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Rheological and microstructural investigation of oat β-glucan isolates varying in molecular weight

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ABSTRACT

The rheological properties and microstructure of aqueous oat β-glucan solutions varying in molecular weight were investigated. The structural features and molecular weights (MW) were characterized by ^{13}C NMR spectroscopy and high performance size-exclusion chromatography (HPSEC), respectively. The microstructure of the β-glucans dispersions was also examined by atomic force microscopy (AFM). The samples with β-glucan content between 78 and 86% on a dry weight basis had MW, intrinsic viscosity ($[\eta]$) and critical concentration (c^*) in the range of 142–2800 × 10^3 g/mol, 1.7–7.2 dl/g and 0.25–1.10 g/dl, respectively. The flow and viscoelastic behaviour was highly dependent on MW and on the concentration of the β-glucans dispersions. Pseudoplastic behaviour was exhibited at high concentrations and Newtonian behaviour was evident at low concentrations. At the same concentration, the viscosity was higher for higher MW samples. The Cox–Merz rule was applicable for the lower molecular weight samples at higher concentrations whereas the high molecular weight sample deviated at concentrations greater than 1.0%, w/v. The mechanical spectra with variation of both MW and concentration were typical of entangled biopolymer solutions. AFM images revealed the formation of clusters or aggregates linked via individual polymer chains scattered heterogeneously throughout the system. The aggregate size increased with the molecular weight of the samples investigated and has been linked to the rheological behaviour of the samples.

1. Introduction

Oat (*Avena sativa* L.) and its products exhibit significant positive health effects including cholesterol lowering, modulation of glucose and insulin responses, weight management and improved gastrointestinal function. This is attributed to β-glucans, which have been accepted as a functional, bioactive ingredient [1–6].

β-Glucans in cereals are the predominant component of the endosperm cell walls of the grains. They are located throughout the starchy endosperm and are concentrated in the sub-aleurone layer. Their content ranges from 1% in wheat grains to 3–9% in oats, and 5–11% in barley [7,8]. β-Glucans are linear homopolysaccharides of consecutively linked (1→4)-β-D-glucosyl residues that are separated by single (1→3) linkages. The polysaccharide is neutral and made up of approximately 70% β-(1→4) and 30% β-(1→3)-linkages [9] and the molecular weight (MW) values for β-glucans range between 20 and 3100 × 10^3 g/mol depending on the botanical source, extraction/isolation protocols, and the analytical methodology used in the determination of these values [1,10–12].

Solubility in water and the capacity to form highly viscous solutions is a fundamental characteristic of oat β-glucans due to their high molecular weight, conformation, and self-association characteristics [2,12]. Fundamental studies on the rheological properties of β-glucans have been performed previously and the functionality of β-glucans was found to be highly dependent on polysaccharide structure, molecular weight and concentration [7,12–16]. However, limited information exists on the link between the microstructure and the rheological properties of this polysaccharide. Application of atomic force microscopy (AFM) has enabled visualization of individual polysaccharide molecules and their interactions in solution [17–27]. Imaging biopolymers by AFM to characterize important factors such as molecular weight, morphology and the nature of self-association can provide useful information to explain functionality.

The objective of this investigation, therefore, is to find a relationship between rheological and microstructural properties of oat

