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Determination of the molecular weight of barley β-glucan using intrinsic viscosity measurements

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Abstract

Molecular weight (MW) of polysaccharides determines their solution properties such as intrinsic viscosity [η], flow and aggregation behavior. Determination of polysaccharide MW usually involves specialized equipment, extensive sample preparation and equipment calibration. Barley β-glucan gum (BBG) was extracted at laboratory (LAB) and pilot plant (PP) scale. Its MW and critical concentration (c^*) were determined using [η] at infinite dilution. Solutions of β-glucan standards (32–443 K), PP and LAB gums were prepared at different concentrations (0.025–0.20% w/w) and viscosity was measured at 25.8 s^{-1}. Intrinsic viscosity was calculated from linear and exponential extrapolation of reduced viscosity. PP and LAB gums had MW of 198 and 598 K, respectively. Mark–Houwink relationship between MW and [η] was found to be different from previously reported findings. Calculation of c^* was not applicable to low-viscosity BBG gums. Determination of MW through [η] measurements is a simple method for characterization of β-glucan, which is a soluble fiber component receiving increased attention due to its health benefits.

Keywords: Critical concentration; β-Glucan; Intrinsic viscosity; Molecular weight

1. Introduction

Molecular weight (MW) is one of the most fundamental parameters characterizing a macromolecule (Varum, Smidsrod, & Brant, 1992). MW is related to solution properties of polysaccharides such as intrinsic viscosity at low concentrations and flow behavior at higher concentrations (Robinson, Ross-Murphy, & Morris, 1982). Data on MW of β-glucan are variable (Dawkins & Nnanna, 1995), and to a great extent depend on the testing method and MW standards used for calibration. Proper determination of β-glucan MW is important for enhanced characterization of this soluble fiber component, which is receiving increased attention as a food ingredient due to its health benefits, such as cholesterol reduction and regulation of blood glucose levels (Bhatty, 1999; Wood & Beer, 1998).

Molecular weight of barley and oat β-glucan (BBG and OBG, respectively) can be determined by different techniques. Wood, Weisz, Fedec, and Burrows (1989) used high performance gel chromatography to determine the MW of OBG. The column was calibrated with pullulan standards. Later, Wood, Weisz, and Mahn (1991) found that the MW standards should also be OBG since the use of pullulan led to significant overestimation of OBG MW. Then, the challenge is proper determination of MW of standards. Morris (1989) stressed the need to determine the parameters in the Mark–Houwink relationship by calibration against an absolute method of MW measurement such as light scattering. Using light scattering, Varum et al. (1992) determined the average MW of seven OBG fractions (4.4×10^4–18.0×10^4 g/mol) and concluded that approximately 10% of OBG was in the form of reversible aggregates, which were largely dissociated at concentrations <0.2 g/l.

Another method of MW determination is through intrinsic viscosity [η]. Intrinsic viscosity is independent of concentration (c) by virtue of extrapolation of reduced viscosity to $c = 0$ and is usually related to molecular weight (Boucher & Alves, 1973). Grimm, Kruger, and Burchard (1995) determined the intrinsic viscosity of BBG from beer at 20 °C and a shear rate of 30 s^{-1}. They found that beer BBG completely dissociated in cuoxam (copper (II) tetramine-hydroxide) giving an approximate

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MW of 175,000. Because of a relatively small increase in the gyration radius of aggregates compared to MW growth, they proposed a fringed micelle type of β-glucan aggregation with a degree of association <17 for short beer β-glucan, while longer OBG analysed by Varum et al. (1992) had a degree of association 4–5. Similarly, reversible dissociation of (1 → 3)-β-D-glucan from *Poria cocos* sclerotium in cadoxen (saturated CdO solution in 29% ethylenediamine) was described by Zhang, Ding, Zhang, Zhu, and Zhou (1997). They speculated that the regularity of structure may be responsible for aggregation of this type of β-glucan.

Intrinsic viscosity depends on solvent type. The presence of sugars generally increases apparent viscosity while decreasing intrinsic viscosity (Elfak, Pass, Philips, & Morley, 1977). Grimm et al. (1995) tested the influence of maltose on beer β-glucan and reported minimum $[\eta]$ at a maltose concentration of 5%. A drop in temperature increased both apparent and intrinsic viscosity of beer β-glucan while the presence of 5% ethanol increased intrinsic viscosity and enhanced precipitation (Linemann & Kruger, 1998a).

