Carbohydrate Polymers 86 (2011) 1732–1738

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Diffusion and rheology characteristics of barley mixed linkage β-glucan and possible implications for digestion

Kinnari J. Shelat[a], Francisco Vilaplana[a], Timothy M. Nicholson[b], Michael J. Gidley[a], Robert G. Gilbert[a,*]

[a] The University of Queensland, Centre for Nutrition & Food Sciences, Queensland Alliance for Agriculture and Food Innovation, Brisbane, Qld 4072, Australia
[b] The University of Queensland, Centre for High Performance Polymers, School of Chemical Engineering, Brisbane, Qld 4072, Australia

ARTICLE INFO

Article history:
Received 24 May 2011
Received in revised form 5 July 2011
Accepted 5 July 2011
Available online 12 July 2011

Keywords:
Diffusion
(1,3
1,4)-β-Glucan
Viscosity
Size exclusion
Fluorescence recovery after photobleaching

ABSTRACT

β-Glucan is one of the most studied soluble dietary fibres, and is known for its positive effects on human health such as lowering glycemic responses and reducing serum cholesterol levels. Viscosity and diffusion phenomena are thought to play an important role in imparting these beneficial effects through interactions with digestive enzymes and bile salt micelles in the digestive tract. Correlations between viscosity, probe diffusivity, and molecular structure for three barley β-glucans are studied here to enhance understanding of the molecular basis for these nutritional effects. Fluorescence recovery after photobleaching (FRAP) is used to measure the diffusion coefficients of a dextran probe similar in size to both digestive enzymes and bile salt micelles in β-glucan solutions. Diffusion coefficients are found to decrease with an increase in the viscosity, but showed systematic deviations from Stokes–Einstein behaviour, similar to those found for cereal arabinoxylans, and thus indicating that bulk viscosity measurements cannot be reliably used as sole indicator of diffusion processes, due to local aggregation and microviscosity effects. The diffusion coefficient values are 10–100 times slower than predicted for diffusion in the absence of β-glucan, consistent with a functional role in retarding digestion and absorption processes in the small intestine.

1. Introduction

Cell wall polysaccharides such as mixed-linkage (1,3; 1,4) β-glucans and arabinoxylans are the major source of soluble dietary fibre in cereals. They are a major component of cell walls in the starchy endosperm of cereals such as oat, barley, rye and wheat (Cui & Wang, 2009; Lazaridou & Biliaderis, 2007). β-Glucan is one of the most studied soluble dietary fibres, mainly due to its wide range of applications in the food industry (Brennan & Cleary, 2005) and demonstrated beneficial effects to human health (Brownlee, 2011; Jenkins et al., 1978; Wood, 1994). Barley mixed-linkage β-glucan consists predominantly of linear chains of cellotetraosyl units randomly connected by cellotriosyl units (Gomez, Navarro, Manzanare, Hortab, & Carbonell, 1997a, 1997b; Woodward, Fincher, & Stone, 1983). The physicochemical properties of β-glucans are governed by their structural aspects, primarily average molecular weight (and probably molecular weight distributions) and the ratio of cellotetraosyl (DP3) and cellotriosyl units (DP4) (Lazaridou & Biliaderis, 2007). The water solubility of β-glucan largely depends on β-(1–3) and β-(1–4) linkages, and the presence of β-(1–3) linkage increases the flexibility of the chain enhancing water solubility (Bulinga, Brant, & Fincher, 1986; Lazaridou & Biliaderis, 2007).

It is now established that soluble dietary fibres such as β-glucan can play an important role in improving human health and help to prevent diseases such as type2 diabetes and cardiovascular disorders (Brownlee, 2011). It has been observed that the presence of β-glucan affects digestion by increasing viscosity of gut content (Dikeman et al., 2006), potentially resulting in slowing down the absorption of nutrients through the unstirred mucosal layer (Lund, Gee, Brown, Wood, & Johnson, 1989). For instance, it was demonstrated that in healthy human volunteers, oat β-glucan reduced the postprandial glucose response to an oral glucose load similarly to guar gum (Wood, 1994). Blackburn and Johnson (1981) reported reduced blood cholesterol correlated with increased viscosity in rat models on diets containing guar galactomanan. Jenkins et al. (1978) observed high viscosity dietary fibre had positive effect on decreasing postprandinal glucose level in human subjects. In human studies, reduction of molecular size (and hence viscosity) of oat β-glucan has been shown to limit the lowering of serum LDL cholesterol (Wolever et al., 2010), confirming that physicochemical properties of β-glucan need to be taken into account when

* Corresponding author. Tel.: +61 7 3365 4809; fax: +61 7 3365 1188.
E-mail address: b.gilbert@uq.edu.au (R.G. Gilbert).

