Molecular Weight Determination of 83% Degree of Decetylation Chitosan with Non-Gaussian and Wide Range Distribution by High-Performance Size Exclusion Chromatography and Capillary Viscometry

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ABSTRACT: Molecular weight determination of 83% degree of deacetylation (DD) chitosan with non-Gaussian and broad molecular weight distribution by high-performance size exclusion chromatography (HPSEC) and by capillary viscometry were proposed. The relationships between weight average retention volumes (RVw) of HPSEC and intrinsic viscosities ([η]) measured by capillary viscometer and the weight average molecular weight (Mw) measured by static light scattering were established for routine molecular weight determination of chitosans either by HPSEC or by the capillary viscometry method, respectively. These results showed: relationships of RVw and Mw for different Mw of 83.0% DD chitosans can be expressed by the equation Log Mw $= -0.433$ RVw $+ 11.66$. The RVw of other DD chitosans do not correlate well with this equation. It indicated that DD of chitosan affected the relationship of RVw and Mw of chitosans studied. The Mark–Houwink constant a decreased from 0.715 to 0.521, as the solution ionic strength increased from 0.01M to 0.30M, whereas constant k increased from 5.48×10^{-4} to 2.04×10^{-3} over the same range of ionic strength solutions. The established RVw and Mw equation and [η] and Mw equation (Mark–Houwink equation) can be routinely used to determine the molecular weight from RVw or [η] of chitosan by HPSEC or by capillary viscometer, respectively, without the need of expensive instrumentation. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 1905–1913, 1999

Key words: chitosan; weight average molecular weight; retention volume; Mark–Houwink viscometric constants; HPSEC

INTRODUCTION

Chitosan [(1-4)-2-amino-2-deoxy-*b*-D-glucan] is a versatile, environmentally friendly biopolymer. It can be applied in food processing, agriculture, biomedicine, biochemistry, wastewater treatment, membranes, and microcapsule applications.[1–11] The molecular weight and its distribution affect physical and chemical properties of polysaccharides,[12] such as the rheological properties of chitosan,[11,13] and mechanical properties and pore size of membranes and microcapsules of chitosan.[2,5,6,14] Therefore, molecular weight determination is very important for elucidating the characteristics of the chitosan itself and the products made from it. Gel forming, osmotic pressure modification, viscosity enhancing, or fiber formation also depend on molecular weight and its distribution and have been use in food industrial, pharmaceutical, and medical applications.[12]

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Harding and colleagues[12] pointed out that it is difficult to determine the molecular weight of polysaccharides accurately due to a wide range of molecular weight distribution, thermodynamic deviations from ideal conditions, structural diversity, and strong intermolecular interactions.

Static light scattering is an absolute method to determine the molecular weight of matter. It is an ideal method because it does not need a standard compound (marker) for molecular weight determination.[15,16] Wang and colleagues,[13] Muzzarelli and colleagues,[17] Terbojevich and colleagues,[18] and Yomota and colleagues[19] have successfully determined the weight average molecular weight (Mw) of chitosan by static light scattering. However, contamination from dust or the propensity of some polymers to aggregate may interfere with the determination.[12,20,21] If the sample to be determined is a polyelectrolyte, the Zimm plot obtained becomes irregularly shaped, and the molecular weight determination may not be accurate[15,21]; besides, sample concentration and purity have to be carefully controlled.[21] Therefore, the technique of light scattering is difficult and complex; furthermore, the required instrumentation is expensive. It is far from being an ideal or fast method for molecular weight determination.

High-performance size exclusion chromatography (HPSEC) has been applied in molecular weight determination of chitosan. However, it needs markers to determine the calibration curve of the retention volume. If the markers used are not chitosan, differences in structure and thermodynamic properties will cause errors. Furthermore, chitosan markers are not commercially available. Yomota and colleagues[19] reported using different molecular weight pullulans as markers and applied universal calibration to eliminate the deviation. However, this calibration method is too troublesome. It is easier to use chitosan as marker. However, the Mw of those chitosans should be determined by an absolute method beforehand. Terbojevich and colleagues[22] established calibration curve by the integral-MWD method and used broad molecular weight distribution chitosan as marker. But, the integral-MWD method only suitable for those polymers their molecular weight distribution are Flory distribution ($Mw/Mn = 2$).[23] It is a perfect method if only the calibration curve of HPSEC can be established by chitosan standards.

