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Calculation of Mark–Houwink–Sakurada (MHS) equation viscometric constants for chitosan in any solvent–temperature system using experimental reported viscometric constants data

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Abstract

Mark–Houwink–Sakurada (MHS) equation constants, a and K, for chitosan in several solvent–temperature systems have been already reported. In this article, two equations are proposed to determine, a and K, for chitosan in any solvent–temperature system using the previous reported viscometric constants data. This study resulted in two following equations:

$$\text{Exponent } a = 0.6202 + 0.699x/(0.4806 + x)$$

$$\log K \cdot 10^{-5} = -5.7676 \cdot \text{Exponent } a + 5.9232$$

where x equals to [DA/pH·μ], with DA, degree of acetylation of chitosan, pH of chitosan solution in a solvent with ionic strength of μ. The two equations are used to determine the constants of chitosan solution without urea between 20 and 30 °C. The validity of prediction of the constants from the knowledge of the DA, pH and ionic strength were examined using the above equations. The constants were obtained by a deviation in the range of ±0.40–29.0% for the exponent a, and ±6–71% for most of K values. The deviation of experimental and calculation data is reasonable. This is because several factors affecting the correct values for the constants were not taken into consideration by authors whose reported experimental data. The effects of temperature and urea on the constants have been described and taken into consideration in this article. The author of this manuscript described the various parameters affecting the constants. The procedures to determine the correct values of the constants for chitosan were also explained. The author of this study introduced the empirical function for chitin/chitosan chain conformation, (DA/μ·pH), as a measure of chain stiffness in solution. The empirical value or hydrodynamic volume of the polyelectrolyte molecule in solution increased with an increase in DA and a decrease in μ of solvent and pH of solution. The quality of different solvents was compared using the latter empirical parameter. Different solvents, which were used to determine the intrinsic viscosities and the viscometric constants, a and K (published in the literature for chitosan), were compared. The latter empirical parameter was also compared with B-parameter defined by Smidsrørd & Haug as chain stiffness; $d\ln[\eta]/d(1/T)$, and $d\ln[\eta]/dT$ as chain flexibility of the polymer.

Keywords: Chitosan; Viscometric constants; Intrinsic viscosity; Molecular weight

1. Introduction

Chitosan is a polysaccharide possessing various physicochemical and biological properties (Allan & Hadwiger, 1979; El Ghaouth, Arul, Wilson, & Benhamou, 1994; Tasi & Su, 1999). It is used in food, pharmaceutical, and cosmetics industries due to its several interesting properties (Sandford & Hutchings, 1987). The effectiveness of chitosan depends on its molecular weight (Allan et al., 1984; Hadwiger, Fristensky, & Riggleman, 1984; Olsen, Schwartzmiller, Weppner, & Winandy, 1989; Wu & Bough, 1978), and molecular weight distribution, MWD. If the molecular weight is known along with good understanding

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of the polymer conformation, many properties such as rheological and mechanical properties can be estimated. Average molecular weight determination (M_n, M_w or M_z) is time-consuming. The determination of viscosity-average molecular weight is relatively quick method. The intrinsic viscosity of a polymer solution is related to the polymer molecular weight according to the Mark–Houwink–Sakurada (MHS) equation (Flory, 1953; Tanford, 1961).

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

where $[\eta]$ is the intrinsic viscosity, M_v the viscosity-average molecular weight, and K and a, are the constants for a given solute–solvent system.

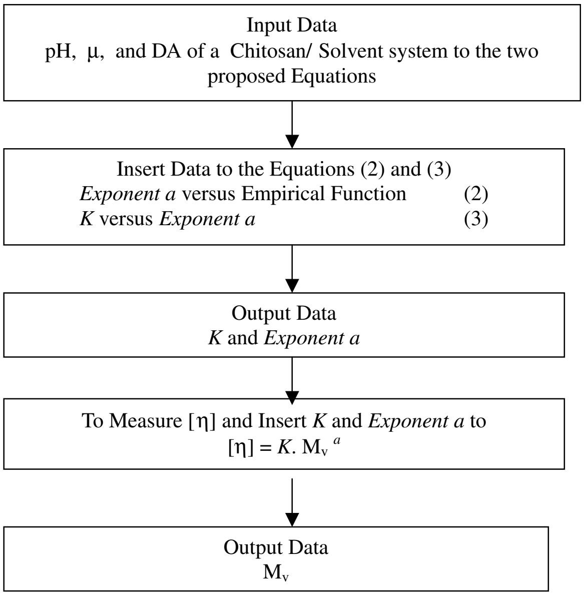
The constants are required: to determine viscosity-average molecular weight, M_v; to construct SEC universal calibration curve; to determine hydrodynamic volume of polymers, $[\eta]M_v$; to interpret the data in SEC; and to transform MWD and M_W data from standard polymers to unknown polymer samples having structurally different from standard polymers, where the constants are necessary for both standard and unknown characterized polymer to perform transformation procedure.

The viscosity measurement needs a simple; easy-to use apparatus; and takes a short time for intrinsic viscosity determination compared to other methods (HPLC/SEC equipped with light scattering detector, osmometry and sedimentation). The estimation of molecular weight for the polymer samples produced in a manufacture during polymer processing in a short time is a critical aspect. Thus, estimation of viscosity-average molecular weight of a polymer from measurements of its intrinsic viscosity is an economical, practical and useful method for a routine analysis.

The present work was aimed at extending the latter studies (Kasaai, Arul, & Charlet, 2000). The objectives of this work were: to propose two equations as a model to calculate, a and K, for chitosan in any solvent–temperature system using viscometric constants data, which have been reported by several research groups; to discuss parameters affecting the constants; to describe parameters and procedures to determine correct values of the constants and the reasons of the deviation between experimental and calculated values; to introduce an empirical function for chitin/chitosan chain conformation in solution, (DA/μ·pH), as a measure of chain stiffness or chain flexibility; and to compare various solvents; their quality and interaction of polymer–solvent using determination of intrinsic viscosity; to discuss parameters and procedures affecting the constants and determine correct values of the constants.

2. Calculation methods

In the previous report (Kasaai et al., 2000), it was described that the exponent a roughly increases with an increase in the value of DA/(pH·μ). In the present work, the author proposed two equations by using reported experimental data on exponent a, intercept K, and empirical function, [DA/(pH·μ)]. The model was the best curve fits for the plots (exponent a versus [DA/(pH·μ)], and log K versus exponent a), as shown in Eqs. (2) and (3), respectively (see Section 3.1). The procedure of calculation is illustrated in Scheme 1 as follows:



Scheme 1. Calculation procedure for chitosan viscometric constants.

The constants have been estimated from the knowledge of the DA, pH and ionic strength, μ, using the proposed equations: exponent a versus [DA/(pH·μ)]; and log K versus exponent a. The deviation of the calculation values from corresponding experimental values was determined. The plots of experimental values against calculated values for the constants yielded roughly straight lines whose slopes provided values close to the unity.

The effects of temperature and urea concentration have been taken into consideration using literature information and two related correction factors were added to the empirical function of DA/(pH·μ) (see Section 3.3).

