Effects of Ionic Strength and pH on the Diffusion Coefficients and Conformation of Chitosans Molecule in Solution

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ABSTRACT: The effects of ionic strength and pH on the diffusion coefficients and gross conformation of chitosan molecules in solution were studied. Chitosan with 83% degree of deacetylation (DD) was prepared from red shrimp (Solemocera prominenitis) processing waste. Ten different molecular weight chitosans were prepared by ultrasonic degradation, and their molecular weights were determined by static light scattering. The weight-average molecular weight (M_w) were between 78 to 914 kilo dalton (KDa). Solution of different ionic strengths ($I = 0.01$, 0.10, and 0.20) but the same pH (2.18) and different pHs (2.37, 3.10, and 4.14) but the same ionic strength ($I = 0.05$) were prepared to measure their mutual diffusion coefficient (D_m). The diffusion coefficients for standard condition ($D_{20,w}$) were derived from D_m. Intrinsic viscosities ($[\eta]$) were determined by a capillary viscometer in different pH solutions. The Mark–Houwink exponents a and ε were obtained from plots of Log $[\eta]$ and Log $D_{20,w}$ versus Log M_w, respectively. The results show that diffusion coefficients increased with increasing ionic strength or with increasing pH or with decreasing M_w. Value of ε and a were between 0.503 to 0.571 and ranged from 0.543 to 0.632, respectively. The results indicates that chitosans conformation were in random coil in solutions in the ranges of ionic strength and pH studied. The values of a^*, ε^* and a^{**}, ε^{**}, Mark–Houwink exponents of smaller and higher than 223 KDa chitosans, respectively, were between 0.752 to 0.988 and 0.585 to 0.777 for smaller M_w chitosans and 0.406 to 0.428 and 0.430 to 0.518 for larger M_w chitosans, respectively. Molecular-weight-induced conformational transition occurred because smaller M_w chitosans was more extended than higher M_w chitosans. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 2041–2050, 1999

Key words: chitosan; diffusion coefficient; conformation; pH; ionic strength; light scattering; intrinsic viscosity

INTRODUCTION

Hydrodynamic behavior or molecular gross conformation in solution of a neutral polymer depends on its primary structure, such as bond length, bond angle, and rotational potential; on

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the thermodynamic properties of the solvent system, such as solvent power and temperature; and on the nature of the interaction, such as the concentration effect.[1] Hydrodynamic behavior of a polyelectrolyte depends on solution composition, especially pH, ionic strength, and small electrolytes, besides the above-mentioned parameters.[2] Intrinsic viscosity, translational frictional coefficient, and rotational frictional coefficient are parameters frequently employed alone or combined to characterize the hydrodynamic behavior of a

molecule in solution.[3,4] These parameters are derived from viscosity, sedimentation or diffusion, and flow birefringence or non-Newtonian viscosity, respectively.

Chitosan behaves as a polycationic electrolyte in acidic solution. The rheological properties of chitosan depends on the molecular weight, degree of deacetylation (DD), the distribution of the acetyl group, solution pH, and ionic strength.[5] Many reports use Mark–Houwink exponent parameter,[5–13] some use diffusion coefficient,[9,14] flow birefringence,[8,15] sedimentation coefficient,[6,9,14] light scattering[10,13,14,16] to character the gross solution conformation of chitosan.

Hydrodynamic behavior can be employed to relate the chain flexibility. By manipulating the chain flexibility of chitosan, different mechanical properties and porosity membrane or capsule can been prepared.[17–22] Hydrodynamic behavior can also be used to calculate the molecular weight or to characterize the hydrodynamic behavior of a macromolecule by using the Mark–Houwink equations, such as the following.