The viscosity method is the simplest, fastest way to determine the molecular weight of a polymer.[13] After determining the intrinsic viscosity of a polymer and applying it to the Mark–Houwink equation, the viscosity average molecular weight (Mv) can be obtained directly. The Mark–Houwink equation is as follows[12,16,24–27]:

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

The parameters a and k are empirical constants and depend on the solvent system (ionic strength and pH), temperature, and molecular weight etc.[12,24,27] To determine a and k, a series of markers, their weight average molecular weight (Mw) or number average molecular weight (Mn) were determined and used. If the molecular weight distribution of a polymer was smaller than 3 and its Mv only smaller than Mw by a few percent, then Mw can be used directly to determine k and a.[16,28] Even when a similar solvent system, temperature, and degree of deacetylation (DD) of chitosan were employed, a and k values reported differed significantly.[13,23,25,28–33] Therefore, systematic and accurate a and k values are badly needed.

In this study, we used static light scattering to measure the Mw of a series of chitosans of different molecular weight and DD, and chitosans of the same DD but different molecular weight. The above chitosans were used as markers to determine the weight average retention volume (RVw) by HPSEC and intrinsic viscosity ([η]) by capillary viscometer. Plots of Mw and RVw, and Mw and [η] were made. The relationship between Mw and RVw, and Mw and [η] were established. These equations can be used to determine molecular weights of chitosans more easily and faster, and no expensive instrumentation is required.

MATERIALS AND METHODS

Preparation of Chitosan

Chitin was prepared by the method of Chen and colleagues[1] from shrimp (*Solemocera prominenitis*) waste. Chitosans of different degrees of deacetylation and different molecular weights were prepared by alkali deacetylation with 50% NaOH at 100° and 140°C for 1–12 h, at the ratio of chitin to 50% NaOH of 1 : 20.[11]

Same DD, Different Molecular Weight Chitosans

Chitosans of the same 83% degree of deacetylation (DD), but different molecular weights were obtained by ultrasonic degradation.[13] One per-

cent chitosan was dissolved in an acetic acid aqueous solution (5%, v/v) and then ultrasonically degraded at 400 Watts (CREST, 950E, USA) for various times (0–60 h) at 80°C. After degradation, the chitosans were precipitated with a 2*N* NaOH solution and washed with water, then lyophilized (Virtis, UNITOP 800L, USA).

Determination of DD

Infrared spectrometry was used to determine the degree of deacetylation of the chitosans.[34] Chitosan powder was mixed with KBr (1 : 100) and pressed into a pellet. The absorbances of amide 1 (1,655 cm^{-1}) and the hydroxyl band (3450 cm^{-1}) were measured using an Bio-Rad FTS-155 infrared spectrophotometer. The band of the hydroxyl group at 3,450 cm^{-1} was used as an internal standard to correct for disc thickness and differences in chitosan concentration in making the KBr disc. The percentage of the amine group's acetylation in a sample is given by $(A_{1655}/A_{3450}) \times 115$. Herein, A_{1655} and A_{3450} are the absorbances at 1,650 cm^{-1} and 3,450 cm^{-1}, respectively.

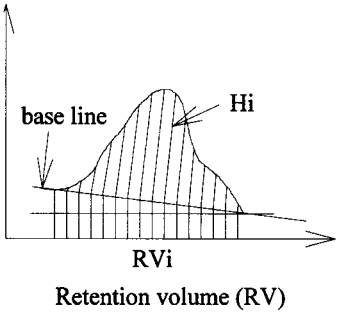
Determination of *Mw*

The static light scattering method was used to measure the *Mw* of these chitosans.[13,17,18,20] Different concentrations (0.001–0.01 g L^{-1}) of chitosan in 0.01*N* HCl solutions were prepared. Adjust solution ionic strength to 0.01, 0.05, 0.10, and 0.20*M* by adding different NaCl concentration. The solvents and solutions were filtered through 0.02 μm (Whatman, Anotop 25, USA) and 0.45 μm (Lida, USA) filters, respectively. The scattered light intensity of the solutions between 30° and 140° was measured by a Malvern light scattering photometer (Malvern 4700, UK) at 632.8 nm and 30 ± 0.1°C. The *Mw* was calculated from the Zimm plot processed by Malvern software (version 1.26 for Windows). Every sample measurement was repeated five times. Refractive index increments (dn/dc) of chitosan solutions equaled 0.189 mL g^{-1} and were determined by an interferometric refractometer (Wyatt/Optilab 903, Santa Barbara, CA).