Intrinsic viscosity may also be used to calculate critical concentration, c^*, which is the concentration at which molecular entanglement begins. For most hydrocolloids, it is calculated according to the formula $c^* \approx 4/[\eta]$ (Morris, 1989), with the exception of galactomannans, guar and locust bean gum, where $c^* \approx 2.5/[\eta]$. In all cases, viscosity at c^* is about 10 mPa s. Gum concentrations above c^* may suppress the taste and flavor of the product the gum is incorporated into due to an increase in viscosity (Morris, 1989).

Determination of $[\eta]$ and c^* would contribute to an understanding of BBG behavior in solution or more complicated food systems and perhaps lead to successful product applications. The goal of this study was to determine the $[\eta]$, MW and c^* of pilot plant (PP) and laboratory (LAB) obtained BBG gums based on intrinsic viscosity of BBG standards of known MW.

2. Materials and methods

2.1. Materials

Pure BBG MW standards (99.5% purity, w/w, dry wt; 32,000, 143,000, 212,000, 327,000 and 443,000) were obtained from Megazyme International Ireland Ltd. (Bray, Ireland). MW, as specified by the supplier, was determined by multi-angle laser light scattering in NaOH as solvent. PP and LAB gums were obtained as described by Burkus and Temelli (2000) and contained 78.92 and 71.14% (w/w, as is) β-glucan, respectively, as determined according to McCleary and Glennie-Holmes (1985). Distilled water was from the local supply.

2.2. Preparation of β-glucan solutions

Stock solutions for intrinsic viscosity determination were prepared in duplicate at a concentration of 0.2% (w/w). A dry 50 ml beaker was weighed and tared, water was added in the amount required for stock solution preparation, and the beaker with water and a magnetic stirring bar was weighed precisely to ± 1 mg. The beaker was emptied and dried with compressed air. BBG was weighed precisely into the beaker and the magnetic stirring bar was added. The required amount of water to achieve the desired concentration was added simultaneously with the beginning of stirring. After 5 s, the beaker was transferred onto a pre-heated hot plate, covered with aluminum foil and quickly brought to a boil while stirring. Heating was continued for 1 h at 85 °C in a water bath. After cooling to room temperature, the beaker was wiped carefully to remove excess condensation and the weight was adjusted to compensate for evaporative losses. The beaker was immediately covered with Parafilm® (American Can Company, Greenwich, CT) and aluminum foil and stirred for an additional 30 s. Each stock solution was further diluted into duplicate lines of samples having concentrations of 0.100, 0.075, 0.050 and 0.025% (w/w). The concentration was adjusted by weighing the necessary amounts of sample and adding distilled water into capped vials on the balance (i.e. 4.000 g of β-glucan solution + 4.000 g of distilled water). Capped vials were previously pasteurized by wet heat to prevent any microbial growth during measurements. Diluted samples were vigorously stirred on a vortex mixer and left to equilibrate at least 15 min prior to viscosity measurements.

For the concentration range 0.25–1%, solutions were prepared using the same method as for the intrinsic viscosity determination. For each concentration, the amount of gum was weighed directly without further dilution.

2.3. Viscosity measurements

Viscosity was determined by a fixed speed test at 20 rpm (25.8 s^{-1}) using a PAAR Physica UDS 200 rheometer (Glenn Allen, VA) equipped with a Peltier heating system. The instrument was calibrated with S3 standard oil (3.89 mPa s at 20 °C, Cannon Instrument Co., State College, PA) for low viscosity (LV) measurements and Brookfield 500 cps standard oil (482 mPa s at 25 °C, Brookfield Engineering Laboratories, Inc., Middleboro, MA) for high viscosity (HV) measurements. Temperature was calibrated with a thermocouple. Tests were performed at 20 ± 0.02 °C using the DG 27 cup and bob geometry with double gap and 7 ml sample size. Sample size was not measured by volume but by weight. The clean DG 27 cup was placed on the balance, tared, and 7.000 ± 0.005 g of sample was measured directly into the cup.

