

Intrinsic Viscosity–Molecular Weight Relationship and Hydrodynamic Volume for Pullulan

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ABSTRACT: A numerical method for determination of Mark–Houwink–Sakurada (MHS) equation constants, a and K , was tested with success for two series of pullulan standard samples having narrow and broad molecular weight distributions (MWD) and taken into account their polydispersity. Different solvents, which were used to determine the intrinsic viscosities and the viscometric constants, a and K (published in the literature for pullulan), were compared. The various parameters affecting the constants are discussed. The procedure to determine the correct value of the hydrodynamic volume for pullulan was also described. This study resulted in the following MHS equations for narrow and broad MWD series of pullulan samples with M_w in the range of 5–1000 kDa:

$$[\eta] = 1.990 \times 10^{-4} M_w^{0.667} = 1.990 \times 10^{-4} q_{\text{MHS}} M_w^{0.667} \\ = 1.956 \times 10^{-4} M_w^{0.667} \text{ (Narrow MWD)}$$

$$[\eta] = 2.263 \times 10^{-4} M_w^{0.657} = 2.263 \times 10^{-4} q_{\text{MHS}} M_w^{0.657} \\ = 2.056 \times 10^{-4} M_w^{0.657} \text{ (Broad MWD)}$$

where q_{MHS} is the polydispersity correction factor and $[\eta]$ is the intrinsic viscosity in dL g^{-1} . The plot of $\log K$ versus exponent a was linear and inversely related. This curve was used to estimate the constant K for pullulan with a known exponent a . Among various reported solvents, the diluted aqueous salt solutions have more advantages than other solvents. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 4325–4332, 2006

Key words: pullulan; viscometric constants; intrinsic viscosity; molecular weight; polydispersity

INTRODUCTION

Pullulan, a neutral glucan, is an extracellular polysaccharide produced by the fungus *Aureobasidium pullulans* (*Pullularia pullulans*). It consists of linear chains of D-glucopyranosyl units that alternate regularly between one α -D-1,6- and two α -D-(1,4) glycoside linkages, or it can be in the form of a linear polymer of maltotriosyl units connected by α -D-1,6-glycoside linkages.^{1,2}

Pullulan is insoluble in methanol and acetone, but it dissolves in water and forms a transparent, viscous adhesive solution. Pullulan and its derivatives exhibit film- and fiber-forming properties. The film is non-toxic, biodegradable, and edible.^{3,4} The polymer is used for personal-care products, such as hand and facial lotions, shampoos, and cosmetics.⁵ Because of its analogous chemical structure to linear polysaccharides, a narrow molecular weight distribution (MWD) pullulan is used as the standard in size-exclusion chromatography (SEC)^{6–11} for linear polysaccharides. It may be also useful as standard sample for studies of

water-soluble polymers. The usefulness and applications of the polymer depends on its molecular weight. With knowledge on polymer conformation and molecular weight value, many properties, such as mechanical and rheological properties, can be predicted. But determining the average molecular weights (M_n , M_w , and M_z) of the polymer is time consuming. Among them, the viscometry method is relatively quick and simple, requiring easy-to-use apparatus.

The determination of viscosity–average molecular weight, M_v , requires measurement of intrinsic viscosity of the polymer, $[\eta]$, in a solvent with known Mark–Houwink–Sakurada (MHS) equation constants, a and K . The value of $[\eta]$ varies with M_v for a homologous series according to^{12–14}:

$$[\eta] = K M_v^a \quad (1)$$

Accurate values for the constants are determined when all parameters affecting them are taken into consideration. The value of M_v , which cannot be obtained experimentally, can be calculated, once the values of the constants are determined. The correct value of hydrodynamic volume, which is critically important to construct universal calibration curve of SEC, can be determined using the value of M_v . Viscometric

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TABLE I
 M_n , M_w , and M_z of Pullulan Samples Provided by American Polymer Standard Corporation