Determination of RV*w*

The HPSEC method was used to measure the retention volume of the chitosans. The TSK gel columns in series of G4000 PWXL and G5000 PWXL (Tosoh Co., 7.8 × 300 mm, Japan) were used. Their analysis molecular weight range were 1–700 kDa and 50–700 kDa for dextran, respectively. The mobile phase consisted of 0.2*M* CH_3COOH/0.1*M* CH_3COONa, and 0.008*M* NaN_3 as antimycotics. Sample concentrations of 1 g L^{-1} were loaded and eluted with a flow rate of 0.6 mL min^{-1} by an LDC Analytical ConstaMetric 3500 pump. The elute peak was detected by an RI detector (Gilson, model M132, USA). Because the elution profile was not Gaussian distribution, the RV*w* was used to alleviate the error caused by using retention volume obtained from a asymmetric distribution. The RV*w* was calculated as follows:

$$RVw = \frac{\sum Hi \times RVi}{\sum Hi}$$



Herein, Hi and RVi are height of elute peak and retention volume of the *i*th fraction, respectively. The calibration curve obtained can be used to establish the relationship of Log *Mw* and RV*w* of chitosans. Data were then analyzed by ChemLab software (Scientific Information Service Corporation, version 1.0 for Win 95, Taiwan).

Determination of Intrinsic Viscosity and Mark–Houwink Constants

A Cannon–Fenske capillary viscometer was used to measure the passage time of solutions flowing through the capillary. Chitosans were dissolved in two solvent systems. System 1 was 0.01*M* and 0.30*M* HCl, 0.01*M* HCl and various concentrations of NaCl to adjust ionic strength to 0.01, 0.05, 0.10, and 0.20. System 2 was composed of different concentrations of acetic acid and sodium acetate buffer. These solutions were cleared through a 0.45 μm filter (Lida, USA). The capillary viscometer was filled with 5 mL of sample and equilibrated in a water bath (Tamson TMV-40, Holland) at 30 ± 0.1°C for 15 min. The sample was passed through the capillary once before the running time was measured. Running time was used to calculate the relative viscosity, then the reduced viscosity. The reduced viscosity was plotted against the concentration, with the intercept being the intrinsic viscosity.[27]

Log [η] was plotted against log Mw to obtain the Mark–Houwink constants a and k. The slope is the exponent a; the antilogarithmic value of the intercept is the constant k.[12,16,24–27]

RESULTS AND DISCUSSION

The Reduced Viscosities of Chitosan

Figure 1 showed reduced viscosities of different concentrations of 83.0% DD chitosans in different concentrations of acetic acid and sodium acetate buffer at 30 ± 0.1°C. The lines for chitosan in 0.05–0.50*M* acetic acid (curves a–e) are curvature because of electroviscous effect. At the right-hand side of the peak, the reduced viscosities decreased, whereas at the left-hand side of the peak, the reduced viscosities increased with increasing chitosan concentration due to the third and the second electroviscous effects, respectively.[11,27,35–38] Adding sodium acetate in chitosan–0.20*M* acetic acid solutions, the curvature line transformed into linear because the electroviscous effect was repressed. The higher the concentration of sodium acetate added, the stronger the suppression effect resulted.

Figure 2 showed the reduced viscosities of different concentrations of 83.0% DD chitosan in HCl/NaCl solutions of different isoionic strength (0.01–0.20*M*) solution. The electroviscous effect shown in Figure 1 (curves a–e) was suppressed. The reduced viscosities also decreased because the chitosan molecules become contracted as ionic strength increasing.

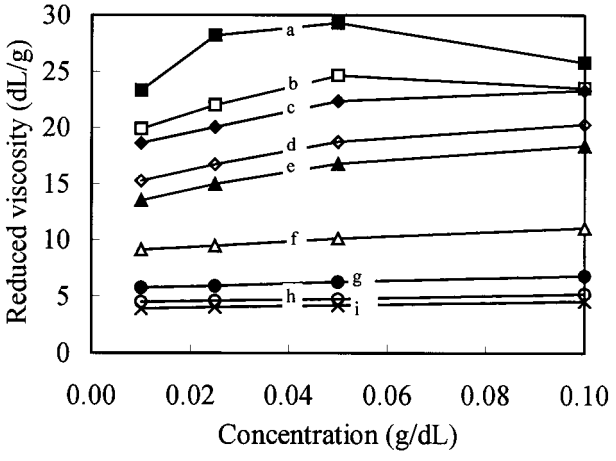


Figure 1 Reduced viscosity of different concentration chitosans (83.0% DD, 914 kDa) in different acetic acid and sodium acetate solutions at 30 ± 0.1°C. (a) 0.05*M* HAc, (b) 0.10*M* HAc, (c) 0.20*M* HAc, (d) 0.30*M* HAc, (e) 0.50*M* HAc, (f) 0.20*M* HAc/0.01*M* NaAc, (g) 0.20*M* HAc/0.05*M* NaAc, (h) 0.20*M* HAc/0.10*M* NaAc, (i) 0.20*M* HAc/0.20*M* NaAc.