Viscosity was measured 10 times in one run for each sample, the rheometer was stopped and started anew 9 times so that each viscosity value used for the calculation of

reduced viscosity represented an average of 100 measurements (i.e. ten measurements for each of ten runs).

2.4. Determination of intrinsic viscosity, molecular weight and critical concentration

Reduced viscosity was calculated as described by Linemann and Kruger (1998b):

$$\eta_{red} = (\eta - \eta_0)/\eta_0 c \quad (1)$$

where η is the sample viscosity, η_0 is the viscosity of the solvent (distilled water) and c is the concentration of pure hydrocolloid (g/ml). The concentration of standard BBG gums was adjusted according to manufacturers specifications of 7% moisture and 99.5% purity. The presence of pentosans in BBG gum also contributes to viscosity (Bhatty, MacGregor, & Rossnagel, 1991). Therefore, for PP and LAB gums, the total concentration of pure β-glucan (78.92 and 71.14%, w/w as is basis, respectively) and pentosans (1.64 and 3.75%, w/w as is, respectively) was used in the calculations as 80.6 and 74.9%, respectively.

Intrinsic viscosity $[\eta]$ was calculated by linearly extrapolating the reduced viscosity to zero concentration from concentrations of 0.025–0.1%, or by polynomial extrapolation from concentrations of 0.025–0.2%. Linearly extrapolated $[\eta]$ was used to determine the relative MW of LAB and PP gums from the linear plot of MW vs. $[\eta]$. Polynomially determined $[\eta]$ was further used in the Mark–Houwink relationship (Eq. (2)) to determine BBG relative MW and BBG solution behavior. Mark–Houwink equation is

$$[\eta] = K' M_r^{\alpha} \quad (2)$$

where M_r is relative molecular weight and K' and α are 'stiffness' parameters calculated from a double logarithmic plot of $[\eta]$ against M_r.

Critical concentration (c^*) was first determined as

$$c^* \approx 4/[\eta] \quad (3)$$

as described by Robinson et al. (1982), and then as

$$c^* \approx 2.5/[\eta] \quad (4)$$

as suggested by Doublier and Wood (1995) for high viscosity OBG.

3. Results and discussion

3.1. Intrinsic viscosity and molecular weight

The viscosities of β-glucan MW standards and PP and LAB gums in the concentration range 0.25–1% (w/w) are depicted in Fig. 1. LAB gum and the 443 K standard had similar viscosities at different concentrations (at 25.8 s^{-1}), but, due to its lower purity, the reduced viscosity of LAB gum was much higher (Fig. 2). The highly viscous 443 K

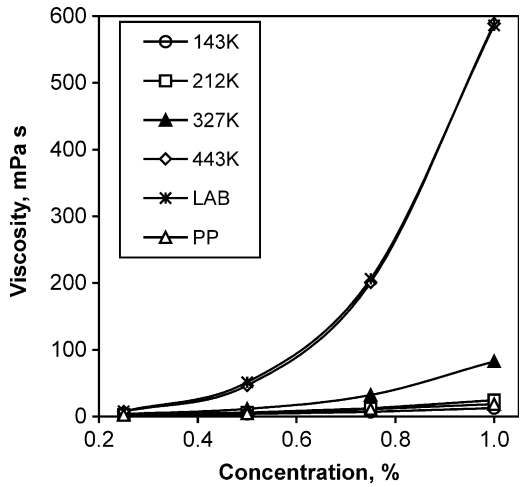

Fig. 1. Viscosity of MW standards, PP and LAB gums in the concentration range 0.25–1% (w/w, as is). The 32 K standard was not included due to its very low viscosity (<2 mPa s).

gum exhibited an exponential increase in viscosity, even at a concentration of 0.1%, which was also reflected in the non-linearity of reduced viscosity (Fig. 2). The reduced viscosities of the other gums were quite linear below 0.1% concentration. Intrinsic viscosity of the different gums and standards at $c \rightarrow 0$, as determined by linear and polynomial extrapolation of η_{red}, is shown in Table 1. The intrinsic viscosities of MW standards were plotted in a linear standard curve (Fig. 3) from which the relative MW of PP and LAB gums were determined to be 198 and 598 K, respectively.