3. Results and discussion

3.1. Viscometric constants

The constants have been determined in various solvents by many authors over the past 30 years. The values of MHS equation constants, a and K, published in the literature (Anthonsen, Varum, & Smidsrød, 1993; Berkovich, Timofeyera, Tsyurupa, & Davankov, 1980; Errington, Harding, Värum, & Illum, 1993; Gamzazade et al., 1985; Kasaai et al., 2000; Lee, 1974; Podogina et al., 1986; Rao, 1993; Rinaudo, Milas, & Dung, 1993; Roberts & Domszy, 1982; Yomota, Miyazaki, & Okada, 1993), are presented in Table 1. The values for a ranges from 0.58 to 1.26 in various solvents. Similarly, the values for K ranges from 5.6×10^{-3} to 3.0×10^{-7}. Fig. 1 shows the exponent a versus the empirical function, [DA/(pH·μ)]. The open symbols are experimental points, which have been reported

Table 1
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 g^{-1})	a	Molecular weight range (kDa)	Reference
0.02 M HAc/0.1 M NaAc/0.1 M NaCl	20	0	4.5	0.2	559.0	0.58	15–310	Anthonsen et al. (1993)
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)
0.3 M HAc/0.2 M NaAc	25	2	4.6	0.2	82.0	0.76	100–600	Rinaudo et al. (1993)
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)
0.3 M HAc/0.2 M NaAc	25	21	4.6	0.2	74.0	0.76	100–600	Rinaudo et al. (1993)
0.02 M HAc/0.1 M NaAc/0.1 M NaCl	20	15	4.5	0.2	58.5	0.78	35–245	Anthonsen et al. (1993)
0.25 M HAc/0.25 M NaAc	25	21–26	4.7	0.25	15.7	0.79	35–2220	Kasaai et al. (2000)
0.2 M HAc/0.1 M NaAc	30	0	4.4	0.1	16.8	0.81	194–937	Wang et al. (1991)
2% HAc/0.2 M NaAc	25	15 ± 3	4.5	0.2	13.8	0.85	61–150	Gamzazade et al. (1985)
0.2 M HAc/0.1 M NaAc	30	9	4.4	0.1	6.59	0.88	211–1260	Wang et al. (1991)
0.1 M HAc/0.2 M NaCl	25	≈20	2.8	0.2	1.81	0.93	48–630	Roberts and Domszy (1982)
0.2 M HAc/0.1 M NaAc	30	16	4.4	0.1	1.42	0.96	536–1850	Wang et al. (1991)
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)
0.02 M HAc/0.1 M NaAc/0.1 M NaCl	20	60	4.5	0.2	2.18	1.06	15–164	Anthonsen et al. (1993)
0.2 M HAc/0.1 M NaAc	30	31	4.4	0.1	0.104	1.12	477–2510	Wang et al. (1991)
0.1 M HAc/0.02 NaCl	25	≈20	2.9	0.02	0.0304	1.26	48–630	Roberts and Domszy (1982)
0.2 M HAc/0.1 M NaCl/4 M urea	20	9	2.6		89.3	0.71	163–492	Lee (1974)
1% H Ac	30		2.8	0.01	4.74	0.72	205–657	Rao (1993)
0.2 M HAc/0.2 M NaAc	25	58	4.3	0.2		1.14	4.3–64.1	Errington et al. (1993)
0.17 M HAc/0.47 M NaCl	25	⩽10	2.5	0.47	1115	0.147	1.6– 15	Berkovich et al. (1980)

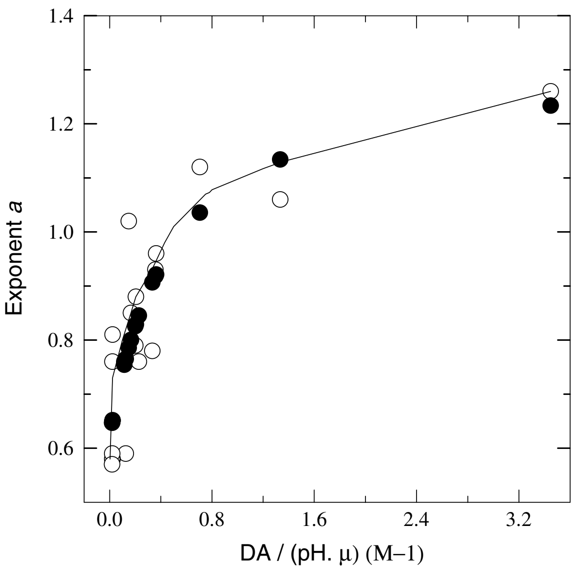


Fig. 1. Exponent a as a function of the [DA/(pH·μ)]. Open symbols represent literature results; full symbols represent the calculated data; and full line refers to the best curve fit.

by several research groups. The full symbols are calculated points from the proposed equation [Eq. (2)]. The full line was the best curve fit for the relationship between the exponent a and the empirical function, [DA/(pH·μ)]. The fit for the plot was passed through the calculated points. The following equation was best described for the curve fit, exponent a versus [DA/(pH·μ)].

$$\text{Exponent } a = 0.6202 + 0.699x/(0.4806 + x) \tag{2}$$

where x equals to [DA/pH·μ].

Berkovich et al. (1980) reported a value of 0.147 for exponent a. The concentration of salt in the solvent and the value of μ, which have been used by the latter authors, were high and thus resulted in small and compact conformation. But the reported value for exponent a is dramatically low and it is highly deviated from other reported exponent a and thus it was eliminated from the processed data. Lee (1974) calculated the constants in a solution containing urea. This data was also eliminated from the processed data.

The Mark–Houwink constant a decreased as the ionic strength of solution increased from 0.01 to 0.30 M, whereas constant K increased over the same range of the ionic strength (see Table 1). This is understandable that an increase in ionic strength results in a decrease in electrostatic repulsions, and reduction in hydrodynamic volume and exponent a. The pH of the polymer solutions varied from 2.5 to 4.7 (Table 1). At constant ionic strength and DA, the exponent a increases with a decrease in pH value of the polymer solutions, because of a higher degree of expansion of chitosan due to electrostatic repulsions.

This study resulted in Eq. (3) for chitosan samples. The plot of $\log K$ versus exponent a, was linear and the following equation was obtained from the linear plot (Fig. 2).

$$\log K \cdot 10^{-5} = -5.7676 \cdot \text{Exponent } a + 5.9232 \tag{3}$$

Thus, if a chitosan sample having a known DA is dissolved in a solvent, one enables to determine the empirical value, [DA/(pH·μ)], through the measurement of pH of the chitosan solution and ionic strength of the solvent. The

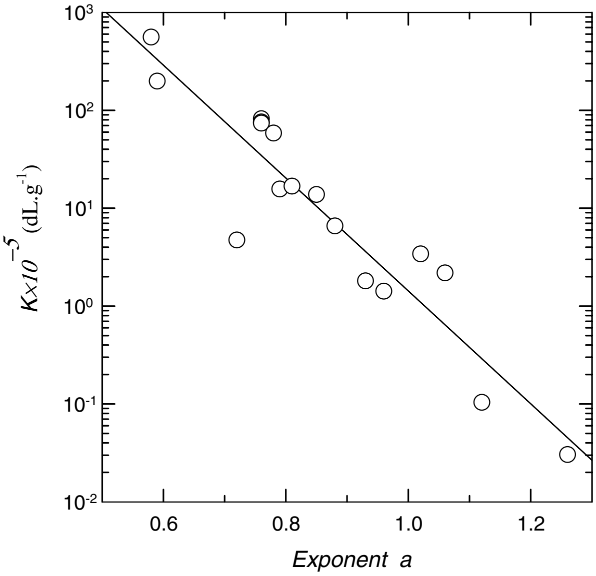


Fig. 2. Values of K versus exponent a. Open symbols represent literature results and a full line refers to the best curve fit.

constants, *a* and *K*, are determined from Eqs. (2) and (3), respectively.

