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DILUTE SOLUTION PROPERTIES OF CHITOSAN IN PROPIONIC ACID AQUEOUS SOLUTIONS

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ABSTRACT

Dilute solution properties of chitosan in propionic acid aqueous solutions with various pH values were studied using intrinsic viscosity, static light scattering (SLS), and dynamic light scattering (DLS). We demonstrated that the particle sizes (intrinsic viscosity [η], average hydrodynamic radius $\langle R_h \rangle$, and average radius of gyration $\langle R_G \rangle$) of chitosan molecules in dilute propionic acid/water solutions increased with decreasing pH value. SLS data also demonstrated that second virial coefficient (A_2) increased with decreasing pH value suggesting that solubility of chitosan in water increased with increasing propionic acid concentration. Differential refractive index increment (dn/dC), depolarization ratio (ρ_v), and ratio of $\langle R_G \rangle / \langle R_h \rangle$ increased with decreasing pH value indicating the increment of chitosan molecular chain anisotropy with increasing propionic acid concentration. Increasing propionic acid concentration in chitosan aqueous solution caused an increase of $-NH_3^+$ group on chitosan molecules leading to an increase in intra-molecular electrostatic charge repulsion and chain expansion of chitosan molecules. Thus, the molecular size, A_2, ρ_v, dn/dC, and $\langle R_G \rangle / \langle R_h \rangle$ of chitosan increased with increasing propionic acid concentration.

Key Words: Chitosan; Propionic acid aqueous solution; Intrinsic viscosity; Light scattering

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INTRODUCTION

Chitosan, partially deacetylated chitin, is made by alkaline *N*-deacetylation of chitin, which is structural polysaccharide in the exoskeleton of many arthropods. It is an important kind of natural biopolymer composed of mainly two kinds of common sugars, β-(1,4)-2-deoxy-2-amino-*D*-glucopyranose (*D*-glucosamine) and β-(1,4)-2-deoxy-2-acetamido-*D*-glucopyranose (*N*-acetyl-*D*-glucosamine).[1−3] The recent increased understanding of their functions in biology and their potential in technological and biotechnological applications[3−5] have attracted a great deal of attention and have brought into vogue in research in recent years.[6−13] In neutral or basic pH, chitosan contains free amino groups and is insoluble in water, while in acidic pH, chitosan is soluble in water due to protonation of amino functions. The solubility depends on the distribution of free amino and *N*-acetyl groups.[11] The solution properties of chitosan are governed by the relative content of the two monomers as designated by the fraction of amino units (or degree of deacetylation) and by the distribution of the monomers along the chain, the molecular weight, the pH, and the ionic strength of the solution.[14]

In the past few years, aqueous solution properties of chitosan had been reported by several researchers. Wang et al.[15,16] and Anthonsen et al.[17] established the Mark-Houwink equations for chitosans with different degrees of deacetylation. Matsumoto et al.[18] investigated the influence of degree of deacetylation (degrees of deacetylation were 0.62, 0.79, and 0.96) on the heterogeneous molecular aggregation for chitosan solution in 2 wt% acetic aqueous solution using rheological and small angle X-ray scattering. Rheological measurements showed that chitosans aqueous solutions with 0.62 and 0.96 degrees of deacetylation were homogeneous while that with 0.76 degree of deacetylation was heterogeneous and had a fractal dimension of about 1.3. The rheological properties of chitosan solutions were also reported by Chen et al.[19] Their results showed that the viscosity of chitosan was strongly affected by the pH, ionic strength, and types of counter ions. Using a combination of static and dynamic light scattering (SLS and DLS), Wu et al.[20] measured weight average molecular weight and molecular weight distribution of chitosan. With 91% deacetylated chitosans in 0.2 M CH_3COOH/0.1 M CH_3COON_a aqueous solution, Wu et al. showed that chitosan had an extended chain conformation and a tendency to aggregate both in the static and dynamic light scattering data. The aggregation behavior of chitosans having fraction of acetylation (F_A) ranging from 0 to 60% and different molecular weights had also been observed by Anthonsen et al.[21] using static light scattering. The intensity and dynamic light scattering (ILS and DLS) study of semi-dilute acid aqueous solutions of unmodified chitosan and of hydrophobically modified chitosan was reported by Kjoniksen et al.[22] The ILS and DLS measurements suggested the formation of associated structure with a fractal dimension about 2. DLS and SLS were also performed by Buhler and

Rinaudo[23] on chitosan in dilute and semi-dilute acetic acid/sodium acetate aqueous buffer solutions. In the dilute regime, typical good solvent behavior was found. However, in the semi-dilute regime, the time autocorrelation function of the scattered electric field was found to be bimodal. Both the short and long time relaxation were scattering vector q^2 dependent. The fast mode was attribute to the relaxation of concentration fluctuations characterized by a cooperative diffusion mechanism of the polymer network meshes. The slow relaxation time appeared to be related to the diffusion of polymer associations.