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doi:10.1016/j.carbpol.2011.07.004

assessing potential health benefits. An increase in viscosity of gut contents may also affect bile salt-binding capacity (Lund et al., 1989). As the viscosity of gut contents increases in the presence of viscous dietary fibres, there is resistance to flow, which may affect fat and cholesterol as well as bile acid absorption. The increase in viscosity may also affect the transit time of digesta in the gut lumen.

The physico-chemical properties of the two major cereal cell wall polysaccharides arabinoxylan and β-glucan have been recently studied, to understand the role of macromolecular characteristics such as molecular weight, molecular structure and viscosity in imparting beneficial effects on human health. Xu et al. (2007) studied micro-heterogeneity and micro-rheological properties of high viscosity oat β-glucan using well dispersed polystyrene microspheres by video fluorescence microscopy. A detailed investigation was performed on physical and structural properties of barley β-glucan by Gomez et al. (1997a, 1997b) indicating a stiff worm-like cylindrical conformation. Wang, Wood, and Cui (2002) reported that dissolution of β-glucan in water is an important step, which has implications for its characterisation. Recently, Pitkanen, Virkki, Tenkanen, and Tuomainen (2009) reported macromolecular differences between arabinoxlyan from different sources (wheat and rye), which may affect their role as dietary fibres. In our recent study (Shelat et al., 2010), we focused on diffusion and viscosity characteristics of arabinoxylans in relation with their macromolecular structure. Interestingly, anomalous comparative diffusion characteristics were observed for rye and wheat arabinoxylan, due to different substitution patterns and consequent macromolecular properties. Diffusion and viscosity were shown to be independent of each other, most likely due to differential microviscosity effects arising from the presence of aggregates in the arabinoxylan solutions. In the present study, correlations between diffusion, viscosity and macromolecular structure of three mixed-linkage barley β-glucans are investigated to probe molecular origins for potential nutritional benefits. Size exclusion chromatography is used to determine conformational and macromolecular characteristics. Fluorescence recovery after photo bleaching (FRAP) is used to determine the diffusion coefficient of a FITC-dextran probe in β-glucan solutions of varying concentrations. Viscoelastic characteristics for a range of concentrations of different β-glucan solutions are studied by steady-state and oscillatory rheological measurements, and the particle sizes of any aggregates are characterised by laser light scattering. Comparison of results from this range of techniques is used to test relationships between solution structure and probe diffusion as a model for interactions involving β-glucans in the digestive tract.

2. Materials and methods

2.1. Materials

Three different mixed linkage barley β-glucan samples, with manufacturer stated kinematic viscosities at 1% (w/v) of 10, 28 and 100 cSt (corresponding dynamic viscosities of 10, 28 and 100 mPa s; 1 cP = 1 mPa s for fluid density of 1 g cm^{-3}), were purchased from Megazyme International Ltd. These are denoted here as B10, B28 and B100, respectively. As stated by the supplier, the purity of these samples is ~95–97% and there is ~0.12% starch, ~2–3% of moisture and ~1% of protein. Fluorescein isothiocyanate conjugated dextran (FITC-dextran) (manufacturer-reported molecular weight 7×10^4) was purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). Dimethyl sulfoxide (DMSO, GR for analysis ACS) was purchased from Merck & Co., Inc. (Kilsyth, VIC, Australia).

2.2. Diffusion coefficient measurement using fluorescence recovery after photobleaching (FRAP)

The diffusion coefficient of FITC-dextran probe in varying concentrations of β-glucan solutions was measured using FRAP. The detailed description of this method and details of protocols are described elsewhere (Perry, Fitzgerald, & Gilbert, 2006; Shelat et al., 2010). For diffusion coefficient measurements, the β-glucan samples were dissolved in 0.2% (w/v) FITC-dextran/deionised water solution at concentrations ranging from 0.5% to 1% (w/v). About 40 μL of the prepared solution was placed into the cavity of a concave glass slide and sealed with a cover slip. FRAP experiments were performed using a Zeiss LSM 10 META confocal microscope with a uniform bleach radius of 25 μm using a 10× objective at ambient temperature. For each measurement, 250 scans were performed with a delay of 2 s between each scan. For all the measurements, freshly prepared samples were used. FRAP measurement was only performed on concentrations ≤1%, due to difficulties in transferring samples of higher concentration (and hence viscosity) to the microscope slide. All measurements were made in at least triplicate. The diffusion coefficient was obtained by curve fitting using the Braeckmans model for the normalised recovery curve at time t, $F\mathrm{tot}(t)/F_0$ (Eq. (1)) (Braeckmans, Peeters, Sanders, De Smedt, & Demeester, 2003).

$$\frac{F_{\mathrm{tot}}(t)}{F_0} = 1 + [1 - e^{-2y}(I_0(2y)) + (I_1(2y))]\sum_{n=0}^{\infty}\left[\frac{(-K_0)^n}{n!}\frac{1}{\sqrt{1+n}}\right] \quad (1)$$

where $y = \omega^2/4Dt$, D is the two-dimensional lateral diffusion coefficient, ω is the e^{-2} radius of the Gaussian bleach spot, I_0 and I_1 are modified Bessel functions of the zero and first order, respectively, and K_0 is an instrumental parameter.

