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Intrinsic Viscosity–Molecular Weight Relationship for Chitosan

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ABSTRACT: The intrinsic viscosity–molecular weight relationship for chitosan was determined in 0.25 M acetic acid/0.25M sodium acetate. Chitosan samples with a degree of acetylation (DA) between 20 and 26% were prepared from shrimp-shell chitosan by acid hydrolysis (HCl) and oxidative fragmentation ($NaNO_2$). Absolute molecular weights were measured by light scattering and membrane osmometry. Size exclusion chromatography (SEC) was used to determine average molecular weights (M_n, M_v, and M_w) and polydispersity. The following Mark–Houwink–Sakurada equation (MHS) is proposed for chitosan of M_w in the range of 35–2220 kDa:

$$[\eta] = 1.57 \times 10^{-4}\, M_v^{0.79} = 1.57 \times 10^{-4}\, q_{\mathrm{MHS}}\, M_w^{0.79} = 1.49 \times 10^{-4}\, M_w^{0.79}$$

The value of the MHS exponent a suggests that chitosan behaves as a flexible chain in this solvent. Examination of MHS constants obtained in this work and those available in the literature with other solvents indicates that a and K are inversely related and that they are influenced by DA, and pH and ionic strength of the solvent. J Polym Sci B: Polym Phys 38: 2591–2598, 2000

Keywords: chitosan; Mark–Houwink–Sakurada equation; intrinsic viscosity; molecular weight; polydispersity

INTRODUCTION

The intrinsic viscosity of a polymer solution is related to the polymer molecular weight according to the Mark–Houwink–Sakurada (MHS) equation:[1,2]

$$[\eta] = KM_v^a \qquad (1)$$

where $[\eta]$ is the intrinsic viscosity, M_v the viscosity-average molecular weight, and K and a are constants for given solute-solvent system and temperature. The determination of constants K and a from the intrinsic viscosity data requires either a series of monodisperse polymers of known molecular weight or a series of polydisperse polymer samples with known viscosity-average molecular weights, M_v. In general, M_v, is not experimentally accessible. When polymer fragments are used as samples of varying molecular weight, the constants can be obtained by a statistical method using the number-average molecular weight (M_n) data and a statistical function with an assumption that fragmentation occurs by random scission.[3–5] Alternatively, the constants can be evaluated by a numerical method. The viscosity-average molecular weight can be substituted with the weight-average molecular weight, M_w , and a polydispersity correction factor, q_{MHS}, can be introduced in eq 1, giving a modified MHS equation:[6]

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$$[\eta] = Kq_{\mathrm{MHS}}M_w^a \tag{2}$$

The value of q_{MHS} varies from one sample to another. It is a function of a, M_n, M_w, and M_z and can be evaluated according to:[7]

$$q_{\mathrm{MHS}} = (M_w/M_n)^b(M_z/M_w)^c \tag{3}$$

where M_z is the z-average (diffusion average) molecular weight. The constant c depends only on a, while the constant b depends on a as well as (M_z/M_w):[8]

$$c = 0.113957 - 0.844597\,a + 0.730956\,a^2 \tag{4}$$

$$b = k_1 + k_2\left[\left(\frac{M_z}{M_w}\right) - 1\right]^{k_3} \tag{5}$$

where constants k_1, k_2 and k_3 are least square polynomials functions of a:

$$k_1 = 0.048663 - 0.265996\,a + 0.364119\,a^2 - 0.146682\,a^3 \tag{6}$$

$$k_2 = -0.096601 + 0.181030\,a - 0.084709\,a^2 \tag{7}$$

$$k_3 = -0.252499 + 2.31988\,a - 0.889977\,a^2 \tag{8}$$

The method was tested with success for linear polydisperse polymer samples having different molecular weight distributions.[7,9] Viscometric constants for chitosan have been reported in various solvents.[5,10–18] In most reports, various average-molecular weights (M_n, M_w, and M_z) other than M_v were substituted in eq 1 ignoring polydispersity effects.