When the pH value is less than 5.0, chitosan in solution carries a positive charge along with its backbone. It is this cationic nature that makes it possible to be used in many biomedical applications. As a linear polyelectrolyte, chitosan has both reactive amino and hydroxyl groups. Its physical and solution properties change with the surrounding chemical environment. In this paper, using intrinsic viscosity, SLS, and DLS measurements, we investigate the dilute solution properties of chitosan in propionic acid/water solutions with various propionic acid concentrations. Based on the experimental data, the dependence of chitosan structural conformation on propionic acid concentration is discussed.

EXPERIMENTAL

Materials

Chitosan

Chitosan (from shell and head of spear shrimp) was purchased from Kaohsung Applied Chemical Co. Ltd., Taiwan. Its degree of deacetylation determined by UV spectrometry[24] was 85%.

Propionic Acid

Propionic acid, purchased from Riedel de Haen Co Ltd., Germany, had a purity of 99.5 wt%.

Sodium Chloride

Sodium chloride (reagent grade), purchased from Riedel de Haen Co. Ltd., Germany, was used as a calibrated NaCl aqueous solutions in differential refractive index increment *dn/dC* measurements.

Instrumentation

pH Meter

Knick model-761 pH meter (Berlin) was used to measure the pH value of chitosan propionic acid aqueous solutions.

Ubbelohde Viscometer

A Ubbelohde viscometer with a flow time of 90 sec. for distilled water was used for intrinsic viscosity measurements.

Refractometer

Nippon Optical Works (Tokyo) Abe refractometer was used to measure the refractive indexes of propionic acid/water solutions with various pH values.

Differential Refractometer

A C. N. Wood (PA, USA) RF600 differential refractometer was used to determine the differential refractive index increment dn/dC, which is also referred to as the specific increment, ν. This was calibrated using aqueous NaCl solutions[25] All of the dn/dC data were measured at both 436 and 546 nm. The value at 633 nm was determined by linear extrapolation.

Dynamic Light Scattering (DLS)

A Brookhaven BI-200SM goniometer with BI9000 correlator was used to investigate the chitosan particles size distribution in aqueous solution with various propionic acid concentration. DLS measurements were made at a scattering angle of 90° and at a temperature of 25°C ± 0.5°C. The laser was a 25 mW He-Ne (633 nm) Spectra Physics model. The particle size distribution was calculated from DLS correlation functions by using CONTIN software.

Static Light Scattering (SLS)

A Brookhaven BI-200SM goniometer with BI2030AT correlator was used to carry out SLS measurements. SLS measurements were made at a temperature of 25 ± 0.5°C. The laser was a 10 mW He-Ne (633 nm) Spectra Physics model. Depolarization ratios ρ_v, second virial coefficients A_2, radius of gyration R_G, and molecular weight $\overline{M}_w$ of chitosan in various pH values of propionic acid/water solutions were obtained from this measurements.

Sample Preparation

Chitosan Propionic Acid/Water Solutions

The chitosan/propionic acid/water solutions were prepared by dissolving chitosan into propionic aqueous solutions with various propionic acid concentrations and diluted to chitosan concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mg/mL. The pH values of propionic acid/water solvents for preparing chitosan solutions were 1.55, 2.00, 2.50, 3.00, and 3.25. All of the solutions were filtered through a 0.8 μm AAWP 02500 Millipore filter before measurements. These solutions were used for refractive index increment, SLS, and intrinsic viscosity measurements. The solutions with a concentration of 0.1 mg/mL were used for DLS measurements.

RESULTS AND DISCUSSION

The chitosan used in this work composed 85 mol% of β-(1,4)-2-deoxy-2-amino-*D*-glucopyranose units, and 15 mol% of β-(1,4)-2-deoxy-2-acetamido-*D*-glucopyranose. The presence of propionic acid causes the transformation of $-NH_2$ group of β-(1,4)-2-deoxy-2-amino-*D*-glucopyranose units to positively charged $-NH_3^+$ group. It is obvious the propionic acid concentration strongly affect the charge density of chitosan molecules and thus the chitosan chain conformation in aqueous solutions.

Intrinsic Viscosity Study

The flow times of chitosan/propionic acid/water solutions and their solvents with various pH values and chitosan concentrations were measured using an Ubbelohde viscometer at 25°C. The reduced viscosities η_{sp}/C vs. chitosan concentration C in propionic acid/water solutions with various pH values are plotted and shown in Fig. 1. The intrinsic viscosities of chitosan/propionic acid/water solutions calculated using Huggins and Kraemer equations[26] are summarized in Table 1. In Table 1, the viscosities of propionic acid/water solutions with various pH values are also listed. These results showed that the intrinsic viscosity of chitosan in propionic acid/water solutions increased with decreasing pH suggesting a chain expansion of chitosan molecules with increasing propionic acid concentration in water solutions.

