Abstract

In the past, intrinsic viscosity and sedimentation velocity analyses have been used separately to assess the conformation and flexibility of guar and locust bean gum galactomannans based on worm-like chain and semi-flexible coil models. Publication of a new global method combining data sets of both intrinsic viscosity and sedimentation coefficient with molecular weight, and minimising a target (error) function now permits a more robust analysis. Using this approach, values for the persistence length of (10 ± 2) nm for guar and (7 ± 1) nm for locust bean gum are returned if the mass per unit length $M_L$ is floated as a variable. Using a fixed mass per unit length based on the known compositional data of each galactomannan yields a similar value for $L_p$ in both cases, (8 ± 1) nm for guar and (9 ± 1) nm for locust bean gum, with combined set of data yielding (9 ± 1) nm: within experimental error the flexibilities of both galactomannans are very similar.

Keywords: Galactomannan; Guar; LBG; Worm-like chain; Semi-flexible coil; Persistence length; Mass per unit length

1. Introduction

Recent studies have demonstrated the usefulness of the so-called pressure cell method for the solubilisation of galactomannans and subsequent analysis of molecular conformation and flexibility (guar gum and locust bean gum (LBG)) (Patel, Picout, Ross-Murphy, & Harding, 2006; Picout, Ross-Murphy, Errington, & Harding, 2001; Picout, Ross-Murphy, Jumel, & Harding, 2002). It was found that intrinsic viscosity, $[\eta]$; sedimentation coefficient, $s_{20,w}$; z-average radius of gyration, $R_g$; and weight average molar mass, $M_w$ all decrease with increased temperature and heating times, although at temperatures > 100 °C the concomitant application of pressure appears to have a protective effect on polysaccharide chain degradation. Data sets of intrinsic viscosity versus molecular weight, radius of gyration versus molecular weight or sedimentation coefficient versus molecular weight then permit not only simple estimates of chain conformation type (sphere rod, coil, etc.) from power law or “Mark–Houwink–Kuhn–Sakurada” types of analysis but also estimates of the flexibility via the chain persistence length $L_p$ from more sophisticated representations such as the Burchard-Stockmayer–Fixman (BSF) (Stockmayer & Fixman, 1963), Hearst (1963) and Bohdanecky (1983) equations based on data sets of $[\eta]$ versus $M_w$ and the Hearst and Stockmayer (1962) relation of $s_{20,w}$ versus $M_w$, later refined by Yamakawa and Fujii (1973).

Table 1 gives a comparison of some of the values for $L_p$ returned. The way these approaches are implemented can lead to significant variability in the results, i.e. contrary to expectation, $L_p$ is model dependent (Bohdanecky &
2. Combined (Global) analysis method (HYDFIT)

The linear flexibility of polymer chains is represented in terms of the persistence length, \( L_p \), of equivalent worm-like chains (Kratky & Porod, 1949) where the persistence length is defined as the average projection length along the initial direction of the polymer chain and for a theoretical perfect random coil \( L_p = 0 \) and for the equivalent extra-rigid rod (see for example Harding, 1997) \( L_p = \infty \), although in practice limits of \( \pm 1 \text{ nm} \) for random coils (e.g. pullulan) and \( 200 \text{ nm} \) for an extra-rigid rod (e.g. schizophyllan) are more appropriate (see for example Tombs & Harding, 1998). Chain persistence lengths, \( L_p \) can be estimated using several different approaches using either intrinsic viscosity (Bohdanecky, 1983; Hearst, 1963; Stockmayer & Fixman, 1963) or sedimentation coefficient (Hearst & Stockmayer, 1962; Yamakawa & Fujii, 1973) measurements. For example the Bohdanecky relation (Bohdanecky, 1983):

\[
\left( \frac{M_w}{[\eta]} \right)^{1/3} = A_0 M_L \Phi^{1/3} + B_0 \Phi^{1/3} \left( \frac{2L_p}{M_L} \right)^{-1/2} M_p^{-1/2} \tag{1}
\]

where \( \Phi \) is the Flory-Fox constant \((2.86 \times 10^{23} \text{ mol}^{-1})\) and \( A_0 \) and \( B_0 \) are tabulated coefficients (Bohdanecky, 1983) and the Yamakawa and Fujii (1973) form of the Hearst and Stockmayer (1962) equation:

\[
s^0 = \frac{M_L (1 - T \rho_0)}{3 \pi \eta_0 N_A} \times \left[ 1.843 \left( \frac{M_w}{2M_L L_p} \right)^{1/2} + A_2 + A_3 \left( \frac{M_w}{2M_L L_p} \right)^{-1/2} \right] + \ldots \tag{2}
\]