The complete linearity of the plot with excellent R^2 value (0.9985) was somewhat surprising. Robinson et al. (1982) also obtained excellent linearity of intrinsic viscosity for guar gum ($R^2 > 0.99$), but the intercept $[\eta]$ on the y-axis

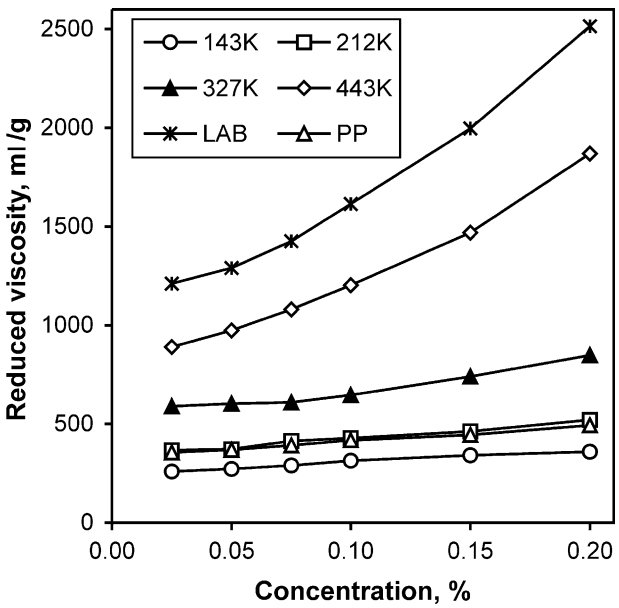

Fig. 2. Reduced viscosities of BBG MW standards, PP and LAB gum in the concentration range 0.025–0.2% (w/w, as is). 32 K not shown.

Table 1
Intrinsic viscosity [η] of MW standards, PP and LAB gums obtained by linear extrapolation from $\leq 0.1\%$ concentration to $c \rightarrow 0$, or polynomial extrapolation from 0.2% to $c \rightarrow 0$

Gum (K)	Linear [η] (ml/g)	Polynomial [η] (ml/g)
32	44	44[a]
143	238	233
212	339	347
327	570	588
443	776	835
LAB	1049	1125
PP	333	338

[a] The same as linear for MW calculations; polynomial extrapolation not applicable.

when MW $\rightarrow$ 0 was 151 ml/g, which is impossible. After fitting their data to the Mark–Houwink relationship (Eq. (2)), Robinson et al. (1982) obtained $\alpha = 0.723$ for guar gum, while such a fitting in this study resulted in $\alpha = 1.09$ for BBG. This finding is much different from that of Grimm et al. (1995) who determined $a = 0.725$ ($a = \alpha$) for beer β-glucan. In their case, the same β-glucan was tested in different solvents and the exponent a represented aggregation behavior of beer β-glucan. This exponent value ($a = 0.725$) was used by Linemann and Kruger (1998b) to determine the MW of their β-glucan.

In an effort to explain the large discrepancy between the previously reported value for $\alpha = 0.725$ for BBG (Grimm et al., 1995) and $\alpha \simeq 1.1$ obtained in this study, the impact of concentration and MW range used for calculations of α was evaluated. The concentration range used by Grimm et al. (1995) and specifics of extrapolations to determine [η] were not described. In addition, the MW data used for the calculation of α were in the 3×10^6–1.2×10^7 range obtained for partially hydrolyzed beer β-glucan in 2–10% maltose solutions where it was shown that β-glucan formed aggregates.

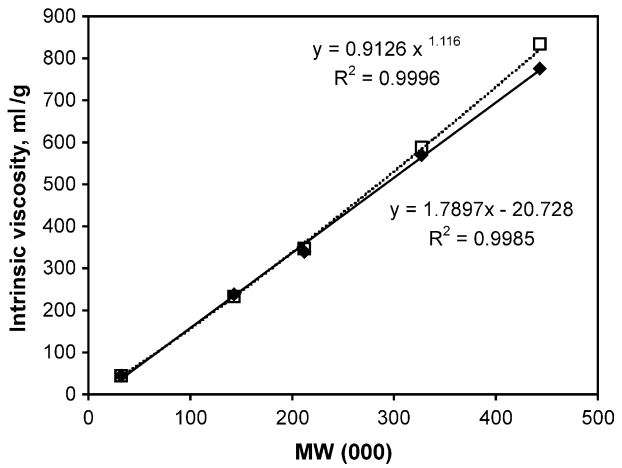

Fig. 3. MW-[η] standard curve determined linearly (solid line) from the linear extrapolation of [η] to $c \rightarrow 0$ (solid symbols), or determined from Mark–Houwink relationship (dotted line) from exponential extrapolation of [η] to $c \rightarrow 0$ (open symbols).