SLS Study

SLS measurements were carried out at 25°C. A classic graphic technique for simultaneously extrapolating light-scattering data in the dilute regime is the Zimm plot which is given by Eq. (1):[27,28]

$$\frac{KC}{\Delta R_\theta} = \frac{1}{\overline{M}_w}\left[1 + \frac{16\pi^2\langle R_G{}^2\rangle}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right) + \cdots\right] + 2A_2C + \cdots \tag{1}$$

where ΔR_θ is the excess Rayleigh ratio of solution at scattering angle θ, λ the wavelength of incident light in vacuum, A_2 the second virial coefficient, $\langle R_G^2\rangle$

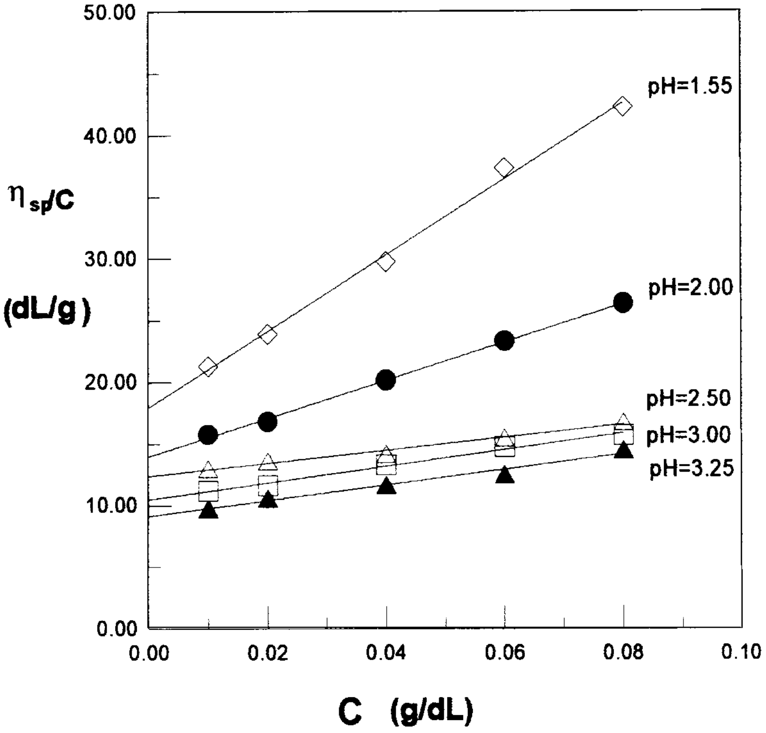

Figure 1. Reduced viscosities η_{sp}/C vs. chitosan concentration C in propionic acid/water solutions with various pH values.

Table 1. Viscosity of Propionic Acid Aqueous Solutions and Intrinsic Viscosity of Chitosan/Propionic Acid Aqueous Solutions

pH	η_o (cps)	$[\eta]$ (cm^3/mg) (Huggins eq)	$[\eta]$ (cm^3/mg) (Kraemer eq)
1.55	1.05	1.84	1.87
2.00	1.02	1.40	1.40
2.50	0.987	1.24	1.24
3.00	0.943	1.05	1.07
3.25	0.916	0.91	0.94

the mean square radius of gyration of polymer, $\overline{M}_w$ the weight average molecular weight of polymer, and C the polymer concentration. For vertically polarized incident radiation, the optical constant, K, is given by:

$$K = \frac{4\pi^2 n_0^2 (dn/dC)^2}{\lambda^4 N_A} \tag{2}$$

where n_0 is the index of refraction of the solvent, i.e., propionic acid/water solution; N_A the Avogadro's number; and dn/dC the differential refractive index increment. Zimm plots were constructed in order to extrapolate to zero concentration and zero scattering angle in Eq. (1).

The Zimm plot of chitosan/propionic acid/water solutions at pH = 2.5 is shown in Fig. 2. This is achieved by plotting $KC/\Delta R_\theta$ vs. $\sin^2(\theta/2)+ 1000\,C$. The factor of 1000 is a chosen constant to give a convenient spacing of the data points on the graph. The data points on the grid corresponding to a given angle are extrapolated to zero concentration, and similarly the points at a given concentration are extrapolated to zero angle. The inversion of the $\overline{M}_w$ is obtained from the intercept of the $\sin^2(\theta/2) = 0$ curve, and A_2 is obtained from the slope ($2A_2$ = slope). The intercept of C=0 curve again gives inverse of $\overline{M}_w$ and the initial slope is proportional to $\langle R_G^2\rangle$. The linear extrapolation of $KC/\Delta R_\theta$ to $C \to 0$ and $\theta \to 0$ are shown in Fig. 2. Similar Zimm plots of chitosan/propionic acid/water solutions with other pH values were also obtained.