Sample	Narrow MWD			Broad MWD			
	M_n (kDa)	M_w (kDa)	$[\eta]$ (dL g ⁻¹)	M_n (kDa)	M_w (kDa)	M_z (kDa)	$[\eta]$ (dL g ⁻¹)
1	640.6	788.0	1.676	368.0	959.2	1789	1.898
2	357.5	404.0	1.075	388.0	925.8	1701	1.808
3	164.0	212.0	0.700	46.20	120.0	215.0	0.379
4	100.0	112.0	0.458	45.20	114.9	178.6	0.467
5	44.6	47.3	0.258	39.20	98.20	215.0	0.342
6	21.3	22.8	0.159	12.40	30.80	52.80	0.224
7	10.7	11.8	0.103				
8	5.4	5.9	0.065				

constants for pullulan have been reported in various solvents. In all reports, different average molecular weights (M_p , M_n , M_w , and M_z), instead of viscosity-average molecular weight, M_v , were substituted in eq. (1).

The objectives of this work were to determine the values of K and a , for pullulan samples with both narrow and broad MWD, taking into account the polydispersity of the polymer using a numerical method; to compare various solvents used by several researchers to determine intrinsic viscosity; to discuss the parameters affecting the constants; to determine viscosity-average molecular weight, M_v , using other weight-average molecular weights (M_n , M_w , M_z) and intrinsic viscosity data; and to describe the procedure to determine correct value of hydrodynamic volume.

METHODS

Materials

Pullulan standards with a broad ($30 \leq M_w \leq 960$ kDa; $2.39 \leq M_w/M_n \leq 2.61$) and narrow ($6 \leq M_w \leq 788$ kDa; $1.06 \leq M_w/M_n \leq 1.29$) molecular weight distributions provided by the American Polymer Standards Corp. (Mentor, OH) were used in this study. The intrinsic viscosities of the polymer samples in 0.05M Na₂SO₄ aqueous solutions at 30°C were measured by the supplier. The average molecular weights (M_n , M_w , M_z), and intrinsic viscosities of the polymer samples provided by the supplier are listed in Table I.

Calculation of polydispersity correction factor, q_{mhs}

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. Equation (1) can be rearranged and resulted in a modified MHS equation as follows:

$$[\eta] = KM_v^a = K(M_v/M_w)^a M_w^a = K_{q_{MHS}} M_w^a \quad (2)$$

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 MWD. It is a measure of the width of MWD as well as probability of MWD curve. It can be calculated using M_w , MWD (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 to¹⁵⁻¹⁷:

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

where b and c are empirical polynomial functions of MHS exponent a . Constant c depends only on a according to¹⁷⁻¹⁹:

$$c = 0.113957 - 0.844597a + 0.730956a^2 \quad (4)$$

Constant b depends on a and $(M_z/M_w)^{17}$:

$$b = k_1 + k_2[(M_z/M_w) - 1]^{k_3} \quad (5)$$

where constants k_1 , k_2 , and k_3 depend on a through the following least-square polynomials:

$$k_1 = 0.048663 - 0.265996a + 0.364119a^2 - 0.146682a^3 \quad (6)$$

$$k_2 = -0.096601 + 0.181030a - 0.084709a^2 \quad (7)$$

$$k_3 = -0.252499 + 2.31988a - 0.889977a^2 \quad (8)$$

The numerical coefficients have been already calculated empirically¹⁷ for eqs. (6)–(8). Thus, the calculation of viscometric constants using the numerical method involves several consecutive steps [calculation of numerical coefficients of eqs. (6)–(8); calculation of b

TABLE II
Polydispersity Correction Factor, q_{MHS} , M_v , and $[\eta]M_v$ for Each Polymer Sample