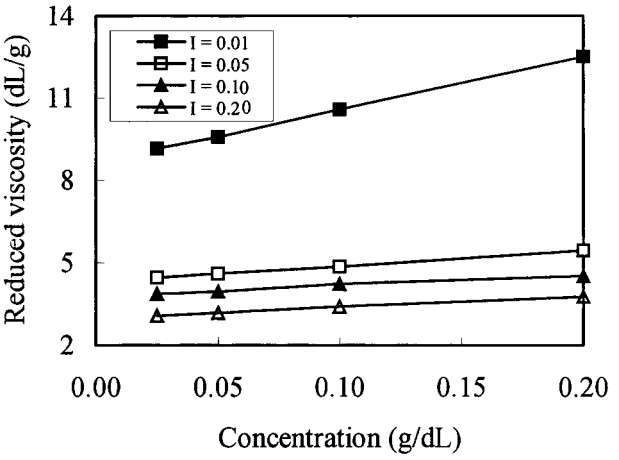


Figure 2 Reduced viscosity of different concentration of chitosans (83.0% DD, 914 kDa) in different ionic strength solutions at 30 ± 0.1°C. I = 0.01 (0.01*M* HCl); I = 0.05 (0.01*M* HCl/0.04*M* NaCl); I = 0.10 (0.01*M* HCl/0.09*M* NaCl); I = 0.20 (0.01*M* HCl/0.19*M* NaCl).

The DDs and *Mw*s of Chitosans Used

The DDs and Mws of chitosans used are listed in Table I; DDs of chitosans used ranged between 66.8% and 90.0%. Mws of those chitosans ranged between 199 and 2,788 kDa determined by static light scattering in 0.01*M* solvent of HCl/0.19*M* NaCl. DDs of chitosans after different ultrasonic treatments did not change significantly and were 83% ± 1%. However, Mws decreased from 914 kDa to 78 kDa.

The Mw range of these 15 chitosans used in this article were 78–2,788 kDa determined by static light scattering. Molecular weight ranged from 78 to 2,788 kDa, and ranged from 78 to 914 kDa were used to determine RVws and intrinsic viscosities by HPSEC, and by viscosity method, respectively. Molecular weights of chitosan used in molecular weight determinations reported in the literature were smaller than those used in this article. The widest molecular weight range used for SEC on line with multiple-angle laser light scattering (SEC-MALLS) or SEC on line with low-angle laser light scattering (SEC-LALLS) was reported by Ottøy and colleagues[28]; their molecular weights ranged between 50–1,778 kDa. The widest molecular weight range used for SEC only was reported by Yomota and colleagues[19]; their molecular weights ranged between 117 to 1,424 kDa. The widest molecular

Table I RV*w* and Intrinsic Viscosity of Six Different DD and Different Molecular Weight (*Mw*) Chitosans and 10 Different *Mw*s, But with the Same (83%) DD of Chitosans

DD (%)	*Mw* (kDa)	RV*w* (mL)	Intrinsic Viscosity (dL g^{-1})				
			$I = 0.01$[a]	$I = 0.05$	$I = 0.10$	$I = 0.20$	$I = 0.30$
66.8	2,788	11.568					
74.3	1,376	12.557					
79.4	1,078	12.693					
83.0	914	13.173	8.642	4.314	3.785	2.975	2.411
82.7	680	13.492	7.511	3.809	3.272	2.521	2.198
83.3	481	13.718	6.306	3.286	2.924	2.273	1.802
82.5	362	14.041	5.480	2.810	2.524	1.960	1.663
83.1	322	14.272	5.238	2.651	2.387	1.871	1.612
82.7	280	14.377	4.829	2.424	2.231	1.809	1.529
82.3	223	14.612	4.260	2.236	1.984	1.622	1.351
83.0	148	15.002	2.626	1.547	1.416	1.101	0.982
83.2	120	15.155	2.335	1.345	1.216	0.951	0.879
82.8	78	15.624	1.449	0.942	0.862	0.741	0.677
86.7	1,005	13.411					
90.0	199	15.084					

DD, *Mw*, RV*w*, and intrinsic viscosities were determined by infrared spectroscopy, static light scattering, HPSEC, and Cannon–Fenske capillary viscometer, respectively.