For optically anisotropic molecules the polarization of the scattered light has components both parallel and perpendicular to the incident polarization.

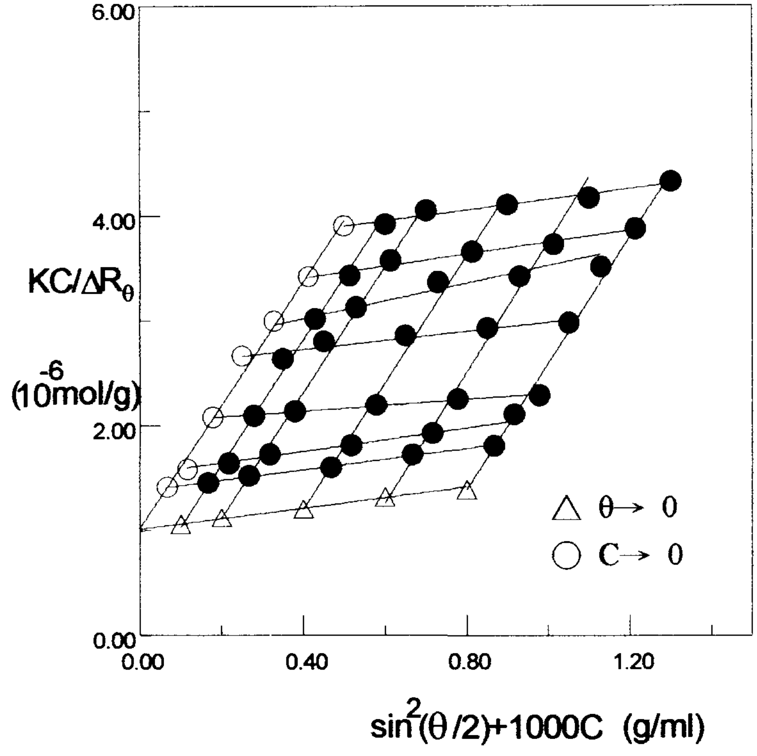

Figure 2. Zimm plot of chitosan/propionic acid/water solutions at pH = 2.5.

The anisotropic part of the scattering was expressed using depolarization ratio defined as follows:[27]

$$\rho_v = H_v(90^\circ)/V_v(90^\circ) \tag{3}$$

where the subscript v indicates measurements involving a vertically polarized incident laser light, $H(90^\circ)$ the horizontally scattered light at the scattering angle $\theta = 90^\circ$, and $V(90^\circ)$ the vertically scattered light at the scattering angle $\theta = 90^\circ$. For polyelectrolytes which have electrostatic charge repulsion within the polymer chains, depolarization effects should not be neglected. The refractive indexes n_0 of pure propionic acid/water solutions, dn/dC, and ρ_v values of chitosan in propionic acid aqueous solutions with various pH values are listed in Table 2. Inspecting dn/dC and ρ_v values shown in Table 2, similar trend of these two data were found. Both dn/dC and ρ_v decreased with increasing pH value. The depolarization ratio is a function of polymer particle shape and anisotropy in solvent. This permits one to investigate the polymer shape and anisotropy.[29,30] The more anisometric the shape of a particle, the larger optical anisotropy can be expected. Thus appreciable depolarization of scattered light, due to solute has been observed as rod like particles. On the other hand, for polymers with conformation of coils in solvents, the depolarization of scattered light, i.e., ρ_v, is quite low. In propionic acid/water solutions with lower pH value, the chitosan molecules have higher $-NH_3^+$ charge density and behave more like to rigid rods due to higher intra-molecular electrostatic charge repulsion. However in solutions with higher pH value, the chitosan molecules have lower $-NH_3^+$ charge density and behave more like to flexible polymers due to lower intra-molecular electrostatic charge repulsion.

SLS measurements of polyelectrolyte solutions with linearly polarized incident light perpendicular to the scattering plane and no analyzer, $\overline{M}_w R_G$, and A_2 calculated from Eq. 1 should be considered apparent, but not true values. In a propionic acid/water solution with a low pH value, the chitosan molecules behave like rigid rod polymers. However, in a propionic acid/water solution with a high pH value, the chitosan molecules behave like flexible