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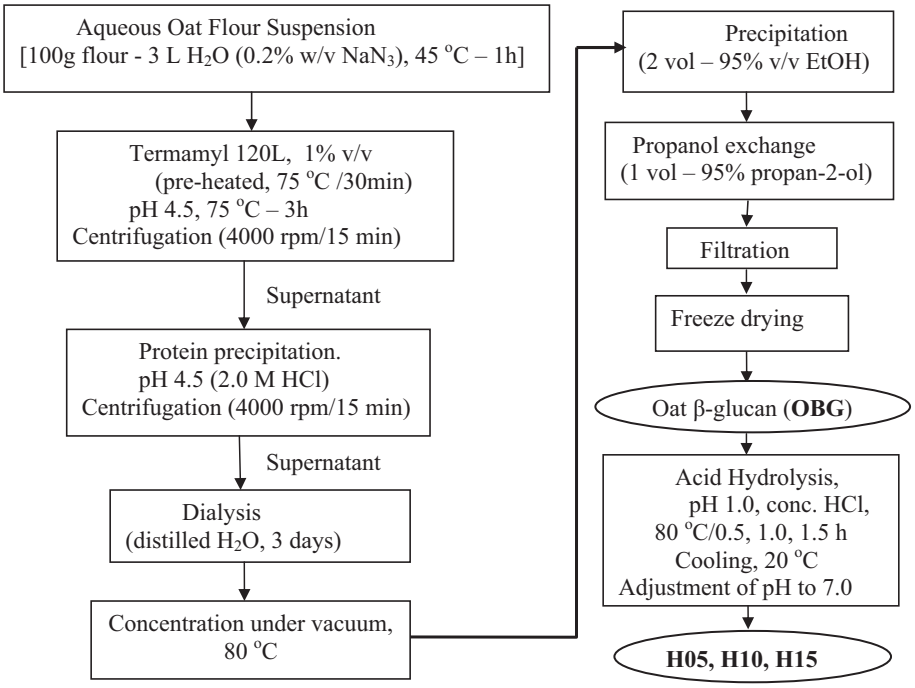

Fig. 1. Extraction and purification of β-glucan from oat flour.

β-glucans as a function of molecular weight using rheometry and AFM.

2. Materials and methods

2.1. Materials and chemicals

The Megazyme mixed-linkage beta-glucan assay and Quick Start™ Bradford protein assay kits were purchased from Megazyme International Ltd (Bray, Ireland) and Bio-Rad Laboratories Inc. (Hercules, California, USA), respectively. Proteinase K-Agarose (from *Tritirachium album*), sodium azide (NaN_3), dialysis membrane tubing (MWCO 12000) and closures were purchased from Sigma–Aldrich (Poole, Dorset, UK) whereas ethanol and isopropanol were obtained from Fisher Scientific (Loughborough, UK). Termamyl 120L Type L (heat-stable alpha-amylase) was purchased from Univar (Bradford, UK). Oat flour (OatWell® 28%, an oat bran with 28% β-glucan content) was obtained from CreaNutrition (Swedish Oat fibre, Sweden). Distilled water was used throughout the experiments. All chemicals used were analytical grade reagents.

2.2. Extraction and purification of β-glucans

Extraction of oat β-glucans from oat flour was performed by adapting previously published isolation protocols with slight modifications [7,12,14]. Fig. 1 presents a schematic diagram of the extraction procedures employed for the isolation and purification of β-glucans. The extraction resulted in the initial β-glucan isolate that was denoted as OBG.

2.3. Acid hydrolysis

Three more samples (H05, H10, H15) were obtained from the initial isolate (OBG) by controlled acid hydrolysis. The β-glucan sample was dispersed in double-distilled water (1.5%, w/v) at 80 °C under continuous stirring in a sealed vial. When the polysaccharide was fully dispersed, the temperature was lowered to 70 °C and concentrated HCl added to bring the concentration to 0.1 M. The polysaccharide was hydrolysed for 0.5, 1, and 1.5 h and, immediately after the end of hydrolysis, the solutions were cooled in running water to room temperature, and the pH was adjusted to 7.0 with 5 M NaOH. The hydrolysates were precipitated with three volumes of 95% (v/v) ethanol and left standing for 1 h at 4 °C. The precipitate was collected by filtration, washed with isopropyl alcohol, freeze dried and ground to a powder of 600 μm mesh size.

