Effect of Molecular Weight and NaCl Concentration on Dilute Solution Properties of Chitosan

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Abstract

Solution properties of polyelectrolytic biopolymers such as chitosan, pectin, alginate and etc. are significantly influenced by molecular weight and salt concentrations. The effect of NaCl concentration on the hydrodynamic properties of chitosan in dilute region was investigated for chitosans of varying molecular weights. Intrinsic viscosity($[\eta]$) of chitosans with 5 different molecular weight was determined by glass capillary viscometer, and the viscosity average molecular weight was calculated using Mark-Houwink equation. Intrinsic viscosity decreased with increasing NaCl concentration for all chitosan samples, and it was proportional to the logarithmic NaCl concentration, i.e., $[\eta] \propto \log(C_{NaCl})^a$. Decreasing trend of $[\eta]$ with NaCl concentration became more pronounced with increasing molecular weight. It was also found that the a values, indicating C_{NaCl} dependence of $[\eta]$, were linearly correlated with the logarithmic molecular weight($R^2=0.980$). The chain stiffness parameters (B) were calculated by $B=S/([\eta]_{0.1})^{1.32}$, in which S was obtained from slope of $[\eta]$ vs $I^{-1/2}$. The B values of chitosan samples were determined to be 0.113~0.071 with a average of 0.09.

Key words: chitosan, intrinsic viscosity, rheology, chain stiffness

INTRODUCTION

Chitin is a linear polymer of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucopyranose(GlcNAc). In nature, chitins are widely distributed in the shells of crustacea (crab, shrimp, etc.), in the cuticles of insects(cockroach, silkworm, etc.), in the shells & skeletons of mollusks (krill, squid, etc.) and in the cell walls of fungi(mushroom, baker's yeast, etc.)(1,2). The shells of crab, shrimp and krill, which are now waste materials of the food processing industry, are the main sources of industrial chitin production due to their apparent abundance(3).

Chitosan, which is partially N-deacetylated chitin, consists of 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc: A-unit) and 2-amino-2-deoxy-β-D-glucopyranose(GlcN: D-unit) residues linked through β-(1 →4) glycosidic linkages and thus may be considered a binary heteropolysaccharide(4,5). The A- and D-units have been shown to be randomly distributed in water-soluble partially N-acetylated chitosans(6). Chitosan is positively charged due to amino groups at acidic pH, whose magnitude is dependent on the degree of deacetylation, and it is thus classified as one of polyelectrolytes.

Commercial chitosan is obtained from chitin by heterogeneous alkaline deacetylation, and commercial chitosans usually contain a fraction of acetylated units(F_A) between 0 and 0.2(5,7). Chitosan is distinguished from chitin by its solubility in dilute aqueous acid solutions (8). Chitosan and their derivatives have found numerous applications in food, biotechnology, pharmacology, medicine and environmental technology during the last two decades(9-11).

Molecular size of polysaccharides is a crucial factor affecting various functional properties(12,13). At low molecular size, depolymerized polysaccharides confer the bifidogenic effect in most cases as oligosaccharides and also provide a wide range of physiological and medical functions(14-16). In contrast, polysaccharides of high molecular weight act as thickening and gelling agents, and in the case of non-starch polymers the physiological roles can be anticipated as dietary fiber sources(17-19). This indicates that various food and physiological functions of polysaccharides greatly depend on the molecular weight of polysaccharides. Thus, the accurate characterization of molecular weight dependence on functional properties is of primary impor-

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tance to find a wide range of application of polysac-harides.

Natural polyelectrolytes include multi-charged func-tional groups such as carboxyl(pectin, alginate), sulfate (carrageenan) or amino(chitosan) groups. In charged state, the anionic or cationic functional groups in bio-polymers impart the intramolecular electric repulsive force, which results in increasing the hydrodynamic volume by the extended chain conformation(20,21). How-ever, in the presence of salt the charged groups are sc-reened, and thus the chain rather possesses compact structure, leading to the decreased hydrodynamic vol-ume. In addition, the charge density of polyelectrolytes is greatly dependent on pH. Thus, understanding the effects of salt concentration and pH is crucial to op-timize the functional properties of polyelectrolytic bio-polymers.