$$[\eta] = k\, M^a \tag{1}$$

$$s = k'\, M^b \tag{2}$$

$$D = k''\, M^{-\varepsilon} \tag{3}$$

$$Rg = k'''\, M^\nu \tag{4}$$

Where k, k′, k″, and k‴ and a, b, ε, and ν are empirical parameters, which are constants at a given temperature, solvent, and a limited range of molecular weights. In molecular weight calculation, the empirical parameters have to determined beforehand. For characterization, the hydrodynamic behavior of a macromolecule in solution, a, b, ε, and ν are used as the conformational indicators. The a between 0.5–0.8, b between 0.4–0.5, ε between 0.5–0.6, and ν between 0.5–0.6, respectively, indicating that the macromolecule is in random coil conformation, while a is 0, b is 0.7, ε is 0.3, and ν is 0.3, indicating compact sphere, and a is 1.8, b is 0.2, ε is 1.0, and ν is 1.0, indicating that the molecule is a stiff rod conformation.[14,23–28]

Although there are many reports using exponent a of the Mark–Houwink equation to characterize the gross solution conformation of chitosan in the literature,[5,6,9–14,29,30] these data are not in accord with each other (ranged from 0.147 to 1.29), due to using different solvent systems (different solution ionic strength and/or pH), even chitosan, with a similar degree of deacetylation were used. Although the effect of DD on the gross conformation have been explored,[5,10,12] the results were controversy. Anthonsen et al.[5] and Wang et al.[12] reported that the a value decreased with increasing DD of chitosan used, while Rinaudo et al.[10] reported that the persistence length is independent of DD of the used chitosan in the range studied. The discrepancy that may be due to the effect of solvent system have not been systematically studied. Anthonsen et al.[5] and Roberts and Domszy[11] investigated the effect of ionic strength on chitosan conformation. However, so far, the effect of solution pH on the hydrodynamic behavior of chitosan has not been explored systematically.

Most studies used intrinsic viscosity to characterize the hydrodynamic behavior of chitosan.[5,6,9–14,29,30] However, shear thinning may occur as solution flow through the capillary, which may affect the intrinsic viscosity determination. For larger molecular weight ones, this effect was more manifest.[23] Ottøy et al.[30] reported that the intrinsic viscosity of the larger molecular weight chitosan molecule was anomalously small. In order to avoid the uncertainty caused by the shear-thinning effect, the diffusion coefficient determined by dynamic light scattering as a hydrodynamic parameter of chitosan have been used, although the diffusion coefficient is rarely used.

The hydrodynamic radius (Rh) can be calculated from the translational diffusion coefficient (D_{tr}) by using the following Stokes–Einstein equation[24,31–33]:

$$Rh = \frac{k \cdot T}{6\pi \cdot \eta \cdot D_{tr}} \tag{5}$$

where k is Boltzmann's constant, T is the absolute temperature, and η is the viscosity of the solvent. Equation (5) can be applied for the nonsphere conformation polymer. The hydrodynamic radius calculated from eq. (5) can be used to define the effective hydrodynamic radius,[24] so diffusion coefficient can be applied to macromolecules that are not only spherical but also other shapes, such as the shape of a rod, to characterize their hydrodynamic behavior. Although there are two merits in using the diffusion coefficient to characterize the hydrodynamics of a macromolecules, only Pogodina et al.[9] and Errington et al.[14] used

diffusion-sedimentation and dynamic light scattering, respectively, so far to determine the diffusion coefficient of chitosan. However, the types of solvents they used were limited. Therefore, the effect of solvent system, especially the solution's pH and ionic strength on the hydrodynamics properties, need to be systematically explored.

The mutual diffusion coefficient measured by dynamic light scattering of chitosan in different ionic strengths but the same pH (2.18) and different pHs but the same ionic strength (0.05) solution were determined. Diffusion coefficient for standard condition ($D_{20,w}$) were then derived. Intrinsic viscosities measured by capillary viscometer in different pH solutions were obtained. The effect of ionic strength and pH on diffusion coefficient and gross conformation of chitosans in solution of different ionic strength and pH were then elucidated.

MATERIALS AND METHODS

Chitosans with the Same Degree of Deacetylation but Different in Molecular Weight

Chitin was prepared from shrimp (Solemocera prominenitis) waste by the method of Chen et al.[17] Chitin was alkali deacetylation with 50% NaOH at 100°C for 3 h to get 83 ± 1% DD chitosan.[22] Chitosan (1%) was dissolved in 5% (w/v) acetic acid aqueous solution and then was ultrasonically degraded (CREST, 950E, USA) at various times (0–60 h) at 80°C. After ultrasonic degradation, the chitosans were precipitated with 2M NaOH solution and were washed with water, then freezing dried (Virtis, UNITOP 800L, USA) to obtain the chitosans with the same DD but different molecular weight products.[12] The degree of deacetylation of chitosans was determined by infrared spectroscopy[34] and was 83 ± 1%.