The objective of this work was to determine the MHS constants, a and K, for chitosan in a wide range of molecular weight, taking into account the polydispersity of the polymer. The solvent 0.25M acetic acid (HAc)/0.25M sodium acetate (NaAc), was selected because it is a high resolution eluent for chitosan in chromatography.

EXPERIMENTAL

Materials

Two high viscosity shrimp-shell chitosans, with a nominal degree of acetylation (DA) of 20 and 25%, were purchased from Nova-Chem. Ltd (Halifax, Nova Scotia, Canada) and purified. Pullulan standards ($5.88 \leq M_w \leq 1660$ KDa; $1.06 \leq M_w/M_n \leq 1.19$) were purchased from American Polymer Standards Corporation (Mentor, Ohio, USA). Acetic acid (HAc) and sodium acetate (NaAc) were of HPLC grade, sodium chloride (NaCl), and sodium nitrite ($NaNO_2$) were of analytical grade; and all other chemicals were of reagent grade.

Fragmentation

The fragments were prepared from these two original chitosan samples by oxidative fragmentation with $NaNO_2$ or hydrolysis with HCl. Oxidation with $NaNO_2$ was carried out at 23 °C for 4 h with 1.0% chitosan solution in 0.1 M HAc using different $NaNO_2$ concentrations ($1.45 \times 10^{-4} - 3.15 \times 10^{-3}$ M). Acid hydrolysis was performed in a spherical flask equipped with a reflux condenser at 65 °C on 1.0% chitosan solution in 0.5 M HCl for 35 min. In both hydrolysis and oxidative methods, the reaction mixture was subsequently neutralized with 1.0 N NaOH to a pH of 8.0 to precipitate the chitosan. The precipitated chitosan was recovered by centrifugation, washed with deionized water several times, and dried by lyophilization.

Characterization

Structural Analysis and Degree of Acetylation of the Fragments

In order to assess any structural modification occurring during fragmentation, the structure of the original chitosan and a few fragments were determined by elemental analysis and ^{1}H NMR spectroscopy. The elemental composition was determined using a Carlo–Erba 1108 Elemental Analyzer (Model EA 1109, CHN, FISONS Instruments). The degree of acetylation was calculated from the carbon/nitrogen ratio (C/N). The latter varies from 5.145 in completely *N*-deacetylated chitosan ($C_6H_{11}O_4N$ repeat unit) to 6.861 in chitin, the fully *N*-acetylated polymer ($C_8H_{13}O_5N$ repeat unit). The degree of acetylation was therefore calculated according to:

$$DA = \frac{C/N - 5.145}{6.861 - 5.145} \times 100 \tag{9}$$

^{1}H NMR spectra of chitosan solutions in 2%(w/w) CD_3COOD/D_2O were obtained at 70 °C on a NMR

Table I. Degree of Acetylation (DA) of the Original Chitosans and Selected Fragments as Determined by Elemental Analysis (EA) and/or ^{1}H NMR Spectroscopy

	DA (%)	
Chitosan Preparation	EA	NMR
Original (A)	23 ± 5	22 ± 4
Original (B)	26 ± 5	26 ± 4
Fragment, hydrolysis (F2)	—	21 ± 4
Fragment, oxidative (F3)	—	22 ± 4
Fragment, oxidative (F6)	26 ± 5	—

spectrometer (Model AC-300 MHz spectrometer Bruker, Billerica, MA). The experimental conditions were essentially the same as described by Hirai et al.[19] except for the polymer concentration (2.5 mg/mL for the original chitosan), the pulse repetition delay (6s), the use of a single pulse sequence (40°) and the number of scans (16 and 64 for fragments and the original chitosan, respectively). The degree of acetylation was determined using the following relation:

$$DA = \frac{\frac{1}{3}(\text{surface area at 2.1 ppm})}{\text{surface area at 3.2 ppm}} \times 100 \quad (10)$$

where the resonances at 2.1 and 3.2 ppm correspond to methyl protons and the main-chain proton closer to the *N*-acetyl group, respectively. The values of DA determined by ^{1}H NMR and elemental analysis, EA, are presented in Table I. The results indicate that the chemical structure was not altered significantly by the fragmentation processes.