Table 2
Values of α and R^2 after fitting [η] data Mark–Houwink equation with and without 32 K MW standard

Concentration range (%)	Description	With 32 K		Without 32 K	
		α	R^2	α	R^2
Polynomial extrapolation					
0.025–0.200	All 6 values	1.116	0.9996	1.140	0.9983
Linear extrapolation					
0.025–0.075	First 3	1.099	0.9988	1.071	0.9938
0.025–0.100	First 4	1.092	0.9994	1.065	0.9970
0.025–0.150	First 5	1.077	0.9990	1.010	0.9988
0.025–0.200	All 6	1.052	0.9967	0.924	0.9985
0.050–0.200	Last 5	1.024	0.9934	0.844	0.9989
0.075–0.200	Last 4	0.993	0.9841	0.720	0.9986
0.100–0.200	Last 3	0.953	0.9675	0.574	0.9973

As demonstrated in Table 2, depending on the concentration range, MW range (with and without 32 K standard) and the type of extrapolation (linear vs. polynomial) used for the determination of [η], it is possible to obtain values for α ranging from 0.57 to 1.14 with an apparently excellent fit for all cases ($R^2 > 0.99$) except for two correlations. An increase in the concentration range for [η] determination resulted in the decrease of α and was more pronounced when 32 K standard was excluded from calculations. The particularly interesting case is when concentration range was 0.1–0.2% and 32 K standard was excluded as shown in Fig. 4. Obtained $\alpha = 0.574$ gave an excellent fit with $R^2 = 0.997$. These findings demonstrate the importance of avoiding linear extrapolation of η_{red} data to c $\rightarrow$ 0 from relatively high concentration range ($c > 0.1\%$), especially for high MW β-glucan. It is also critical to use pure β-glucan MW standards of as large MW range as possible for accurate determination of α.

The value of $\alpha \simeq 1.1$ implies that barley β-glucan behaves like a partially stiffened coil or wormlike chain

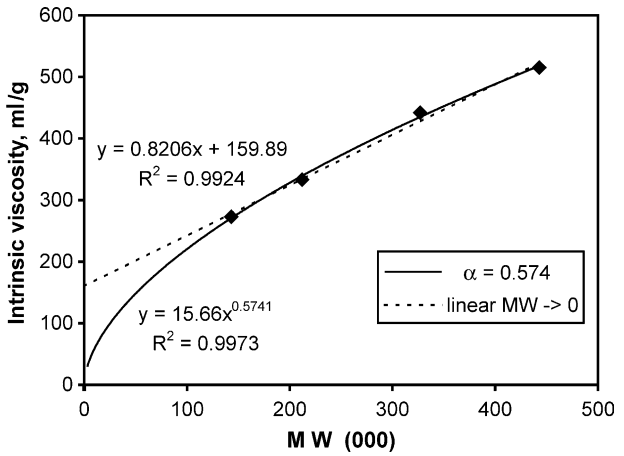

Fig. 4. MW-[η] standard curve obtained after linear extrapolation of η_{red} values for concentrations $c = 0.10$–0.20% results in $\alpha = 0.574$. Dotted line represents linear extrapolation of [η] to MW $\rightarrow$ 0 with [η] $\simeq$ 160 ml/g at the intercept.