To date only a few studies have been conducted on solutions properties of chitosan in dilute and concen-trated domains(3,22,23). To our knowledge, however, no attempts have been made to systematically inves-tigate solution properties considering the molecular size of chitosans in the presence of various salt con-centrations. Accordingly, this research aims to inves-tigate the effects of NaCl concentration on the hy-drodynamic properties of chitosan in dilute region re-presenting the polymer concentration below physical intermolecular contact. In addition, we also studied how much the molecular size of chitosan affects the molecu-lar response of chitosan in the presence of NaCl.

MATERIALS AND METHODS

Preparation of chitosans

Three major steps, i.e., demineralization, deproteini-zation and deacetylation are required to prepare chi-tosans from chitinous raw materials(7). In this research,

chitosan samples of 5 different molecular weights were prepared according to the procedures shown in Table 1. First, chitin was prepared by treating *Chitinonecetes opilio* with 5~15% HCl at 30~50°C for 3~24hr(demin-eralization) and subsequently with 5% NaOH at 90°C for 12hr(deproteinization). Then, chitin was treated with 40~60% NaOH at 70~140°C for 6~48hr to remove the acetyl residues(deacetylation).

Determination of intrinsic viscosity

The intrinsic viscosity of chitosan was measured by Cannon-Fenske glass capillary viscometer(Cannon In-strument Co., USA; size 50) at 25°C. A certain amount of chitosan was dissolved in 0.05, 0.1, 0.2, 0.3M NaCl/0.1M CH₃COOH solution. The chitosan solutions were filtered through a 0.45μm Millipore filter to get rid of dusts and insoluble impurities. Then, 10ml solution was pipetted into the capillary viscometer, which was equ-ilibrated at 25°C water bath for 30min prior to mea-surements.

Specific viscosity(η_{sp}) and intrinsic viscosity($[\eta]$) were determined as follows(24):

$$\eta_{sp}=(\eta-\eta_s)/\eta_s \tag{1}$$

and

$$[\eta]= \lim_{c\to 0} \eta_{sp}/C \tag{2}$$

where η is the solution viscosity, η_s is the solvent viscosity, and C is the solution concentration.

Determination of molecular weight

Molecular weight of chitosan was calculated by the following Mark-Houwink equation in 0.2M NaCl/0.1M acetic acid(25):

$$[\eta] = 1.81 \times 10^{-3} M_v^{0.93} \tag{3}$$

Table 1. Preparation of chitosans with various molecular weights

Samples	HCl (demineralization)			NaOH (deproteinization)			NaOH (deacetylation)		
	Conc.(%)	Temp.(°C)	Time(hr)	Conc.(%)	Temp.(°C)	Time(hr)	Conc.(%)	Temp.(°C)	Time(hr)
CTS-1	3	30	3	5	90	12	40	70	6
CTS-2	5	30	6	5	90	12	50	100	12
CTS-3	10	30	12	5	90	12	60	110	24
CTS-4	10	40	12	5	90	12	60	130	36
CTS-5	15	50	24	5	90	12	60	140	48

where [η] is the intrinsic viscosity(ml/g), and M_v is the viscosity average molecular weight.

RESULTS AND DISCUSSION

Table 2 shows the intrinsic viscosity of chitosans at 0~300mM NaCl concentrations in conjunction with the viscosity average molecular weight calculated from Eq. 2 at 0.2M NaCl/0.1M CH₃COOH. The intrinsic viscosity of chitosans significantly decreased with increasing NaCl concentration regardless of molecular weight of chitosans. This can be attributable to the screening effects of sodium ions for the cationic charges of chitosans(23). Due to the reduced electrostatic repulsive force, chitosan molecules are changed from the extended structure to the more compact one. This results in decreasing the intrinsic viscosity([η]), representing the hydrodynamic volume of a polymer per unit mass. The similar observations were also reported for anionic polysaccharides such as alginate, pectin and etc.(21,26).