Sample	Narrow MWD			Broad MWD		
	q_{MHS}	M_v (g mol ⁻¹)	$[\eta]M_v$ (dL mol ⁻¹)	q_{MHS}	M_v (g mol ⁻¹)	$[\eta]M_v$ (dL mol ⁻¹)
1	0.972	767,695	1,286,657	0.90	937,587	1,779,540
2	0.983	394,489	424,076	0.91	870,762	1,574,338
3	0.966	207,352	145,147	0.91	80,739	30,600
4	0.985	109,773	50,276	0.93	110,944	51,811
5	0.992	46,432	11,979	0.88	69,053	23,616
6	0.991	22,472	3,573	0.92	36,262	8,123
7	0.987	11,720	1,207			
8	0.988	5,877	3,820			

and c ; calculation of polydispersity correction factor; and calculation of the constants]. The precision of the output from a numerical function depends on the precision of the input and progressively degrades as a result of round off error. The precision of an approximate real number is the number of decimal digits. Therefore, to prevent small errors from rounding at each step to affecting the final result, it is necessary to retain additional digits of significant figures for intermediate steps. When the numerical precision increases, these errors become correspondingly smaller. Accuracy of final result of calculation depends on whether additional digits are chosen for each preceding step of calculations.²⁰ The number of digits is adjusted during calculations. Therefore, six decimal digits were chosen for three preceding steps of the constants calculations [eqs. (6)–(8)], to retain three decimal digits for the final results (i.e., the viscometric constants).

The calculation of q_{MHS} requires prior knowledge of the exponent a . To circumvent the difficulty, an iterative procedure was used. A detailed description on the procedure appeared elsewhere.²¹

RESULTS AND DISCUSSION

Viscometric constants

The polydispersity correction factor, q_{MHS} , for pullulan samples with wide MWD were calculated and are given in Table II. The plot of $(\log [\eta] - \log q_{\text{MHS}})$ versus $\log M_w$, is shown in Figure 1. This plot yielded a straight line whose slope and intercept provided the constants a and K , respectively. The constants can be used to determine M_v (Table II) by using eq. (1). The resulting modified MHS equation for pullulan in the M_w range of 3.0×10^4 – 1.0×10^6 Da and average value of 0.91 for q_{MHS} was:

$$[\eta] = 2.263 \times 10^{-4} M_w^{0.657} = 2.263 \times 10^{-4} q_{\text{MHS}} M_w^{0.657} \\ = 2.056 \times 10^{-4} M_w^{0.657} \text{ (Broad MWD).} \quad (9)$$

where $[\eta]$ is expressed in dL g⁻¹.

Number-average molecular weights, M_z , are not available for narrow MWD series. The polydispersity correction factor, q_{MHS} , for this series was calculated by assuming the following equation:

$$M_z/M_w = M_w/M_n. \quad (10)$$

This equation is valid for a Gaussian concentration elution curve. Such curve is usually obtained for narrow MWD polymers. The values of q_{MHS} and M_v are listed in Table II. Figure 2 shows the plot of $(\log [\eta] - \log q_{\text{MHS}})$ versus $\log M_w$. The following modified MHS equation ($q_{\text{MHS}} = 0.983$) was obtained:

$$[\eta] = 1.990 \times 10^{-4} M_w^{0.667} = 1.990 \times 10^{-4} q_{\text{MHS}} M_w^{0.667} \\ = 1.956 \times 10^{-4} M_w^{0.667} \text{ (Narrow MWD).} \quad (11)$$

The value of exponent a suggests that pullulan behaves as a flexible random coil in 0.05M Na₂SO₄. The