(Gomez, Navarro, Manzanares, Horta, & Carbonell, 1997; Robinson et al., 1982). Using this higher value of $\alpha = 1.116$ (obtained using polynomial extrapolation of η_{red} data for 0.025–0.2 concentration range and 32–443 K MW range) in Eq. (2) for MW estimation results in a relative MW for PP gum of 199 K, which is similar to the estimate of 198 K based on linear extrapolation. Extrapolation of the Mark–Houwink equation for LAB gum results in a MW estimate of 585 K, or about 2% less than 598 K obtained from the linear relationship of MW vs. $[\eta]$. Therefore, the linear standard curve can be applied to estimate BBG MW in the range 30–600 K. For higher MW samples, the Mark–Houwink relationship should be used. Simple linear relationship between MW and $[\eta]$ might be applicable even for higher MW ranges if higher MW standards were available.

The coefficient $a = 0.725$ determined by Grimm et al. (1995) for beer β-glucan actually shows a decreasing trend as viscosity increases with MW, meaning that at concentrations above the critical value c^*, β-glucan micelles grow more laterally, slowly increasing in diameter. For LV β-glucan with shorter chain length, this coefficient may be even lower due to pronounced lateral aggregation. It was observed in this study that the 32 K sample at 1% concentration had approximately the same reduced viscosity as at 0.75% concentration, probably due to lateral aggregation. This measurement was repeated with another similar sample having a MW of 31 K. Both samples had viscosity <2 mPa s at 1% concentration (w/w).

It is clearly visible from Fig. 3 that intrinsic viscosity, if extrapolated, will reach zero before MW drops to zero, which is logical since the viscosity of oligosaccharide solutions at low concentrations will be close to that of water. Extrapolation of the linear standard curve in Fig. 3 gives an intercept for $[\eta] = 0$ at MW = 11.58 K, which corresponds to approximately 71 glucose units in a polymer. However, if the data for only the three lower MW standards were used, an intercept on the MW axis at 3.38 K is obtained, which is approximately 21 glucose units. This would be the point when BBG loses solubility because of very low viscosity and enhanced aggregation into an insoluble precipitate. This is in agreement with Doublier and Wood (1995) who found that, after digestion with lichenase, OBG yielded an insoluble precipitate composed mostly of glucose oligosaccharides with 9–15 glucose units.

Conversely, extrapolation of the MW vs. $[\eta]$ standard curve obtained after determination of $[\eta]$ from $c \geq 0.1$ ($\alpha = 0.574$ in Table 2) toward MW $\rightarrow 0$ (Fig. 4) results in a relatively high $[\eta]$ for low MW, which is impossible. If one were to perform such linear extrapolation of $[\eta]$ values for $\alpha = 0.574$ toward MW $\rightarrow 0$, the result would be $[\eta] \simeq 160$ ml/g (again impossible), which is very close to 151 ml/g obtained similarly using the data from Robinson et al. (1982) for guar gum. However, this whole calculation is based on the wrong approach, which is determination of $[\eta]$ from a very high concentration range. Therefore, extrapolation of the MW standard curve to MW $\rightarrow 0$ can always serve as a verification of the accuracy of the whole method. Some preliminary measurements for OBG (unpublished data) in the concentration range similar to that in Fig. 1. (0.25–1%) indicated similar behavior and MW not much different from barley β-glucan.

3.2. Critical concentration

The critical concentration c^* for PP and LAB gums was determined (Eq. (3)) to be 12 and 3.8 g/l, respectively, or 1.2 and 0.38% of pure β-glucan, or 1.49 and 0.51% of gum. If the value of $[\eta] = 1125$ ml/g for LAB gum, obtained after polynomial extrapolation of reduced viscosities, is used for c^* calculation, then $c^* \approx 0.36\%$ for pure β-glucan. Both gums should have a viscosity of about 50 mPa s (or slightly higher) at c^*.

Applying the same formula, $c^* \approx 4/[\eta]$, to HV 327 K β-glucan yields $c^* \approx 0.76\%$. The viscosity of this gum at a similar concentration of 0.75% was 32.8 mPa s, which was sharply increased from 11 mPa s at 0.5% concentration. This finding indicates that the critical concentration was already achieved. Doublier and Wood (1995) distinguished first and second entanglement points in the behavior of OBG corresponding to $c^*[\eta] = 0.7$ and 2.5, respectively. Using equation $c^*[\eta] \approx 0.7$ results in $c^* \approx 0.13\%$ for 327 K β-glucan with a viscosity of <2 mPa s. It is hard to believe that such a low viscosity would affect taste perception during sensory evaluation. Application of the second entanglement point of $c^*[\eta] \approx 2.5$ results in critical concentration at 0.47% with viscosity estimated between 10 and 11 mPa s, which is in the expected region. The same calculation for LAB gum results in $c^* \approx 0.30–0.32\%$, which probably yields viscosity in the vicinity of 10 mPa s since a 0.25% solution had viscosity of 7.7 mPa s.