Table 2. Refractive Index of Propionic Acid Aqueous Solutions and Differential Index of Refraction and Depolarization Ratios of Chitosan Solutions

pH	n_0	dn/dC (cm^3 g^{-1})	ρ_v
1.55	1.376	0.289	0.27
2.00	1.360	0.238	0.24
2.50	1.350	0.216	0.19
3.00	1.345	0.201	0.18
3.25	1.339	0.190	0.12

polymers. Polarization corrections of $\overline{M}_w, A_2$, and R_G for flexible polymers are shown in Eqs. 4, 5, and 6, respectively:[27–29]

$$\overline{M}_w = \overline{M}_{w,\mathrm{app}}[(3+3\rho_v)/(3-4\rho_v)] \quad \text{(for flexible polymers)} \tag{4}$$

$$A_2 = A_{2,\mathrm{app}}/[(3+3\rho_v)/(3-4\rho_v)] \quad \text{(for flexible polymers)} \tag{5}$$

$$R_G = R_{G,\mathrm{app}}/[(3+3\rho_v)/(3-4\rho_v)]^{0.5} \quad \text{(for flexible polymers)} \tag{6}$$

The polarization correction of R_G for rigid rod polymers is:[27–29]

$$R_G = R_{G,\mathrm{app}}[1-(4/5)\delta+(47/35)\delta^2]/[1+(7/5)\delta^2)]^{0.5} \quad \text{(for rigid rod polymers)} \tag{7}$$

In Eq. (7),

$$\delta^2 = 5\rho_v/(3-4\rho_v) \tag{8}$$

The apparent $\overline{M}_{w,\mathrm{app}}, A_{2,\mathrm{app}}$, and $R_{G,\mathrm{app}}$ of chitosan solutions were obtained from the intercepts and slopes of extrapolated data, respectively, from Zimm plots using Eq. 1. The uncorrected $\overline{M}_w$ and A_2, data (assume rigid rod polymers) and $\overline{M}_w$ and A_2, data (assume flexible polymers) corrected using Eqs. 4 and 5, respectively, are summarized in Table 3. The R_G data corrected using Eqs. 6 (assume flexible polymers) and 7 (assume rigid rod polymers) are also shown in Table 3. The variations of A_2 and R_G with pH value are shown in Figs. 3 and 4 respectively. In high pH solutions (pH = 3.00 and 3.25) chitosan molecules behave like flexible molecules, A_2 and R_G data corrected using flexible polymer model are used and plotted in Figs. 3 and 4, respectively. In low pH solutions (pH = 1.55 and 2.00) chitosan molecules behaved like rigid rod molecules, A_2 and R_G data calculated using rigid rod models are used and plotted in Figs. 3 and 4, respectively. In a solution with pH = 2.5,

Table 3. Static Light Scattering Data of Chitosan Solutions

	A_2 (flex.)	A_2 (rod)	R_G (flex.)	R_G (rod)	M_w (flex.)	M_w (rod)
pH	(10^{-4}cm^3 mol g^{-2})		(nm)		(10^6 g mol^{-1})	
1.55	2.34	4.65	228	256	2.15	1.09
2.00	2.27	4.13	222	240	1.91	1.05
2.50	1.47	2.34	164	170	1.61	0.98
3.00	1.45	2.24	91	92	1.25	0.83
3.25	1.22	1.62	75	72	1.10	0.83

flex: calculated using flexible polymer model.
rod: calculated using rigid rod model.

chitosan molecules have conformations between flexible polymers and rigid rods, A_2 and R_G plotted in Figs. 3 and 4, respectively, are the averages of the data obtained using flexible polymer and rigid rod models. The experimental data of Figs. 3 and 4 revealed that both A_2 and R_G increased with decreasing pH value. It is obvious increasing propionic acid concentration resulted in an increase in the concentration of $-NH_3^+$ group of chitosan molecules which caused an increase of solubility of chitosan in propionic acid/water solutions. The increase in $-NH_3^+$ group on chitosan molecules also lead to an increase of R_G due to the increase of intra-molecular electrostatic charge repulsion by $-NH_3^+$ group.

DLS Study

The DLS measurements were also carried out at 25°C with a scattering angle $\theta = 90°$ for chitosan/propionic acid/water solutions with a chitosan concentration of 0.1 mg/mL and various pH values. The normalized intensity autocorrelation functions $g^{(2)}(t)$ of 0.1 mg/mL chitosan in propionic acid/water solutions with various pH values are shown in Fig. 5. The hydrodynamic radius (R_h) distributions calculated from Fig. 5 using CONTIN

