

Synthesis and Rheological Behavior of a Novel N-Sulfonate Ampholyte Chitosan

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ABSTRACT: Modified chitosan derivatives with different degrees of substitution varying from 0.17 to 0.96 were synthesized by the heterogeneous reaction of chitosan with 3-chloro-2-hydroxy propanesulfate in a neutral aqueous solution. The modified chitosans with a degree of substitution higher than 0.36 were soluble in water, whatsoever the pH. The structures of the polymers were characterized by Fourier transform infrared and ¹H-nuclear magnetic resonance spectroscopies. The potentiometric titration revealed that the modified chi-

tosan had an isoelectric point at around pH = 5.7, and its rheological behaviors in aqueous solution were studied under different conditions. The results showed that the modified chitosan had typical polyampholyte characteristics, which exhibited polyelectrolyte effect at pH = 1.7 or 13 and antipolyelectrolyte effect at pH = 8. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 113: 3382–3387, 2009

Key words: chitosan; polyampholytes; rheology; turbidity

INTRODUCTION

Chitosan is a polysaccharide derived from deacetylation of chitin, which has been used in a wide variety of fields because of its good biocompatibility and biodegradability.^{1–3} However, chitosan can only be dissolved in acidic aqueous solution because of the protonation of the –NH₂ groups; this greatly limits its promotion and application. The conversion of chitosan to water-soluble derivatives is an important goal, heading for further applications of chitosan as a novel material. Considerable interest has grown in the introduction of new and improved properties by chemical modification of the chitosan, and numerous works have been dedicated to the synthesis of water-soluble chitosan derivatives.^{4–8} Polyampholytes^{9,10} are copolymers containing both positively and negatively charged units on the same polymer chain. The properties of polyampholytes solutions are based on complex multiscale interaction mechanisms involving the primary structure of the polyions, counterions, co-ions, and solvent.^{11–13} Therefore, polyampholytes can serve as biosensors, act as amphoteric buffers for electrophoresis, and can be used as a simple model for understanding the complex behavior of proteins.^{14–16}

Chemical modification of the amino and hydroxyl groups in chitosan with sulfate is of great importance to produce chitosan-based polyampholytes, which generate water-soluble chitosan derivatives useful as biomaterials in pharmaceutical applications.^{3,17} These processes often require organic solvents, such as tetrahydrofuran or dimethylformamide, to dissolve both the sulfating agents and chitosan and relatively strong sulfating agents, which lead to a nonselective sulfation on the O-6, O-3, and N-2 positions.^{18–21}

In this article, a novel N-sulfated amphiphilic chitosan by the substitution reaction of the 3-chloro-2-hydroxy propanesulfate (CHPS) with chitosan was prepared, and its solution behaviors were studied.

EXPERIMENT

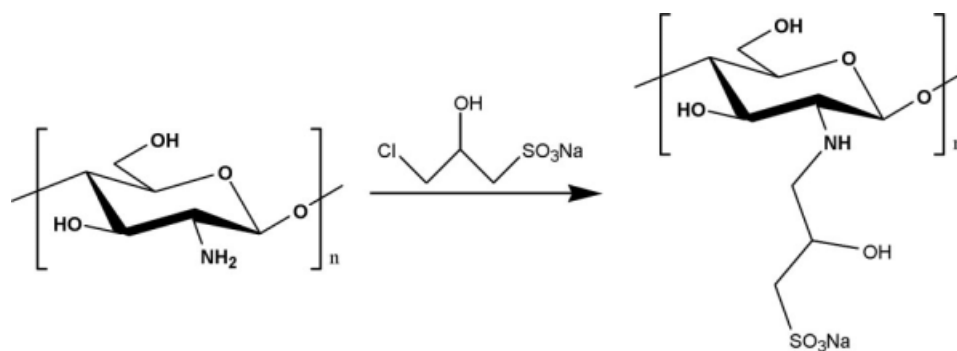
Materials

Chitosan was supplied by Yuhuan Ocean Biochemistry, in Zhejiang province in China, with a degree of deacetylation of 82% and viscosity average molecular weight of 65,000 g/mol. All other chemicals were commercial products of analytical pure grade and were used without further purification.