Solvent Systems

Solvents of the Same pH but Different Ionic Strength

Solvent consisted of 0.01M HCl and 0, 0.09, and 0.19M NaCl to make a solution of pH 2.18 ± 0.03, ionic strength of 0.01, 0.10, and 0.20, respectively.

Solvents of 0.05 Ionic Strength but Different in pH

pH 2.37 solvent was consisted of 3.00M acetic acid, 0.01M sodium acetate, and 0.04M potassium chloride, pH 3.10 solvent was composed of 1.00M acetic acid, 0.03M sodium acetate, and 0.02M potassium chloride, whereas pH 4.14 solvent was prepared with 0.20M acetic acid and 0.05M sodium acetate.

Weight-Average Molecular Weight (M_w) Determination

Static light scattering method was used to measure the M_w of prepared chitosans.[12,35–38] Different concentrations (0.001–0.1 g/L) of chitosans solution were prepared by dissolving chitosan in 0.01M HCl/0.19M NaCl solvent. The solvent and solutions were filtered through 0.02 μm (Whatman, Anotop, USA) and 0.45 μm (Lida, USA) filter, respectively. The scattered light intensity between 30–140 degrees was measured by a Malvern light scattering photometer (Malvern 4700, UK) with 632.8 nm at 30 ± 0.1°C. The Zimm plot and M_w calculation was processed by Malvern software (version 1.26 for Windows). Every sample was repeated five times of measurement. Refractive index increments (dn/dc) of chitosan solutions were 0.189 mL/g. It was determined by an interferometric refractometer (Wyatt/Optilab 903, USA).

Mutual Diffusion Coefficient (D_m) Determination

The dynamic light scattering method was used to measure the D_m of different M_w chitosans.[14,24,26,33] Three chitosan (0.5–2.5 g/L) solutions of different ionic strengths but the same pH and three solutions of different pHs but the same ionic strength were prepared. The solvents and solutions were also filtered through 0.02 μm (Whatman, Anotop, USA) and 0.45 μm (Lida, USA) filter, respectively, to remove dust and other supramolecular particles from solutions and the D_m was measured at 90 degrees by a Malvern light scattering photometer (Malvern 4700, UK) with 632.8 nm at 30 ± 0.1°C. For every sample, the measurement was repeated three times.

Translational Diffusion Coefficient Calculation

The translational (tracer) diffusion coefficient (D_{tr}) is defined as diffusion coefficient obtained in the absence of a concentration gradient, that is, having no intermolecular interaction effects on diffusion coefficient. D_{tr} related to D_m through equation (6),[14,24,26,33] as follows:

$$D_m = D_{tr}(1 + k\,c) \qquad (6)$$

where c is the polymer concentration. The interception on the ordinate of the plot of D_m versus concentration was D_{tr}.

The diffusion coefficients obtained for the chitosans in solvents at 30°C were converted to standard conditions (20°C and water as solvent) by using the following Van Holde equation[3]:

$$D_{20,w} = \frac{293.1}{T} \times \frac{\eta_{T,S}}{\eta_{20,w}} \times D_{tr} \qquad (7)$$

where $D_{20,w}$ is the diffusion coefficient in the standard condition, T is the absolute temperature, $\eta_{T,S}$ is the viscosity of solvent at temperature T, and $\eta_{20,w}$ is the viscosity of water at 20°C.