Pilot plant gum at $\geq 1\%$ concentration displays time dependent behavior e.g. a gelling tendency stimulated by low shear rates, as described by Burkus (2001). Therefore, any conclusions about determination of c^* from Eq. (3), as proposed by Robinson et al. (1982), may be premature for LV BBG gums. The calculation of c^* for PP gum from Eq. (4) resulted in $c^* \approx 0.93\%$, with an expected viscosity of about 17 mPa s. PP gum at 0.93% concentration is still prone to gelling over time, as discussed by Burkus (2001). Due to the formation of a network, its solution actually becomes a weak gel with a yield point and much higher viscosity. Applying the same equation (Eq. (4)) to 143 K β-glucan results in $c^* \approx 1.14\%$. At that concentration, 143 K gum would gel even faster than PP gum because of its lower viscosity. Therefore, using the equation $c^*[\eta] \approx 2$ may give better results for low viscosity, gel forming BBG. However, it is questionable how PP gum behaves at 0.74% concentration and proper determination of critical concentration for this type of BBG gum may require additional experimental

measurements. Application of even this lowered c^* estimate on 32 K gum yields $c^* \approx 4.9\%$. At that concentration, this very LV BBG gum will probably gel in a matter of hours, if not minutes. Therefore, determination of c^* for gel-forming LV BBG is probably out of the question. Above c^*, flavor and taste release are retarded for almost all food hydrocolloids (Morris, 1989). This is probably true for fresh β-glucan gum solutions as well.

4. Conclusions

The intrinsic viscosity of BBG MW standards follows a linear relationship of MW vs. $[\eta]$ with $R^2 > 0.99$, based on linear extrapolation of reduced viscosity at concentrations $\leq 0.1\%$. The relative MW of PP and LAB gums was determined to be 198,000 and 598,000, respectively, while α in Mark–Houwink relationship was calculated as $\alpha = 1.099$ with $R^2 = 0.9988$. Polynomial extrapolation of η_{red} resulted in the even better fit to Mark–Houwink relationship with $\alpha = 1.116$ and $R^2 = 0.9996$, while MW of PP and LAB gums was estimated to be 198 and 585 K, respectively.

The value of α in the Mark–Houwink relationship is highly dependent on the chosen concentration range, the type of η_{red} extrapolation to obtain $[\eta]$ at $c \rightarrow 0$ and the range of MW standards. It is highly recommended to use concentrations $c \leq 0.10\%$ for the determination of $[\eta]$ by linear extrapolation of η_{red}, or to use polynomial extrapolation for the $[\eta]$ determination. This would result in higher values of $[\eta]$ for higher MW standards and more accurate determination of α in Mark–Houwink relationship, which is in the vicinity of $\alpha = 1.1$ for BBG. It is recommended to use concentrations $c < 0.025\%$ for the MW determination of standards by methods such as light scattering because of the observed problems with β-glucan chain association or disassociation when $c \rightarrow 0$. One possible way to assess the accuracy of $[\eta]$ vs. MW standard curve is its extrapolation to MW $\rightarrow 0$, where $[\eta]$ should have very low values.

Critical concentration c^* for PP and LAB gums was calculated from $c^* \approx 4/[\eta]$ to be approximately 1.49 and 0.51% of gum, respectively. Viscosity at critical concentration would be about 50 mPa s, which is higher than the commonly accepted value of about 10 mPa s. The equation $c^*[\eta] \approx 2.5$ seems to be suitable for the calculation of critical concentration for HV BBG, as already determined by Doublier and Wood (1995) for OBG, while determination of c^* for LV BBG probably requires experimental confirmation of applied formulas. Time-dependent aggregation–gelation of PP gum and, generally, LV BBG gums, may void any practical application of c^* in certain types of products.

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