[a] Solvent systems: $I = 0.01$ (0.01*M* HCl); $I = 0.05$ (0.01*M* HCl/0.04*M* NaCl); $I = 0.10$ (0.01*M* HCl/0.09*M* NaCl); $I = 0.20$ (0.01*M* HCl/0.19*M* NaCl); $I = 0.30$ (0.30*M* HCl).

weight range used for light scattering only was reported by Terbojevich and colleagues[18]; their molecular weights ranged between 10 to 1,000 kDa. Those used for the viscosity method reported by Wang and colleagues[13] have similar molecular weight ranges (211–1,260 kDa) as in this article, and they were the widest molecular weight range that was found in the literature. Overall, the widest molecular weight range was 2 orders in the literature and was smaller than 3 orders in this article.

The choice of solvent is very important and crucial to successfully determine the polymer molecular weight by light scattering. If the solvent used is not a good solvent, the polymer is prone to aggregate easily and the molecular weight will be overestimated.[15] Because chitosan is a cationic polyelectrolyte, some small molecular weight electrolyte should be added to the solution to alleviate the polyelectrolyte effect. Figure 3(a) showed KC/R values were independent of the indent angle (arrow indicated) and implied the loss of the colligative property due to the polyelectrolyte effect. A small molecular weight electrolyte, such as NaCl, was added to the solvent to increase the ionic strength to 0.20*M*. Then, the Zimm plot is changed as shown in Figure 3(b). Kratochvil[15] reported that the concentration of the salt added should be higher than 0.10*M*. In this article, the concentration of NaCl was 0.19*M*. Results in Figure 3(b) show the polyelectrolyte effect was supressed effectively. Salt concentration of 0.12*M* CH_3COONa, 0.20*M* NaCl, and 0.10*M* CH_3COONa were used in solvents by Wang and colleagues,[13] Muzzarelli and colleagues,[17] and Terbojevich and colleagues,[18] respectively. Even the solvent system used in SEC-MALLS contains salt concentrations, greater than or equal to 0.1*M*. Therefore, in molecular weight determinations by light scattering, the solvent used should contain the proper amount of salt to alleviate the polyelectrolyte effect.

Relationship Between *Mw* and RV*w*

Figure 4 shows the elution patterns of the high-performance size exclusion chromatograms of three different molecular weight chitosans were asymmetry. The maximum retention times of those different chitosans were ~ 32 min; however, higher molecular weight ones has shorter retention time. Because the chromatograms were asymmetric, the peak of chromatograms cannot be used to calculate the average molecular weight directly; therefore, the RV*w* of chitosans was calculated and listed in Table I. Figure 5 was plots of the calculated RV*w* and logarithmic *Mw*. The hollow sphere represented the RV*w* of different

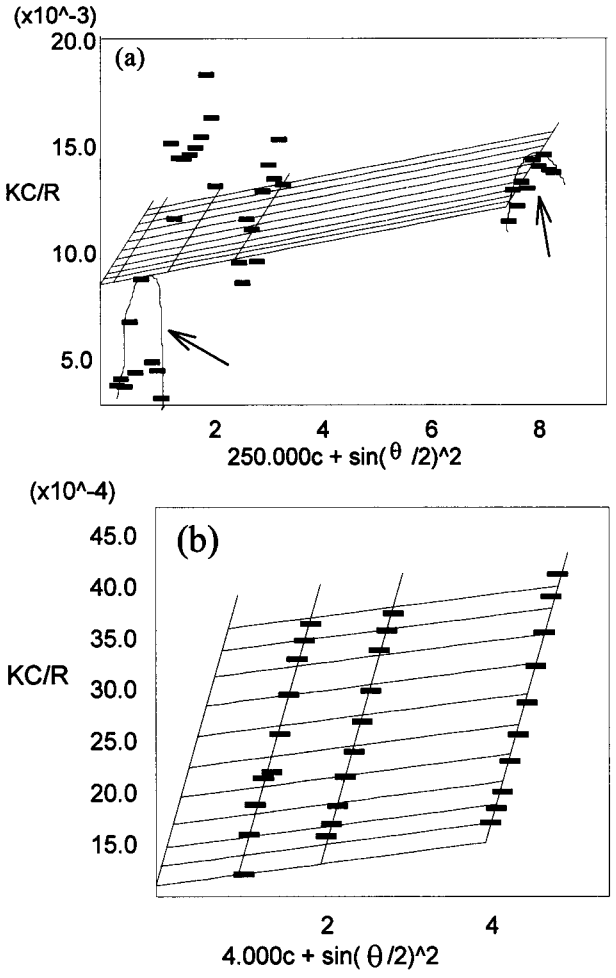


Figure 3 Zimm plot of 83% DD chitosan in: (a) 0.01*M* HCl and (b) 0.01*M* HCl/0.19*M* NaCl.

