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A simple and rapid method for evaluation of Mark–Houwink–Sakurada constants of linear random coil polysaccharides using molecular weight and intrinsic viscosity determined by high performance size exclusion chromatography: application to guar galactomannan

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Abstract

A rapid method for establishing the constants in the Mark–Houwink–Sakurada equation, relating intrinsic viscosity and molecular weight (MW), of guar galactomannan is described. Following partial acid hydrolysis, the galactomannan was analyzed using high performance size exclusion chromatography employing viscosimetry and right angle light scattering detectors. In this way, a large number of samples of polysaccharides with a wide range of MW distributions were prepared, without need for isolation, and intrinsic viscosity and MW rapidly determined. The *a* and *K* values found for guar galactomannan were 0.72 and 5.13×10^{-4} ([η] in dl/g) respectively, in good agreement with previously published values.

Keywords: High performance size exclusion chromatography; Polysaccharide; Guar galactomannan; Partial acid hydrolysis

1. Introduction

Solution properties of polymers are controlled by fundamental molecular characteristics such as molecular weight (MW), intrinsic viscosity [η], and chemical structure. The Mark–Houwink–Sakurada (MHS) equation relates the intrinsic viscosity [η] and the MW:

$$[\eta] = K \times \mathrm{MW}^a, \quad (1)$$

where both *K* and *a* are constants for a given well-defined polysaccharide–solvent system. Despite the considerable commercial use of guar galactomannan, and its beneficial physiological effects (Blake, Hamblett, Frost, Judd & Ellis, 1997), the MHS relationship has only been evaluated in two studies (Doublier & Launay, 1981; Robinson, Ross-Murphy & Morris, 1982). The values of *K* and *a* reported in these two studies differ markedly, with the latter showing an *a* value (0.98) rather higher than that which would be expected for a random coil conformation. A third study (Sharman, Richards & Malcolm, 1978) which quotes an *a* value similar to Robinson et al. (1982) but a significantly higher *K*, was done with different sources of galactomannans and may not be comparable. The infrequent attempts to determine the MHS relationship probably reflects the difficulties in obtaining pure samples of different molecular size as well as in the determination of intrinsic viscosity and MW. The objective of the present study was to exploit recent developments in high-performance exclusion chromatography (HPSEC) in which right-angle light scattering (RALLS) and viscometric detectors, in conjunction with refractive index (RI) allow rapid determination of MW and intrinsic viscosity [η] using small amounts of sample.

2. Materials and methods

2.1. Material

Standard food grade guar gum (M150) was obtained from Meyhall Chemical AG (Kreuzlingen, Switzerland). The guar gum was purified by stirring in water (60 min at 70°C) till it dissolved, followed by clarifying it by centrifugation (8000*g*, 15 min), and then slowly adding ethanol to a final concentration of 50%. The precipitate was recovered by centrifuging; and then washed with 95% ethanol and

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dried. Monosaccharide residues were analyzed by acid hydrolysis (1 M H_2SO_4 at 100°C for 3 h), followed by high performance anion exchange chromatography on a Dionex system (Dionex, Sunnyvale, CA) as described by Wood, Weisz and Blackwell (1994).

2.2. Partial acid hydrolysis of guar galactomannan

Approximately 200 mg of guar gum (M150) was dissolved in 50 ml water by stirring constantly for 4 h at 70°C. The solution was allowed to cool to room temperature (RT), after which 50 ml of HCl (0.02 M) was added and aliquots of 5 ml were heated at 80 or 100°C for up to 180 min (intervals of 1 min up to 10 min; and then intervals of 10 min from 10 to 180 min). Samples were neutralized with 5 ml NaOH (0.01 M), cooled in an ice bath, and aliquots taken to determine MW and intrinsic viscosity by HPSEC.