Relations between NaCl concentration and intrinsic viscosity of chitosans are demonstrated in Fig. 1. The intrinsic viscosity of chitosans exhibited the linear relation with logarithmic NaCl concentration as follows:
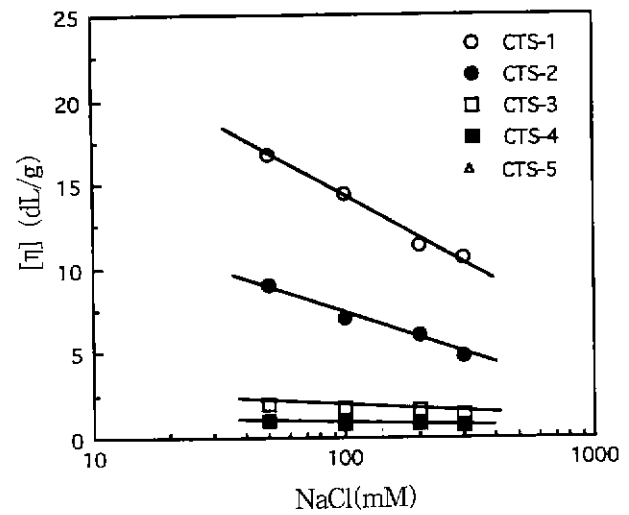
$$[\eta] \propto \log (C_{NaCl})^{\alpha} \qquad (4)$$

where C_{NaCl} is the NaCl concentration(mM), and α is the constant. Table 3 exhibits α values and correlation coefficients for chitosan samples. The α values, representing the slopes of [η] vs log(C_{NaCl}), increase with molecular weight of chitosans. This indicates that chitosans with higher molecular weight shows the greater reduction in intrinsic viscosity as the NaCl concentration increases. As shown in Fig. 2, the linear correlation
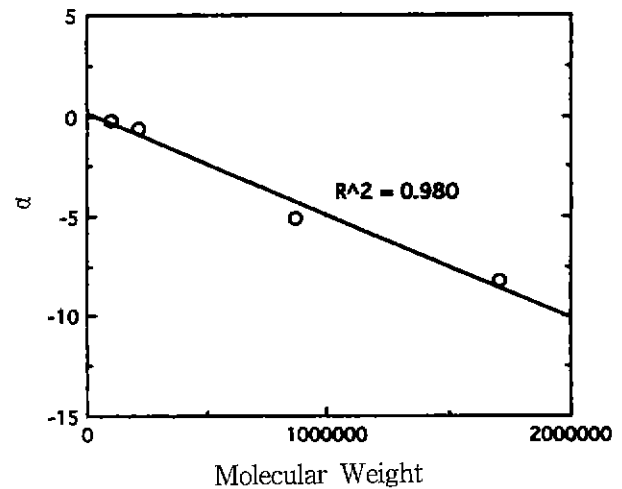

Fig. 1. Intrinsic viscosity of chitosan samples at different NaCl concentrations.

Fig. 2. Correlation of molecular weight and α values of [η] log(C_{NaCl})$^{\alpha}$.

(R^2=0.980) was observed between the logarithmic molecular weights of chitosans and α values obtained from Eq. 4.

For polyelectrolytes as typically exemplied by chitosan

Table 2. Intrinsic viscosity and molecular weight of chitosans as a function of NaCl concentration

NaCl (mM)	Intrinsic viscosity(dl/g)				
	CTS-1	CTS-2	CTS-3	CTS-4	CTS-5
0	59.760	39.075	6.729	3.998	3.045
50	16.736	8.966	1.949	0.938	0.908
100	14.536	7.074	1.736	0.890	0.848
200	11.342	6.009	1.634	0.812	0.780
300	10.644	4.785	1.389	0.762	0.699
$M_v(\times 10^{-4})^a$	170.98	86.36	21.29	10.04	9.61

$^aM_v=(552.49\times[\eta])^{1.0752}$ at 0.2M NaCl/0.1M CH₃COOH([η]: ml/g)

Table 3. Correlation of NaCl concentration and intrinsic viscosity

Samples	α^a	R^2
CTS-1	-8.17	0.987
CTS-2	-5.14	0.986
CTS-3	-0.66	0.954
CTS-4	-0.26	0.985
CTS-5	-0.23	0.967

$^a[\eta] \propto \log(C_{NaCl})^{\alpha}$

in this research, the important source of coil expansion is segment-segment electrostatic repulsive expansion (27). The more expanded structure can impart the higher intrinsic viscosity. It has been well documented that the intrinsic viscosity decreases almost linearly with the square root of ionic strength as follows(28):

$$[\eta] = A + S \ I^{-1/2} \tag{5}$$

where A is the constants, I is the ionic strength, and S is the slope of intrinsic viscosity vs $I^{-1/2}$. As shown in Fig. 3, the plot of intrinsic viscosity as a function of $I^{-1/2}$ exhibited straight lines, in which the slopes increased with increasing molecular weights. This indicates that the intrinsic viscosity is more significantly influenced at higer molecular size in the presence of salts.