Viscometry

Intrinsic viscosities in 0.25 M HAc/0.25 M NaAc were measured using an automated capillary viscometer (Model AMV-200, Paar Physica USA Inc., Edison, N.J.). The capillary diameter was 0.9 mm, and the inclination angle was 15°. These conditions, along with the use of solution concentrations lower than 1% (w/V), were selected so that corrections for kinetic energy and shear were negligible. The intrinsic viscosity was determined by both Huggins and Kraemer plots.

Light Scattering

The weight-average molecular weight (M_w) of five chitosan samples was determined by light scattering in 0.25 M HAc/0.25 M NaAc using a laser light-scattering photometer (Dawn-Model F, Wyatt Technology, Santa Barbara, CA). The measurements were performed at room temperature using a 5 mW He-Ne linearly polarized laser (wavelength 632.8 nm). Pure solvents of known Rayleigh ratio were used to calibrate the instrument. The intensity of scattered light was measured at different angles (θ) from 21 to 160°. Zimm or Berry plots were constructed by plotting the values of (KC/ Rθ) versus [$\sin^2(\theta/2)+$ AC], where Rθ is the excess Rayleigh ratio, K the optical constant containing the refractive index increment (dn/dC), and A is constant. The average-molecular weight was determined by double extrapolation to zero concentration and zero angle. The refractive index increment (dn/dC) of chitosan in 0.25 M HAc/0.25 M NaAc at 632.8 nm was determined using a differential refractometer (Model RF-500, CN. Coop, Newtown, PA). A value of 0.158 mL · g^{-1} was obtained, in good agreement with literature values.[20,21]

Membrane Osmometry

The number-average molecular weight (M_n) of three chitosans was determined by membrane osmometry (Model, 090-B, Gonotec GmbH) using a regenerated cellulose acetate membrane with limits of 5 and 1000 kDa in 0.1 HAc/0.02 NaCl at 25 °C. The plot of reduced osmotic pressure (π/C) versus concentration yielded a straight line whose intercept provided RT/ M_n according to:[1]

$$\frac{\pi}{C} = RT\left(\frac{1}{M_n} + A_2C\right) \quad (11)$$

where R is the universal gas constant, T, the absolute temperature and A_2 is the second virial coefficient.

Size Exclusion Chromatography

Size exclusion chromatography (SEC) was used to determine average-molecular weights (M_n, M_v, M_w, and M_z) and polydispersity parameters (M_w/M_n, and M_z/M_w). The chromatograms were obtained using a HPLC/SEC (Hewlett-Packard, Model 1050) equipped with a refractive index detector. Separation was performed at 35 °C using a Toso Haas-TSK gel column (GMPW$_{XL}$, 30 cm × 7.8 mm) with a flow rate of 0.4 mL · min^{-1}. The appropriate mobile phase was selected among three solvents; 0.1 M HAc (pH = 2.9); 0.1 M

HAc/0.1 M NaAc (pH = 4.7); and 0.25 M HAc/0.25 M NaAc (pH = 4.7). A comparison showed that the resolution was improved with an increase in ionic strength of the solvent, yielding to the selection of 0.25 M HAc/0.25 M NaAc as eluting solvent. The intrinsic viscosity and SEC chromatograms of well-characterized monodisperse pullulan samples were measured in order to construct a universal calibration curve of the hydrodynamic volume of the polymer as a function of the elution volume; V_e:

$$[\eta]M = f(V_e) \tag{12}$$

The same curve was used for chitosan samples. The basic assumption of universal calibration is that polymers of identical hydrodynamic volumes are detected at identical elution volumes:

$$[\eta]_i M_i = [\eta]_1 M_1 = f(V_e) \tag{13}$$

where 1 and i refer to pullulan and chitosan, respectively. Substitution of the MHS relationship yields:

$$K M_i^{a+1} = f(V_e) \tag{14}$$

where K and a are the viscometric constants for chitosan in the eluant. The preceding equation can be rearranged into:

$$\log M_i = \frac{\log f(V_e) - \log K}{a + 1} \tag{15}$$

which shows that molecular weight determination by SEC requires a prior knowledge of K and a. However, determination of the latter is precisely the objective of the molecular weight characterization. The following procedure was followed to determine the viscometric constants:

1. A preliminary estimate of K and a was obtained from a double-logarithm plot of intrinsic viscosity versus M_w measured by light scattering.
2. The number, weight, and viscosity average-molecular weights of chitosan samples were determined from SEC chromatograms using equations A2, A3 and A4 (appendix) and the preliminary estimate of K and a.
3. Final values of the viscometric constants were extracted from a log-log plot of intrinsic viscosity as a function of the viscosity-average molecular weight determined in step 2.

Polydispersity Correction Factor, q_{MHS}

As in the case of the determination of M_v by SEC, the calculation of q_{MHS} requires prior knowledge of the viscometric constant a. In order to circumvent the difficulty, an iterative procedure was used. An initial value of q_{MHS} was computed for each sample, assuming a is equal to unity. To determine q_{MHS}, the number, weight, and z-average molecular weights of chitosan samples were determined from SEC chromatograms using equations A2, A3, and A5 (appendix), and substituted in eq 3. Values of ($\log [\eta] - \log q_{MHS}$) plotted against $\log M_w$ (as obtained from SEC) yielded a straight line whose slope provided a new value of a. The latter value was used to calculate the new value of q_{MHS}. The procedure was repeated until two successive values for a differed by less than 0.001. The final values of the slope and intercept of the linear ($\log [\eta] - \log q_{MHS}$) versus $\log M_w$ plot provided a and $\log K$, respectively.

RESULTS AND DISCUSSION

Figure 1(A) shows $\log [\eta]$ versus $\log M_w$, where M_w values were determined by light scattering. The values of 0.776 and 1.81×10^{-4} were obtained as preliminary estimates of a and K, respectively. The average-molecular weights (M_n, M_v, and M_w) of chitosans were then calculated from chromatograms of chitosan using eq 10, and the preliminary estimate of the of a and K values. The resulting average-molecular weights M_n, M_v, and M_w, are given in Table II. The difference between SEC averages and M_n or M_w measured independently by membrane osmometry and light scattering are within the experimental uncertainty, indicating that the method used should give reliable values of M_v. Figure 1(B) shows $\log [\eta]$ versus $\log M_v$. Values of 0.794 and 1.567×10^{-4} are respectively obtained for a and K from the slope and intercept. The polydispersity correction factor method was also used to calculate the viscometric constants. The value of q_{MHS} for chitosan was found to be 0.95. Figure 2 shows the final plot of ($\log [\eta] - \log q_{MHS}$) versus $\log M_w$, obtained upon convergence of the iterative procedure (see Experimental section). Values of 0.794 and 1.565×10^{-4} dL $\cdot$ g^{-1} were obtained for a and

