



Short communication

A novel method to estimate the stiffness of carbohydrate polyelectrolyte polymers based on the ionic strength dependence of zeta potential

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ABSTRACT

Polysaccharides have received a great deal of attention from, for example, the food, cosmetic and pharmaceutical industries. Their conformations (flexibility/stiffness) span a wide range of conformational flexibilities with large hydrated volumes, these properties are important in relation to polysaccharide structure–function relationships.

Perhaps the simplest parameter available to estimate the dilute solution conformation of polysaccharides is the Smidsrød–Haug stiffness parameter (B) where the stiffness of polyelectrolytes can be estimated by measuring the intrinsic viscosity at a number of different ionic strengths.

In this paper we propose an alternative method for estimating the Smidsrød–Haug stiffness parameter (B) using the ionic strength dependency of zeta potential. For this purpose we have studied a number of different polysaccharides.

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1. Introduction

Polysaccharides have received a great deal of attention from, for example, the food, cosmetic and pharmaceutical industries. Their conformations (flexibility/stiffness) have been characterised using a variety of hydrodynamic techniques including sedimentation velocity, size exclusion chromatography–multi-angle light scattering (SEC–MALS) and viscometry. Results have shown that polysaccharides span a wide range of sizes and conformational flexibilities with large hydrated volumes, these properties are important in determining polysaccharide structure–function relationships.

Perhaps the simplest parameter available to estimate the dilute solution conformation of polysaccharides is the Smidsrød–Haug stiffness parameter (B). This is a simple conformational parameter based on the intrinsic viscosity; however it is only applicable to polyelectrolytes. In brief, the stiffness of polyelectrolytes can be estimated by measuring the intrinsic viscosity at a number of different ionic strengths and then extrapolation to infinite ionic strength as shown in Eq. (1) (Pals & Hermans, 1952):

$$[\eta] = [\eta]_{\infty} + (S)^{1/2} \quad (1)$$

where $[\eta]_{\infty}$ is the intrinsic viscosity at infinite ionic strength and S is stiffness parameter which can be used to estimate the conformation of different polyelectrolyte polymers, with the constraint however, that they be of the same molar mass and in identical solvent conditions. Smidsrød and Haug (1971) suggested a new parameter (B) which removed these restrictions by comparing the intrinsic viscosity at a fixed ionic strength (typically 0.1 M) and B is defined in Eq. (2):

$$S = B([\eta]_{0.1\text{ M}})^{\nu} \quad (2)$$

where ν has been shown experimentally to be approximately 1.3 ± 0.1 . Therefore B can be estimated from a plot of $[\eta]$ versus $I^{-1/2}$.

Furthermore in addition to assessing the screening of charges on a polyelectrolyte by measuring intrinsic viscosity at a number of different ionic strengths, it is also possible to measure the zeta potential of polyelectrolyte under the same conditions. The zeta potential is defined as the electrical potential at the hydrodynamic shear plane of charged particle with respect to the bulk solvent (McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008). When a small amount of electrolyte is added to a biopolymer solution it does not greatly affect the solvation of the biopolymer and therefore it is probable that the reduction in viscosity

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due to the electroviscous effect may be due to decreased potential at the electrical double layer and hence a decrease in zeta potential (Masuelli & Sansone, 2012). This is demonstrated in Eq. (3) (Masuelli & Sansone, 2012; Rubio-Hernandez, Carrique, & Ruiz-Reina, 2004; Rubio-Hernandez, Gomez-Merino, Ruiz-Reina, & Carnero-Ruiz, 1998).

$$\eta_{sp} = \nu\phi \left[1 + \frac{1}{\eta_0 \kappa R_H^2} \left(\frac{\epsilon_r \epsilon_0 \zeta}{2\pi} \right)^2 \right] \quad (3)$$

where ν is Einstein shape factor, which has a theoretical minimum of 2.5 for a compact sphere (Einstein, 1911), ϕ is the volume fraction, η_0 is the viscosity of the solvent, κ is the thickness of the electrical double layer, R_H is the hydrodynamic radius, ϵ_r is the dielectric constant of the liquid medium, ϵ_0 is the vacuum permittivity and ζ is the zeta potential.