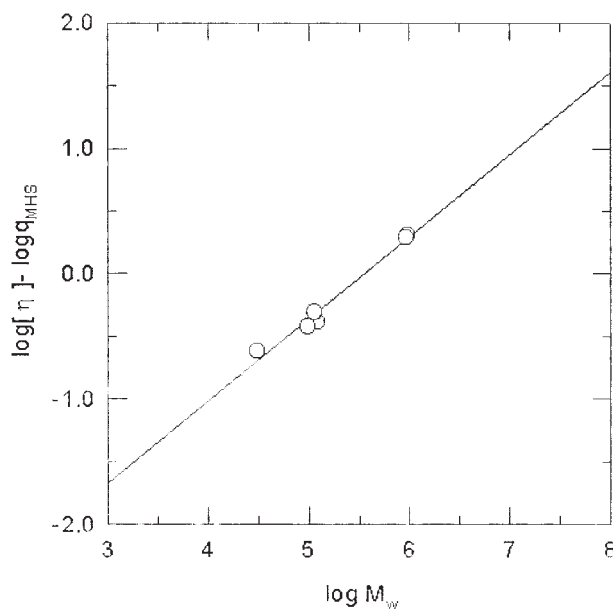


Figure 1 $(\log [\eta] - \log q_{\text{MHS}})$ as a function of $\log M_w$ for broad MWD pullulan samples in 0.05M Na₂SO₄ at 30°C.

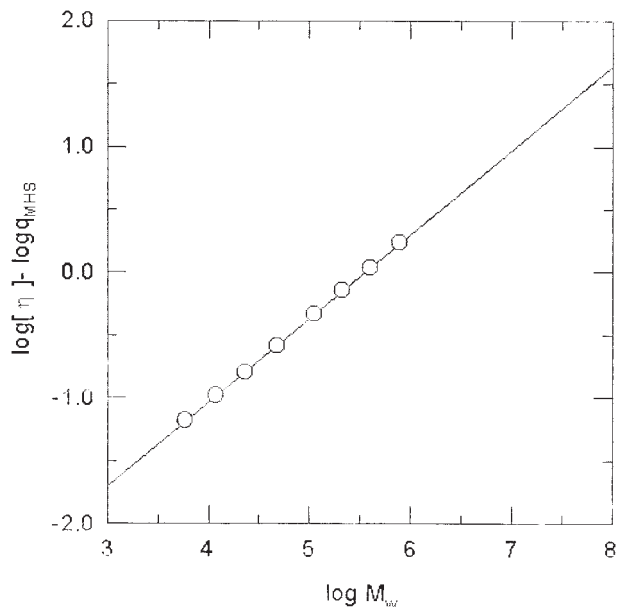


Figure 2 ($\log [\eta] - \log q_{\text{MHS}}$) as a function of $\log M_w$ for narrow MWD pullulan samples in 0.05M Na_2SO_4 at 30°C.

supplier²³ reported the values ($a = 0.665$ and $K = 2.01 \times 10^{-4}$) for both narrow and broad MWD. The disparity between the values obtained from this work ($a = 0.657$, and $K = 2.263 \times 10^{-4}$) for wide MWD series and the values provided by supplier is due to substituting M_w instead of M_v and ignoring polydispersity effects; in the narrow MWD series, the constants obtained from this work ($a = 0.667$, and $K = 1.990 \times 10^{-4}$) and the values provided by supplier are identical. This work shows that the numerical method is valid and can be used to determine viscometric constants for both narrow and broad MWD. In addition, the use of the polydispersity correction factor results in accurate values for viscometric constants.

The plots of ($\log [\eta] - \log q_{\text{MHS}}$) versus $\log M_w$ were linear for both narrow ($R^2 = 0.999$) and broad ($R^2 = 0.978$) MWD. The values of linear regression, which are close to unity, indicate that the plots are quite linear in the experimental molecular weight ranges, and all of the experimental points, including the highest and lowest molecular weights points, lie at or close to the straight lines.

Comparison of various solvents used to determine intrinsic viscosity and hydrodynamic volume

Owing to the hydrophilic nature, pullulan is soluble in polar, hydrogen-bonded solvents such as water and aqueous salt solutions. There are weak interactions between the polymer chains, which result in its high solubility in the solvents. Solvents are classified as good or poor on the basis of the polymers dissolution in dilute solutions. The intrinsic viscosity of polymer

solution and exponent a change with the quality of solvent.^{21,22} A high value of intrinsic viscosity and exponent a indicate that the secondary forces between the polymer and the solvent molecule are strong, in other words, a better solvent.