Intrinsic Viscosity Determination

Different concentration (0.01–0.1%) solutions of chitosan in three different pH but same ionic strength solvents were prepared. These solutions were passed through filter (Lida, USA) of 0.45 μm to remove insoluble materials. The capillary viscometer (Cannon–Fenske, No 75) was filled with 5 mL of sample and equilibrated in a water bath (Tamson, TMV 40, Holland), which was double-controlled with a thermosta (Firstek B403, Taipei) to maintain the temperature at 30 $\pm$ 0.1°C. The sample was passed through the capillary once before the running time was measured. Each sample was measured three times. The running time of solution and solvent were used to calculate the specific viscosity and reduced viscosity. The reduced viscosity was plotted against the concentration with the intercept being the intrinsic viscosity,[27] as follows.

$$\text{Specific viscosity }(\eta_{sp}) = (t/t_s) - 1 \qquad (8)$$

$$\text{Reduced viscosity }(\eta_{red}) = \eta_{sp}/c \qquad (9)$$

$$\text{Intrinsic viscosity }([\eta]) = (\eta_{red})_c \rightarrow 0 \qquad (10)$$

Where t is the running time of chitosan solution, t_s is the running time of the solvent, and c is the chitosan concentration in g/dL.

Mark–Houwink Exponent a and ε Calculation

Logarithmic $[\eta]$ and logarithmic $D_{20,w}$ were plotted against logarithmic M_w, respectively, with the ab-

Figure 1 Effect of (a) ionic strength (in pH 2.18 solution) and (b) pH (in 0.05 ionic strength solution) on the mutual diffusion coefficient (D_m) of the chitosan molecule (83% DD, 914 KDa) at 30 $\pm$ 0.1°C. Solvents: I = 0.01 (0.01M HCl), 0.10 (0.01M HCl/0.09M NaCl), and 0.20 (0.01M HCl/0.19M NaCl); pH 2.37 (3.00M HAc/0.01M NaAc/0.04M KCl); 3.10 (1.00M HAc/0.03M NaAc/0.02M KCl); 4.14 (0.20M HAc/0.05M NaAc). HAc: CH$_3$COOH; NaAc: CH$_3$COONa.

solute values of slopes of the plots being the Mark–Houwink exponents a and ε, respectively.[14,23,25–28]

RESULTS

Effect of Ionic Strength and pH on Mutual Diffusion Coefficient

Figure 1(a) shows the effect of ionic strength, and Figure 1(b) shows the effect of pH on the mutual diffusion coefficient (D_m) of 83% DD chitosan (M_w = 914 KDa), respectively. The results show that D_m increased with increasing solution ionic strength or with increasing solution pH at the same chitosan concentration. D_m also increased

Table I   Effect of Ionic Strength on the Translational Diffusion Coefficients (D_{tr}, $\times 10^8$ cm^2/s) and Diffusion Coefficient of Standard Conditions ($D_{20,\,w}$, $\times 10^8$ cm^2/s) of 83% Deacetylated Chitosans with Different Molecular Weight (M_w) in pH 2.18 Solutions at 30 $\pm$ 0.1°C

M_w (KDa)	$I = 0.01$		$I = 0.10$		$I = 0.20$	
	$D_{tr}{}^a$	$D_{20,w}{}^b$	D_{tr}	$D_{20,w}$	D_{tr}	$D_{20,w}$
914	1.524	1.162	2.195	1.708	2.635	2.076
680	1.755	1.338	2.785	2.167	3.047	2.401
481	2.333	1.779	3.259	2.536	3.575	2.817
362	2.487	1.896	3.715	2.891	4.210	3.317
322	2.733	2.084	3.858	3.002	4.570	3.601
280	2.978	2.271	4.126	3.210	4.718	3.718
223	3.029	2.310	4.571	3.556	5.109	4.026
148	3.822	2.914	6.112	4.756	6.483	5.108
120	4.808	3.367	6.532	5.082	7.210	5.681
78	6.753	5.149	8.764	6.663	9.473	7.465

^{a} The translational diffusion coefficient data are the mean of triplicates.
^{b} The $D_{20,w}$ data were derived from D_{tr}.

with decreasing chitosan concentration in solution of the same pH or same ionic strength.

Effect of Ionic Strength and pH on D_{tr} and $D_{20,w}$

D_{tr} was obtained by extrapolated D_m to infinite dilution from Figure 1(a) and Figure 1(b) and is listed in Table I and Table II. The $D_{20,w}$ calculated from eq. (7) was also listed. The data showed that D_{tr} and $D_{20,w}$ increased with increasing ionic strength or with increasing pH. Data in Table I also showed that the higher the

M_w of chitosan used, the smaller the D_{tr} and the $D_{20,w}$ are in the solution of the same solvent system.