The validity of prediction of the constants from the knowledge of the DA, pH and μ were examined using Eqs. (2) and (3) (see Table 2). The constants were obtained by a deviation in the range of ±0.40–29.0% for all of the exponent *a*, and ±6–71% for most of *K* values, suggesting that the curve fits and corresponding equations can be useful for estimation of the constants. While pH measurement of chitosan solution needs only a single measurement and determination of ionic strength of the solvent takes a short time, the determination of the constants using (HPLC/SEC equipped with light scattering detector, osmometry and sedimentation) are time-consuming.

Fig. 3 shows the experimental values for exponent *a*, versus corresponding calculated exponent *a*. The linear relationship between the experimental and calculated exponent *a* appeared as follows:

$$a_{\text{Exp}} = a_{\text{Model}}(0.90) + 0.099 \tag{4}$$

Fig. 4 shows the experimental values for *K* versus corresponding calculated *K* values. The following equation was obtained from the linear relationship.

$$\log K_{\text{Exp}} \cdot 10^{-5} = \log K_{\text{Model}} \cdot 10^{-5}(0.88) + 0.047 \tag{5}$$

Some of experimental points are on or close to the curve fits (see Figs. 1 and 2). A few experimental points are far from the curve fits. The accuracy of results limited by the

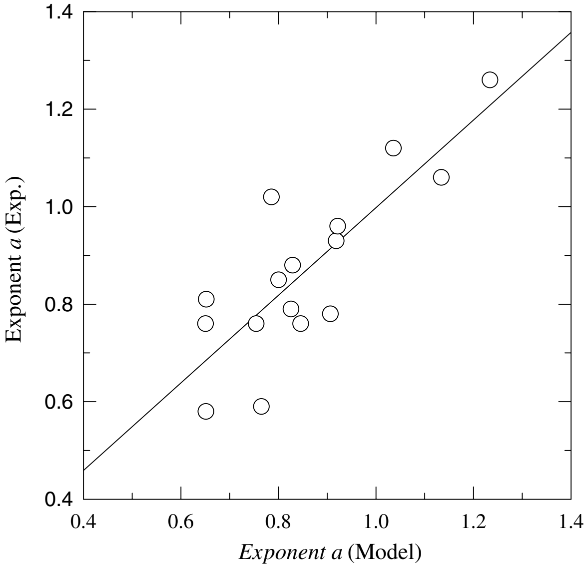


Fig. 3. Experimental exponent a as a function of calculated exponent a determined from the mathematical model.

Table 2
Experimental, estimated and deviation (%) of viscometric constants for chitosan

Solvent	Exponent *a*			$K{\cdot}10^{-5}$ (dL g^{-1})		
	Experimental	Estimated	Deviation (%)	Experimental	Estimated	Deviation (%)
0.02 M HAc/0.1 M NaAc/0.1 M NaCl	0.58	0.651	−12.25	559.0	378.4	32.30
0.5 M HAc/0.5 M NaAc	0.59	0.765	−29.65	199.0	331.4	−66.52
0.3 M Hac/0.2 M NaAc (DA = 2)	0.76	0.650	14.42	82.0	34.66	47.34
0.3 M HAc/0.2 M NaAc (DA = 10.5)	0.76	0.754	0.75	76.0	34.66	41.34
0.3 M HAc/0.2 M NaAc (DA = 21)	0.76	0.845	−11.23	74.0	34.66	39.34
0.02 M HAc/0.1 M NaAc/0.1 M NaCl	0.78	0.906	−16.21	58.5	26.57	54.60
0.25 M HAc/0.25 M NaAc	0.79	0.826	−4.5	15.7	23.27	−48.2
0.2 M HAc/0.1 M NaAc	0.81	0.652	−19.54	16.8	17.84	−6.20
2% HAc/0.2 M NaAc	0.85	0.800	5.90	13.8	10.49	24.0
0.2 M HAc/0.1 M NaAc	0.88	0.829	5.81	6.59	7.04	−6.86
0.1 M HAc/0.2 M NaCl	0.93	0.918	1.3	1.81	3.63	−100.3
0.2 M HAc/0.1 M NaAc	0.96	0.921	4.04	1.42	2.43	−71.4
0.33 M HAc/0.3 M NaCl	1.02	0.786	23.0	3.41	1.097	67.8
0.02 M HAc/0.1 M NaAc/0.1 M NaCl	1.06	1.13	−6.98	2.18	0.645	70.4
0.2 M HAc/0.1 M NaAc	1.12	1.04	7.52	0.104	0.291	−179.6
0.1 M HAc/0.02 NaCl	1.26	1.23	2.09	0.0304	0.0453	−49.0

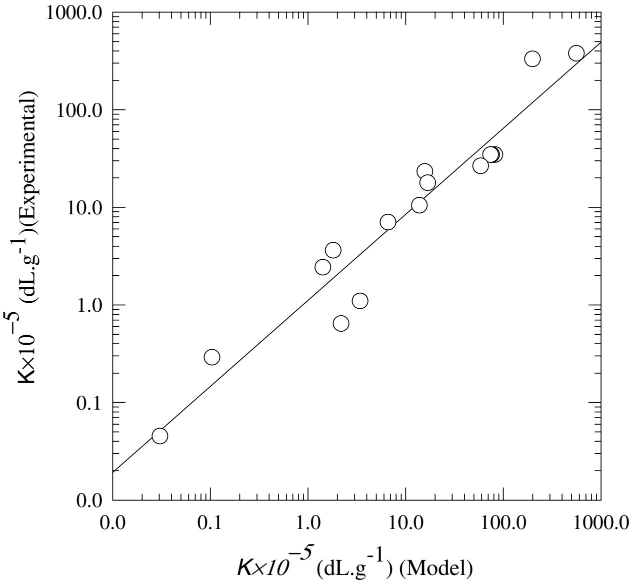


Fig. 4. Experimental value of K versus calculated K from the proposed model.

experimental errors, which is linked to the techniques employed for molecular weight determination. The disparity between the experimental and the calculated data obtained from Eqs. (2) and (3) depend on several factors as follows:

(1) The molecular weight of chitosan samples were obtained by a variety of methods including light scattering, HPLC/SEC, sedimentation, osmometry and end-group analysis. Various average-molecular weights (M_p, M_n, M_w, and M_z) instead of M_v were substituted in MHS equation. The value of M_v is different from M_n, M_w, and M_z in a polydisperse polymer sample (Kasaai et al., 2000). Determination of constants, K and a, from the intrinsic viscosity data, requires a series of monodisperse polymer samples with 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, whereas other-average molecular weights are accessible. The Eq. (1) can be rearranged and resulted in a modified MHS equation as follows:

$$[\eta] = KM_v^a = K(M_v/M_w)^a M_w^a = Kq_{\mathrm{MHS}} M_w^a \qquad (6)$$

where the value $(M_v/M_w)^a$ as well as q_{MHS} is polydispersity correction factor. The value of q_{MHS} is statistical function of molecular weight distribution. It is a measure of the width of molecular weight distributions (MWD) as well as probability of molecular weight distribution curve. It can be calculated using M_w, molecular weight distribution (M_v/M_w) and exponent a. The value of q_{MHS} varies from one sample to another one, because it is a function of a and average-molecular weights (M_v, M_w). Alternatively, the value of q_{MHS} can be calculated using numerical method and other-average molecular weights (M_n, M_w, M_z) according to (Bareiss, 1999; Kurata & Tsunashima, 1999):

$$q_{\mathrm{MHS}} = (M_w/M_n)^b (M_z/M_w)^c \qquad (7)$$

where b and c are empirical polynomial functions of the exponent a. A few research groups have been taken into account the polydispersity of the polymer. Roberts and Domszy (1982) used a statistical function to describe polydispersity of the polymer samples, assuming that degradation of chitosan occurred by random scission. Kasaai et al. (2000), was used combined methods (HPLC/SEC, light scattering and osmometry), to determine average-molecular weights and the polydispersity of the polymer. Therefore, most of the pair constants were calculated by a wrong substitution of average- molecular weight and the effect of polydispersity was ignored. Thus, accurate values for the constants should not be obtained under above-mentioned conditions. The accurate values are obtained according to the following procedures: The polydispersity of the polymer determined by HPLC/SEC; M_w by light scattering; M_n by osmometry or end-group analysis; and M_z by sedimentation methods. If each average-molecular weight determined by a correct method and substitution is performed in a correct equation, and then, the correct values for the constants are obtained. The error is significant when the polymer samples have a broad MWD and the values of M_n or M_z is substituted instead of M_v. The advantage of the light-scattering method is that it gives M_w, which is much closer to M_v than M_n and M_z, because the value of exponent a for chitosan in most of solvents falls in the range of 0.7 and 1.0. The closer a to the unity, the smaller the error for the constants. When the value of a equals to the unity, the value of M_v and M_w are identical.

(2) Wang, BO, Li, and Qin (1991), reported different pairs of constants for chitosans with identical pH and μ but different DA values, whereas Rinanudo et al. (1993), reported identical exponent a for chitosans with DA between 2 and 21. The intrinsic viscosity and hydrodynamic volume of chitosan depend on the DA. An increase in DA reduces the number of positive charges NH_3^+ on chitosan and electrostatic repulsions, leading to a higher degree of expansion and hydrodynamic volume as well as a larger value for exponent a. The exponent a reported by Rinanudo et al. (1993) reveal that the effect of DA on conformation as well as exponent a have not been taken into consideration. For example, chitosan with DA = 21 should have the exponent a larger than that of chitosan with DA = 2. The reported pairs of constants by Wang et al. (1991) is reasonable, because the effect of DA on chitosan conformation and exponent a was taken into account. In addition, some of chitosan samples, which were used to determine viscometric constants, were obtained from degradation of chitosans. The corresponding authors assumed that the original and the samples resulting from degradation process have the same DA, whereas the DA may not be identical. Two major degradation processes may be occurred for chitosan: (1) degradation of glucosidic linkage; and degradation of side chains such as N-deacetylation. The chemical structure and conformation of the macromolecule will not change by degradation of glucosidic linkage. The latter process results in reduction of the

molecular size. While the chemical structure, the DA, and consequently the conformation of macromolecule will change by degradation of its side chains. If the change in the value of DA is small and negligible, one can use identical values of viscometric constants for original and the samples resulting from degradation process. If the change in the values of DA is significant, the viscometric constants change as a function of the DA.

(3) The reported authors of the constants constructed universal calibration curve (for HPLC/SEC method) based on the product of $[\eta]M_n$ or $[\eta]M_w$ instead of $[\eta]M_v$. The value of M_v is different from other average-molecular weights and it is not a unique value, and varies from one solvent to another, since it is a function of a, K, and $[\eta]$.

The deviation of K was greater than that of exponent a. This is because the values of K is more sensitive than that of the exponent a to MWD (Whitehouse, 1971; and Moore & Brown, 1959). The latter authors obtained larger deviation for K than exponent a.

The slope was 0.9 for Fig. 3 and 0.88 for Fig. 4. In an ideal case, the slope of Fig. 3 or 4 equals to the unity. Since several above-explained parameters affected the slope values and most of the experimental data were not obtained from precise procedures, and thus the slopes became less than 1, which is reasonable. If the calculation were performed on only the corrected data (the data obtained from precise procedures), the results should not be covered a wide range of experimental conditions. In addition, the number of correct values is very limited. Thus, almost data were taken into account for the proposed model. It is understandable that the calculated data can not be accurate, where the processed data (experimental data) was not.

3.2. Chain conformation of chitosan in solution

The slope $d\ln[\eta]/dT$; $d\ln\eta_{rel}/d(1/T)$; or $d\ln\ [\eta]/\ d(1/T)$; has been already used as an index of polymer stiffness or chain flexibility of polymers including polysaccharides and polyelectrolytes (Kamide & Saito, 1987; Milas & Rinaudo, 1986; Rinaudo & Domard, 1989). Based on the latter definition for chain stiffness, stiffer polymers have larger slopes. The slope can be used to evaluate chain flexibility for both neutral and ionic polymers (polyelectrolytes). The Smidsrørd–Haug parameter B (1971) has been introduced as characteristics of stiffness for polyelectrolytes. The B parameter is based on the fact that the chain dimensions and the intrinsic viscosity of polyelectrolytes are related to the salt or ionic strength variation in solutions. The B-parameter, which has been defined by Smidsrød and Haug (1971), is based on theories of Flory (1953) and Fixman (1964). B value is a relative characteristic, and it is suitable for comparative studies. The determination of B-parameter requires two following information: (1) $[\eta]$ versus μ and; (2) $\log S$ versus $\log[\eta]$ at $\mu = 0.1$, where S is the slope of the plot obtained from the following equation:

$$S = d[\eta]/d(1/\mu^{1/2}) \tag{8}$$

The B-parameter is then related to S by following equation:

$$\log S = \log B + v \log[\eta]_{(\mu=0.1)} \tag{9}$$

where v is a factor, which is changed by chain flexibility (Smidsrød & Christensen, 1991; Smidsrød & Haug, 1971).The values of v and B can be obtained as slope and intercept of the plot, respectively.