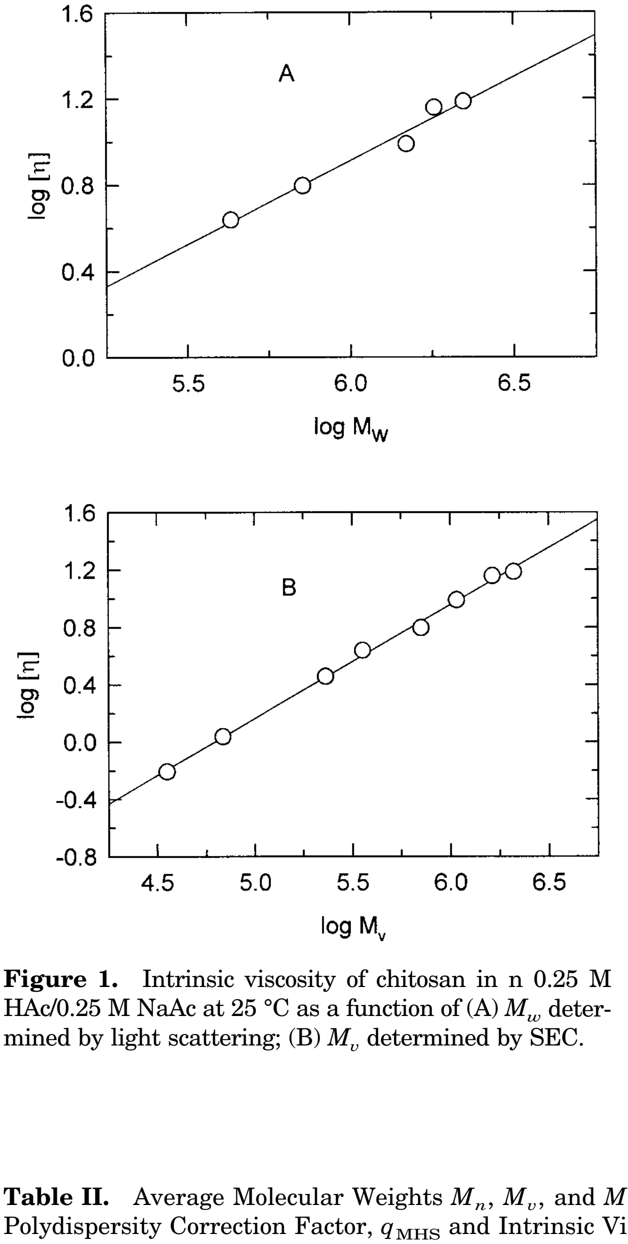

Figure 1. Intrinsic viscosity of chitosan in n 0.25 M HAc/0.25 M NaAc at 25 °C as a function of (A) M_w determined by light scattering; (B) M_v determined by SEC.

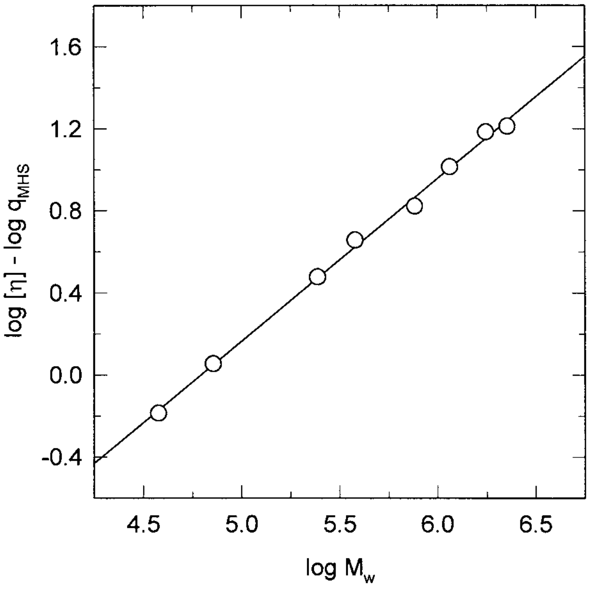

Figure 2. (Log [η] − log q_{MHS}) as a function of log M_w determined by SEC in 0.25 M HAc/0.25 M NaAc at 25 °C.

K, respectively, in excellent agreement with the above results. In summary, the following MHS equation is proposed for chitosan ($q_{\mathrm{MSH}} = 0.95$) in the M_w range of 35–2200 kDa:

$$[\eta] = 1.57 \times 10^{-4}\, M_v^{0.79} = 1.57 \times 10^{-4}\, q_{\mathrm{MHS}} M_w^{0.79} \tag{16}$$

Table III compares the values of MHS equation constants, *a* and *K*, obtained in this work with those published in the literature. The values for *a* range from 0.58 to 1.26 in various solvents, com-

Table II. Average Molecular Weights M_n, M_v, and M_w, Polydispersity Parameters, M_w/M_n, M_z/M_w, Polydispersity Correction Factor, q_{MHS} and Intrinsic Viscosity ([η]) of Chitosan Samples in 0.25*M* HAc/0.25*M* NaAc at 25 °C