Synthesis of the N-(2-hydroxy-3-propane sulfonate)-chitosan

A typical synthetic method of the N-(2-hydroxy-3-propane sulfonate)-chitosan (NSCs) is described as

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Scheme 1 The synthetic pathway of NSCs.

follows: 9.825 g CHPS was dissolved in 100 mL aqueous solution and 2.046 g (0.012 mol with respect to monomeric unit) chitosan was added. The obtained heterogeneous solution was heated for 48 h at 70°C under continuous stirring. As the reaction proceeds, the reaction medium becomes translucent. At the end of the reaction, the solution was neutralized to pH \sim 7. The modified chitosan was precipitated by the addition of excess ethanol, washed, and dried in vacuum at 50°C.

Methods

The degree of substitution (DS) was determined by potentiometric titration: 0.2 g of polymer was dissolved in 40 mL 0.01N HCl and then titrated by 0.01N NaOH. Fourier transform-infrared spectra were recorded on a Perkin Elmer IR 2000 series (KBr pellet method). ^1H -Nuclear magnetic resonance (NMR) spectra were obtained in $\text{D}_2\text{O}/\text{DCl}$ at room temperature on JEOL JNM-LA 300 WB FTNMR (300 MHz). Element analysis was performed on an Exeter Analytical CE-440 Element analyzer.

Determination of the isoelectric point

Because of the simultaneous existence of the positive and negative charges at isoelectric point (IEP), the copolymer is in its most compact conformation at this point; the coil size of the polyampholytes have minima at the IEP. So the turbidity data was used to determine the IEP of the NSCs: the NSCs' solutions at different concentrations of 0.5, 1, and 2% were adjusted to different pH, then transmittance was measured by PerkinElmer Lambda 25 ultraviolet-vis spectrometer under different wavelengths at $\lambda = 500$ nm; the minimum of the curves is the IEP.

Rheology studies

The rheological tests were performed using BROOKFIELD DV-III ultra programmable rheometer. Meas-

urements were carried out at the temperature of 25°C with a rotation rate 40 rpm. The rotator is LV1, 61#.

RESULTS AND DISCUSSION

Structure and degree of substitution of N-sulfonated chitosan

A typical synthesis pathway is given in Scheme 1. A heterogeneous reaction system was designed to carry out the modification process. The chitosan was in suspension in early stage of the reaction, and as the reaction proceeded, the chitosan dissolved gradually, and the reaction mixture gradually clarified and the viscosity of the solution increased. The advantages of the heterogeneous reaction system were that the shielding effect of the H^+ toward the nucleophilic electron of nitrogen atoms were greatly reduced. This enhanced the nucleophilic effect of the nitrogen atoms, and higher selectivity of the reaction can be expected. The advance of the reaction could be easily monitored by identifying the variation of the viscosity and the clarification of the reaction mixture. But on the other hand, this reaction procedure could be expected to give a rather broad distribution of DS. The effect of reaction conditions on the distribution of DS within the polymer sample will be reported in a future publication.

^1H -NMR spectra of chitosan and N-sulfonate chitosan were shown in Figure 1. Typical signals at 3.2–3.4 (s, 2H) assigned to the methene hydrogen ($-\text{CH}_2-\text{SO}_3\text{H}$) of the N-sulfonate group were observed. In the IR spectra (Fig. 2), compared with chitosan, the appearance of a large shoulder at 1227 cm^{-1} ($\text{V}_{\text{as}}\text{O}[\text{DBond}]\text{S}[\text{DBond}]\text{O}$)²² associated with a peak at 1038 cm^{-1} ($\text{V}_s\text{O}=\text{S}=\text{O}$)²³ and the appearance of the absorption S[SBond]O bond stretching vibration peaks at 619 cm^{-1} were observed. These facts confirmed the existence of the N-sulfonate substituted group in the modified chitosan, and the N-2 position of the chitosan was selectively sulfonated under present experimental conditions with the CHPS.