2.4. β-Glucan and protein content determination

The β-glucan content of the isolates produced was determined by the McCleary method using the Megazyme mixed-linkage beta-glucan assay kit [28] and calculated using the Megazyme Mega-Calc™ Software. The protein content was determined according to the Bradford protein assay [29]. A standard calibration curve was generated with bovine serum albumin (BSA) standards (125–1000 μg/ml) and used to estimate the protein content of samples. Absorbance readings were taken at 595 nm using a spectrophotometer (Shimazu UV-VIS 160A).

2.5. ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy

The ^{13}C NMR spectroscopy was performed with a Bruker AV 500 Spectrometer at 125.76 MHz using a 5 mm PABBO probe. The samples were dispersed (2%, w/v) in pure deuterated methylsulphoxide (d6-DMSO) by heating and continuous stirring at 90 °C for 3 h. The proton-decoupled spectra were recorded at 70 °C overnight by applying 12,800 pulses with a delay time of 2 s and a radio frequency angle of 30°. Chemical shifts were expressed in parts per million (ppm) relative to d6-DMSO at 39.5 ppm and reported relative to tetramethylsilane (Me_4Si).

2.6. Molecular weight determination

Purified gums were solubilized (~1 mg/ml) in deionised water for 3 h at 90 °C. Solutions were diluted with deionised water, filtered through a 0.45 μm filter, and the peak molecular weight (M_p) was measured by high-performance size-exclusion chromatogra-

phy (HPSEC). The sample was injected into a Shodex (Showa Denko K.K., Tokyo, Japan) OHpak SB-806 M column (with OHpak guard) followed by a Waters Ultrahydrogel linear column (40 °C) using a Waters 717plus autosampler and eluted at 1 ml/min in 0.1 M Tris buffer (pH 8.0) with a Shimadzu model LC-20AT pump. A Shimadzu LC-10ATVP pump was used for post-column addition of Calcofluor (20 mg/L in 0.1 M Tris buffer, pH 8.0, at 1 ml/min) (Calcofluor-WhiteM2R New, C.I. 40622, fluorescent brightener 28, American Cyanamid Co., Bound Brook, NJ), enabling fluorescence detection in a Shimadzu RF-10Axl fluorescence detector (excitation, 360 nm; emission, 450 nm). Fluorescence intensity was collected by a Viscotek DM 400 data manager and data integration was performed using TriSEC 3.0 (Viscotek, Houston, TX) software. Five β-glucan molecular weight standards (20,000–1,200,000 g/mol), both prepared in-house and obtained commercially (Megazyme International), were used to construct a calibration curve for β-glucan by plotting retention time versus log M_p.

2.7. Atomic force microscopy (AFM)

Prior to AFM imaging, β-glucan sample dispersions (0.1%, w/v) were treated with 1.0 mg/ml of proteinase K-agarose (Sigma Chemicals, UK) at 37 °C for 48 h to further reduce the protein content of the samples. The AFM used was manufactured by East Coast Scientific Ltd. (Cambridge, UK). The instrument was operated in contact mode under butanol and the cantilevers used were 100 μm 'SiNi Budget Sensors' (Innovative Solutions, Bulgaria Ltd.) with a quoted force constant of 0.27 N/m. The β-glucan samples were diluted in pure water Barnstead Nanopure (Triple Red, UK) to 2 μg/ml. Heating to 90 °C in a sealed tube for 30 min was necessary to ensure complete dissolution of the samples. After returning to room temperature a pipette was used to deposit 3 μl single droplets onto freshly cleaved mica (Agar Scientific, UK). The deposit was left for 10 min to dry before imaging.