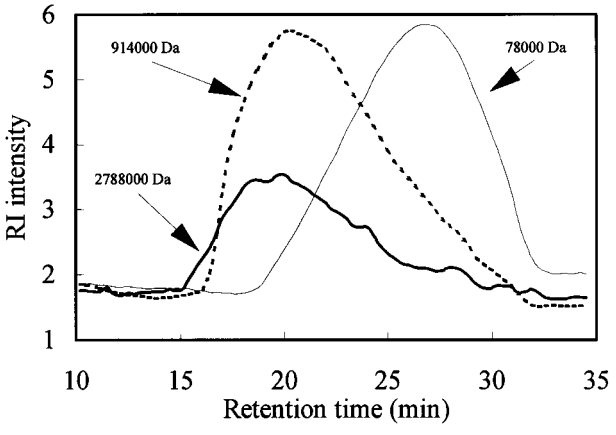


Figure 4 Elution patterns of HPSEC of different *Mw* chitosans. Columns: TSK gel G4000PWXL and G5000PWXL; mobile phase: 0.20*M* acetic acid/0.10*M* sodium acetate/0.008*M* sodium azide.

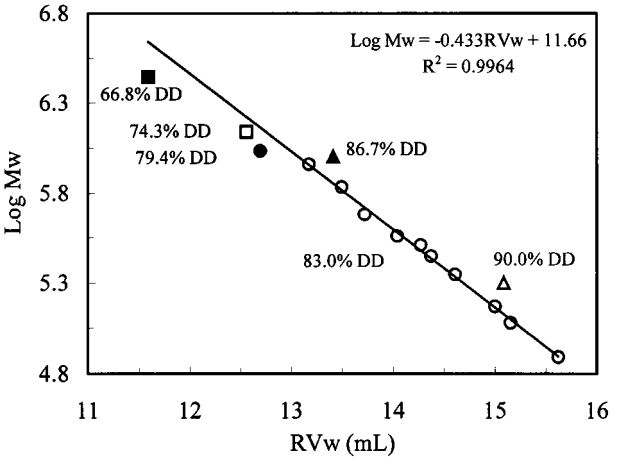


Figure 5 Relationship between logarithmic *Mw* and RV*w* of different DD and different molecular weight chitosans. ■, 66.8% DD; □, 74.3% DD; ●, 79.4% DD; ○, 83.0% DD; ▲, 86.7% DD; △, 90.0% DD.

molecular weight chitosans of 83.0% DD. The relationship of RV*w* and Log *Mw* of those chitosans are as follows:

$$\text{Log } Mw = -0.433\ \text{RV}w + 11.660$$

$$R^2 = 0.966 \quad (2)$$

Chitosan with DD other than 83.0%, the relationship of the RV*w* and log *Mw* did not correlate well (Fig. 5). For those chitosans, their DDs are higher than 83% DD, such as 86.7% and 90.0% DD ones; the points were located above the regression line. But, for those chitosans, their DDs were lower than 83% DD, such as 66.8%, 74.3%, and 79.4% DD ones; the points were located below the regression line. It may be because the molecules of higher DD chitosans are more flexible and tend to interact with gel of the column firmer than those of lower DD ones. Ottøy and colleagues[28] reported higher DDs of chitosan shows higher weak reversible adsorption for the gel and resulted in higher molecular weight. Their results are consistent with results shown in Figure 5. Therefore, the relationship established is useful to 83% DD chitosan only. It indicated not only the molecular weight, but also that DD affected the relationship of RV*w* and log *Mw* of chitosans.

The molecular weight determined by HPSEC depends on the accuracy measurement of the retention volume. However, the interaction of sample and gel used in the analysis column affects the retention volume determined to prevent the tendency of chitosan molecules from interacting with

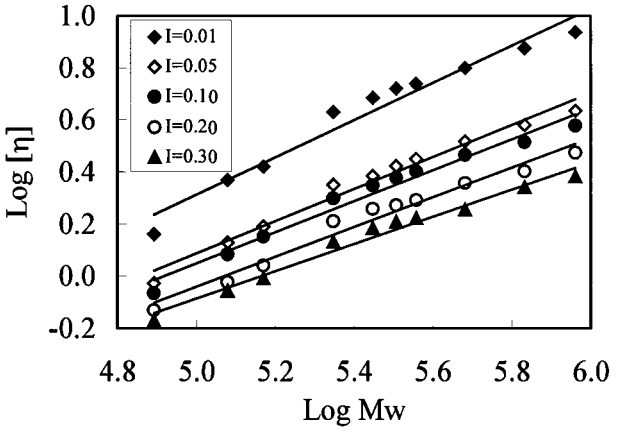


Figure 6 Plot of log [η] *versus* log Mw for chitosan in various ionic strength (I) solutions at 30 ± 0.1°C.