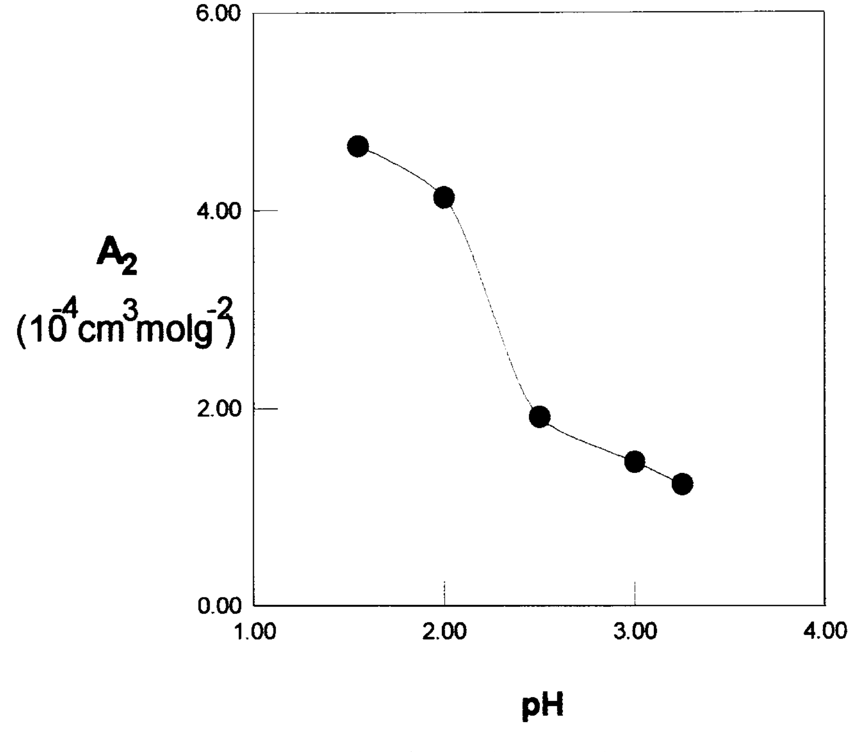

Figure 3. Plot of A_2 against pH value.

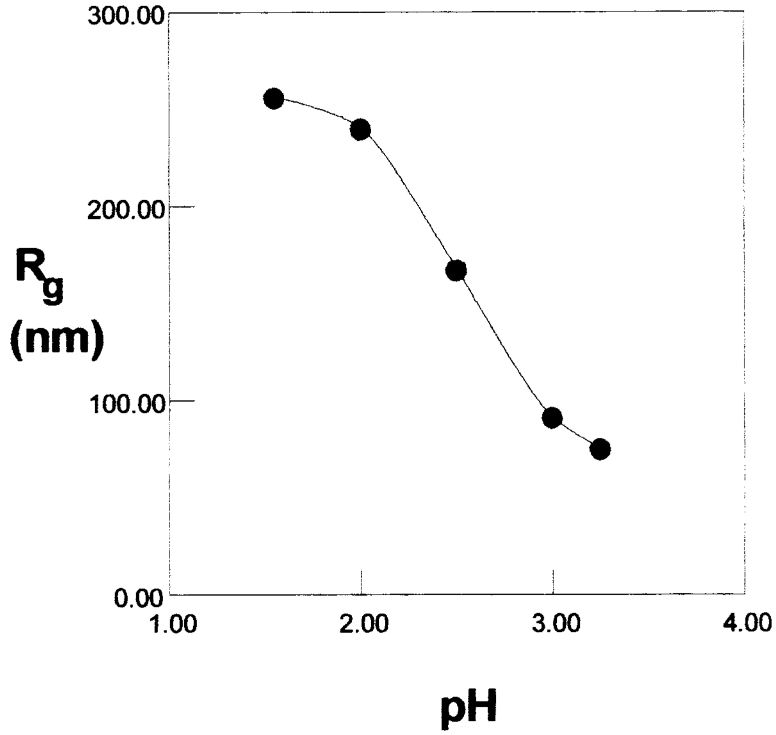

Figure 4. Plot of R_G against pH value.

software are shown in Fig. 6. The average hydrodynamic radius of chitosan in propionic/water solutions with various pH values are summarized in Table 4. As shown in Table 4, the average hydrodynamic radius $\langle R_h \rangle$ had a similar trend as $\langle R_G \rangle$ value, i.e., $\langle R_h \rangle$ increased with decreasing pH value. These results suggested that increasing propionic acid concentration would result in an increase in $-NH_3^+$ group concentration of chitosan molecules which caused an increase in chitosan chain expansion due to the increase of intra-molecular electrostatic charge repulsion from NH_3^+ groups.

Combination of SLS and DLS

In Table 4, average value of radius of gyration $\langle R_G \rangle$, and ratio of $\langle R_G \rangle / \langle R_h \rangle$ of chitosan/propionic acid/water solutions at various pH values are also listed. During translational motion of the particles, the solvent can penetrate deeply into the fairly open molecular chains leaded R_h to be smaller than R_G. However, if the particle is highly compact then only the outer shell is drained by solvent. Thus R_h is larger than R_G for compact molecules. The ratio $\langle R_G \rangle / \langle R_h \rangle$ is an indicator of the compactness of polymers in solvents. The relation of polymer architecture with $\langle R_G \rangle / \langle R_h \rangle$ in solvents had been