Most of the solvents used to determine the intrinsic viscosity of pullulan consisted of water, aqueous salt and buffer solutions. The presence of salts in aqueous solutions weakens the interactions between polymer chains and allows better and facile solubility.²² Most of the solvents used to determine intrinsic viscosity for pullulan (Table III) are thermodynamically similar due to nearly identical values of exponent a (between 0.65 and 0.67). This data shows that the polymer in different solvents have similar polymer-solvent interactions. The values of exponent a for this polymer (0.65–0.67) are relatively low compared with that of most of the linear polysaccharides. This is attributed to both the flexibility and the neutral nature of the polymer. The low values should not be attributed to the poor quality of the solvents. In general, polysaccharides having α -D-1,4 linkages have a more extended conformation in aqueous solutions than those with α -D-1,6-glycoside linkages. The value of 0.83 is equivalent to an overestimated value of exponent a , due to the same reason indicated earlier. Kumar et al.²⁴ reported a value of 0.45 for exponent a , in 5% NaH_2PO_4 /3% CH_3CN , which is a small value and can be attributed to the poor quality of the solvent.

Pullulan is used as a standard in SEC, to determine the average molecular weights (M_n , M_w , and M_z) and MWD, by constructing a universal calibration curve. Till now, there is no commercially available β -glucans or other similar polysaccharide standards that are comparable with pullulan with respect to the narrow polydispersity. Generally, to have a good resolution as a function of molecular size, an appropriate solvent should be selected for the investigated polymers (polysaccharides or water-soluble polymers). Pullulan both as a standard and investigated polymer must be dissolved in the same solvent. The solute may be neutral or ionic (cationic and anionic). Several solvents are used in the mobile phase to prepare solutions of polysaccharides and water-soluble polymers. The solvent may be water, an aqueous salt, acid, base, or a buffer solution. Weak acids, bases, or buffers of weak acids or bases are considered to be appropriate solvents for pullulan. Appropriate conditions to determine size-exclusion chromatograms and hydrodynamic volumes are the latter solvents; low concentration of salt or acid in the solvents; low polymer concentration; and quick preparations of polymer solutions for measurements of their viscosity. This is due to the fact that the hydrolysis of glycoside linkages should be negligible at the aforementioned conditions. The viscosity of a dilute polymer solution decreases after a long-term storage due to either a slight chain

TABLE III
MHS Equation Constants for Pullulan at Different Solvents

Solvent	T (°C)	$K \times 10^4$ (dL g ⁻¹)	a	Molecular weight range (kDa)	References
H ₂ O	25	2.36	0.658	2.8–1238	32
0.1M NaCl	25	1.79	0.67	48–1500	10
H ₂ O	25	1.91	0.67	48–2180	31
0.05M Na ₂ SO ₄	30	2.01	0.665	5.8–1660	23
0.15M NaCl	35	2.31	0.65	5.8–853	34
H ₂ O	25	2.21	0.66	107–2360	30
0.05M Na ₂ SO ₄	30	2.263	0.657	31–959	This work
0.05M Na ₂ SO ₄	30	1.990	0.667	5.9–788	This work
5% NaH ₂ PO ₄ /3%CH ₃ CN	25	24.66	0.454	5.9–112	24
NaH ₂ P ₄ /Na ₂ HP ₄ (pH 7.0)	25	2.76	0.65	5.9–1600	9
H ₂ O	20	2.0	0.678	20–800	8
H ₂ O	25		0.65	5.3–1015	33
			0.83		29

degradation or a change in interactions between polymer chains. The degradation process depends on concentrations of the solvent and polymer, temperature, and the time of contact between the polymer and solvent.²²