Yamakawa and Fujii (1973) showed that \( A_2 \) can be considered as \(-\ln(d/2L_p)\) and \( A_3 = 0.1382 \) if the \( L_p \) is much larger than the chain diameter, \( d \). Difficulties arise if the mass per unit length is not known, although both relations have now been built into an algorithm Multi-HYDFIT (Ortega & García de la Torre, 2007) which estimates the best values (or best range of values) of \( L_p \) and \( M_L \) based on minimisation of a target (error) function, \( \Delta \). The chain diameter \( d \) can also be floated as a variable but extensive simulations (Ortega & García de la Torre, 2007) have shown that the results returned for \( L_p \) are relatively insensitive to the value chosen for \( d \) (taken here as \( 1 \text{ nm} \) for each galactomannan). As before the assumption is made that hydrodynamic interaction between chain elements is so strong as to exclude intramolecular draining effects (Tanford, 1961).

We considered two possible cases:

1. The chain diameter, \( d \), was fixed at 1.0 nm for each galactomannan and the mass per unit length, \( M_L \), was taken as \( 490 \text{ g mol}^{-1} \text{ nm}^{-1} \), \( 410 \text{ g mol}^{-1} \text{ nm}^{-1} \) and \( 450 \text{ g mol}^{-1} \text{ nm}^{-1} \) for guar gum, locust bean gum and combined data sets for both galactomannans (Picout et al., 2002).

2. Only the chain diameter, \( d \), was fixed at 1.0 nm.

The Multi-HYDFIT program then “floats” the variable parameters \( (L_p \text{ in case } 1; L_p \text{ and } M_L \text{ in case } 2) \) in order to find a minimum of the multi-sample target (error) function, \( \Delta \) (Ortega & García de la Torre, 2007).

In this procedure as defined by Ortega and García de la Torre (2007) \( \Delta \) is calculated using equivalent radii (or the ratio of equivalent radii), where an equivalent radius is defined as the radius of an equivalent sphere having the same value as the determined property. These ‘determined properties’ include the translational frictional coefficient, \( f \) (calculated from either the diffusion or sedimentation coefficients); intrinsic viscosity, \([\eta]_\text{g,z}\); radius of gyration, \( r_{g,z} \) or the rotation relaxation time, \( \tau \). In the present study, we are interested in the equivalent radii resulting from the sedimentation coefficient i.e. translational frictional coefficient and from the intrinsic viscosity:

\[
a_{\tau} = \frac{f}{6 \pi \eta_0} \tag{3}
\]

where \( \eta_0 \) is the viscosity of water at 20.0 °C, and

\[
a_{\tau} = \left( \frac{3[\eta]M_w}{10\pi N_A} \right)^{1/3} \tag{4}
\]

where \( N_A \) is Avogadro’s number.
The target function, $A$, can be evaluated from respectively:

$$A^2 = \frac{1}{N_i} \sum_{i=1}^{N_i} \left[ \left( \sum W_T \right)^{-1} \sum W_T \left( \frac{a_{T\text{cal}} - a_{T\text{exp}}}{a_{T\text{exp}}} \right)^2 \right]$$

and

$$A^2 = \frac{1}{N_i} \sum_{i=1}^{N_i} \left[ \left( \sum W_1 \right)^{-1} \sum W_1 \left( \frac{a_{1\text{cal}} - a_{1\text{exp}}}{a_{1\text{exp}}} \right)^2 \right]$$

where $N_i$ is the number of samples in multi-sample analysis, $W_T$ and $W_1$ are the statistical weights for equivalent radii $a_T$ and $a_1$ (from translation frictional coefficient and intrinsic viscosity data, respectively) and the subscripts cal and exp represent values from calculated and experimental values respectively. $A$ is thus a dimensionless estimate of the agreement between the theoretical calculated values for the translational frictional coefficient (consequently the sedimentation coefficient) and the intrinsic viscosity for a particular persistence length, $L_p$ (and mass per unit length, $M_L$ in case 2) and the experimentally measured parameters (Ortega & García de la Torre, 2007).