The author of the present article introduces the empirical function of (DA/pH·μ) as a measure of chain stiffness for chitosan with a polyelectrolyte nature. This empirical function includes intrinsic parameter related to chemical structure of chitosan, DA, and two extrinsic parameters related to the solvent and solution behavior of chitosan (ionic strength of the solvent and pH of chitosan solution). A combined function at a constant temperature can be used to estimate the chain stiffness of chitosan. Larger value of the DA causes stiffer chain conformation. Higher values of μ or higher values of pH as well as smaller values of acidity yield more flexible conformation. On a whole a larger value of $(\mathrm{DA}\cdot\mathrm{pH}^{-1}\cdot\mu^{-1})$, yields stiffer chain conformation. The advantage of the latter empirical function over B-parameter defined by Smidsrord & Haug is: both the structure parameter affected the rigidity of chitosan (DA) and the quality of solvent affected the chain conformation of chitosan (as a polyelectrolyte in nature) are taken into consideration in a unique function. The exponent a as a function of the empirical factor (DA/pH·μ), can be also presented in another way as shown in Fig. 5. In this way, a bi-linear relationship was observed, where the slope of the initial linear portion was higher, and the change in

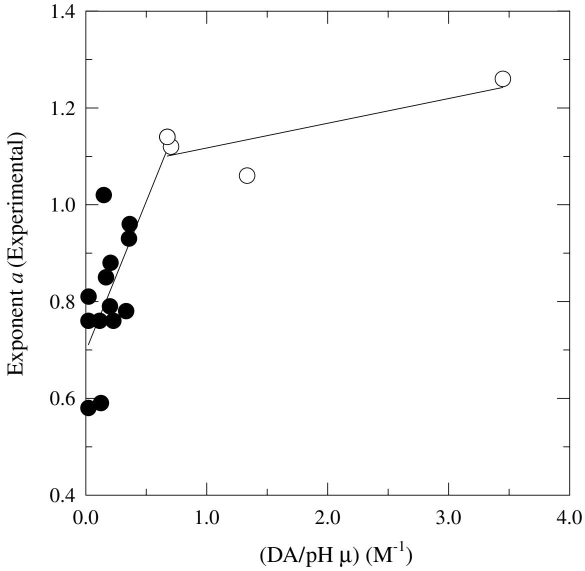


Fig. 5. Experimental exponent a as a function of the [DA/(pH·μ)]. Full symbols represent the experimental points with the value of μ greater than 0.1 and/or the value of DA smaller than 50. Open symbols represent the experimental points with the value of μ smaller than 0.1 and/or the value of DA greater than 50.

slope occurred when the value of μ was smaller than 0.1 and/or the value of DA was greater than 50. The exponent a in initial slope is smaller than unity and in the second portion the exponent a is greater than unity, in other word, the conformation in the second portion is stiffer than that of initial one.

Table 3 shows several parameters related to chain conformation as well as hydrodynamic volume of chitosan at various experimental conditions in solution, i.e. chain flexibility parameter, $\mathrm{d}\ln[\eta]/\mathrm{d}T$; the value of $\mathrm{d}(\ln[\eta]/\mathrm{d}\ 1/T)$; B-parameter (Smidsrød & Haug (1971)); empirical parameter for chitin/chitosan chain conformation, (DA/pH·μ), introduced in this article. The value of B-parameter found to be equal to 0.1 and independent of DA for a series of chitosans with DA smaller than 0.21 (Trzciński, Varum, Staszewska, Smidsrød, & Bohdanecky, 2002). The latter authors concluded that the effect of counter-ions on B-parameter was not significant. B values decreased from 0.1 for chitosan with DA = 0.15 to the value of 0.02 for chitosan with DA = 0.6 (Anthonsen et al., 1993). This is because the effect of intra-molecular hydrogen bond is promoted with an increase in DA value and result in more extended and stiffer chain conformation (Anthonsen et al., 1993). B value slightly was smaller for chitosan with DA = 0 (B = 0.09) than that of chitosan with DA = 15. This is because there is strong repulsion between free amine groups in chitosan with a low DA. The charge or electrostatic effect is the highest for chitosan with DA = 0 (Anthonsen et al., 1993) at low ionic strengths, where the electrostatic repulsion is the major effect. Thus, chitosan with DA = 0 is an extended conformation and the value of B is smaller than that of chitosan with higher value of DA. Chitin/chitosan chain conformation parameter, DA/pH·μ, increased with an increase in DA (see Table 3), i.e. chitosan with larger DA is stiffer, which is consistent with the data reported by Anthonsen et al. (1993) for chitosans with DA between 0 and 0.6.

The chain flexibility parameter, $\mathrm{d}\ln[\eta]/\mathrm{d}T$, and the value of $\mathrm{d}(\ln[\eta]/\mathrm{d}\ 1/T)$ for two series of chitosans with DA = 0.1–0.28, and DA = 0.09–0.21 in 0.1 M HAc/0.2 M NaCl were reported to be independent with varying DA. The chain flexibility, $\mathrm{d}\ln[\eta]/\mathrm{d}T$, for the both series was negative (see Table 3) (Trzciński et al., 2002). Lee (1974) and Podogina et al. (1986) have also reported negative values for $\mathrm{d}\ln[\eta]/\mathrm{d}T$.

Chitin/chitiosan chain conformation parameter (DA/pH·μ), varies with ionic strength of the solvent and pH of the solution (see Table 3). Smaller value for ionic strength or pH yields in greater value for the parameter; stiffer chain conformation for chitosan or larger value for exponent a. This result is consistent with the results of Tsaih and Chen (1999). The latter authors studied on the effects of ionic strength and pH on exponent a and chain conformation of chitosan in solution. One enables to make clear and direct conclusion from the effect of ionic strength or pH on chain conformation using (DA/pH·μ) parameter, whereas using B or chain flexibility parameter, $\ln[\eta]/\mathrm{d}T$, or using the value of $\mathrm{d}(\ln[\eta]/\mathrm{d}\ 1/T)$ does not. B-parameter

Table 3
Chain flexibility; $\mathrm{d}\ln[\eta]/\mathrm{d}T$; $\mathrm{d}\ln[\eta]/\mathrm{d}(1/T)$; B- parameter; and DA/(pH·μ) parameter at different experimental conditions and different molecular weight ranges

Experimental conditions	Molecular weight (kDa)	$\mathrm{d}\ln[\eta]/\mathrm{d}T$	$\mathrm{d}\ln[\eta]/\mathrm{d}(1/T)$	Chain stiffness (B-parameter)	DA/(pH·μ)[a]	References
0.1 M HAc/0.2 M NaCl, DA = 0.10–0.28	$M_v = 300–490$	-5.0×10^{-3}	480	–	–	Trzciński et al. (2002)
0.1 M HAc/0.2 M NaCl, DA = 0.09–0.21	$M_v = 30$	-4.5×10^{-3}	429	0.1	–	Trzciński et al. (2002)
0.33 M HAc/0.3 M NaCl, DA = 0.20	$M_w = 134$	-5.3×10^{-3}	488	–	–	Podogina et al. (1986)
0.2 M HAc/0.1 M NaCl–4 M urea, DA = 0.21	$M_v = 135–92$	-3.2×10^{-3}	–	–	–	Lee (1974)
0.2 M HAc/0.1 M NaAc DA = 0.21	$M_v = 135–492$	-4.0×10^{-3}	–	–	–	Lee (1974)
DA = 0.0, $\mu = 0.1$, pH = 4.5	$M_n = 15–310$	–	–	0.09	–	Anthonsen et al. (1993)
DA = 0.15, $\mu = 0.1$, pH = 4.5	$M_n = 35–245$	–	–	0.1	–	Anthonsen et al. (1993)
DA = 0.6, $\mu = 0.1$, pH = 4.5	$M_n = 30–164$	–	–	0.02	–	Anthonsen et al. (1993)
0.01 M HCl, DA = 0.17	$M_w = 78–914$	–	1334–666	0.11–0.152	–	Chen and Tsaih (1998)
DA = 0.01–0.2 $\mu = 0.01$, pH = 4.5	$M_w{}^{a} = 50–1000$	–	–	–	0.22–4.45	Kasaai et al. (2000)
DA = 0.01, pH = 4.5 $\mu = 0.01–0.2$	$M_w{}^{a} = 50–1000$	–	–	–	0.22–0.011	Kasaai et al. (2000)
DA = 0.5, pH = 4.5 $\mu = 0.1–0.5$	$M_w{}^{a} = 50–1000$	–	–	–	1.11–0.22	Kasaai et al. (2000)