2.8. Rheological measurements

β-Glucan samples were dispersed at 0.01–8.0% (w/v) in distilled water in sealed glass-vials at 80 °C by continuous stirring to ensure complete solubilization. The intrinsic viscosity [η] of β-glucan solutions was determined at 20 °C with a Ubbelohde capillary viscometer and calculations made according to the Huggins equation. All rheological measurements (oscillatory and viscometry) were carried out at 20 °C using a Bohlin Gemini 200HR nano rotational rheometer (Malvern Instruments, Malvern, UK) equipped with cone-and-plate geometry (55 mm diameter, cone angle 2°). The oscillatory measurements were performed at a strain of 0.1%, which was found to be within the linear viscoelastic range of the material after strain sweep measurements, and a range of angular frequencies (ω) of 0.06–628 rad/s. The flow curves were measured at a range of shear rates in the range 0.01–1000 s^{-1}. Thixotropic loop experiments were also conducted over a shear rate of 0.1–1000 s^{-1} for a total cycle time of 5–60 min to monitor the viscosity changes. The data were analyzed with the supporting rheometer software. All rheological measurements were carried out on freshly prepared samples.

3. Results and discussion

3.1. Purity and molecular weight determination of oat β-glucan isolates

Initially the purity and the molecular weight (MW) were determined for all the samples. The protein content of the β-glucan isolate (OBG) and the hydrolysates (H05, H10, H15) was about 13% and the β-glucan content was found to be in the range 78–86% on a dry weight basis (Table 1). The original oat β-glucan isolate (OBG) had a very high peak molecular weight of 2.8×10^6 g/mol whereas the acid hydrolysis process yielded lower MW samples varying between 142 and 252×10^3 g/mol (H05 > H10 > H15) (Table 1). Controlled acid hydrolysis caused depolymerization of the initial β-glucan sample, thereby generating products of different MW but with the same structural characteristics: the longer the acid hydrolysis treatment time employed, the lower the MW of the resulting material. Similarly, other researchers also utilized acid hydrolysis to degrade oat gums and reported a reduction in the average MW of the initial material [14,30,31]. After isolation, purity and molecular weight determination of the samples, their structural characteristics were investigated as described in the following section.

Table 1
Content, molecular and structural features of oat beta-glucan isolates on dry basis (d.b).

Sample	β-Glucans (% d.b)	Protein (% d.b)	MW[a] × 10^3 (g/mol)	(1→4)/(1→3)[b]
OBG	83	13.7	2800	2.15
H05	86	13.2	252	2.20
H10	78	13.0	172	2.18
H15	78	12.9	142	2.07

[a] Peak molecular weight obtained from the HPSEC chromatograms.
[b] From ^{13}C NMR spectra (relative intensities of the two C-6 resonances).

3.2. Structural features of oat β-glucans

The ^{13}C NMR spectra obtained for all samples (Fig. 2) was typical of a mixed linkage cereal β-glucan and the peaks were assigned using relevant information previously reported in the literature [7,9,12,32–36]. The presence of only a single resonance for C-3 and C-4 of the O-3-β-glucopyranosyl residue (at 86.9 ppm and 68.2 ppm, respectively) confirms that there is no consecutive β-(1→3)-linkages in the β-glucan chain [7,9,12,32–36]. The non-consecutive occurrence of β-(1→3) linkages along the polymeric chain has been suggested to contribute to solubility and flexibility of (1→3),(1→4)-β-glucans whereas the regularity of the (1→4) linkages is considered to be responsible for insolubility and aggregation phenomena [37,38]. The purity of the β-glucan is also confirmed by the presence of only three resonances for the C-1 carbons. Thus the absence of starch and arabinoxylan is indicated by the absence of resonances due to the alpha configuration of the anomeric carbons (α-Glc) which would resonate at ~100 ppm whereas the anomeric β-Glc carbon resonances slightly downfield (at ~104 ppm) [33,35]. Finally, the relative intensities of the two C-6 resonances at 60.3 ppm and 60.7 ppm gives an index of the ratio of the (1→4)/(1→3) linkages in the β-glucan chain [7] and this was found to be between 2.07 and 2.20 (Table 1).