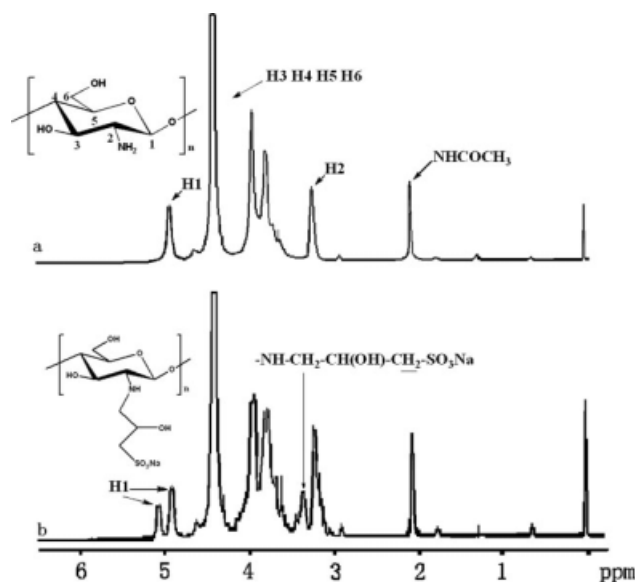


Figure 1 The $^1\text{H-NMR}$ of the chitosan and NSCs: (a) Chitosan; (b) NSCs.

The potentiometric titration in excess of HCl or NaOH gave three well-defined bends and peaks in the integral and differential titration curves, respectively. The first peaks in differential titration curves correspond to titration of the excess of HCl. The other two peaks correspond to titration of $-\text{SO}_3\text{H}$ and NH_2^+ . Furthermore, the third peak corresponds to titration of NH_2^+ was not distinguishable because of the low content of tertiary amino groups. Figure 3 gives a typical pH-titration curve of NSCs with the concentration of 0.5%. The DS of NSCs could be calculated through the consumption of sodium hydroxide. The DS was calculated using

$$\text{DS} = [(V_2 - V_1) \times C_{\text{NaOH}}] / (m/M) \quad (1)$$

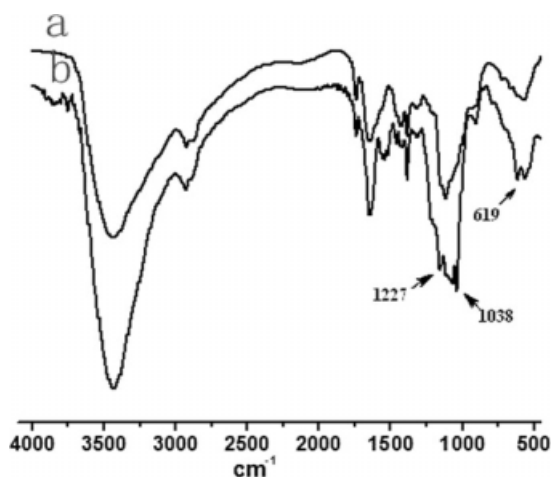


Figure 2 The IR of the chitosan and NSCs: (a) Chitosan; (b) NSCs.

where V_1 was the volume consumption of sodium hydroxide at first transition of the titration curve, which represented the neutralization of HCl in excess, V_2 was volume consumption of sodium hydroxide at second transition of the titration curve, and $V_2 - V_1$ represented the neutralization of $-\text{SO}_3\text{H}$ groups. The $[(V_2 - V_1) \times C_{\text{NaOH}}]$ represented the mol value of the $-\text{SO}_3\text{H}$ groups.

The mass of NSCs was denoted by m , and M was repeat unity molecular weight of NSCs, which was 321. And the m/M represented the repeat unity of the NSCs. In Figure 3, the V_1 was 15.09 mL, and the V_2 was 19.14 mL. From eq. (1) the DS could be calculated, and it is equal to 0.67.

In the same time, the DS values of several modified chitosan samples were also confirmed by element analysis of sulfur content of the samples; the DS values were calculated as

$$\text{DS}(s) = 168.7 \times x / (32.1 - 160.2 \times x) \quad (2)$$

where x was the sulfur content of the sample determined by element analysis. The DS values of modified chitosan by titration and by element analysis, respectively, were in good agreement.