2.3. z-Average diameter

The z-average diameter of particles in B10, B28 and B100 in water was measured using a zetasizer (Nano-ZS, Malvern, UK) for a range of concentrations from 0.0625% (w/v) to 1% (w/v). The samples were prepared by dissolving the β-glucan samples in deionised water at 80 °C for 8 h in an oil bath.

2.4. Rheological investigation

An Advanced Rheometric Expansion System (ARES, TA Instruments) was used to investigate the viscoelastic characteristics of β-glucan solutions of varying concentrations. A range of concentrations from 0.5% to 2% (w/v) (0.5%, 0.8%, 1%, 1.5% and 2% (w/v)) of each of β-glucan (B10, B28 and B100) was prepared by dissolving in deionised water at 80 °C for 8 h in an oil bath; it was found that samples were often not dissolved with visible insoluble particles if left for shorter time. Parallel plate geometry (50 mm diameter) was used at ambient temperature for oscillatory and steady-state experiments. The linear viscoelastic region was determined for each sample using a dynamic strain sweep at 10 rad s^{-1} frequency. This was followed by frequency sweep experiments for 1–100 rad s^{-1} at a suitable strain within the linear viscoelastic region. Although steady shear viscosity was measured in the range of 1–100 s^{-1} due to sensitivity of the rheometer used, the Newtonian plateau value could be estimated from the data obtained as the curves are close to linear. All the rheological measurements were performed in duplicate and on freshly prepared samples.

2.5. Molecular size distributions of β-glucan

Size separation of the three β-glucan samples was performed using an Agilent 1100 Series SEC system (PSS GmbH, Mainz,

Germany), equipped with a column set-up consisting of a GRAM preColumn, a GRAM 100 and a GRAM 1000 column in series (PSS GmbH, Mainz, Germany), in a column oven at 80 °C and a flow rate of 0.6 mL min^{-1}. Dimethyl sulfoxide (DMSO) with 0.5% (w/w) LiBr was employed to dissolve the samples and as the mobile phase in the SEC, to ensure complete dissolution and analysis of the macromolecular properties without the occurrence of aggregation; this solvent system disrupts hydrogen bonds. NMR studies (Schmitz, Dona, Castignolles, Gilbert, & Gaborieau, 2009) have shown that this gives molecular dissolution of even hard-to-dissolve starches without aggregation. A volume of 100 μL of the β-glucan samples with a concentration of 0.2% (w/v) concentration was injected in the system. Detection was performed by multi-angle laser light scattering (MALLS) (BIC-MwA7000, Brookhaven Instrument Corp., New York, USA) and a refractive index detector (RID) (Shimadzu RID-10A, Shimadzu Corp., Japan).

SEC separates by size (hydrodynamic volume, V_h) and not by molecular weight; the size distributions are therefore presented in terms of an equivalent size parameter, the corresponding hydrodynamic radius R_h, with $V_h = 4/3\pi R_h^3$. Size calibration was implemented by the empirical Mark–Houwink relation, using pullulan standards (PSS GmbH, Mainz, Germany), with a molecular weight range of 342–2.55 × 10^6 g/mol. The method for calibration to obtain V_h has been given elsewhere (Cave, Seabrook, Gidley, & Gilbert, 2009). The Mark–Houwink parameters for pullulan in DMSO/LiBr (0.5 wt%) at 80 °C are $K = 2.427 \times 10^{-4}$ dL g^{-1} and $\alpha = 0.6804$ (Kramer and Kilz, PSS, Mainz, private communication) (Cave et al., 2009). The upper limit of the size of the standards corresponds to $Rh \sim 50$ nm.

Different size distributions, including the SEC weight distribution $w(\log V_h)$, the size dependence of the weight-average molecular weight, $\bar{M}_w(V_h)$ and the root mean square radius (the radius of gyration, R_g) were obtained from the detector signals after size separation (see (Vilaplana & Gilbert, 2010) for further details). The Zimm method with a differential refractive index (dn/dc) value of 0.0869 mL g^{-1} for pullulan in DMSO/LiBr (0.5% w/w) was used to calculate the $\bar{M}_w(V_h)$ and average radius of gyration $\bar{R}_g(V_h)$ for β-glucans and to obtain semi-quantitative data for comparison among the samples. Because this value of dn/dc is for a somewhat different polysaccharide, the molecular weight values are only semiquantitative, but permit meaningful comparisons of samples of similar composition.