Chitosan Preparation	M_w[a] (kDa)	M_n[b] (kDa)	M_n[c] (kDa)	M_v[c] (kDa)	M_w[c] (kDa)	(M_w/M_n)[c]	(M_z/M_w)[c]	q_{MHS}	[η] (dL/g)
Original (A)	2220	—	1034	2105	2259	2.19	1.73	0.941	15.33
Original (B)	1810	—	820	1636	1755	2.14	1.74	0.941	14.37
Fragment, oxidative (F1)	1490	—	558	1076	1151	2.06	1.69	0.944	9.76
Fragment hydrolysis (F2)	715	—	356	711	763	2.14	1.73	0.941	6.25
Fragment, oxidative (F3)	429	—	213	358	379	1.77	1.53	0.955	4.34
Fragment, oxidative (F4)	—	114	137	231	244	1.78	1.59	0.951	2.86
Fragment, oxidative (F5)	—	40	42	68	72	1.71	1.43	0.962	1.09
Fragment, oxidative (F6)	—	14	19	36	38	2.0	1.53	0.951	0.62

[a] Light scattering.
[b] Membrane osmometry.
[c] Six exclusion chromatography with pullulan standards.

Table III. MHS Equation Constants for Chitosans with Varying of DA and Solvents of Different pH and Ionic Strength, μ

Solvent	T (°C)	DA (%)	pH	μ (M)	$K \times 10^5$ ($dL \cdot g^{-1}$)	a	Molecular Weight Range (kDa)	Reference[a]
0.02*M* HAc/NaAc/0.1*M* NaCl	20	0	4.5	0.1	559.0	0.58	15–310	Anthonsen et al., 1993[1]
0.5*M* HAc/0.5*M* NaAc	25	29.5	4.7	0.5	199.0	0.59	115–1590	Yomota et al., 1993[2]
0.3*M* HAc/0.2*M* NaAc	25	2	4.6	0.2	82.0	0.76	100–600	Rinaudo et al., 1993[3]
0.3*M* HAc 0.2*M* NaAc	25	10.5	4.6	0.2	76.0	0.76	100–600	Rinaudo et al., 1993[4]
0.3*M* HAc/0.2*M* NaAc	25	21	4.6	0.2	74.0	0.76	100–600	Rinaudo et al., 1993[5]
0.02*M* HAc/NaAc/0.1*M* NaCl	20	15	4.5	0.1	58.5	0.78	35–245	Anthonesn, et al., 1993[6]
0.25*M* HAc/0.25*M* NaAc	25	21–26	4.7	0.25	15.7	0.79	35–2220	This work[7]
0.2*M* HAc/0.1*M* NaAc	30	0	4.4	0.1	16.8	0.81	194–937	Wange et al., 1991[8]
2% HAc/0.2*M* NaAc	25	15 ± 3	4.5	0.2	13.8	0.85	61–150	Gamzazade et al., 1985[9]
0.2*M* HAc/0.1*M* NaAc	30	9	4.4	0.1	6.59	0.88	211–1260	Wange et al., 1991[10]
0.1*M* HAc/0.2*M* NaCl	25	≈ 20	2.8	0.2	1.81	0.93	48–630	Roberts et al., 1982[11]
0.2*M* HAc 0.1*M* NaAc	30	16	4.4	0.1	1.42	0.96	536–1850	Wange et al., 1991[12]
0.33*M* HAc/0.3*M* NaCl	21	20–22	4.7	0.3	3.41	1.02	13–193	Podogina et al., 1986[13]
0.02*M* HAc/NaAc/0.1*M* NaCl	20	60	4.5	0.1	2.18	1.06	15–164	Podogina et al., 1986[13]
0.2*M* HAc/0.1*M* NaAc	30	31	4.4	0.1	0.104	1.12	477–2510	Wange et al., 1991[15]
0.1*M* HAc/0.02 NaCl	25	≈ 20	2.9	0.02	0.0304	1.26	48–630	Roberts et al., 1982[16]
0.2*M* HAc/0.1*M* NaCl/4*M* urea	20	9	2.6	0.1	89.3	0.71	163–492	Lee, 1974
1% HAc	30		2.8	0.01	4.74	0.72	205–657	Rao, 1993
HAc/0.2*M* NaAc	25	58	4.3	0.2		1.14	4.3–64.1	Errington et al., 1993