The effect of the molar ratio of CHPS to the chitosan on the DS was shown in Table I. The DS of the product increased with the amount of CHPS added in the reaction medium. By using 1–3 equivalent of CHPS, the DS values of the products were in the range of 0.17–0.28, and the modified chitosan was partially soluble in water at neutral pH. It was normal to observe that higher concentration (4–5 equivalent) of CHPS used in the reaction leading to products with higher DS, and the modified chitosan with high DS value becomes completely soluble in neutral water.

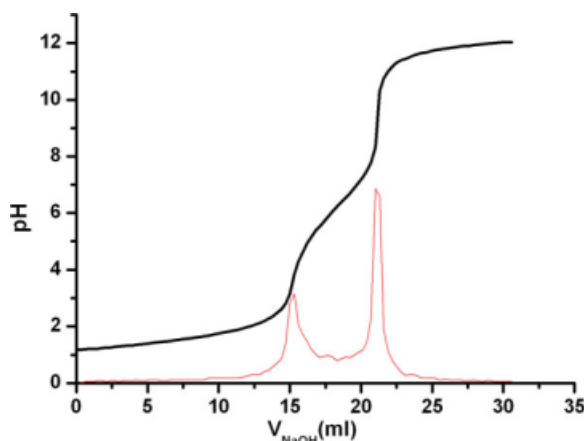


Figure 3 pH-titration curves of NSCs (Sample 9). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

TABLE I
Effect of Molar Ratio of 3-chloro-2-hydroxy propanesulfate (CHPS)

Sample no.	CHPS (equivalent)	T (°C)	Time (d)	DS ^a	DS (s) ^b	Solubility ^c
1	1	70	4	0.17		Partially soluble
2	2	70	4	0.28	0.29	Partially soluble
3	3	70	4	0.36		Soluble
4	4	70	4	0.61	0.63	Soluble
5	5	70	4	0.89	0.84	Soluble

^a Calculated from the potentiometric titration.

^b Calculated from the element analysis.

^c Soluble: the solution is translucent; partially soluble: some solid residues remain in the solution.

The effects of temperature and reaction time on DS values were summarized in Table II for the given ratio of the reactants. It is evident that the DS increased with increasing temperature and reaction time. At 25°C, the reaction was slow and the DS value of the product was only 0.21 after 4 days even with a very high equivalent of CHPS in the reaction medium.

The DS was easily controlled by changing the ratio of the reactants, the temperature, and reaction time. The solubility of the modified chitosan in neutral water increased with increasing DS. The modified chitosan samples with a DS higher than 0.36 were all soluble in water at neutral pH. This was mainly due to that the change of chitosan structure decreases the hydrogen bonds form of intermolecules and damages the ability of crystallization.²⁴

Isoelectric point

A characteristic feature of polyampholytes, in particular those composed of weak acid and base species, was that they possess an IEP. The specificity of the IEP is that the number of cationic groups within polymer chains is equal to that of anionic groups and consequently the polymer is exactly intermediate between a polycation and a polyanion.²⁵ The IEP could be predicted theoretically. At certain compositional ratios, such as the acidic residues was equal to basic residues in the polymer, the IEP was simply

the arithmetic average of the respective pK_a and pK_b values of the acidic and basic residues¹⁰:

$$\text{IEP} = (pK_a + pK_b)/2 \quad (3)$$

The IEP also could be determined experimentally by titration or viscosity. Because of the simultaneous existence of the cationic and anionic group, the aggregation process of the polymers was changed by the pH change in aqueous solution (Fig. 4). Figure 4 illuminated the transmittance change of NSCs (the mass concentration of NSCs are 0.5, 1, and 2%) at different pH. From Figure 4, the IEP of the NSC was found to be at pH = 5.7 by titration. In contrast, Rosica²⁶ measured the reduced viscosity, as a function of pH, for a statistical polyampholyte composed of *N*-carboxyethylchitosan, and the IEP was pH = 5.4. At the IEP, the number of the negative charge SO_3^- was equal to the number of the positive charge NH_3^+ ; the copolymer is in its most compact conformation at this point, and the coil size of the polyampholytes has minima at the IEP; the light transmittance of the solution drastically declines; and polymer precipitates because of the electrostatic attraction of the positive and negative charge. The aggregation process of the polymers was changed by the pH change in aqueous solution. Away from the IEP, the polymer chains dissolved in the water due to the electrostatic repulsions.