3.3. Rheological measurements

The viscosity of a polymer solution depends on the molecular weight (MW), concentration and also on the interaction between the solvent and neighbouring polymeric chains. The fractional increase in viscosity due to the presence of a polymer in solution is expressed as the specific viscosity (η_{sp}) and the intrinsic viscosity ([η]) is used to characterize the volume occupied by the individual polymer molecules in isolation. The intrinsic viscosities at 20 °C were determined by the extrapolation of the experimental viscometric data to zero concentration according to the Huggins equation:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \quad (1)$$

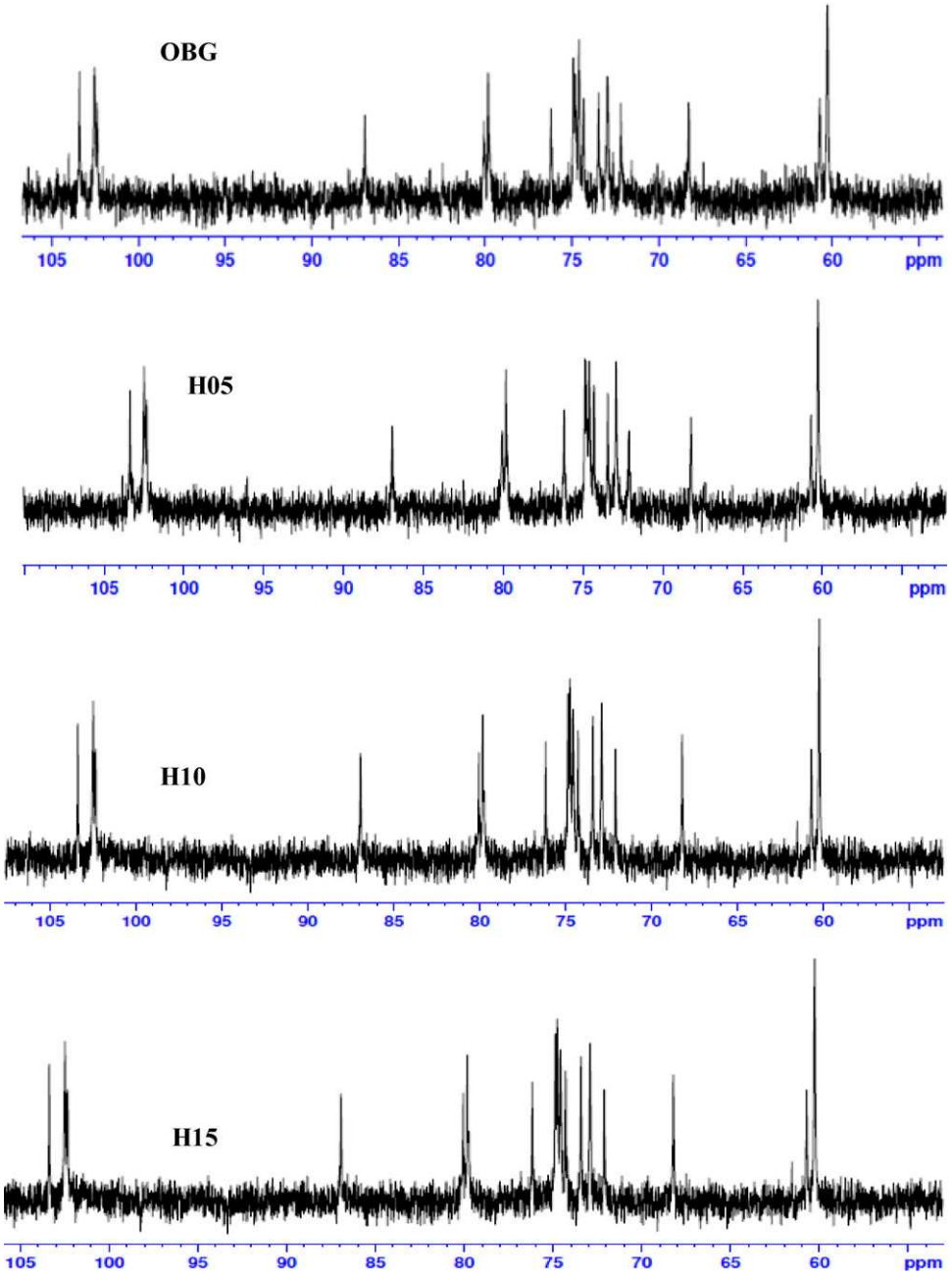

Fig. 2. ^{13}C NMR spectra of oat β-glucan isolates.

where $\eta_{sp} = (\eta_{solution}/\eta_{solvent}) - 1$ and k_H is the Huggins constant. The calculated $[\eta]$ values of β-glucan samples varied between 1.7 and 7.2 dl/g (Table 2) and increased markedly with increasing MW. Similar values of $[\eta]$ namely, 0.67–3.83 dl/g (MW between 35 and 350×10^3 g/mol), 1.15–4.6 dl/g (MW between 40 and 375×10^3 g/mol), 4.9–6.4 dl/g (MW between 0.27 and 0.78×10^6 g/mol) and 2.58–9.63 dl/g (MW between 100 and 1200×10^3 g/mol) have been reported previously for other β-glucan preparations [7,12,31,39,40]. The product of intrinsic viscosity and concentration gives an index of the total