3. Results and discussion

3.1. Diffusion coefficient measurement using FRAP

Fig. 1 plots the diffusion coefficients of the FITC-dextran probe (a molecular probe similar in size to both digestive enzymes and bile salt micelles) in different aqueous solutions of the three β-glucan samples as measured by FRAP, as a function of β-glucan concentration. The diffusion coefficient (D) of the probe in the absence of β-glucan was measured to be 1.55 × 10^{-7} cm^2 s^{-1}. As expected, D values were found to decrease with increasing β-glucan concentration for all samples. Moreover, B10 aqueous solutions exhibit the highest diffusion coefficient of FITC-dextran at all experimental concentrations while sample B100 display the lowest; this indicates that the values of D decrease with increasing sample viscosity. Xu et al. (2007) reported similar decrease in values of D for a range of concentrations (0.25–2%) for high-viscosity barley β-glucan. This decrease in diffusion coefficient with increasing sample viscosity of β-glucan is as expected, being qualitatively consistent with the Stokes–Einstein relation. This trend was however not observed for arabinoxylans (Shelat et al., 2010), where the corresponding anomalous behaviour suggested the existence of more complex correlations between the molecular structure, aggregation phenomena, and macroscopic behaviours in terms of local microviscosity and diffusional behaviour.

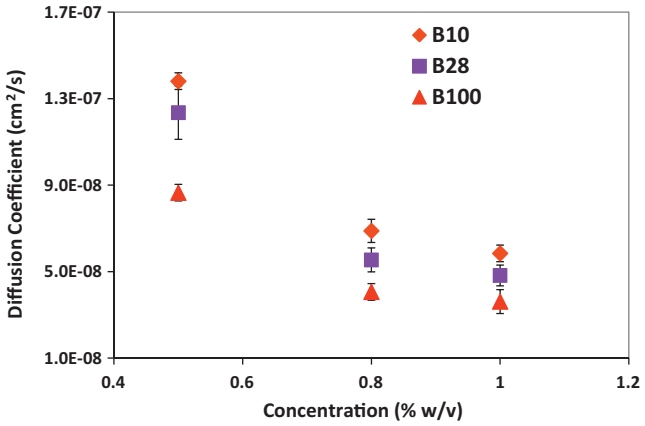

Fig. 1. Diffusion coefficient of FITC-dextran probe at varying β-glucan concentrations.

3.2. Viscoelastic characteristics of β-glucan solutions

The values of the storage (G') and the loss modulus (G'') increase with frequency, as shown in Fig. 2 for different aqueous concentrations of sample B100 as an example. In the case of B10 and B28 β-glucan samples, liquid-like characteristics are observed at all concentrations over the range of frequencies measured, i.e. with $G'' > G'$. For B100 sample solutions a typical liquid-like behaviour is also observed at 0.5% (w/v) to 1.5% (w/v) concentrations. However in the case of 2% (w/v) β-glucan solution a cross-over point is observed, showing weak solid-like behaviour at higher frequencies. Fig. 3 shows the plot of dynamic and steady state viscosity vs. frequency or shear rate respectively for the B100 sample. The measured values of dynamic viscosity at low shear rate at 1% concentration are 98 mPa s, 11 mPa s and 29 mPa s, similar to values as quoted by manufacturer 100, 10 and 28 mPa s for B100, B10 and B28 respectively; this suggests that our techniques do not cause significant degradation. In the case of B100, close to Newtonian behaviour is observed for concentrations below 2%, while shear thinning is observed for 2% at high frequency. As seen in this plot, the dynamic viscosity (η^*) and steady state viscosity (η) closely overlap with each other (i.e. the Cox–Merz rule is obeyed). This indicates that there is only physical entanglement present in the aqueous solutions of β-glucans over the length scale of

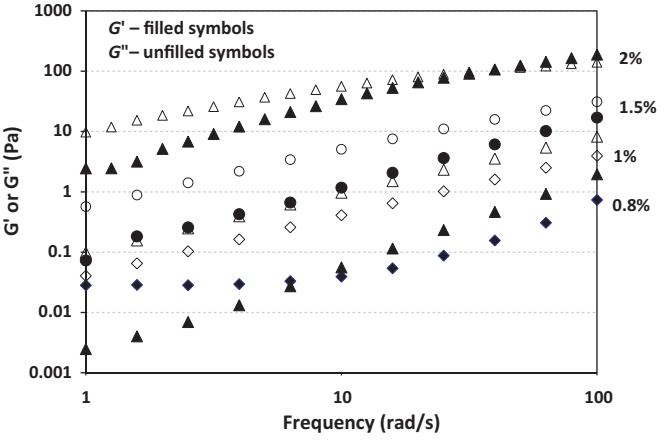

Fig. 2. G' and G'' of B100 at varying concentrations.