[a] An average value was chosen for molecular weight ranges presented in Table 2.

is a relative characteristic for biopolymers and polyelectrolytes. It is a suitable and an interesting parameter for comparative studies of chain stiffness of polyelectrolytes and polysaccharides. Smidsrød and Christensen (1991) have found a good correlation between B-parameter and chain stiffness for several polyelectrolytes based on the theories of Flory (1953) and Fixman (1964). Smidsrød and Haug (1971) have concluded that the more flexible chain results in higher value for B-parameter. But B-parameter is not useful for specific and direct evaluation of chain stiffness for chitin and chitosans having a definite molecular structure parameter (DA); and solvent and solution (μ, pH, and T) information. This is because B-parameter does not directly depend on μ and pH of solvent and solution. And some variations in the stoichiometric charge density of polyelectrolytes do not lead to variation in B.

3.3. Factors affecting the constants

3.3.1. Effect of molecular weight

Some parameters other than the parameters those explained in Section 3.1 affect the constants. The values of a and K may vary with molecular weight range, since the flexibility of polymers conformation as well as exponent a may change with its molecular size. Over a very wide range of molecular weights, neither a nor K is constant (Kasaai, 2006).

Contradict results have been reported for the effect of molecular weight on: (i) conformation; (ii) intrinsic viscosity; or (iii) exponent a. Chain flexibility parameter, $\mathrm{d}\ln[\eta]/\mathrm{d}T$, and/or the value of $\mathrm{d}(\ln[\eta]/\mathrm{d}\ 1/T)$ for chitosan samples with different molecular weights in various experimental conditions are given in Table 3. The values reported for $\mathrm{d}\ln[\eta]/\mathrm{d}T$, and/or $\mathrm{d}(\ln[\eta]/\mathrm{d}\ 1/T)$ were independent with molecular weight of chitosan (Trzciński et al., 2002; Podogina et al., 1986; Lee, 1974). While the values of $\mathrm{d}(\ln[\eta]/\mathrm{d}\ 1/T)$ reported by Chen and Tsaih (1998) varied with molecular weight (see Table 3). These authors noted that the lower molecular weight chitosans were stiffer than higher molecular weight ones. No significant change in the value of exponent a was reported for chitosans in a wide range of molecular weights (DA = 35–2250 kDa) (Kasaai et al., 2000). The B-parameter reported by Tsaih and Chen (1997) for chitosans with M_w between 78 and 914 kDa were between 0.110 and 0.152. One may conclude that higher molecular weight chitosan are slightly more flexible than that of smaller molecular weight ones (see Table 3, Tsaih & Chen, 1997). However, a clear conclusion cannot be made that the B-parameter increased with an increase in molecular weight. In other word, B-parameter is independent of chitosan molecular weight. Most of the data indicates that the effect of molecular weight on chain conformation is negligible and its effect on exponent a can be ignored in the range of 50–1000 kDa. The latter values were chosen as average value for molecular weight ranges presented in Table 1.

3.3.2. Effect of temperature

The constants of MHS equation for polymers may vary with polymer solution temperature. This is because the effective dimensions of macromolecules (hydrodynamic volume) change with solution temperature via change in their chain flexibility (Kasaai, 2002; Moore & Brown, 1959). In a good solvent, a temperature increase results in a decrease in intrinsic viscosity and a less-extended conformation; because the entropy value increases with an increase in temperature and it is unfavorable for an extended conformation, whereas in a poor solvent, a temperature increase causes an increase in entropy and intrinsic viscosity and is favorable for the extended conformation. The temperatures, which have been used as solution temperature for intrinsic viscosity and constants determination by several authors and presented in Table 1, were between 20 and 30 °C. This is relatively a narrow range (see Table 1).

The effect of temperature on intrinsic viscosity and chain conformation can be evaluated using the value of slope for the plot either $\ln[\eta]$ versus $1/T$ or $\ln\eta_\mathrm{rel}$ versus $1/T$ (the value of $\mathrm{d}\ln[\eta]/\mathrm{d}[1/T]$ or $\mathrm{d}\ln\eta_\mathrm{rel}/\mathrm{d}[1/T]$). Stiffer polymers have larger slopes. If the polymer is a stiff chain, the effect of temperature on exponent a is limited (Bohdanecky & Kovar, 1982; Rinaudo & Domard, 1989). Several research groups showed: the intrinsic viscosity of chitosan decreased with an increase in solution temperature; negative values obtained for chain flexibility; and chain flexibility enhanced with an increase in temperature (see Table 3) (Lee, 1974; Pogodina, et al., 1986; Trzciński et al., 2002). Chen and Tsaih (1998) demonstrated that the intrinsic viscosity of chitosans (with the same DA and ionic strength but different molecular weights: DA% = 17; $\mu = 0.01$; $M_\mathrm{w} = 78$–914) decreased linearly with an increase in solution temperature. Fig. 6 shows the exponent a versus temperature (between 10 and 50 °C) for three ranges of molecular weights ($M_\mathrm{w} = 78$–914; 78–223; and 223–914 kDa). These data indicates that exponent a increased linearly with an increase in solution temperature. There was a slight difference in their slope values. The average value of the slopes was 0.003. The Eq. (2) was corrected as follows:

$$\text{Exponent } a = [0.6202 + 0.699x/(0.4806 + x)] + [0.003(T - 20)] \qquad (10)$$

The Eq. (10) can be used to determine the exponent a at different temperatures range between 10 and 50 °C. The average temperature for the data presented in Table 1 was 20 °C. The later temperature was chosen as an average temperature for determination of the constants using Eqs. (2) and (3).

3.3.3. Effect of urea

The constants for chitosan in solution with ionic strength of 0.01 M HCl ($\mu = 0.01$) at 30 °C were found to be ($a = 0.715$ and $K = 5.48 \times 10^{-4}$), whereas in a chitosan solution with the same ionic strength and temperature but containing 4 M urea were found to be ($a = 1.004$ and

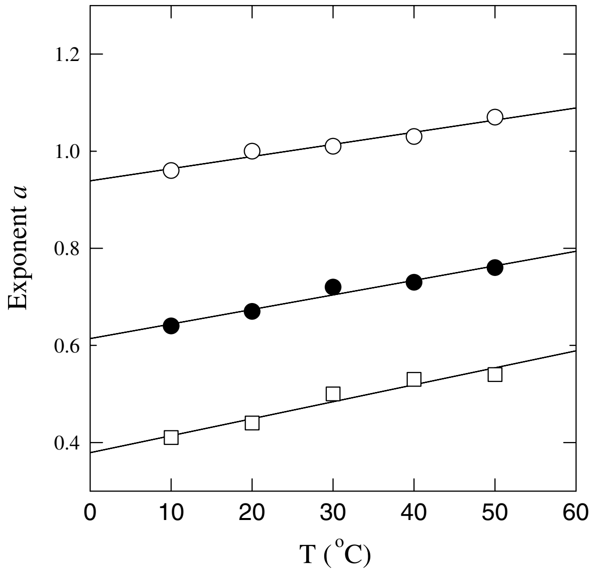


Fig. 6. Values of exponent *a* (reported by Chen and Tsaih (1998)) versus solution temperature (between 10 and 50 °C). Open circle symbols represent chitosan samples with molecular weight range of 78–223 kDa; full circle refers to chitosan samples with molecular weight range of 78–914 kDa; and open rectangular symbols represent chitosan samples with molecular range of 223–914 kDa.