[a] The number in superscript refers to the point in Figure 3.

pared to 0.79 obtained in 0. 25 M HAc/0.25 M NaAc. Similarly, the values for *K* ranges from 2.0×10^{-3} to 3.0×10^{-7} compared to our value in the order of 10^{-4}. Chitosan being a cationic polyelectrolyte with a pK_a value of about 6.2–6.7,[22] the polymer conformation and the polymer–solvent interactions depend on the number of positive charges (NH_3^+) on chitosan. The latter, in turn, depends on DA, the pH and the ionic strength (μ) of the solvent. The number of positive charges increases when DA decreases. High value of degree of acetylation results in a rigid conformation, leading to a higher degree of expansion of chitosan. The number of positive charges on chitosan will be higher at low pH of the solvent, leading to a higher degree of expansion of chitosan due to electrostatic repulsions. Low ionic strength of the solvent may not completely neutralize the fixed positive charges on chitosan either through specific binding or through nonspecific attraction of the Debye–Hückel type, thus resulting in higher degree of expansion of chitosan. On the whole, the exponent *a* should roughly increase with an empirical function, [DA/(pH. μ)]. Figure 3 shows the reported values of *a* as a function of the latter parameter; a parabolic relationship was observed. A plot of *K* as a function of [DA/(pH. μ)] also shows a good correlation, but *K* decreases as [DA/(pH. μ)] increases. The MHS constants appear to

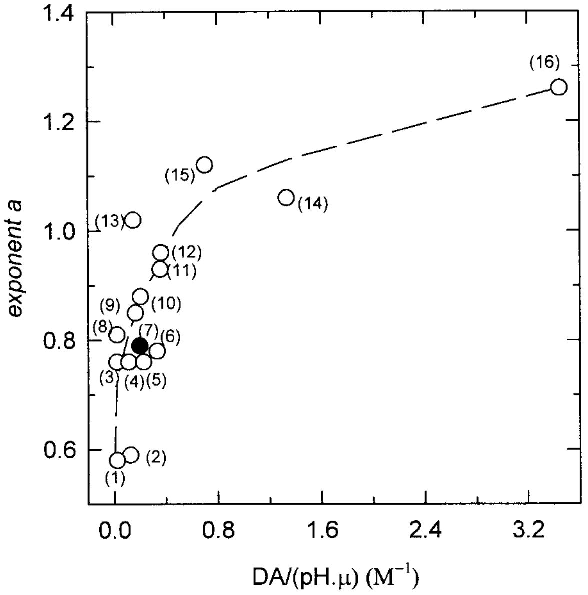

Figure 3. Exponent *a* as a function of the [DA/(pH. μ)]. Open symbols represent literature results and filled symbol refers to this work.

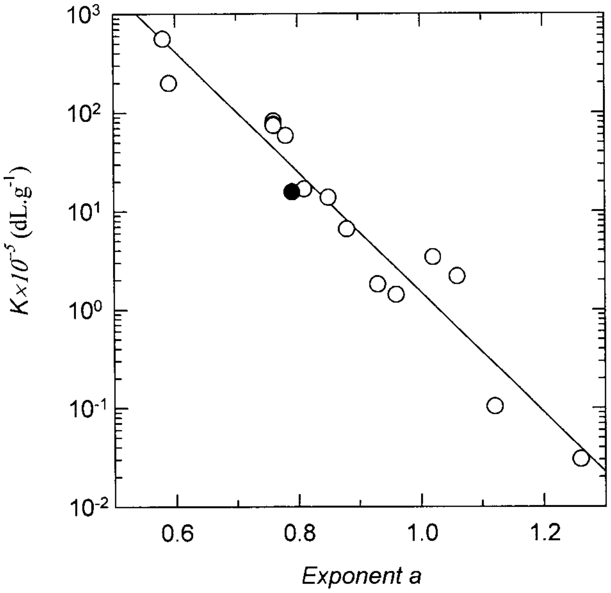

Figure 4. The value of K versus exponent a. Open symbols represent literature results and filled symbol refers to this work.