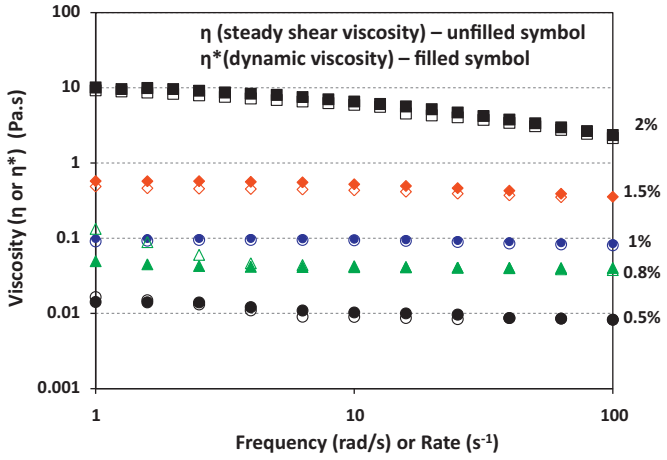

Fig. 3. Dynamic (η^*) and steady shear (η) viscosities of B100.

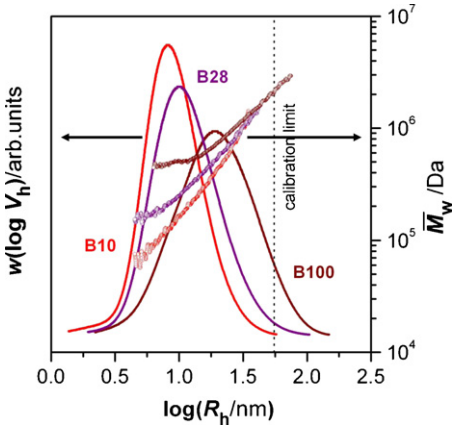

Fig. 5. SEC size distribution $w(\log V_h)$ and $\bar{M}_w$ as a function of R_h.

the rheological measurement (mm) (Cox & Merz, 1958; Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). The Cox–Merz rule was also observed to hold for B10 and B28 at all concentrations. In another study, Papageorgiou, Lakhdara, Lazaridou, Biliaderis, and Izydorczyk (2005) observed that the Cox–Merz rule was followed for different varieties of barley β-glucans except for one variety of high molecular weight at higher concentrations.

3.3. *z*-Average size for β-glucans using zetasizer

The *z*-average diameter increases for all the samples with increasing concentrations (Fig. 4), with sample B10 having the lowest value at all concentrations and B100 with the highest. For lower concentrations (<0.2%), the *z* diameter for samples B10 and B28 is around 200 nm, which are in the order of magnitude of the average R_g from SEC in DMSO. For B100 at low concentrations (<0.2%), the values of the *z* diameter are around 400 nm, which are slightly higher than twice the R_g values that we observe from SEC (around 150 nm). It is important to note that quantitative comparison between *z*-average and SEC size data is limited, since they differ in the solvent employed (water in the zetasizer, DMSO in SEC), and also because the average R_g and the *z*-average are different averages over the underlying size distribution. For higher concentrations, there is an increase in the *z*-average size, especially noticeable at concentrations above 0.5% (w/v%) for all samples, indicating a marked increase in β-glucan aggregation as the concentration increases. These size measurement results indicate the presence of aggregates for all β-glucan samples in aqueous solution. It seems that it is primarily concentration that determines the measured *z*-average particle sizes of a given sample (Fig. 4). The length scale of these aggregates is micron or lower, which explains why there is no evidence for structure in the system at the longer (mm) rheological length scale.

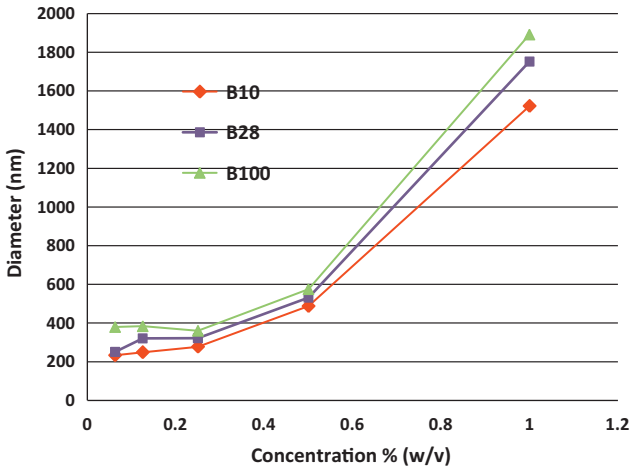

Fig. 4. : *z*-Average diameter as a function of concentration for B10, B28 and B100 in water.

3.4. Molecular size distributions of β-glucan

The macromolecular structural features of the three β-glucan samples were studied using SEC coupled with differential refractometry and light scattering detection. Fig. 5 shows the SEC weight distribution $w(\log V_h)$ and the size dependence of the weight-average molecular weight ($\bar{M}_w(V_h)$) for the three β-glucan samples. All β-glucan samples exhibit similar monomodal $w(\log V_h)$ size distributions, progressively shifted towards higher sizes with increasing viscosity of the samples. The weight-average molecular weight distributions $\bar{M}_w(V_h)$ also display similar features, with the sample with higher 1% (w/v) kinematic viscosity (B100) showing higher $\bar{M}_w$ values in the size distribution range of the data. The calculated overall molar-mass averages ($\bar{M}_n$ and $\bar{M}_w$) the dispersity ($D = \bar{M}_w/\bar{M}_n$), and the weight-average radius of gyration ($\bar{R}_g$) for the three β-glucan samples are presented in Table 1. Again, the average values present good correlation with the kinematic viscosity values, with the samples of higher viscosity showing progressively increasing values of overall molecular weight and radius of gyration.