The effect of salt concentration on the solution behavior of modified chitosan was studied by

TABLE II
Effect of Reaction Temperature and Time on the DS and Solubility

Sample no.	CHPS (equivalent)	T (°C)	Time (d)	DS	DS (s)	Solubility
6	5	25	4	0.21		Partially soluble
7	5	50	4	0.75	0.75	Soluble
8	5	60	4	0.80		Soluble
9	5	70	1	0.67	0.68	Soluble
10	5	70	2	0.83		Soluble
11	5	70	3	0.86		Soluble
5	5	70	4	0.89	0.84	Soluble
12	5	80	1	0.96		Soluble
13	5	90	1	0.95		Soluble

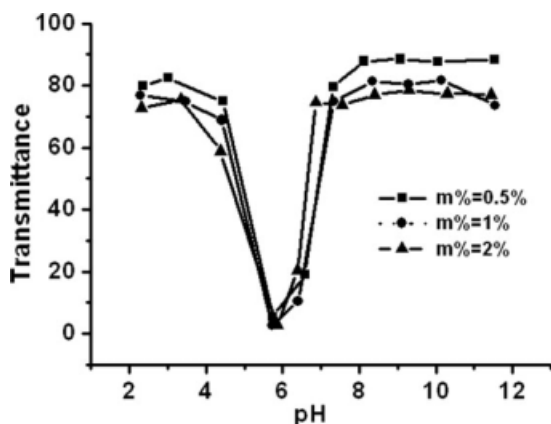


Figure 4 The transmittance of NSCs at different pH (Sample 5).

measuring the light transmission of the solution. The transmittance of NSCs solution (1%) at different NaCl concentration at the IEP is showed in Figure 5. The transmittance of NSCs solution increased when NaCl concentration increased. This process could be explained as follows. At the IEP, the polymer precipitated because of the electrostatic attraction of the positive and negative charges. The addition of the NaCl partly shielded the electrostatic attraction of the positive and negative charge on the polymer chains. This electrostatic shielding effect reduced the efficiency of the polymer aggregation, and the polymer became more soluble resulting in an increase of the transmittance of NSCs solution. The transmittance of the solution increased and reached asymptotically its highest value with the increase of the salt concentration. This phenomenon revealed the typical antipolyelectrolyte property that the addition of salt to an aqueous solution results in a coil size increase of the modified chitosan.

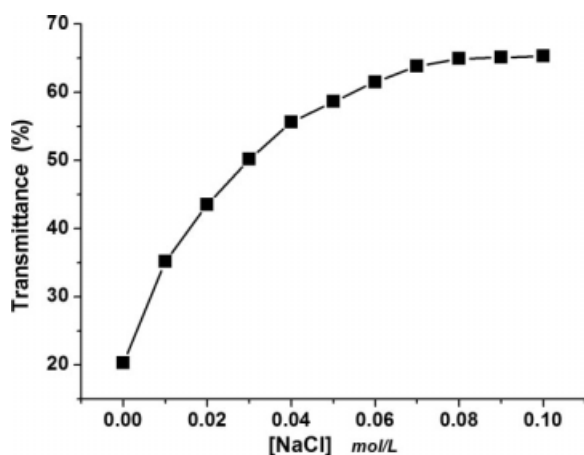


Figure 5 The transmittance of NSCs at different NaCl concentration (pH = 5.8, Sample 5).

Rheological behavior of the NSCs solution

The aqueous solution behavior of polyampholytes was dictated by Coulombic interactions between anionic and cationic species located on different monomer units along and among the polymer chains. Polyampholytes exhibited both polyelectrolyte and antipolyelectrolyte behavior depending on factors such as solution pH, the absence or addition of low molecular weight electrolyte, and the relative strengths of the acidic and basic residues.