Universal calibration curve

Grubisic et al.²⁵ constructed a universal calibration curve by plotting hydrodynamic volume, V_h , versus retention volume, V_e . These authors calculated hydrodynamic volume by using the product of weight-average molecular weight and intrinsic viscosity, $[\eta]M_w$. Hamielec and Ouano²⁶ determined the latter parameter by using the product of number-average molecular weight and intrinsic viscosity, $[\eta]M_n$. Hydrodynamic volume is actually proportional to the product of intrinsic viscosity and viscosity-average molecular weight, $[\eta]M_v$. Inaccurate data for the product is obtained, when M_n or M_w is used instead of M_v . The hydrodynamic volume of pullulan in θ and good solvents can be determined as follows^{27,28}:

$$V_h(\theta) = (R_\theta^2)^{3/2} = [\eta]M_v / \phi_0 \text{ (}\theta \text{ solvent)} \quad (12)$$

$$V_h = (R^2)^{3/2} = [\eta]M_v / \phi \text{ (good solvent)} \quad (13)$$

where ϕ_0 is a universal constant and is equal to $2.84 \times 10^{21} \text{ mol}^{-1}$ (with $[\eta]$ in dL g⁻¹); $(R_\theta^2)^{1/2}$ and $(R^2)^{1/2}$ are root mean square hydrodynamic radii of the polymer chains in θ and good solvents, respectively; and ϕ is a function related to the hydrodynamic behavior of the polymer and is called hydrodynamic factor, or Flory's parameter. It is a constant for a particular solvent at a given temperature, and it depends on the exponent a of the MHS equation as well as the solvent quality. A correct SEC universal calibration curve is obtained by constructing $[\eta]M_v / \phi$ versus elution volume instead of $[\eta]M_w$ versus elution volume. The con-

struction of the former curve ($[\eta]M_v / \phi$ versus elution volume) yields an accurate and more reliable data to determine the molecular weights (M_n , M_v , M_w , M_z), MWD, and the constants of other polymers. To determine the accurate value of hydrodynamic volume, prior knowledge on ϕ is needed. It may be calculated from the following equation:

$$\phi = [\eta]M_v / (R^2)^{3/2} \quad (14)$$

The value of $(R^2)^{1/2}$ can be determined by light scattering; the value of $[\eta]$ for different polymer samples is measured in the same solvent and temperature; and M_v is determined according to the procedure described in this article (see Viscometric Constants section). To accurately measure the molecular weight of polysaccharides by SEC, a polymer standard with the same chemical structure of unknown sample should be used, since the hydrodynamic volume as well as the molecular size is directly related to the type of glycosidic linkages.

Comparison of viscometric constants available in the literature

The values of MHS equation constants, a and K , published in the literature for pullulan^{8–10,23–24,29–34} are presented in Table III. The values of a range from 0.45 to 0.83 and K range from 1.79×10^{-4} to 24.66×10^{-4} . The smaller values of exponent a , correspond to the greater values for K and vice versa. The quality of the solvent is represented by exponent a .^{21,22} Nishinari et al.³³ reported that pullulan, in water, behaves like a random coil. Kato et al.³¹ concluded that the polysaccharide chains with α -1,6-glucosidic linkages behave like a flexible chain in aqueous solution. Dondos and Papanagopoulos²⁹ reported a value of 0.83 for exponent a , compared to most of the reported values of 0.65–0.67. A cursory evaluation would reveal that a

value of 0.83 corresponds to an extended conformation. The values of 0.65–0.67 indicate that pullulan molecules behave like a flexible random coil in most of the solvents. Owing to the neutral nature and flexible chain conformation for pullulan, the values of 0.65–0.67 are more reliable results than the 0.83 value.