In each case we have treated guar and locust bean gum separately and in combination.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>$L_p$ (nm)</th>
<th>$M_L$ (g mol$^{-1}$ nm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar gum</td>
<td>8 ± 1</td>
<td>490</td>
</tr>
<tr>
<td>LBG</td>
<td>9 ± 1</td>
<td>410</td>
</tr>
<tr>
<td>Guar-LBG</td>
<td>9 ± 1</td>
<td>450</td>
</tr>
</tbody>
</table>

Picout et al., 2001, 2002) and with computer modelled data for hypothetical galactomannan molecules of ~8–12 nm (Petkowitz, Reicher, & Mazeau, 1998).

#### 3.2. Fixed diameter, $d$

The best estimates for the persistence lengths and mass per unit lengths for galactomannans are represented on the contour plots shown in Fig. 2a–c. The values of the target (error) function, $A$, are represented by the full colour spectrum ranging from blue ($A = 0.13$) to red ($A = 1.00$). We can see that persistence lengths (Table 3) are in the range 7–10 nm and in good agreement with those calculated when the mass per unit length was fixed and with the results from the Hearst, Bohdanecky and Yamakawa-Fujii approaches in our previous analyses (Patel et al., 2006; Picout et al., 2001, 2002) and with the computer modelled data of Petkowitz et al. (1998).

The calculated values from the mass per unit length differ from the predicted values (Picout et al., 2002) by ~10–20%. It should however be noted that all values of target (error) function, $A$ within each contour in the combined analysis plots vary by ~5% i.e. less than the error in the original experimental data; clearly the results for the case of fixed mass per unit length fit well within these minima.

### 4. Discussion

Whilst giving extra confidence in the values for the persistence length obtained the global method has not given results too different from the intrinsic viscosity and sedimentation coefficient data that had previously been used independently (Patel et al., 2006; Picout et al., 2001, 2002). The combined analysis method Multi-HYDFIT (Ortega & García de la Torre, 2007) has been shown to give unbiased estimates for both the persistence length, $L_p$ and mass unit length, $M_L$.

It may have been expected that the stiffness of the worm-like galactomannan chains would increase with decreasing galactose:mannose (G:M) ratio i.e. locust bean gum (G:M~1:4) being the more stiff and guar gum (G:M~1:2) being the less stiff, however as previously (Picout et al., 2002) this was not observed. This has been most likely due to the reported block-wise (i.e. hairy and smooth regions) distribution of galactopyranosyl residues along the mannopyranan polymer chain. This has been particularly reported for the case of locust bean gum (Clark, Dea, & McCleary, 1985; Dea, 1990; Tombs & Harding, 1998).
Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>$L_p$ (nm)</th>
<th>$M_L$ (g mol$^{-1}$ nm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar gum</td>
<td>10 ± 2</td>
<td>550 ± 70</td>
</tr>
<tr>
<td>LBG</td>
<td>7 ± 1</td>
<td>330 ± 30</td>
</tr>
<tr>
<td>Guar-LBG</td>
<td>8 ± 1</td>
<td>420 ± 20</td>
</tr>
</tbody>
</table>

Fig. 2. The x-axis and y-axis represents $L_p$ (nm) and $M_L$ (g mol$^{-1}$ nm$^{-1}$), respectively. The target function $\lambda$ is calculated over a range of values for $M_L$ and $L_p$. In these representations, the values of $\lambda$ function are represented by the full color spectrum, from blue ($\lambda = 0.13$) to red ($\lambda = 1.00$). The minimum value (indicated for clarity) of $\lambda$ corresponds to the best estimate for $M_L$ and $L_p$. Contour plots from combined sedimentation and viscosity analyses for: (a) guar galactomannan; (b) locust bean gum; (c) guar and locust bean gum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

References


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