degree of space-occupancy: the reduced concentration or coil overlap parameter ($c[\eta]$). Fig. 3 presents double logarithmic plots of η_{sp} versus $c[\eta]$ which illustrates the dependency of specific viscosity at zero shear rates on the reduced concentration of aqueous β-glucan dispersions. The results superimpose closely falling into two distinct linear regions for all samples studied, a behaviour that is typical for most disordered polysaccharide solutions. However, as the specific viscosity is directly related to MW and strongly dependent on concentration, these plots are used to take into account the differences between hydrodynamic dimensions among different samples, and to estimate the transition from the dilute to the concentrated regime. The transition from dilute to concentrated

Table 2
Slopes, intrinsic viscosity $[\eta]$ and critical concentration (c^*) values of β-glucan isolates.

Sample	Slope 1	Slope 2	$[\eta]$ (dl/g)	c^* (g/dl)	$c^*[\eta]$
OBG	1.00	3.97	7.2	0.25	1.80
H05	0.93	3.13	2.1	0.56	1.17
H10	0.69	3.38	1.8	0.97	1.74
H15	0.64	3.62	1.7	1.10	1.85

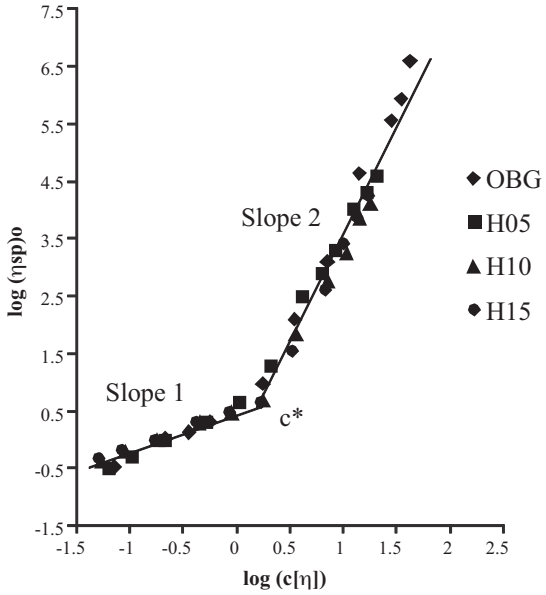

Fig. 3. Zero shear specific viscosity $(\eta_{sp})_o$ versus the reduced concentration $c[\eta]$ for β-glucan isolates. The intercept between the two linear slopes indicates the critical concentration c^* that demarcates the transition from dilute to concentrated solution behaviour.