Effect of pH on the Intrinsic Viscosity of Chitosan

Figure 2 shows intrinsic viscosities of same molecular weight chitosan decreased with increasing pH. Figure 2 also showed intrinsic viscosities of chitosans in same pH solution increased with increasing molecular weight.

Table II   Effect of pH on the Translational Diffusion Coefficients (D_{tr}, $\times 10^8$ cm^2/s) and Diffusion Coefficient of Standard Conditions ($D_{20,\,w}$, $\times 10^8$ cm^2/s) of 83% Deacetylated Chitosans with Different Molecular Weight (M_w) in Ionic Strength 0.05 Solutions at 30 $\pm$ 0.1°C

M_w (KDa)	pH 2.37		pH 3.10		pH 4.14	
	$D_{tr}{}^a$	$D_{20,w}{}^b$	D_{tr}	$D_{20,w}$	D_{tr}	$D_{20,w}$
914	1.456	1.360	1.624	1.392	2.269	1.804
680	1.709	1.597	1.882	1.613	2.756	2.191
481	1.953	1.824	2.240	1.921	3.209	2.552
362	2.156	2.014	2.365	2.028	3.561	2.831
322	2.551	2.383	2.664	2.284	3.666	2.915
280	2.668	2.492	2.783	2.386	3.810	3.030
223	2.755	2.573	3.132	2.685	4.323	3.437
148	3.868	3.614	4.026	3.452	5.230	4.159
120	4.209	3.932	4.830	4.141	6.230	4.954
78	6.178	5.772	6.826	5.852	8.605	6.842

^{a} The translational diffusion coefficient data are the mean of triplicates.
^{b} The $D_{20,w}$ data were derived from D_{tr}.

Figure 2 Effect of pH on intrinsic viscosity of chitosans (83% DD) with different molecular weights in 0.05 ionic strength solutions at 30 ± 0.1°C. Each curve indicates a different molecular weight (KDa) of chitosan: (a) 914; (b) 680; (c) 481; (d) 362; (e) 322; (f) 280; (g) 223; (h) 148; (i) 120; (j) 78.

Effect of Ionic Strength and pH on the Mark–Houwink Exponent a and ε

Figure 3 shows the relationship of $\log M_w$ and $\log D_{20,w}$ of chitosans in three different ionic strength (0.01, 0.10, and 0.20) solutions. Figures 4 and 5 shown the relationship of $\log M_w$ and $\log D_{20,w}$ and $[\eta]$ of chitosans, respectively, in three solutions with different pH (2.37, 3.10, and 4.14). Mark–Houwink equations were obtained by regression analysis. The exponents ε and a, constants k'' and k, and correlation coefficients (R^2)

Figure 4 Double logarithmic plot of weight-average molecular weight (M_w) and diffusion coefficient ($D_{20,w} \times 10^8$) of chitosan molecules in three different pH, same ionic strength (0.05) solutions at 30 ± 0.1°C.

are listed in Table III. Values of ε were between 0.571–0.508 and 0.567–0.503 for chitosans in three different ionic strength (pH 2.18) solutions and three different pH (ionic strength 0.05) solutions, respectively. Values of a were 0.543–0.632 for chitosans in three different pH (ionic strength 0.05) solutions. These data indicated chitosans are all in random coil conformation.

Values of ε of chitosan in ionic strength 0.01 (0.571) was larger than that in 0.10 (0.531), which in turn was larger than that in 0.20 (0.508) (Table III).

Table III shows that ε of chitosan in pH 2.37 solution (0.567) was slightly larger to that in pH

Figure 3 Double logarithmic plot of weight-average molecular weight (M_w) and diffusion coefficient ($D_{20,w} \times 10^8$) of chitosan molecules in three different ionic strength, pH 2.18 solutions at 30 ± 0.1°C.