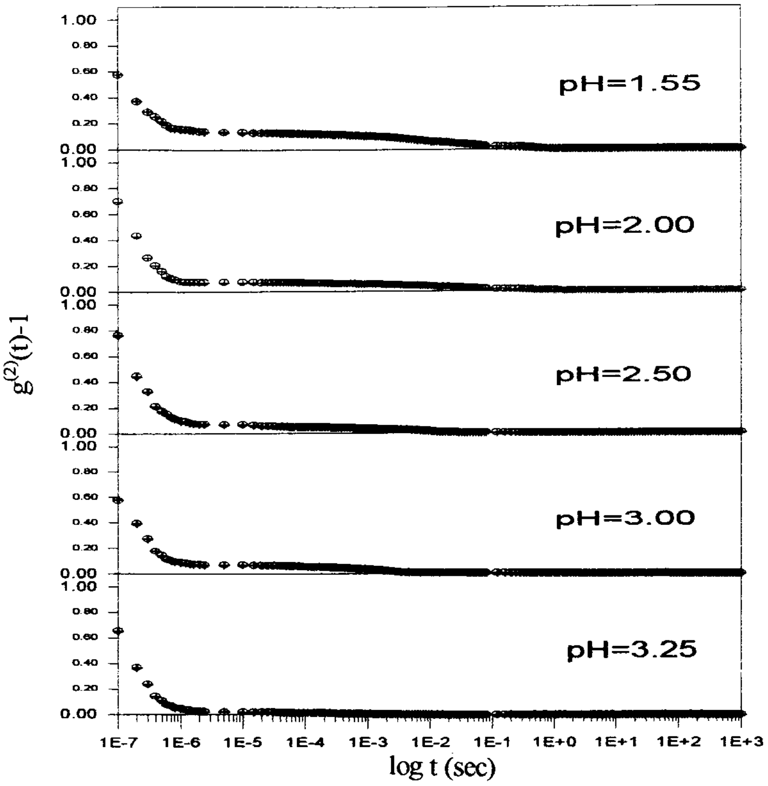

Figure 5. Normalized intensity autocorrelation functions of 0.1 mg/mL chitosan/propionic acid/water solutions with various pH values.

summarized by Burchard[31] and listed in Table 5. Table 5 shows that the ratio of $\langle R_G \rangle / \langle R_h \rangle$ decreases with increasing compactness of polymer chains. Comparing the $\langle R_G \rangle / \langle R_h \rangle$ ratios listed in Tables 4 and 5, we found that the chitosan molecules behaved like random coils at $\mathrm{pH} \geq 3.0$ and behaved like rigid rod at $\mathrm{pH} \leq 2.0$. These results are in consistent with $[\eta]$, ρ_v, and R_G data listed in Tables 2 and 3, respectively.

DISCUSSION

The amino group in chitosan has a $\mathrm{pK_a}$-value of about $6.2 \sim 7$,[17] which makes chitosan a polyelectrolyte at low pH values. The electrostatic charge repulsion of $-\mathrm{NH_3^+}$ group causes a chain expansion of chitosan molecules. However, increasing the concentration of propionic acid in chitosan aqueous solutions results is not only an increment of $-\mathrm{NH_3^+}$ in chitosan molecules but also an increment of negatively charged propionate ($\mathrm{C_2H_5COO^-}$) leading to an increase in the ionic strength of chitosan solutions. It is expected that

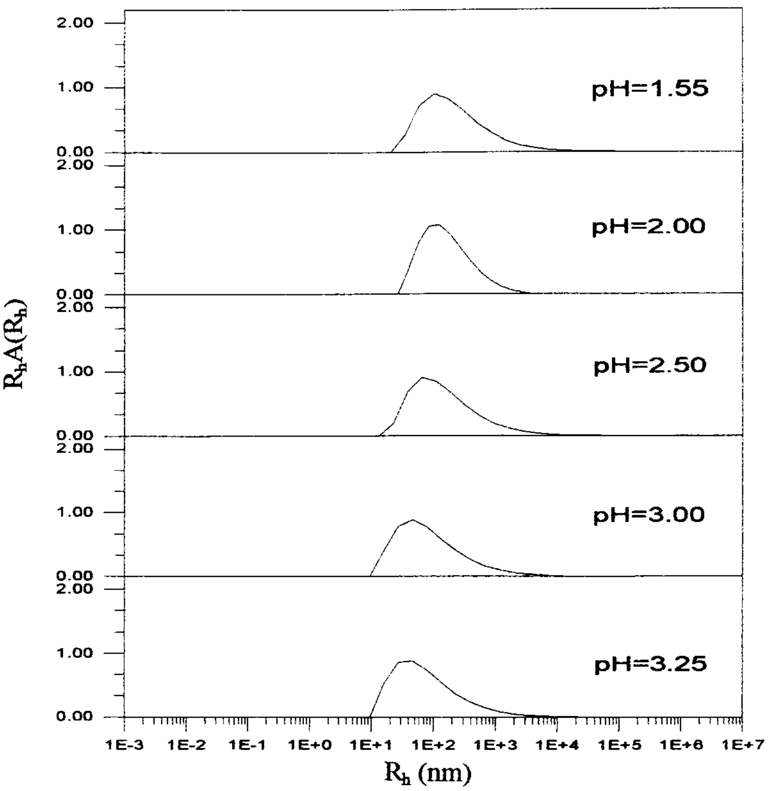

Figure 6. Hydrodynamic radius (R_h) distributions of 0.1 mg/mL chitosan in propionic acid/water solutions with various pH values.