Pullulan is a suitable polymer standard because of the neutral nature of the polymer. Since pullulan is a nonionic polysaccharide, ionic strength does not change its intrinsic viscosity and hydrodynamic volume. The interaction between pullulan as a solute with packing materials does not vary with ionic strength and pH of the mobile phase. Dubin and Principi³⁵ showed that pH and ionic strength do not affect the hydrodynamic properties of pullulan. Guo et al.⁹ reported that intrinsic viscosities of a pullulan sample in 0.1, 0.5, and 2M phosphate buffer solutions were nearly identical. This is understandable, because of the neutral nature of the polymer. Generally, two types of materials, silica- and polymer-based, are used as packing materials in SEC column for determination of molecular weight and MWD of polymers. If silica-based materials were used to separate biopolymers such as pullulan, there should be interaction between silanol groups of silica-based packing materials and biopolymers. The interaction could perturb the validity of the calibration curve for SEC process, and thus, may result in deviation for molecular weight data. Generally, silica-based packing materials are chemically modified to remove the effect of the silanol groups, which tend to have a negative effect on biopolymer separations.³⁶ The polymer-based packaging materials have advantages over silica-based materials because of lack or negligible negative effect on biopolymer separations.

Figure 3 shows the plot of $\log K$ versus a . An inverse linear relationship was observed. This result is not surprising and is similar to what one expects from MHS equation. But from the slope of the plot some conclusions are deduced. The slope obtained from Figure 3 was 5.08. The slopes of the plots for flexible polymers were reported to be between -3.9 and -4.9 , depending on the polymers.³⁷ The slope for cellulose²² having linear conformation was -5.47 and for chitosan²¹ as a polyelectrolyte with relatively extended conformation was -6.1 . These data for the slope suggest that the slope of the plot depends on the quality of polymer–solvent interactions and the size of hydrodynamic volume. The greater value of the slope indicates a higher level of polymer–solvent interactions and larger hydrodynamic volume for the polymers in solution. The slope obtained from Figure 3 (-5.08) is higher than the values reported for synthetic flexible polymers and smaller than the values reported for cellulose and chitosan, suggesting that: (1) the polymer–solvent interaction for pullulan is stronger than that of flexible polymers and weaker than that of

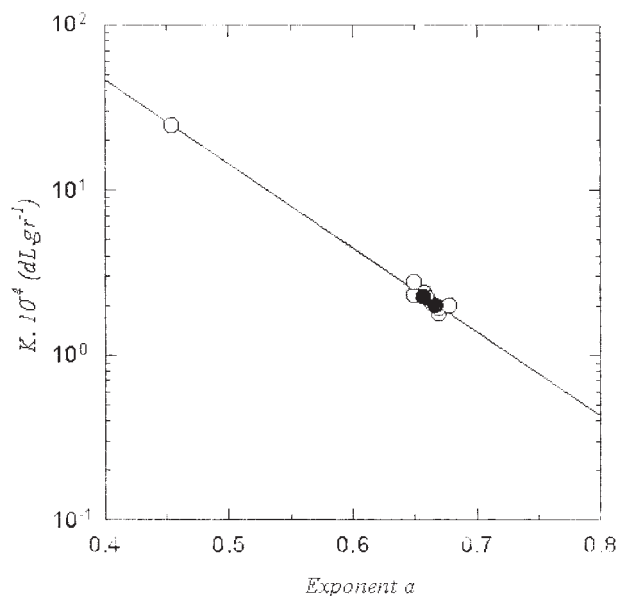


Figure 3 The value of K versus exponent a . Open symbols represents literature results and filled symbol refers to this work.

chitosan and cellulose; and (2) the conformation of pullulan is between synthetic polymers and the two natural polysaccharides (cellulose and chitosan), i.e., less flexible than synthetic polymers and less extended than the two polysaccharides.

This plot can be used to estimate the constant K for a polymer in a solvent if the exponent a in the solvent is known and vice versa. For example, Dondos and Papanagopoulos²⁹ and Nishinari et al.³³ reported values of 0.83 and 0.65 for exponent a , respectively. The corresponding values of K are estimated to be 3.03×10^{-5} and 2.49×10^{-4} , respectively. In addition, this curve can be used to determine whether or not any pair constant values lie on the plot of $\log K$ versus a . In other words, one can identify the suitable solvents among various ones. A large deviation from the linear relationship means that the solvent is unsuitable and these pair constant values (K and a) should be discarded.