In this paper we propose an alternative method for estimating the Smidsrød–Haug stiffness parameter (B) using the ionic strength dependency of zeta potential. For this purpose we have studied several polysaccharides, including chitosan (polycation), pectin and alginate (polyanions).

2. Materials and methods

2.1. Materials

Chitosan medium M_v (~295 000 g/mol) was obtained from Sigma–Aldrich (Gillingham, UK) and reported to have an average degree of deacetylation (DD) of ~75–85%. Low-methoxyl pectin (GENU®) M_v (~120 000 g/mol) and high methoxyl pectin (HM) (GENU®) M_v (~115 000 g/mol) were obtained from CP Kelco (Leatherhead, UK) and were reported to have average degrees of methyl esterification (DM) of 36 and 71% respectively. Alginates were obtained from Sigma–Aldrich (Gillingham, UK) and FMC Biopolymer (Drammen, Norway) respectively and were reported to have M:G ratios of 1:0.6 (low G, M_v ~290 000 g/mol) and 1:2 (high G, M_v ~25 000 g/mol) respectively. Glacial acetic acid, sodium acetate trihydrate and sodium chloride were obtained from Sigma–Aldrich (Gillingham, UK). All materials were used without any further purification.

2.2. Sample preparation

2.2.1. Chitosan sample preparation with different ionic strengths

A stock solution of chitosan (5×10^{-3} g/mL) was prepared by dissolving 500 mg of chitosan in 100 mL of the appropriate ionic strength pH 4.3 sodium acetate buffer (0.05, 0.1, 0.15, 0.2, 0.3, 0.5 and 0.8 M). Dissolution was achieved after 3 h of mild stirring at room temperature. Stock solutions were diluted to appropriate range of concentrations for intrinsic viscosity and zeta potential measurements (2.5×10^{-4} – 2.0×10^{-3} g/mL).

2.2.2. Pectin and alginate sample preparation with different ionic strengths

A stock solution of chitosan (5×10^{-3} g/mL) was prepared by dissolving 500 mg of pectin or alginate in 100 mL of the appropriate sodium chloride buffer (0.05, 0.1, 0.15, 0.2, 0.3, 0.5 and 0.8 M). Dissolution was achieved after 3 h of mild stirring at room temperature. Stock solutions were diluted to appropriate range of concentrations for intrinsic viscosity and zeta potential measurements (2.5×10^{-4} – 5.0×10^{-3} g/mL).

2.3. Determination of intrinsic viscosities

Solutions and reference solvents were analysed using a 15 mL Oswald viscometer (Rheotek, Burnham-on-Crouch, UK) under

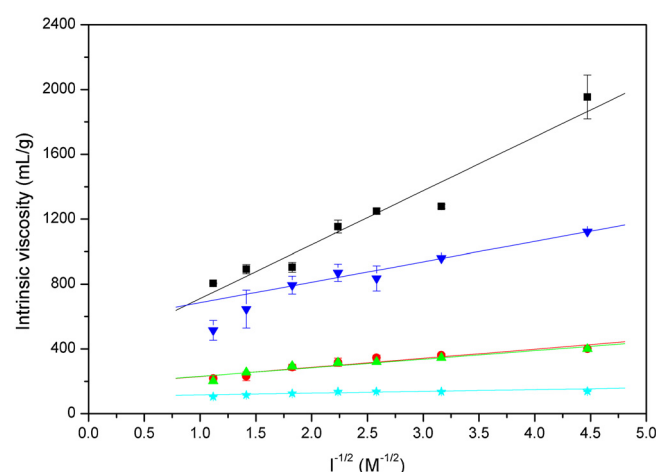


Fig. 1. Observed behaviour for intrinsic viscosity ($[\eta]$) of chitosan (■), low-methoxyl pectin (●), high-methoxyl pectin (▲), low G alginate (▼) and high G alginate (★) as function of inverse square-root of ionic strength.

precise temperature control (25.0 ± 0.1 °C). The relative, η_{rel} and specific viscosities, η_{sp} were calculated as described in Eqs. (3) and (4), respectively:

$$\eta_{rel} = \left(\frac{t}{t_0} \right) \left(\frac{\rho}{\rho_0} \right) \quad (4)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (5)$$

where t is the average (of 3 replicates) flow time of the polyelectrolyte solution at each concentration, t_0 is the flow time for the appropriate buffer, ρ is the density of the polyelectrolyte solution at each concentration, ρ_0 is the density of the appropriate buffer and because of the low concentrations used, $\rho/\rho_0 = 1$ (Harding, 1997).