Further structural information can be obtained from the conformational plots, which display the relation between $\bar{M}_w$ and $\bar{R}_g$ in solution (Burchard, 1999). The de Gennes scaling law concept (de Gennes, 1979), which is a simple power law expression, usually provides an acceptable fit to this relationship for simple polysaccharides:

$$R_g = K_g(\bar{M}_w)^{\nu_g} \quad (2)$$

Here K_g is a constant depending on the monomer structure and the nature of the solvent, and ν_g is an exponent which depends on the polymer architecture, temperature, and polymer–solvent interactions ($\nu_g = 0.5$–0.6 corresponds to a linear random coil conformation in solution; $\nu_g = 0.33$ for a sphere; and $\nu_g = 1$ for a rod). Fig. 6 shows the conformational plots for the three samples, together with the theoretical lines for the ideal geometrical conformations. The values of the exponent ν_g are also presented in Table 1. The conformational plots give information about the intrinsic architecture of the samples. The values of the ν_g exponent for all three samples are quite high, corresponding to a random coil

Table 1
Molar mass averages, dispersity, root-mean-square weight-average radius of gyration, and fractal coefficients from SEC for the β-glucan samples.

Sample	Average $\bar{M}_w$ (g/mol)	Average $\bar{M}_n$ (g/mol)	$\bar{M}_w/\bar{M}_n$	$\bar{R}_{gw}$ (nm)	ν_g
B10	175,190	103,650	1.69	72	0.47
B28	309,630	218,780	1.42	78	0.51
B100	1,003,300	617,840	1.62	120	0.53

structure, as expected for linear polymers. However, sample B10 shows a more compact structure (in DMSO) despite having smaller size and molecular-weight distributions.

3.5. Correlations between diffusion coefficient, viscosity and molecular structure: implications for nutrition

In order to correlate viscosity and diffusion characteristics, a plot of diffusion coefficient and zero-shear viscosity as a function of concentration is shown in Fig. 7(a). The zero-shear viscosity was taken from the lowest frequency data from Fig. 3 ($1\,s^{-1}$) for all samples as the slope of the viscosity curves was close to zero at this point. An attempt was made to use Cross equation (Cross, 1965) to fit the data, but the data range was insufficient to enable this fitting to improve the estimate. Moreover, the values so obtained in most cases were close to the viscosity values as obtained at $1\,s^{-1}$. As shown in Fig. 7(a), the diffusion coefficient decreases with increase in the sample viscosity (10, 28 and 100) at all measured concentrations. This contrasts with the case of arabinoxylan, where there was no generic correlation between solution viscosity and probe diffusion coefficient, an effect ascribed to differences in macromolecular structure and substitution pattern between samples (Shelat et al., 2010). Xu et al. (2007) measured the diffusion coefficient of 0.97 μm polystyrene probes in high viscosity β-glucan solutions for a range of concentrations (from 0.25% to 2%). These authors also observed that the diffusion coefficient decreased with increasing concentration from 0.25% to 2%. Moreover, the phase lag of the probe showed a slight deviation for 2% β-glucan solutions, indicating 'trapping' of the probe due to the elastic characteristics of the high-viscosity β-glucan solution. It was hypothesized that for concentrations ≤1%, probe particles experience diffusive motion while for 2% β-glucan solution, elasticity may also affect probe diffusion. In our present study, diffusion coefficient values are in the range of 10^{-7}–$10^{-8}\,cm^2\,s^{-1}$ whereas for Xu et al. the values were in the range of $10^{-5}\,cm^2\,s^{-1}$. This could be explained by the different types and size of probes used, i.e. FITC-dextran is a flexible probe of approximately 6 nm diameter while Xu et al. used inflexible fluorescent polystyrene microspheres of 0.97 μm diameter. Moreover, the values of diffusion coefficients reported by Xu cannot be compared directly with our diffusion coefficient values due to unavailability of details of the viscosity of the samples used in their work. We chose a small probe because this is comparable in size to both bile salt micelles and digestive enzymes. A simple model system to investigate diffusion of small probe in the presence of β-glucan is used in the present study. This may be relevant to delayed reabsorption of bile salt micelles (and consequent potential to lower plasma cholesterol (Gunness & Gidley, 2010)), and/or reduced rates of protein, carbohydrate or lipid digestion by intestinal enzymes. Both mechanisms are plausible causes for beneficial nutritional effects of soluble dietary fibre (Battilana et al., 2001; Smith & Tucker, 2011).