Influence of pH

The influence of pH on the viscosity (η) of NSCs solution (the mass concentration of NSCs are 0.5, 1, and 2%) was shown in Figure 6. When the pH of the external solution deviated from the isoelectric pH, polyampholyte behave as polycations and polyanions, because of the electrostatic repulsive force between the polycations and the polyanions; the polymer chain took an elongated conformation, which led to an increase of the polyampholytes hydrodynamic volume, and the viscosity of the solution increases. Near the IEP (pH \approx 6), the intraionic attraction between opposite charges made polymer chains collapse, and the polymer was in its most compact conformation. This is reflected by the appearance of a minimum in viscosity.¹⁹ At high pH (pH > 7.5), the polyampholyte behave like polyelectrolyte and takes an elongated conformation because of electrostatic repulsive force between the anionic sulfonate groups. The Coulomb interaction between the charge group and the ions in the solution is dominant. After the increase of the pH, more and more Na^+ or OH^- was absorbed by charged group of the polyampholytes. It weakened the repulsive electrostatic interactions between the polymer units and the polymer coil shrinks, adopting a more

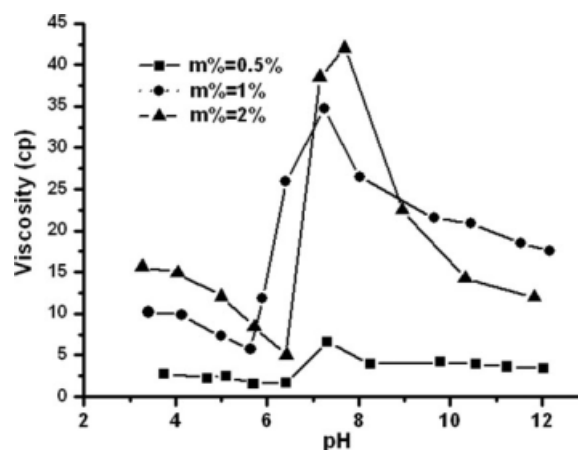


Figure 6 Influence of pH on viscosity of NSCs (Sample 5).

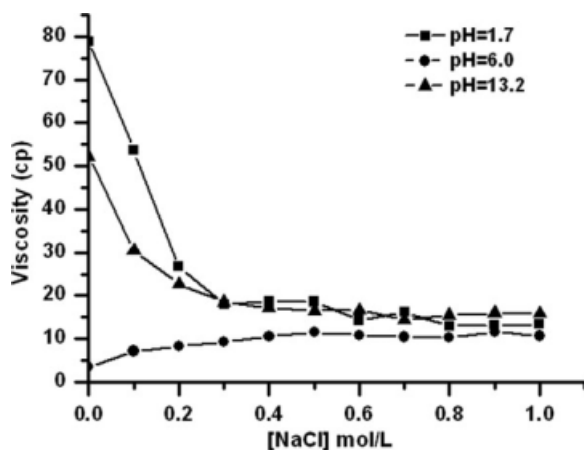


Figure 7 The influence of salt addition on viscosity of NSCs (Sample 5).

entropically favored conformation. So, the viscosity of the solution decreased.

Influence of salt concentration

The influence of salt concentration on viscosity of the NSCs solution (concentration: 1%) at different pH was shown in Figure 7. For polyampholytes with a large net charge, either positive at low pH or negative at high pH, the addition of salt resulted in a drop in solution viscosity.¹⁹ The polyampholytes behave like polyelectrolytes. On the other hand, near the IEP point, a very dense globule conformation was formed because of the interaction of the opposite charge of the group in the polyampholyte. Higher salt concentration in the solution could screen the electrostatic interactions between opposite charges and separated the polymer chains; this caused the dissolution of polyampholytes and led to an increase of the viscosity of the solution.²⁷ The polyampholyte exhibited the antipolyelectrolyte effect in this pH region. This result was in accordance with the light transmission property of the NSCs solution discussed earlier.

CONCLUSIONS

Chitosan could be chemically modified by heterogeneous reaction with CHPS in neutral water. The reaction proceeded smoothly at neutral pH that gives selectively substitution at amine groups of the

sugar unit. The DS could be easily controlled by varying the amount of CHPS added in the reaction mixture, or reaction temperature and time. Potentiometric titration and rheological studies demonstrated that the modified chitosan present typical polyampholytes characteristics with an IEP at around pH = 5.7. These results show that the present method offered an effective way for the preparation of water-soluble chitosan derivatives.

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