Measurements were made at different concentrations and extrapolated to infinite dilution using both Eq. (6) (Huggins, 1942) and (7) (Kraemer, 1938):

$$\frac{\eta_{sp}}{c} = [\eta](1 + K_H[\eta]c) \quad (6)$$

$$\frac{\ln(\eta_{rel})}{c} = [\eta](1 - K_K[\eta]c) \quad (7)$$

where the intrinsic viscosity $[\eta]$ is taken as the mean of the intercepts from Eqs. (6) and (7) and K_H and K_K are the Huggins (1942) and Kraemer (1938) constants respectively.

2.4. Determination of zeta potentials, ζ

Zeta potential (ζ) was determined using Malvern Zetasizer NANO-Z (Malvern Instruments Limited, Malvern, UK). Measurements were performed by using folded capillary cell at 25.0 ± 0.1 °C. Each data value is an average of three measurements. For consistency zeta potential was measured at each concentration, however no concentration dependency of zeta potential was observed over the series of concentrations studied.

3. Results and discussion

The intrinsic viscosity and zeta potential for chitosan, pectin and alginate are shown in Figs. 1 and 2, revealed the expected behaviour for polycations and polyanions. As the ionic strength of the solvent increases the intrinsic viscosity of the polyelectrolyte solution decreases and the net charge on the polymers is reduced. The slopes of the plots in Fig. 1 can be used to calculate the Smidsrød–Haug stiffness parameter, B from Eq. (2) and an alternative version where

Table 1
Estimates for the Smidsrød–Haug stiffness parameter, B , and the corresponding intrinsic persistence length, L_p , for chitosan, pectin and alginate using both the tradition intrinsic viscosity ($[\eta]$) and the novel zeta potential (ζ) methods and their comparison with the previous literature values.

	Smidsrød–Haug stiffness parameter	Intrinsic persistence length, L_p (nm)	Reference
Chitosan	$B_{[\eta]} = 0.03 \pm 0.01$	6–14	This study
	$B_{\zeta} = 0.05 \pm 0.01$	4–6	This study
	$B_{[\eta]} = 0.07$	3	Jiang and Han (1998)
	$B_{[\eta]} = 0.11$	2	Curti and Campana-Filho (2006)
	$B_{[\eta]} = 0.04–0.09$	3–6	Christensen, Vold, and Varum (2008)
	$B_{[\eta]} = 0.11–0.15$	1–2	Tsaih and Chen (1997)
	$B_{[\eta]} = 0.10$	2	Trzcinski, Varum, Staszewska, Smidsrød, and Bohdanecky (2002)
	$B_{[\eta]} = 0.02–0.10$	2–14	Anthonsen, Varum, and Smidsrød (1993)
	$B_{[\eta]} = 0.06–0.12$	2–4	Gartner and Lopez (2012)
	$B_{[\eta]} = 0.06$	4	Morariu, Brunchi, and Bercea (2012)
	$B_{[\eta]} = 0.02–0.15$	1–14	Kasaai (2006)
	$B_{[\eta]} = 0.08–0.11$	2–3	Velásquez, Alborno, and Barrios (2008)
LM-pectin	$B_{[\eta]} = 0.03 \pm 0.01$	6–14	This study
	$B_{\zeta} = 0.10 \pm 0.02$	2–3	This study
	$B_{[\eta]} = 0.03–0.04$	6–9	McConaughy et al. (2008)
	$B_{[\eta]} = 0.02–0.07$	3–14	Axelos and Thibault (1991)
HM-pectin	$B_{[\eta]} = 0.03 \pm 0.01$	6–14	This study
	$B_{\zeta} = 0.16 \pm 0.04$	1–2	This study
Alginate (high G)	$B_{[\eta]} = 0.031$	9	Dentini, Rinaldi, Risica, Barbetta, and Skjåk-Bræk (2005)
	$B_{[\eta]} = 0.020 \pm 0.001$	13–15	This study
Alginate (low G)	$B_{\zeta} = 0.050 \pm 0.002$	5–6	This study
	$B_{[\eta]} = 0.04$	6	Smidsrød and Christensen (1991), Dentini, Rinaldi, Risica, Barbetta, and Skjåk-Bræk (2005)
	$B_{[\eta]} = 0.022 \pm 0.001$	12–13	This study
	$B_{\zeta} = 0.050 \pm 0.003$	5–6	This study