2.3. High performance size exclusion chromatography

The MW and intrinsic viscosity $[\eta]$ of native and partly depolymerized samples were determined using a HPSEC; RI and viscosity (Model 250, Viscotek, Houston, USA); and right angle laser light-scattering (RALLS, Viscotek) detectors. Two columns in series (Shodex OHpak KB-806 M, Showa Denko K.K., Tokyo, Japan; Ultrahydrogel linear, Waters, Milford, USA) and a Waters model 590 pump were used. The columns were maintained at 40°C, the mobile phase was 0.1 M $NaNO_3$ buffer, and the flow rate was 0.6 ml/min. A Perkin–Elmer ISS 100 autosampler and injector was used with an injection volume of 100 μl. Samples were filtered (0.45 μm) before analysis. The system was calibrated with pullulan (Showa Denko KK) and dextran standards (Polymer Standard Service GmbH, Mainz, Germany) of known MW and intrinsic viscosity and a d*n*/d*c* of 0.146 ml/g was used for galactomannan samples.

2.4. Calculation of intrinsic viscosity [η] and MW

Intrinsic viscosity $[\eta]$ and MW (M_p, MW of chromatographic peak; M_w, weight average MW; M_n, number average MW) were calculated using TriSEC software, version 3.0 (Viscotek) on the basis of the following relationship.

The specific viscosity, η_{sp}, in a differential viscometer is defined by the relationship between excess pressure (ΔP) of the polymer solution over that of the solvent (mobile phase) and the inlet pressure (P_i), which is the pressure due to the mobile phase (Haney, 1985a,b).

$$\eta_{sp} = \frac{4 \times \Delta P}{P_i - (2 \times \Delta P)}. \quad (2)$$

Intrinsic viscosity is defined by

$$[\eta] \cong \left[\frac{\eta_{sp}}{c} \right]_{c \to 0}, \quad (3)$$

where c is the concentration of the solute and is usually obtained by extrapolation (Huggins and Kraemer plots). In this case, the concentration of polysaccharide in each slice of the chromatograph, obtained by RI, is very small and it is valid to use the Solomon–Gotesman equation (Solomon & Gotesman, 1967)

$$[\eta] = (1/c)[2\eta_{sp} - 2\, ln(\eta_{rel})]^{1/2}. \quad (4)$$

For normal distributions, the value for the weight average intrinsic viscosity calculated from the HPSEC-viscosity data is equivalent to, and directly comparable with, the laboratory intrinsic viscosity value measured on the bulk polymer solution (Yau & Rementer, 1990

$$[\eta]_{+1} = \sum c_i [\eta]_i / \sum c_i. \quad (5)$$

Molecular weight is determined by light scattering at 90° with the following steps implemented to correct for the light scattering asymmetry using the TriSEC software (Viscotek):

1. Initially, the angular scattering function, P_θ, is assumed to be equal to 1 at 90°, and the initial MW, M_{est}, is calculated for each chromatogram slice from the light scattering intensity, $R(\theta = 90°)$, measured at 90°. Again, because the concentrations, c, in each slice are low, the effect of the second virial coefficient may be neglected, and

$$M_{est} = \frac{R(\theta = 90°)}{K_c}. \quad (6)$$

2. The radius of gyration, R_{FF}, is calculated from the Flory–Fox equation (Flory, 1953) assuming a linear flexible chain molecule. Using the M_{est} value from the MW measurement and the measured intrinsic viscosity, $[\eta]$, for each slice, we obtain

$$R_{FF_{est}} = \frac{1}{\sqrt{6}} \left(\frac{[\eta] M_{est}}{\phi} \right)^{1/3}, \quad (7)$$

where ϕ is the Flory viscosity constant.

3. The estimated radius of gyration, $R_{FF_{est}}$, for each slice is then inserted into the Debye equation (8) to provide a new estimate of P_θ

$$P_\theta = \frac{2}{x^2}(e^{-x} + x - 1), \quad (8)$$

where

$$x = \left(\frac{4\pi n_0}{\lambda_0} \right)^2 R_{FF_{est}} \sin^2 \theta. \quad (9)$$

4. A new estimate of MW is calculated from

$$M = \frac{M_{est}}{P(\theta = 90^\circ)}. \quad (10)$$

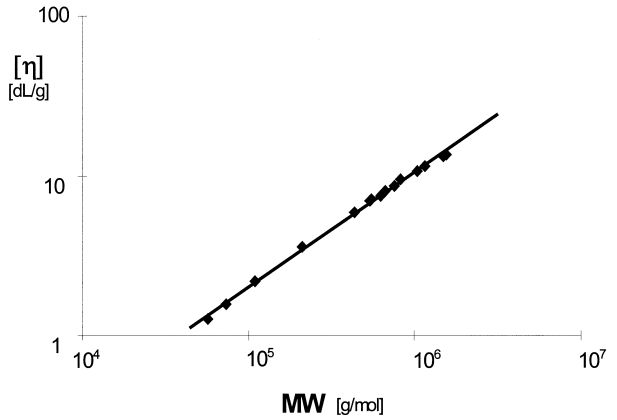

Fig. 1. Plot of log (intrinsic viscosity) against log (MW) from which the Mark–Houwink–Sakurada relationship for aqueous guar galactomannan solutions was derived.