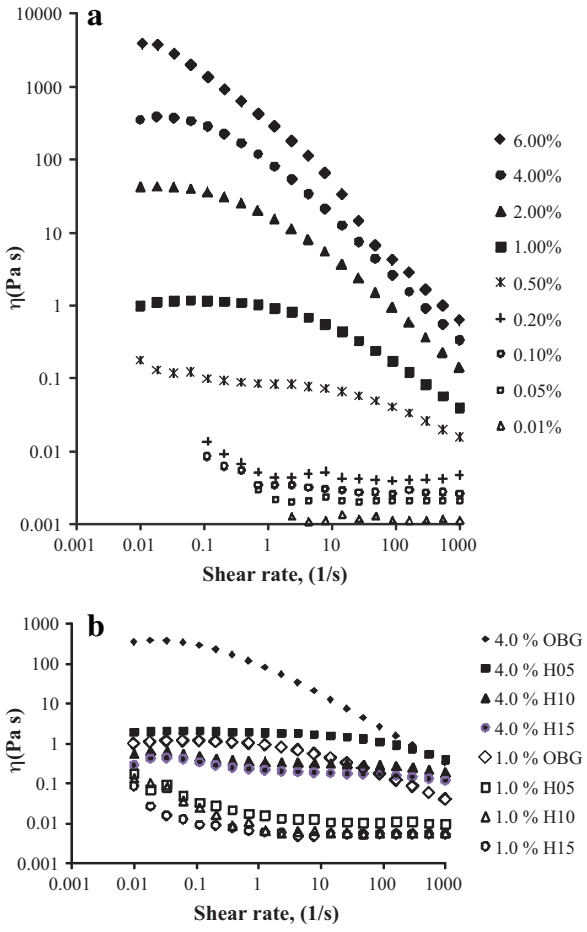

Fig. 4. Viscosity dependence on shear rate for oat β-glucan dispersions differing in (a) concentration (top) and (b) different molecular weights (bottom) at 1% and 4% (w/v).

solution behaviour at the interception between the two linear regions (Fig. 4) is designated as c^*. Thus at concentrations higher than c^* the polymer chains become highly entangled and conformational dynamics are determined by intermolecular/chain–chain interactions, whereas at concentrations less than c^* individual molecules are separated from neighbouring molecules [41,42]. The c^* values varied between 0.25 g/dl and 1.10 g/dl for the different oat β-glucan dispersions due to the differences in the MW of the samples (Table 2). Two linear regions with only one critical concentration c^* varying between 0.5 and 2.0% (g/dl) has also been observed previously for barley β-glucans in the MW range between 375 and 40×10^3 g/mol [43]. Similarly, two distinct linear regimes of slopes that fit the results for β-glucans and disordered chains have been identified previously [40,44]. Other researchers, however, observed an intermediate transition from dilute to semi-dilute and from semi-dilute to a concentrated regime giving rise to two critical concentrations, c^* and c^{**}, respectively (hence three slopes instead of two as in our case) [7,14,33]. However, the estimates are in close agreement with their findings with respect to c^* and c^{**} values for samples with similar MW. The c^* values increased with decreasing MW of the β-glucan samples which shows that coil overlap occurs at lower concentrations in the case of high molecular weight samples (OBG < H05 < H10 < H15) (Table 2).