Figure 5 Double logarithmic plot of intrinsic viscosity ($[\eta]$, dL/g) and weight-average molecular weight (M_w) of chitosan molecules in three different pH, same ionic strength (0.05) solutions at 30 ± 0.1°C.

Table III   Values of Mark–Houwink Equations Exponent a, ε, and Constant k, k″ for Chitosan in Different Ionic Strength (I) and Different pH Solutions at 30 ± 0.1°C

Solvent Conditions		ε	k″ ($\times 10^3$)	R^2	a	k ($\times 10^{-3}$)	R^2
I (pH 2.18)	0.01	0.571	2.87	0.979			
	0.10	0.531	2.58	0.994			
	0.20	0.508	2.20	0.996			
pH (I = 0.05)	2.37	0.567	3.07	0.980	0.632	1.12	0.952
	3.10	0.563	2.96	0.978	0.626	1.08	0.951
	4.14	0.503	1.78	0.979	0.543	2.23	0.967

Solvent systems are as follows. I = 0.01 (pH 2.18): 0.01M HCl. I = 0.10 (pH 2.18): 0.01M HCl/0.09M NaCl. I = 0.20 (pH 2.18): 0.01M HCl/0.19M NaCl. pH 2.37 (I = 0.05): 3.00M CH$_3$COOH/0.01M CH$_3$COONa/0.04M KCl. pH 3.10 (I = 0.05): 1.00M CH$_3$COOH/0.03M CH$_3$COONa/0.02M KCl. pH 4.14 (I = 0.05): 0.20M CH$_3$COOH/0.05M CH$_3$COONa.

3.10 solution (0.563); however, both ε were significantly larger than that in pH 4.14 solution (0.503). The a values have same decreasing tendency (Table III). The a of chitosan was 0.632 in pH 2.37 solution, which was slightly larger than 0.626 of chitosan in pH 3.10 solution; however, both a values were significantly larger than 0.543 of chitosan in pH 4.14 solution.

DISCUSSION

Effect of Ionic Strengths and pHs on Diffusion Coefficients of Chitosan

Diffusion coefficients (D_m, D_{tr}, and $D_{20,w}$) are parameters of hydrodynamics properties of a macromolecules.[3,24] They can be used to characterize the hydrodynamics behavior or gross conformation of chitosans. When the value of diffusion coefficients are smaller, it is indicated that chitosan molecules have a larger hydrodynamic radius or have an extend conformation.

Results of Pogodina et al.[9] and Errington et al.[14] and Tables I and II of this study show diffusion coefficients increased with increasing ionic strength or with increasing pH. It was attributed to chitosan behaved as polycationic electrolyte in dilute acidic aqueous solution. The polyelectrolyte characteristics of chitosan increases with decreasing pH and/or with decreasing ionic strength due to the third electroviscous effect,[9,12,14,17,22,39,40] which in turn increases the hydrodynamic volume of the molecules and decreases the diffusion coefficients.

Data in Tables I and II also showed that the higher the M_w of chitosan used, the smaller the diffusion coefficients (D_{tr}, $D_{20,w}$) are in the solution of the same solvent system. It may be attrib-

uted to chitosans with different M_w's but same the DD (83%) in the same solvent systems were used to determine diffusion coefficient. Therefore, the diffusion coefficient is a function of solution molecular weight only. Van Holde[3] pointed out that solute molecule interaction, molecule size, and shape will affect diffusion coefficient. The larger molecular weight solutes have larger molecular sizes and smaller diffusion coefficients. The results were consistent with that of Errington et al.[14] and Pogodina et al.[9] $D_{20,w}$ shown in Tables I and II are close to that of Pogodina et al.[9] but larger than that of Errington et al.[14]

Figure 1 shows the D_m increased with decreasing chitosan concentration. It may be due to fact that the motility of molecules in solution increased as the chitosan concentration decreases because there is less interference and collision among molecules.[3,26]

Effect of Ionic Strengths on the Conformation

Value of ε of chitosan in ionic strength 0.01 was larger than that in ionic strength 0.10, which in turn was larger than that in ionic strength 0.20 (Table III). It may be due to the electric shielding effects of counterion on the protonated amide group (NH$_3^+$) of chitosan in a higher ionic strength environment.[9,14,17,22,39–41]