Steps 2–4 are repeated using the new estimate of MW until MW values no longer change significantly. This usually requires three iterations.

3. Results and discussion

After purification the guar gum contained 93% galactomannan, consisting of 56.5% anhydromannose and 36.1% anhydrogalactose. Partial acid hydrolysis of galactomannan at 100°C produced rapid depolymerization. After 10 min, M_w was decreased from 1600×10^3 g/mol to 212×10^3 g/mol; after 180 min M_w was decreased to about 14×10^3 g/mol. Samples with an M_w in the range of about $212–46 \times 10^3$ g/mol showed a tendency to form aggregates and made the calculation of MW difficult. Shorter incubation times or lower temperatures (80°C) resulted in less depolymerization.

Determination, using HPSEC, of MHS constants a and K for a single sample of narrow polydispersity (PD) is not possible; a PD > 2.5 is required. In this study, multiple samples were prepared for analysis by partial hydrolysis of a stock solution. Values for $[\eta]$ and MW could then be rapidly obtained for each sample using HPSEC. Previously for galactomannans, relatively few samples were used in batch determinations of $[\eta]$ and MW (Sharman et al., 1978; Doublier & Launay, 1981; Robinson et al., 1982). HPSEC and MALLS were used for cereal β-glucans (Gómez et al., 1997) but batch measurements of intrinsic viscosity still had to be made conventionally. The value obtained for a (0.71) was in good agreement with conventional batch measurements reported by Vårum, Martinsen and Smidsrød (1991) who found 0.75 for a, but the K value was twice that of Gómez, Navarro, Manzanares, Horta and Carbonell (1997). The partial acid hydrolysis method does not require much sample nor do the products require isolation before analysis by HPSEC. The rapidity of the analysis allows many points to be obtained for the double logarithmic plot of MW against intrinsic viscosity, from which a is obtained from the slope, and K from the intercept. With more points, a more reliable estimate of the two parameters are possible. In the study of Doublier and Launay (1981) seven points were used, and in that of Robinson et al. (1982), five. The points are quite close numerically but significantly different slopes and intercepts were determined.

Fig. 1 shows the plot for partly depolymerized guar galactomannans with a MW $> 50 \times 10^3$ g/mol. The variation in intrinsic viscosity (dl/g) with MW followed the relationship $[\eta] = 5.13 \times 10^{-4} \times M_w^{0.72}$. Robinson et al. (1982) reported similar values for a and K of 0.72 and 3.8×10^{-4}, whereas Doublier and Launay (1981) reported 0.98 and 7.76×10^{-4}. The commercial guar samples used by Doublier and Launay (1981) were highly polydispersed ($M_w/M_n > 10$) compared to those used in this study (< 3). With our data, when M_n was used in the plot, the values for a and K were 0.74 and 7.14×10^{-4} respectively, the differences having arisen from polydispersity.

In conclusion, partial hydrolysis of polysaccharides by acid (or enzyme) rapidly provide samples of a wide range of MW distributions, which may be then directly analyzed, without prior purification, by HPSEC. The combination of an in-line viscometer and a right angle light scattering detector then allows measurement of MW and intrinsic viscosity. A single chromatographic analysis takes only 40 min and may be automated, allowing analysis of a sufficient number of samples to complete an MHS plot in a day. The system then becomes a powerful tool for determining MHS constants for linear random coil polysaccharides. These calculations depend on random depolymerization and products retaining similar conformation to the starting material. The system may prove useful for detecting conformational modifications during hydrolysis, but this has not been evaluated. The other polysaccharide successfully studied till date by this methodology (using both acid and enzyme hydrolysis) is cereal β-glucan. This data will be reported elsewhere.

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