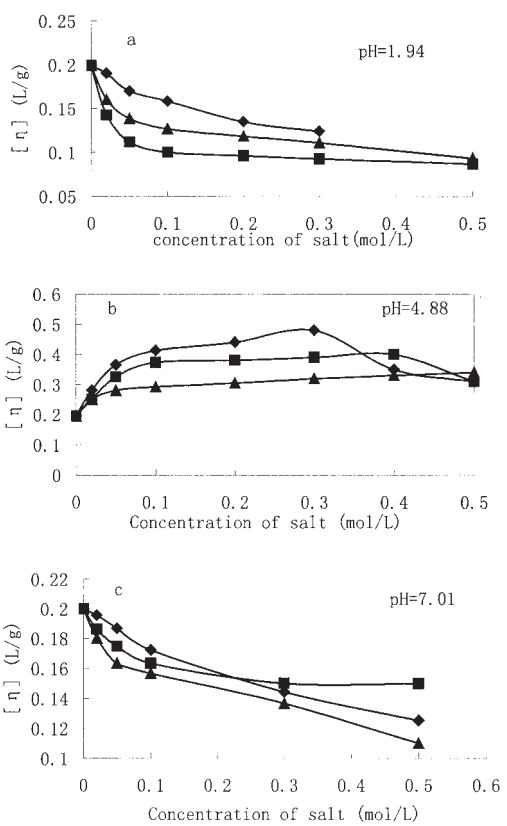

Figure 5 The effects of salts addition on [η] of 6-carboxy-chitosan (DD = 90%, DO = 52.5%) with (a) pH 1.94, (b) pH 4.88, and (c) pH 7.01. (♦) NaCl, (■) $CaCl_2$, (▲) Na_2SO_4.

ability of cations to screen the negative charges on the polymer molecule in aqueous solution decreases in the order $Na_2SO_4 > CaCl_2 > NaCl$, when the pH value of the 6-carboxy-chitosan solution is 7.04 [Fig. 5(c)]. Whereas the effects of salt addition on [η] for 6-carboxy-chitosan at higher salt concentrations are not consistent with those at lower concentrations when the pH value is 7.04. There are probably some other actions between the polymer and ions besides the electrostatic screening and neutralization of some of the charges on the macromolecular backbone when the concentration of the salt addition is high.

When the pH value of 6-carboxy-chitosan solution was 4.88, the salt addition caused the value of [η] for 6-carboxy-chitosan to increase in the order NaCl $> CaCl_2 > Na_2SO_4$ when the salt concentrations were lower than 0.35 mol/L [Fig. 5(b)]. The reason is that the 6-carboxy-chitosan molecule has common characteristics of amphoteric polyelectrolytes and a fold conformation in aqueous solution and the small volume of ions is advantageous to its screening the charges and decreasing the attraction between $-NH_3^+$ and $-COO^-$ groups on the macromolecular backbone. But when the concentration of salt addition is more than 0.35 mol/L, there probably are other actions between polymer molecules and ions besides the screening, which make the effects of salt addition on the value of [η] for 6-carboxy-chitosan at higher salt concentrations not consistent with those at lower concentrations.

CONCLUSION

The oxidization of hydroxymethyl groups of 2-amino-2-deoxy-D-glucose units in chitosan to carboxyl groups with NO_2 gas was a selective reaction. The 6-carboxy-chitosan is an amphoteric polyelectrolyte and has the same macromolecular backbone as chitosan. Its isoelectric point (pI) value is 4.9. Having similar structure and properties as biopolymers, such as protein, nucleic acids, and so on, it is expected that 6-carboxy-chitosan has superior biocompatibility compared to chitosan.

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