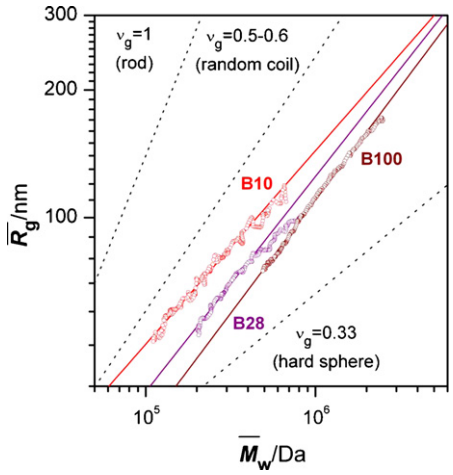

Fig. 6. Fractal dimensions (SEC results for R_g) for B10, B28 and B100.

In our previous study of wheat (AX29) and rye (AX33) arabinoxylans (Shelat et al., 2010), the values of D decreased as the viscosity increased for each individual sample. However, for the arabinoxylan sample with the highest viscosity, the values of D were higher than for the lower viscosity sample at all concentrations. This anomalous behaviour was attributed to differences in the macromolecular aggregation resulting in differences in microviscosity (Shelat et al., 2010). The different macromolecular structure of the different (macro-)viscosity wheat and rye arabinoxylans studied, particularly different substitution patterns of arabinose on the xylan backbone (Pitkanen et al., 2009; Shelat et al., 2010), was proposed to be responsible for different conformations of arabinoxylan in solution, resulting in aggregation and hence anomalous diffusion behaviour. In the present case of barley β-glucan samples, this anomalous behaviour is not observed, presumably because molecular structure is conserved and hence macromolecular size (and molecular weight) is the most important structural parameter, which determines aggregation behaviour in water and macroscopic properties such as viscosity and diffusion coefficient.

The plot of $D\eta/D_0\eta_s$ vs. concentration (Won, Onyenemezu, Miller, & Lodge, 1994) of B10, B28 and B100 shows positive deviations, where $D\eta/D_0\eta_s > 1$, from the Stokes–Einstein equation for the studied concentration range for all the samples (Fig. 7(b)). The deviation does not change largely for B10 with increase in the concentration. For B28 and B100, the deviation increases with increase in the concentration. The z-average size of B10, B28 and B100 increases with increase in concentration, indicating the presence of micron-sized aggregates in aqueous solution. This could be one of the main reasons for deviation from the trend given by the Stokes–Einstein equation. Moreover in the derivation of the Stokes–Einstein equation the probe is considered to be spherical while the probe used in our study is linear with a random coil conformation. For comparison, the data for different arabinoxylans is plotted from our previous study on the same graph. In the case of two different arabinoxylans, AX33 and AX29, qualitative deviations from Stokes–Einstein were observed. The deviation increases with increase in the concentration as also observed in the case of β-glucans. However, for rye vs. wheat arabinoxylan, the higher viscosity sample showed higher values of the diffusion coefficient, owing to a different molecular substitution pattern, aggregation and consequent differences between micro and macro-viscosities. Thus viscosity and diffusion are not necessarily correlated and should be measured independently (Shelat et al., 2010). For β-glucan, the similar chemical nature of the three samples studied precludes such major anomalies. However positive deviation from