Data in Table III also indicates the conformations of chitosans in three different ionic strength (pH 2.18) solutions were all random coil. However, Roberts and Domszy[11] reported that the values for Mark–Houwink exponent a were 1.26 and 0.93 for 80% DD chitosan in ionic strength 0.021 (pH 3.00) and 0.202 (pH 2.85) solution, respectively, that is, in a rodlike shape. The solution

conditions they used and this report were alike, but their a values were larger, and chitosan conformation are more extended than this report. Anthonsen et al.[5] reported Mark–Houwink that the values exponent a were 0.66–0.79 for 85% DD chitosan in ionic strength between 0.013 to 1.007 (pH 4.50), that is, in random coil conformation. The solution ionic strengths they used were similar; however, the pHs were higher than this report. The conformation of chitosan shall be more contracted than this report due to the fact that the degree of protonation of chitosan amide groups is smaller. However, chitosan conformation of both studies are similar. However, results of Anthonsen et al.,[5] Roberts and Domszy,[11] and this report indicated that the higher the solution ionic strength, the more contract the conformation of the chitosan. The discrepancy between Anthonsen et al.,[5] Roberts and Domszy,[11] and this report may be due to the difference in molecular weight, molecular weight distribution, acetyl group distribution, the raw material used for chitosan preparation, and so on.

Our results and these of Anthonsen et al.[5] and Roberts and Domszy[11] indicate that no conformational transition occurred as the ionic strength changed. It may be due to the fact that the ionic strength of 1.007 is not high enough to have a shielding effect and/or the ionic strength of 0.010 is not low enough to have a third electroviscous effect to have conformational transition occurrence.

Effect of pH on the Conformation

Table III shows that the values of ε decreased with increasing pH. The ε of chitosan in pH 2.37 was close to that in pH 3.10, and both values of ε were larger than that in pH 4.10. The effect of pH on Mark–Houwink exponent a is similar to the effect on Mark–Houwink exponent ε. Therefore, a value of chitosan in pH 2.37 was close to that in pH 3.10, and both were lager than that in pH 4.10. It may due to the fact that the degree of protonation of the amide group on chitosan decreased with the increasing pH value (with decreasing hydrogen ion concentration). The conformation becomes contract; thus, a and ε values become smaller.

In the literature, the Mark–Houwink exponents a of these chitosans with similar DD (about 80%) in lower pH solutions of pH 2.55–3.00[9,11] are larger than these in a higher pH solution of pH 4.43–4.56.[5,10,12,29] The overall tendency agree with the results shown in Table III.

The results in Table III show conformation of chitosans in pH 2.37–4.14 and that the ionic strength 0.05 solution was in random coil. However, results of Pogodina et al.[9] and Roberts and Domszy[11] showed chitosans (80% DD) were in a rodlike shape in pH 2.55–3.00, 0.021–0.303 ionic strength solvents. The solvent conditions and DD of chitosan used in Pogodina et al.[9] and Roberts and Domszy[11] are similar to this report. Anthonsen et al.,[5] Gamzazade et al.,[29] and Rinaudo et al.[10] reported that the conformation of chitosan were in random coil and a values were close to this report as chitosan (about 80% DD) in pH 4.44–4.56, 0.013–0.200 ionic strength solution. The DD of chitosan and solution ionic strength they used are similar to this report. However, solution pH were higher than in this report. Wang et al.[12] reported chitosan was in a rodlike shape; however, the solution pH (4.43) and ionic strength (0.10) they used are higher than this report. In general, the chitosan used in this report is more contracted than the reports mentioned above. It may be due to differences in molecular weight, molecular weight distribution, acetyl group distribution, raw material used for chitosan preparation, and so on.

Figure 6 Double logarithmic plot of (a) diffusion coefficient ($D_{20,w} \times 10^8$) and (b) intrinsic viscosity ([η], dL/g) and weight-average molecular weight (M_w) of chitosans in pH 3.10, ionic strength 0.05 solutions at 30 ± 0.1°C.