The magnitude of change in intrinsic viscosity in response to ionic strength increases with increasing flexibility of the polyelectrolyte chain. Smithrød and Haug (29) employed I=0.1M as a reference value to develop a simple method for characterizing chain flexibility without the necessity to prepare samples with different molecular weights. In this case, Eq. 5 can be expressed as follows:

$$[\eta] = A + B([\eta]_{0.1})^\beta \ I^{-1/2} \tag{6}$$

From Eqs. 5 and 6,

$$S = B([\eta]_{0.1})^\beta \tag{7}$$

where the constant B is a measure of chain stiffness(29): the smaller the B value, the higher the chain stiffness.
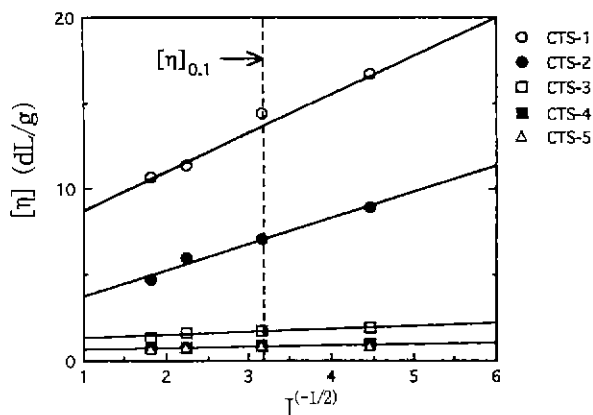
$$\log \ S = \log \ B + \beta \log([\eta]_{0.1}) \tag{8}$$

Table 4. Chain stiffness parameters(B) of chitosans

Samples	B
CTS-1	0.071
CTS-2	0.113
CTS-3	0.091
CTS-4	0.085
CTS-5	0.081
Average	0.088

The slope (S) obtained from Fig. 3 is demonstrated in Fig. 4 as a function of the intrinsic viscosity at I= 0.1M on the double logarithmic coordinates, in which the slope was determined to be 1.32. This result is nearly consistent with the report of 1.3 ± 0.1 for polyelectrolytic polysaccharides such as alginate, carboxymethyl cellulose(CMC) and carboxymethyl amylose(29). The chain stiffness parameter(B), calculated from B= $S/([\eta]_{0.1})^{1.32}$, are presented in Table 4. The B values ranged $0.07 \sim 0.12$, and averaged 0.09. Essentially, cellulose constitutes the backbone of carboxymethyl cellulose and xanthan as well as chitosan(30). The B values of CMC and xanthan were reported to be 0.05 and 0.005, respectively(31), which thus indicate that the chain stiffness is higher than 0.09 of chitosan. This indicates that the carboxymethyl groups of CMC and trisaccharide sidechains of xanthan provide the more rigid conformation for the cellulose backbone. When compared to CMC and xanthan, the more flexible conformation of chitosan can be ascribed to the less bulky amino groups which relatively impart insignificant hindrance to the chitosan molecule. However, the distinct correlation be-
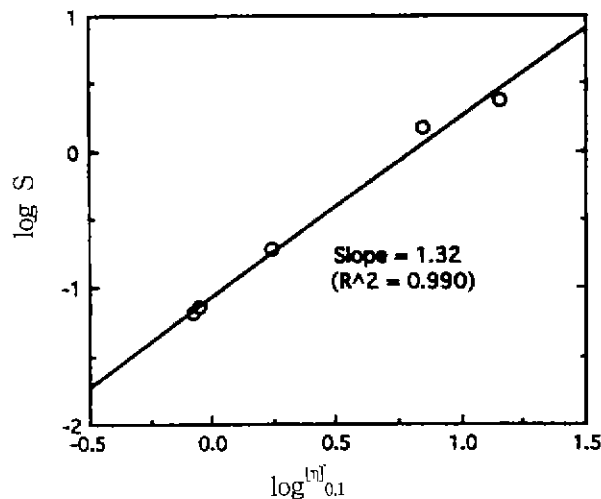

Fig. 3. Ionic strength dependence of intrinsic viscosity for chitosans of different molecular weight.

Fig. 4. Depencence of S values on intrinsic viscosity of chitosans at I=0.1M.

tween molecular weights and B values was not clearly observed in this research. Further studies may be necessary to elucidate the effect of molecular weight on the chain stiffness in terms of molecular weight distribution as well as absolute molecular weight.

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