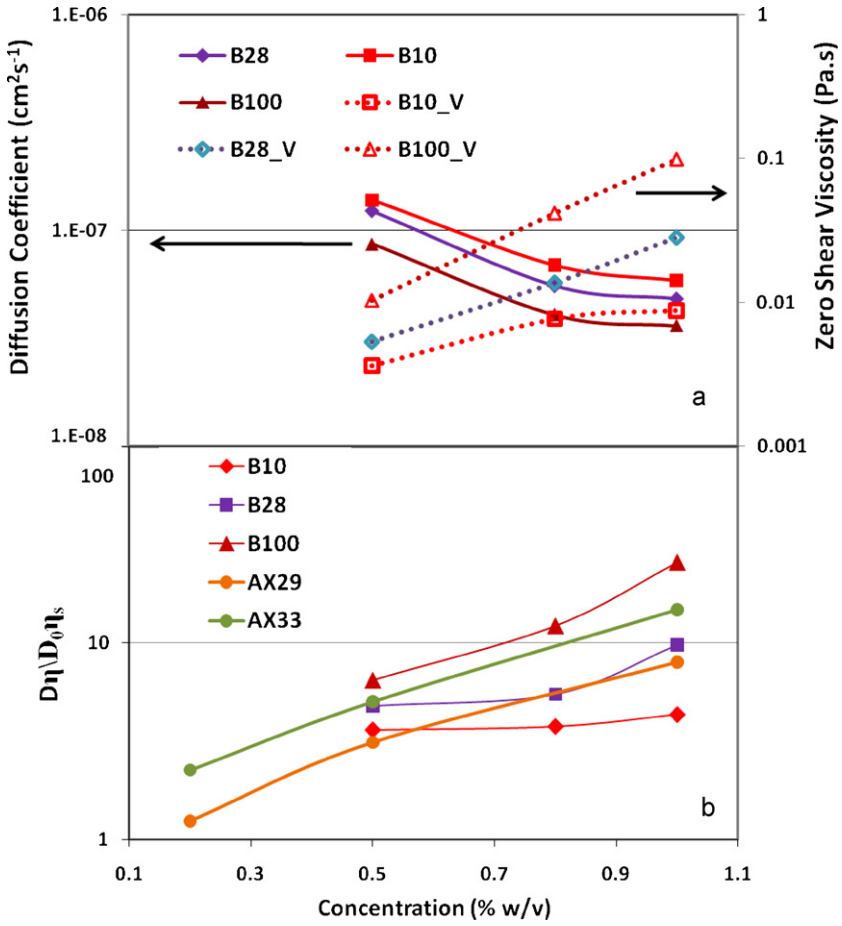

Fig. 7. Correlation of diffusion coefficient and zero shear viscosity of β-glucan (a), deviation from Stokes–Einstein equation for hard spheres (b). Connecting lines are purely for visual guidance and are not intended to imply any non-linear relation.

Stokes–Einstein behaviour of a similar magnitude to that for AX is observed for all samples (Fig. 7(b)), reinforcing the lack of a general relationship between bulk viscosity and probe diffusion for these systems.

In general, increase in digesta viscosity is considered to be one of the main mechanisms responsible for the beneficial effects of dietary fibres (Brownlee, 2011; Gunness & Gidley, 2010; Wolever et al., 2010). The increased viscosity of gut content might be expected to decrease the diffusion of both digestive enzymes and nutrients resulting in slower absorption. However, real food systems are heterogeneous and much more complex than the simple model system studied here. This model system has been chosen to provide baseline data underpinning possible mechanisms for some of the beneficial effects of beta-glucan. The diffusion process is likely to be important in affecting access of digestive enzymes of similar size to the probe used here (e.g. amylases, proteases) to their substrates (e.g. starch, protein) in the lumen of the small intestine. It is possible that diffusion of small molecule digestion products to the gut epithelia may also be affected. However, for small molecular size digestion products, static diffusion as determined here, may be secondary to convective diffusion in reducing transport of e.g. glucose, as inferred from studies with guar gum (Edwards, Johnson, & Read, 1988).

The diffusion coefficient of the probe in the absence of β-glucan (1.55×10^{-7} cm^2 s^{-1}) is 2–4 times faster than the values found for 0.8 and 1.0% β-glucan (Fig. 1). Although this does not appear to be a major reduction, a factor of 4 corresponds to the calculated reduction in diffusion coefficient of α-amylase in milled barley grains (Al-Rabadi, Gilbert, & Gidley, 2009). The consequence of a 4-fold reduction in diffusion coefficient was that particles of 2 mm or greater were hydrolysed less than 25% after 24 h, whereas isolated starch granules were more than 90% hydrolysed after 6 h under the same conditions. Thus a 4-fold reduction in diffusion coefficient has the potential to have a major impact on digestive processes such as starch amylolysis. The implication is that an effective concentration of β-glucan in the gastro-intestinal tract of ca. 1% is sufficient to result in health-benefiting modulation of digesta processing.

Consistent with these conclusions, in a classic paper, Jenkins et al. (1978) reported effects of different dietary fibre on reducing postprandial blood glucose and observed high viscosity dietary fibre was effective in reduction of blood glucose. Dikeman and Fahey (2006) reported changes in the viscosity of various soluble and insoluble dietary fibres and concluded that rice bran, wheat bran and cellulose can be considered as better laxative as these dietary fibres did not increase viscosity during simulated digestion. In the case of psyllium and guar gum, increased viscosity of in vitro digesta was observed which may affect physiological responses such as postprandial glucose uptake.

4. Conclusions

This study examined the correlation between diffusion coefficient and viscosity of barley mixed linkage β-glucans. Our previous study on arabinoxylans suggested that aggregation due to macromolecular structure and substitution pattern affects diffusion coefficient and viscosity, and that these parameters may

not be related. For β-glucans, similar deviations from qualitative Stokes–Einstein behaviour (decreasing bulk viscosity with increasing probe diffusion) were found, although the chemical similarity of the β-glucans used meant that all samples showed qualitatively similar results. This study has reinforced the concept that bulk viscosity cannot be used in isolation to predict diffusion coefficients for dissolved molecules, due to the presence of aggregates creating a micro-environment different from the bulk average. A reduction in diffusion coefficient of the FITC-dextran probe here of up to 4 times at a concentration of 1% of β-glucan is observed.

Acknowledgement

Funding for this work was made available from the Australian Flagship Collaborative Research Program, provided to the High Fibre Grains Cluster via the Food Futures Flagship.

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