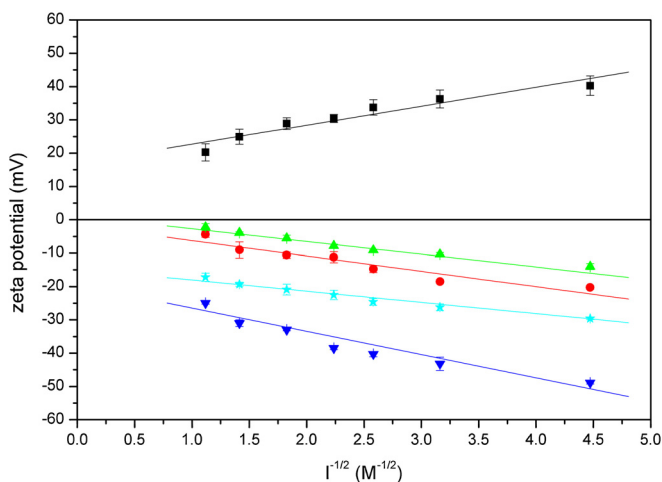


Fig. 2. Observed behaviour for zeta potential (ζ) of chitosan (■), low-methoxyl pectin (●), high-methoxyl pectin (▲), low G alginate (▼) and high G alginate (★) as function of inverse square-root of ionic strength.

the intrinsic viscosity was substituted for zeta potential (Eq. (8)) can be used to estimate B from the slopes of the plots in Fig. 2.

$$S = B(\xi_{0.1} M)^{\nu} \quad (8)$$

The corresponding values of B from both the traditional intrinsic viscosity and novel zeta potential estimation are shown in Table 1. Although the absolute values from both estimations are different and considerably so in the case of both low and high-methoxyl pectin they are generally in good agreement with those previously found in the literature.

Furthermore the Smidsrød–Haug stiffness parameter, B has been related to the intrinsic persistence length, L_p (Smidsrød &

Christensen, 1991) via the following empirical relationship (Eq. (9)):

$$L_p \approx 0.18B^{-1.11} \quad (9)$$

The estimates from both procedures are typical of random or semi-flexible coils and are in general agreement with the current literature values for the intrinsic persistence length calculated from the stiffness parameter B of approximately 1–14 nm (chitosan), ~6 nm (alginate) and 2–14 nm (pectin) although the values estimated from intrinsic viscosity are in *all* cases higher (Table 1).

4. Conclusions, limitations and future perspectives

The novel method of estimating the Smidsrød–Haug stiffness parameter using the ionic strength dependency of zeta potential rather than intrinsic viscosity is a promising development in the estimation of the dilute solution conformation of polysaccharides (provided they are polyelectrolytes). The results from this new approach are consistent with previous estimates in the literature.

We have demonstrated that different approaches (e.g. intrinsic viscosity and zeta potential) used in the estimation of the Smidsrød–Haug stiffness parameter can lead to a bias in the results (Table 1) and therefore it is more appropriate to characterise macromolecules using several techniques. This has been demonstrated previously in the estimation of persistence lengths of for example, pullulan and konjac glucomannan (Morris, Adams, & Harding, 2014; Kök, Abdelhameed, Ang, Morris, & Harding, 2009). The estimation of conformation is sensitive to the choice of model and it is therefore important that when trying to estimate solution conformation of polysaccharides (or any other flexible macromolecule) the quality of the estimate is determined by the amount of experimental data available.

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