the gel of column. Solvents that can repress the polyelectrolyte effect should be used.[19] Figure 1 showed the eluate solvent of 0.20M acetic acid was used. A small amount of sodium acetate (0.01M) was enough to suppress the polyelectrolyte effect. The effectiveness of the suppression increased with an increasing concentration of sodium acetate. Therefore, 0.20M acetic acid/0.10M sodium acetate was used as mobile phase of HPSEC in this studies. Although Kato and colleagues[39] reported TSK gel is very suitable for molecular weight analysis of cationic-soluble polymers because this gel does not interact with the cationic-soluble polymers.

Beri and colleagues,[21] Ottøy and colleagues,[28] and Rinaudo and colleagues[32] all applied the SEC-MALLS or SEC-LALLS techniques to determine the molecular weight of chitosan. These techniques did not need standard markers. It is a straightforward, accurate method for determination of the molecular weight of chitosan. However, these techniques need a multiple- or low-angle light scattering photometer, and these instruments are not common ones. Therefore, the technique of SEC-MALLS cannot be a popular one. The equations established by the present article can be applied widely.

A universal calibration method was used to eliminate the error caused by using pullulan as the marker (Yomota and colleagues[19]). However, their procedure was troublesome. It has to determine the retention volume of chitosan and markers, intrinsic viscosity, too. Terbojevich and colleagues[22] established a calibration curve by integral-MWD method to use broad molecular weight distribution chitosan marker. However, this method was only suitable to those polymers that have Flory distribution ($Mw/Mn = 2$).[23]

Relationship Between *Mw* and Intrinsic Viscosity

The intrinsic viscosity of 10 different molecular weights, but the same (83% ± 1%) DD chitosans were determined and are listed in Table I. The double logarithmic plot of intrinsic viscosities of those 10 chitosans in 5 different ionic strength solutions (0.01–0.30M) whose Mws ranged between 78 and 914 kDa are shown as Figure 6. The Mark–Houwink equations were established, and the constants a and k are listed in Table II. Data in Table II show that constant a decreased from 0.715 to 0.521 as the solution ionic strength increased from 0.01M to 0.30M, whereas constant k increased from 5.48×10^{-4} to 2.04×10^{-3} over the same range of ionic strength solutions. This indicates that the a value decreased, whereas the k value increased with increasing solution ionic strength.

In the literature, CH_3COOH/CH_3COONa or CH_3COOH/NaCl solvent systems were commonly used to determine the viscosity average molecular weight.[13,19,26,28–33] In this article, an HCl/NaCl solvent system was used; therefore, constants a and k determined herein should be significantly different from the constants reported in literature.

Table II Constants a and k of the Mark–Houwink Equations for Chitosan in Solutions of Various Ionic Strengths and Molecular Weight Ranges at 30 ± 0.1°C

Molecular Weight Ranges (Da)	Ionic Strength	a	k	R^2
78,000–914,000	0.01	0.715	5.48×10^{-4}	0.962
	0.05	0.616	1.02×10^{-3}	0.979
	0.10	0.595	1.18×10^{-3}	0.975
	0.20	0.570	1.30×10^{-3}	0.973
	0.30	0.521	2.04×10^{-3}	0.979
78,000–223,000	0.01	1.009	1.69×10^{-5}	0.992
	0.05	0.817	9.47×10^{-5}	0.998
	0.10	0.791	1.17×10^{-4}	1.000
	0.20	0.742	1.67×10^{-4}	0.982
	0.30	0.653	4.28×10^{-4}	0.993
223,000–914,000	0.01	0.497	9.45×10^{-3}	0.999
	0.05	0.479	6.07×10^{-3}	0.997
	0.10	0.450	7.91×10^{-3}	0.996
	0.20	0.420	9.22×10^{-3}	0.994
	0.30	0.404	9.44×10^{-3}	0.990

Intrinsic viscosity in dL g^{-1}.