Table IV   Values of Mark–Houwink Exponents ε^*, ε^{**} and Constants k''^*, k''^{**} for Different Molecular Weight Range Chitosans in Different Ionic Strength (I) and Different pH Solutions at 30 ± 0.1°C

Solvent Conditions		ε^*	k''^*	ε^{**}	k''^{**}
I (pH 2.18)	0.01	0.777	31914	0.518	1455
	0.10	0.587	4949	0.499	1685
	0.20	0.585	5381	0.488	1682
pH (I = 0.05)	2.37	0.755	28138	0.474	920
	3.10	0.747	26050	0.454	709
	4.14	0.633	11733	0.430	684

ε^*, ε^{**} and k''^*, k''^{**} are the Mark–Houwink exponents and constants for 78–223 KDa and 223–914 KDa chitosans, respectively.

Effect of Molecular Weight on the Conformation

Figure 6(a) and (b) shows double logarithmic plots of $D_{20,w}$ and $[\eta]$ and M_w of chitosans in pH 3.10 and 0.05 ionic strength solution, respectively. A break point occurred at 223 KDa for pH 3.10, 0.05 ionic strength solution and also for other solution conditions (pH 2.37 and 4.14, 0.01, 0.10, and 0.20 ionic strength not shown). a^*, k^*, ε^*, and k''^* represent Mark–Houwink exponents and constants of those chitosans in which their M_w were smaller than 223 KDa; whereas, a^{**}, k^{**}, ε^{**}, and k''^{**} represent Mark–Houwink exponents and constants of those chitosans in which their M_w were larger than 223 KDa and are derived and listed in Tables IV and V. In pH 2.37–4.14 (0.05 ionic strength), the ε^* values were between 0.633 to 0.755, ε^{**} values were between 0.430 to 0.474 (Table IV), the a^* values were between 0.752 to 0.988, and the a^{**} values were between 0.406 to 0.428 (Table V). In 0.01–0.20 ionic strength (pH 2.18), the ε^* values were between 0.585 to 0.777, and the ε^{**} values were between 0.488 to 0.518 (Table IV). The results indicate that conformation of those chitosans with M_w smaller than 223 KDa were more stretched than those chitosans with M_w larger than 223 KDa.

Molecular-weight-induced conformational transition occurred in these six solution conditions.

Using intrinsic viscosity as a hydrodynamic parameter, molecular-weight-induced conformational transition, which occurred among larger and smaller molecular weight chitosans, was reported.[38] It was attributed to the difference in intramolecular hydrogen bonds and/or the difference in the charge distribution among larger- and smaller-molecular-weight chitosans.

Intrinsic viscosity decreases with increasing shear rate. The effect is more pronounced for higher-molecular-weight polymer due to shear thinning.[23] Ottøy et al.[30] reports the intrinsic viscosity was smaller anomalously of larger-molecular-weight chitosan molecules; therefore, this anomaly in intrinsic viscosity could not be used for the Mark-Houwink equation. The break of the Mark–Houwink equation in larger-molecular-weight chitosan (smaller slope) may be due to shear-induced conformational transition, not due to molecular weight conformational transition, but Figure 6 showed that the Mark–Houwink equation break obviously either used diffusion coefficient measured by dynamic light scattering or used intrinsic viscosity determined by capillary viscometer as hydrody-

Table V   Effect of pH and Molecular Weight Range on Constants a^*, a^{**} and k^*, k^{**} of the Mark–Houwink Equations for Chitosan in Ionic Strength 0.05 Solutions at 30 ± 0.1°C

pH (I = 0.05)	a^*	k^* ($\times 10^{-5}$)	R^2	a^{**}	k^{**} ($\times 10^{-2}$)	R^2
2.37	0.988	1.69	1.000	0.428	1.60	0.986
3.10	0.962	2.06	0.996	0.421	1.57	0.989
4.14	0.752	18.9	0.993	0.406	1.33	0.990

a^*, a^{**} and k''^*, k''^{**} are the Mark–Houwink exponents and constants for 78–223 KDa and 223–914 KDa chitosans, respectively. Intrinsic viscosity in dL/g.

namic parameter of chitosan. Results in Figure 6 indicated molecular-weight-induced conformational transition indeed occurred for chitosans.

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