$K = 1.91 \times 10^{-5}$) (Tsaih & Chen, 1997). The exponent *a* by addition of 2, 3, 4, and 6 M urea to chitosan solutions increased to the values of 0.839, 0.894, 1.00, and 1.060, respectively. The chain conformation becomes more extended or stiffer when the solution containing 4 M or more urea. When the concentration of urea was higher than 6 M, the solubility of chitosan rapidly decreased. Effects of urea concentration on chain conformation of chitosan can not be studied in a solution with urea concentrations higher than 6 M. This is due to the solubility limitation of chitosan. The intrinsic viscosity of chitosan in solution containing 4 M urea was larger than that of the solution without urea (Tsaih & Chen, 1997). Chitosan as a polyelectrolyte and copolymer with two functional groups, amine and *N*-acetyl groups, hydrogen bonds plays significant role in inter- and intra-molecular interactions in the solid state. For dissolution, solvents which induce either inter-chain repulsion or disturb intermolecular hydrogen bonding are required. Protonation of the amine groups of deacetylated repeat units of chitosan introduce inter-chain columbic repulsions promoting dissolution. External factors such as ionic strength and pH play an important role. Urea disturbs and breaks hydrogen bonds (the intra-molecular hydrogen bond) and yields in a chitosan molecule with an extended chain conformation and a larger hydrodynamic volume as well as a higher intrinsic viscosity. These results are consistent with results of Sklyar et al. (1981), and Watase, Nishinari, Williams, and Phillips (1990). Urea showed similar behavior (hydrogen bond breaking action) to agarose (Watase et al., 1990). Conformation of chitosan in solution depends on the degree of protonation (electrostatic repulsive force) and number of intra- and/or intermolecular hydrogen bonds formed. Intra-molecular hydrogen bonds occur between: (1) the CH_2OH group and the carbonyl of the acetyl group of two neighboring repeat units; and (2) between the OH group and the deoxy of the two neighboring segments (Tsaih & Chen, 1997).

Chen and Tsaih (2000) reported a linear relationship for the plot of exponent *a* versus urea concentration, C_{urea}, (1–6 M) with a slope of 0.06 for chitosans ($M_w = 78$–914 kDa) in 0.01 M HCl containing urea at 30 °C. A new term related to the effect urea ($0.06 \cdot C_{\text{urea}}$) can be added to Eq. (10) as follows:

$$\text{Exponent } a = [0.6202 + 0.699x/(0.4806 + x)] + [0.003(T - 20)] + 0.06 \cdot C_{\text{urea}} \qquad (11)$$

The value of C_{urea} equals to zero for the solutions without urea. Eq. (11) can be used to determine the exponent *a* for chitosans in solutions at different temperature, *T*, with or without urea. For the Eq. (11), it was assumed that the slope of exponent *a* versus urea concentration does not change: (1) in the range of 20–30 °C; and (2) as a function of ionic strength.

3.4. Comparison of various solvents/mobile phase used for determination of intrinsic viscosity, viscometric constants, and hydrodynamic volume, [η]M

For neutral polymers, the values of both constants, *a* and *K*, depend on the nature of polymer, the quality of solvent, the temperature, molecular weight range and molecular weight distribution, MWD. For a polyelectrolyte such as chitosan, the quality of solvent change with pH of chitosan solution and ionic strength of the solvent, μ. The polymer conformation and the polymer–solvent interactions depend on the number of positive charges NH_3^+ on chitosan, and it changes by varying pH and μ. In the previous report (Kasaai et al., 2000), it was shown that the conformation of chitosan as well as the exponent *a* changes with empirical function of ($DA/pH \cdot \mu$).

Solvents are classified based on the quality (good or poor) of solvent. There are strong interactions between polymer–solvent and weak between polymer–polymer chains in a good solvent, whereas the polymer–solvent interactions are weak in a poor solvent. A high value of intrinsic viscosity and exponent *a* are obtained in a good solvent. In the latter case, the polymer–solvent interactions are strong and the conformation of the polymer is extended. A low value of exponent *a* related to a random coil and compact conformation is obtained when a poor solvent is chosen for chitosan. A value of 0.5 for exponent *a* is known as a θ-condition for polymers. The θ-condition for chitosan has not been yet known.

Owing to the hydrophilic and polyelectrolyte nature, chitosan is soluble in a polar solvent. A short-chain organic (formic, acetic or propionic) acids with different concentrations or a buffer solution such as acetic acid/sodium acetate with various concentrations were used as solvents for chitosan. A mixture of a dilute organic acid and a dilute salt aqueous solution such as acetic acid/NaCl with various concentrations has been also used to study solution properties such as measurement of intrinsic viscosity and hydrodynamic volume of chitosan. The presence of salt such as sodium acetate in buffer solution or sodium chloride in dilute acidic aqueous solution weakens the interactions between polymer chains and allows better dissolution of the polymer. Low concentration of salt may not completely neutralized positive charges on chitosan resulting in large conformation as well as large value of exponent *a* for chitosan. High concentration of salt would yield strong interactions between polymer–polymer chains and results in a random coil or a compact conformation. To suppress the polyelectrolyte expansion in solution and the secondary polyelectrolyte effects such as ion exclusion, one may vary the ionic strength and/or pH of the solvent. Chitosan with p*K* value of 6.3 (Muzzarelli, 1977), at pH higher than 6; at high ionic strength; or at a salt concentration exceeds a limited value tend to precipitate.

An acetic acid buffer solution with an equal concentration of acetic acid and its salt (less than 0.25 M) or a mixture of acetic acid and NaCl with a concentration between 0.05 and 0.2 M can be considered as a good solvent for chitosan. The exponent *a* for such solvents should be greater than 0.7. Most of reported exponent *a* for chitosan is larger than 0.7 and it is between 0.7 and 1.0. These values are attributed to conformations from extended to linear chains. Chitosan molecule in a dilute acidic and very low salt concentration behaves as linear stiff and at high salt concentration behaves as random coil. The values of exponent *a* as low as 0.58 is attributed to a random coil conformation.

The pH of aqueous acidic solutions of chitosan containing urea slightly increase due to the consumption of H^+ in solutions through the hydrolysis of urea (Cho, Heuzey, Bégin, Pierre, & Carreau, 2006; Tsaih & Chen, 1997). The ionic strength of the solutions decreases by addition of urea to the solutions. Urea strongly affects polymer–polymer interactions by weakening hydrogen bonds. The reduction of ionic strength yields in a decrease in electrostatic repulsion between protonated glucosamine groups and a decrease in polymer–polymer hydrophobic interactions. The exponent *a* of chitosan with the same DA, but different weight-average molecular weights (78–914 kDa) in 0.01 M HCl containing different concentration of urea (0–6 M) was determined (Chen & Tsaih, 2000). The authors noted that the exponent *a* increased with an increase in concentration of urea. When solutions contained 0, 2, 3, 4, and 6 M urea, the value of exponent *a* increased from 0.715 to 0.894, 1.000, and 1.06, respectively. This indicates the urea-induced conformational changes.