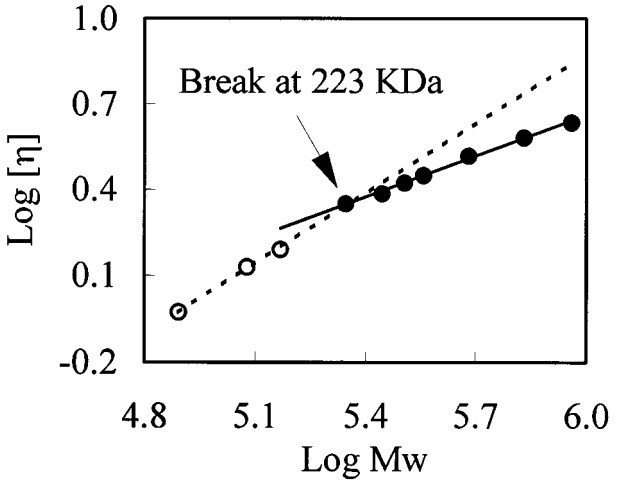


Figure 7 Plot of log [η] *versus* log Mw for chitosan in 0.05M ionic strength solutions at 30 ± 0.1°C; curve break at 223 kDa.

Although a few systematic studies about the effect of DD of chitosan on the a and k constants of the Mark–Houwink equation exist; however, the results are controversial. Maghami and Roberts[40] reported that the a and k constants were not affected by varying the DD of chitosan between 60% and 100%. However, Wang and colleagues,[13] Ottøy and colleagues,[28] and Anthonsen and colleagues[29] reported the a value decreased, but the k value increased with increasing DD of chitosan. Rinaudo and colleagues[32] reported the DD of chitosan did not affect the a value, whereas the k value increased with increasing DD of chitosan. Theoretically, with differences in DD of chitosan, the composition of chitosan differs accordingly and should result in different conformations and, in turn, different a and k values. Therefore, the DD of chitosan should have an effect on the a and k values. However, results of Maghami and Roberts[40] and Rinaudo and colleagues[32] showed no effect. This may be due to different solvents used or for other unknown factors. This article did not address the effects of DD of chitosan on the a and k constants. Therefore, the a and k values reported in Table II are applicable only to those chitosans with a DD of 83%.

From the double logarithmic plot of intrinsic viscosity and molecular weight of chitosan in 0.05M ionic strength solution (Figure 7), the linear curve breaks at a molecular weight of 223 kDa. Bohdanecky and Kovar[25] termed this phenomena a break. The break also occurred in the other four ionic strength solutions. Using 223 kDa as the breaking point, the chitosan curves were divided into two groups. The regression equations were calculated separately, and the corresponding constants a and k are listed in Table II. Data show that the a and k values of larger and smaller molecular weight chitosans were different, and the difference becomes more pronounced with decreasing solution ionic strength. Tsaih and Chen[41] attributed this phenomena due to the molecular weight-induced conformational transition. Therefore, when applying the Mark–Houwink equation to determine the molecular weight of a polymer, one must be aware of the range of molecular weight of the polymer used. The molecular weight range should not be too broad.

CONCLUSIONS

1. The molecular weight of 83% degree of deacetylation chitosan with non-Gaussian and wide-range destribution can be determined routinely by HPSEC or by capillary viscometry without the need of expensive instrumentation.
2. The resolving gel and solvent system used in HPSEC method are TSK gel columns in a series of G4000 PWxL and G5000 PWxL and solvent system consisted of 0.2M CH_3COOH/0.1M CH_3COONa, and 0.008M NaN_3, respectively.
2. The relationship of RVw and Mw for a different Mw of 83.0% DD chitosan can be expressed by the equation Log Mw = −0.443 RVw + 11.66. The RVw of other DDs chitosan do not correlate well with this equation.
3. The Mark–Houwink constant a decreased from 0.715 to 0.521, as the solution ionic strength increased from 0.01M to 0.30M, whereas constant k increased from 5.48 × 10^{-4} to 2.04 × 10^{-3} over the same range of ionic strength solution as follows:

Constant a and k of the Mark–Houwink equation for chitosan in solutions of various ionic strengths

Ionic strength	a	$k \times 10^4$	R^2
0.01	0.715	5.48	0.962
0.05	0.616	10.0	0.979
0.10	0.595	11.8	0.975
0.20	0.570	13.0	0.973
0.30	0.521	20.4	0.979

1. Range of molecular weight of chitosans tested are 78,000–914,000 Da.
2. Chitosans were dissolved in 0.01M and 0.30M HCl and 0.01M HCl and various concentrations of NaCl to adjust ionic strength.

4. When applying the Mark–Houwink equation to determine the molecular weight of a polymer by the viscometry method, one must be aware of the range of molecular weight of the polymer used.

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