increasing the ionic strength of chitosan aqueous solutions would result in an increase in the shielding effect of positively charged $-NH_3^+$ of chitosan leading to shrinkage of chitosan molecular chains. However, our experimental data show that particle sizes of chitosan molecules and second virial

Table 4. The Ratio of DLS $\langle R_h \rangle$ and SLS $\langle R_G \rangle$ Data of Chitosan Solutions

pH	$\langle R_h \rangle$ (nm)	$\langle R_G \rangle$ (nm)	$\langle R_G \rangle / \langle R_h \rangle$
1.55	122	256 (rod)	2.10
2.00	115	240 (rod)	2.09
2.50	86	167 ([rod + flex]/2)	1.94
3.00	50	91 (flex)	1.82
3.25	45	75 (flex)	1.58

rod: data obtained using rod model.
flex: data obtained using flexible polymer model.
[rod + flex]/2: data obtained using average of rod and flexible models.

Table 5. The Ratio $\langle R_g \rangle / \langle R_h \rangle$ for Selected Structures

Architecture	$\langle R_G \rangle / \langle R_h \rangle$
Homogeneous sphere	0.778
Random coil, monodisperse	
theta condition	1.50
good solvent	1.78
Random coil, polydisperse	
theta condition	1.73
good solvent	2.05
Rigid rod	
monodisperse	> 2.0
polydisperse	> 2.0

Summary of ref. 31.

coefficient A_2 increase with increasing propionic acid concentration indicating that the influence of the shielding of negatively charged $C_2H_5COO^-$ group on positively charged $-NH_3^+$ group is less than the electrostatic charge repulsion of $-NH_3^+$ group in chitosan molecules. Chen et al.[19] reported that under a fixed pH value ($pH = 2.0 \sim 4.5$), the intrinsic viscosity of chitosan in water/organic acid solutions increases in the following order: acetic acid $<$ propionic acid $<$ butyric acid. They also reported that in same water/organic acid solutions chitosan intrinsic viscosity increased with increasing organic acid concentration. However, they found that the intrinsic viscosity of chitosan in 0.1 M organic acid/water solutions decreased with decreasing pH value, while the pH value was adjusted by adding extra HCl solution into chitosan/organic acid/water solutions. Their results show that the increase of ionic strength by adding HCl into the chitosan solutions causes the shrinkage of chitosan molecular chains. Though the increase of group may cause a chain expansion, however the influences on the chitosan chains by the Cl^- shielding should be larger than the electrostatic charge repulsion between $-NH_3^+$ groups. The different behavior of chitosan molecules in water by the presence of organic acid and HCl could be due to the solubility difference of chitosan in organic acid/water and in HCl/water solutions. Chitosan backbone is an organic compound with $-NH_3^+$ group distributed along the backbone. Though the $-NH_3^+$ group cause chitosan molecules easy to be dissolved into water, however, its backbone is easier to be dissolved in an organic acid solution than in HCl solution. So in the present study, our experimental data show that increasing propionic acid concentration in chitosan aqueous solution result in not only chitosan $-NH_3^+$ group concentration but also the solubility of chitosan backbone in propionic acid/water mixed solvents. The influence of the shielding of $C_2H_5COO^-$ on $-NH_3^+$ group of chitosan should be lower than those of electrostatic charge repulsion of chitosan –NH_3^+ group and solubility of chitosan backbone in propionic acid/water solutions.

CONCLUSION

By investigating the intrinsic viscosity ([η]), hydrodynamic particle size (R_h) distribution, and radius of gyration (R_G) for chitosan in propionic acid/water solutions with various pH values, we showed that the particle sizes of chitosan molecules increased with decreasing pH value. Similar trends were also observed for differential refractive index increment (dn/dC), depolarization ratio (ρ_v), and second virial coefficient (A_2), i.e., dn/dC, ρ_v and A_2 increased with decreasing pH of propionic acid/water solutions. The experimental data suggested that increasing propionic acid concentration in chitosan aqueous solutions caused an increase of –NH_3^+ concentration on chitosan molecules. Increasing –NH_3^+ concentration improved the solubility of chitosan in water and also resulted in an increase of molecular anisotropy due to the increase of intra-molecular electrostatic charge repulsion from –NH_3^+ group.

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