Factors affecting the constants

Generally, the values of both a and K depend on the nature of polymer, the solvent, the temperature, and the molecular weight range.^{21,22} The values of both constants depend on MWD.^{38,39} However, the value of K is more sensitive to MWD.

Various average molecular weights (M_p , M_n , M_w , and M_z) other than M_v were substituted in MHS equation while ignoring the effect of polydispersity. The value of M_v is different from M_p , M_w , and M_z in a polydisperse polymer sample.^{21,22}

The values of K and a may vary with molecular weight range, since the flexibility of the polymer conformation may change with its size.²¹ Over a very wide range of molecular weights, neither K nor a is constant. A detailed explanation for determination of the constants in a wide molecular weight range appeared elsewhere.²¹ Kato et al.³¹ concluded that the slope of $[\eta]$ versus M_w decreased, as molecular weight of pullulan decreased. Kawahara et al.⁸ reported a higher value for exponent a in water when molecular weight was above 2×10^{-4} (between 2×10^{-4} and 5×10^{-5}) compared to molecular weights in the range of 5×10^{-3} – 2×10^4 .

The constants vary with temperature.²¹ In a good solvent, a temperature increase should result 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 will cause an increase in the intrinsic viscosity, and an increase in entropy, and it is favorable for the extended conformation. Water and aqueous salts solutions are good solvents for pullulan; an increase in temperature results in a less-extended conformation and a negative value for temperature correction factor,²¹ $d[\eta]/dT$. However, the applied range of temperature (25–35°C) was narrow, and thus, thermal effect on the hydrodynamic volume should be insignificant.

CONCLUSIONS

The viscometric constants, a and K , for pullulan standard samples with M_w in the range of 5–1000 kDa, having both narrow and broad MWD were determined in 0.05M Na₂SO₄ at 30°C, taking into account the polydispersity of the polymer. This study resulted in the following MHS equations for both narrow ($q_{MH} = 0.98$) and broad ($q_{MH} = 0.91$) MWD series of pullulan as follows:

$$[\eta] = 1.990 \times 10^{-4} M_w^{0.667} = 1.990 \times 10^{-4} q_{MHS} M_w^{0.667} \\ = 1.956 \times 10^{-4} M_w^{0.667} \text{ (Narrow MWD)}$$

$$[\eta] = 2.263 \times 10^{-4} M_w^{0.657} = 2.263 \times 10^{-4} q_{MHS} M_w^{0.657} \\ = 2.056 \times 10^{-4} M_w^{0.657} \text{ (Broad MWD)}$$

The value of exponent a , suggests that pullulan behaves like a flexible random coil in this solvent. The plot of $\log K$ versus exponent a was linear and inversely related. The plot was used to estimate the constant K for pullulan with a known exponent a . Among various reported solvents (water, aqueous salt and buffer solutions), aqueous salt-diluted solutions have advantages over other solvents because of a complete and fast dissolution of the polymer without a

reduction in its intrinsic viscosity. This work shows the procedure for determination of viscosity-average molecular weight, M_v , using other weight-average molecular weights (M_n , M_w , M_z), and intrinsic viscosity data. The procedure to determine the correct value of hydrodynamic volume of the polymer was also described and its value was compared with those proposed by other researchers. It is desirable to obtain a SEC universal calibration curve, $[\eta]M_v/\phi$ versus elution volume instead of $[\eta]M_v$ versus elution volume. The construction of the former curve ($[\eta]M_v/\phi$ versus elution volume) yields more accurate and reliable data of the molecular weights (M_n , M_v , M_w , M_z), MWD, and the constants for other polymers than that of $[\eta]M_v$ versus elution volume. This needs prior knowledge on ϕ , which may be calculated from the following equation:

$$\phi = [\eta]M_v / (R^2)^{3/2}$$

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