be inversely related as shown in Figure 4, in line with earlier observations on other polymers. For instance, Rai and Rosen[23] observed a linear relation between log K and a for eight synthetic flexible polymers with molecular weight above 10 kDa. The authors reported slopes between −3.6 and −4.9, depending on the polymer. Simha[24] demonstrated that an exponential relation between K and a has theoretical bases. Our observations also show that log K depends linearly on a. The slope deduced from Figure 4 (−6.1 ± 0.4) is significantly higher, suggesting that the relation between K and a depends not only on the polymer, as already reported, but also, in the case of a polyelectrolyte such as chitosan, on the pH and ionic strength of the solvent.

CONCLUSIONS

The viscometric constants, a and K, for polydisperse chitosan samples with DA between 20 and 26% were determined using Mark–Houwink–Sakurada equation (MHS) and an empirically modified MHS equation in the M_w range of 35–2220 kDa at 25 °C. The following MHS equations for chitosan (q_{MHS} = 0.95) in 0.25 M HAc/0.25 M NaAc are proposed:

$$[\eta] = 1.57 \times 10^{-4}\, M_v^{0.79} = 1.57 \times 10^{-4}\, q_{\mathrm{MHS}} M_w^{0.79} = 1.49 \times 10^{-4}\, M_w^{0.79}$$

The value of a (0.79) suggests that the conformation of chitosan in this solvent is that of a flexible chain. Examination of a and K values of chitosan having different DA in various solvents indicates that a and K are inversely related and depend on the function, [DA/(pH · μ)].

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APPENDIX

Calculation of Molecular Weights

The calculation procedure of average-molecular weights was as follows: The height of chromatogram of each fraction, h_i, is proportional to its weight fraction, w_i, which is proportional to the product of number of molecules, n_i , and its molecular weight, M_i according to:

$$h_i = k \cdot w_i = k \cdot n_1 \cdot M_1 \tag{A1}$$

where k is a constant. Practically, each chromatogram was divided into at least 500 equal segments of molecular weight and the corresponding heights were determined. The average molecular weights ($\overline{M_n}$, $\overline{M_v}$, $\overline{M_w}$, and $\overline{M_z}$) were calculated as follows:

$$\overline{M_n} = \frac{\sum_{i=1}^{n} w_i}{\sum_{i=1}^{n} n_i} = \frac{\sum_{i=1}^{n} n_i M_i}{\sum_{i=1}^{n} n_i} = \frac{\sum_{i=1}^{n} h_i}{\sum_{i=1}^{n} \left(\frac{h_i}{M_i}\right)} \tag{A2}$$

$$\overline{M_w} = \frac{\sum_{i=1}^{n} w_i M_i}{\sum_{i=1}^{n} w_i} = \frac{\sum_{i=1}^{n} n_i M_i^2}{\sum_{i=1}^{n} n_i M_i} = \frac{\sum_{i=1}^{n} h_i M_i}{\sum_{i=1}^{n} h_i} \tag{A3}$$

$$\overline{M_v} = \left(\frac{\sum_{i=1}^{n} w_i M_i^a}{\sum_{i=1}^{n} w_i} \right)^{1/a} = \left(\frac{\sum_{i=1}^{n} n_i M_i^{1+a}}{\sum_{i=1}^{n} n_i M_i} \right)^{1/a} = \left(\frac{\sum_{i=1}^{n} h_i M_i^a}{\sum_{i=1}^{n} h_i} \right)^{1/a} \tag{A4}$$

$$\overline{M_z} = \frac{\sum_{i=1}^{n} w_i M_i^2}{\sum_{i=1}^{n} w_i M_i} = \frac{\sum_{i=1}^{n} n_i M_i^3}{\sum_{i=1}^{n} n_i M_i^2} = \frac{\sum_{i=1}^{n} h_i M_i^2}{\sum_{i=1}^{n} h_i M_i} \tag{A5}$$

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