Chitosan solution is stable in presence of various low-molecular weight molecules such as diluted aqueous salts; diluted acidic solutions or urea (as constituent of chitosan solvents) over a period of time. The viscosity, intrinsic viscosity or conformation of chitosan macromolecule changes with change in the media because the polymer–solvent interactions depend on the media. At above-mentioned conditions the size of macromolecule chains remain constant and chain scission can not be occurred (Sklyar et al., 1981). The change in viscosity or intrinsic viscosity is not due to the cleavage of glycoside bonds of the macromolecule chains in a diluted acidic solution.

3.5. Some important points to determine accurate values for viscometric constants

Mark–Houwink equation is used to determine viscosity-average molecular weight of polymers through the measurement of intrinsic viscosity. Intrinsic viscosity in a solvent is measured using a capillary viscometer. The accurate data for viscosity and intrinsic viscosity of polymer solution are obtained by using the following conditions: a capillary with small diameter (less than 1.0 mm) and very low inclination angle; and low concentration of chitosan. These conditions would yield negligible values for kinetic energy and shear rate (Chen, Lin, & Lin, 1994; Kasaai et al., 2000) . The intrinsic viscosity is determined by either Huggins or Kraemer plot and the equations provide a measure of polymer–solvent interactions in a given solvent. The average values of Huggins and Kraemer plots yields in more reliable data for intrinsic viscosity than that of individual plots.

The use of pullulan or dextran as standard polymer in HPLC/SEC on chitosan size characterization without construction universal calibration curve can lead to overestimate value for molecular weight of chitosan. This is because chitosan has more rigid backbone or extended chain conformation than that of the standard polymers (pullulan and dextran) and suggesting that the presence of secondary non-exclusion mechanisms in the separation. Thus, direct conversion of elution volume to molecular weight would give erroneous values, because identical molecular weights of two polymers with different molecular expansions eluted at different elution volumes. To determine accurate molecular weight by SEC, the standard polymer should have the same chemical structure, since hydrodynamic volume is directly related to the type of glycosidic linkages. Chitosan with α-D-(1, 4) glycoside linkages is a ploy-cationic polymer in nature. While pullulan consists of linear chains of D-glucopyranoyl units that alternate regularly between one α-D-(1, 6)- and two α-D-(1, 4)-glycoside linkages or a linear polymer of maltotriosyl units connected by α-D-(1, 6)- glycoside linkages, and it is a neutral glucan (Launay, Doublier, & Cuvelier, 1986). Dextran consists of α-D glucose units with a majority of α-(1–6) glucosidic linkages between them. A few percents of α-(1–3) glucosidic linkages provide side chains, which appear to be short (Nordmeier, Xing, & Lechner, 1993), and thus it is a branch polymer. In conclusion, three latter natural polymers have different chemical structure, and their conformations in identical solvent should be different. In such cases, the molecular weight values of chitosan should be reported as pullulan or dextran equivalents. Pullulan as standard polymer for chitosan size characterization is better than dextran, because pullulan is a linear polymer, whereas dextran is a branched polymer. Pullulan with a narrow distribution commercially is available. However, universal calibration curve based on hydrodynamic volume ($[\eta]M$) would yield reliable results (Crouzet, Martens, & Mangin, 1971 Grubisic, Rempp, & Benoit,1967; Grubisic et al., 1967; Rudin & Hoegy, 1972). The size characterization of unknown polymer can be performed under the same experimental conditions for both chitosan as polyelectrolyte and pullulan or dextran as uncharged polymer. Now, chitosan with a narrow polydispersity as standard polymer is commercially available (American Polymer Standards Corporation, Mentor, Ohio, USA) and can be used for evaluation of average-molecular weight and MWD of unknown chitosan samples. Lower hydrodynamic volume of chitosan macromolecule in an acetic buffer or a mixture of acid with a higher concentration of salt as a mobile phase in SEC/HPLC enables one to analyze chitosan samples with a higher average-molecular weight.

4. Conclusions

Two equations as a model, i.e. exponent a versus empirical function, $[\mathrm{DA}/(\mathrm{pH}\cdot\mu)]$; and log K versus exponent a for chitosan was proposed using the experimental reported data. This study resulted in the following equations for determination of exponent a:

$$\text{Exponent } a = 0.6202 + 0.699x/(0.4806 + x)$$

where x equals to $[\mathrm{DA}/\mathrm{pH}\cdot\mu]$, with DA, degree of acetylation, pH of chitosan solution in the solvent with ionic strength of μ, and a linear plot was obtained between log K and exponent a.

$$\log K \cdot 10^{-5} = -5.7676 \cdot \text{Exponent } a + 5.9232$$

The validity of prediction of the constants from the knowledge of the DA, pH and ionic strength were examined using the above equations. The constants were obtained by a deviation in the range of ±0.40% to 29.0% for the exponent a, and ±6% to 71% for most of K values. The deviation of experimental and calculation data is reasonable and it is because several factors affecting the correct values for the constants were not taken into consideration by authors whose reported experimental data. The effects of temperature and concentration of urea have been taken into consideration and two related correction factors were added to the right side of Eq. (2) as follows:

$$\text{Exponent } a = [0.6202 + 0.699x/(0.4806 + x)] + [0.003(T - 20)] + 0.06 \cdot C_{\mathrm{urea}}$$

The value of C_{urea} equals to zero for the solutions without urea. Eq. (11) can be used to determine the exponent a for chitosans in solutions at different temperature, T, with or without urea. For the Eq. (11), it was assumed that the slope of exponent a versus urea concentration does not change: (1) in the range of 20–30 °C; and (2) as a function of ionic strength.

The author of this manuscript described the various parameters affecting the constants. The procedures to determine the correct values of the constants for chitosan were also explained. The author of this study introduced an empirical function for chitin/ chitosan chain conformation in solution, ($\mathrm{DA}/\mu\cdot\mathrm{pH}$), as a measure of chain stiffness or chain flexibility. The value of empirical function increased with an increase in DA and a decrease in μ of solvent and pH of solution. The quality of different solvents was compared using the latter empirical parameter. The empirical function was also compared with other chain conformation parameters: B-parameter defined by Smidsrørd & Haug as chain stiffness of polyelectrolytes; $\mathrm{d}\ln[\eta]/\mathrm{d}T$; and $\mathrm{d}\ln[\eta]/\mathrm{d}(1/T)$.

Most of reported values for exponent a were between 0.7 and 1.0, suggesting that chitosan behaves like a flexible, extended or linear conformation in the solvents depending on the value of a. Among various reported solvents (acetic acid; buffer of acetic acid; a mixture of acetic acid and sodium chloride with or without urea), a buffer of acetic acid or a mixture of acetic acid/ sodium chloride with ionic strength between 0.05 and 0.2 have advantages over other solvents because of complete dissolution of the polymer, reduce sufficiently intra- and intermolecular interactions between macromolecule chains and no affecting the chemical structure and macromolecular chains (no degradation in main and side chains).

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