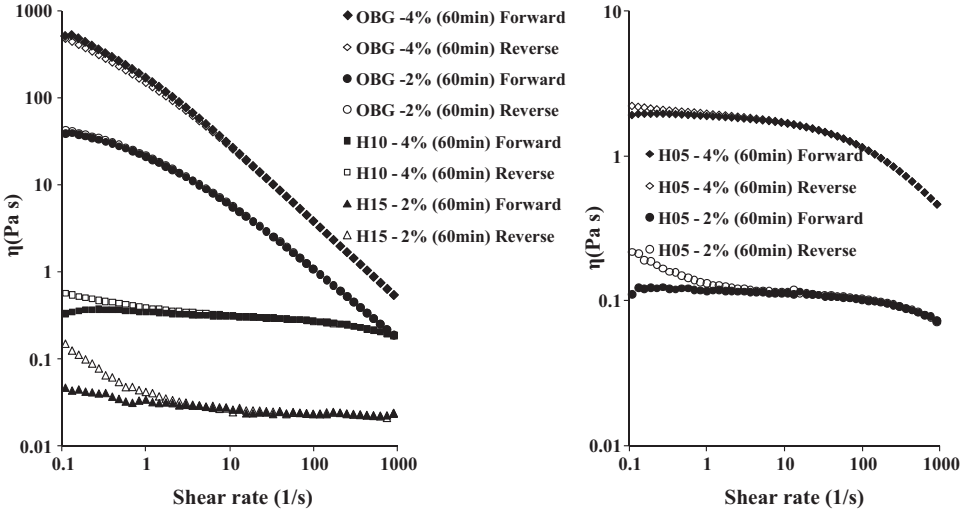

Fig. 5. Thixotropic loop experiments for oat β-glucan dispersions with different molecular weights at 2% and 4% (w/v). The forward and reverse indicate the upward and downward shear ramps, respectively.

The results therefore are indicative of the fact that variation in concentration or MW significantly affects the zero shear rate viscosity. The coil overlap parameter $c^*[\eta]$ ranged between 1.17 and 1.85 and was in close agreement with those reported previously [12,31].

Following dilute solution rheology the flow behaviour of the samples was studied next as a function of MW and concentration at 20 °C. Fig. 4 illustrates the dependence of viscosity on shear rate for the β-glucan dispersions differing in concentration and MW. At higher concentrations, pseudoplastic behaviour was exhibited as the shear rate increased whereas, at lower concentrations, Newtonian behaviour was observed. The molecular weight dependence of viscosity versus shear rate for the four oat β-glucan samples revealed that, at the same concentration, the viscosity was higher for higher MW samples (OBG < H05 < H10 < H15, Fig. 4b). A similar trend has also been observed in previous studies involving β-glucan dispersions varying in MW and concentration [7,12–14]. The thixotropic behaviour of β-glucan dispersions is illustrated in Fig. 5. For OBG, the curves obtained with the increasing shear rate superimposed on the curves obtained with the decreasing shear rate for all the different experimental conditions studied. At high shear rates, the lower MW samples also exhibited similar thixotropic behaviour. However, at low shear rates there was a hysteresis between the upward and downward curves indicating the formation of aggregates during the period of rest. At the points where the loop was manifested, the area was greater for the longer total cycle time. It was also observed that the thixotropic loop area increases as the MW decreases. This is possibly due to the greater diffusion of the low MW chains and aggregates that facilitate intermolecular interactions and aggregation.

The relationship between zero shear viscosity and complex viscosity was also studied for all β-glucan isolates. According to the Cox–Merz rule, the complex dynamic viscosity η^* (as a function of angular frequency, ω, rad/s) can be superimposed onto the plot of shear viscosity as a function of shear rate (1/s), thus according to Cox and Merz [45]:

$$|\eta^*(\omega)|_{\omega\to 0} = |\eta^*(\dot{\gamma})|_{\gamma\to 0} \quad (2)$$

Thus a direct relationship exists between the rheological response to non-destructive and destructive deformation whereby the oscillatory and shear flow curves should be identical if the polymer solutions are free from high-density entanglements or aggregates. Previous studies indicated that polymer solutions follow the Cox–Merz rule at low concentrations but deviations occur at high concentrations whereby η^* was higher than η at high frequencies or shear rates [7,12,14,43,44,46]. At concentrations up to 1% (w/v), OBG followed the Cox–Merz relationship as there were no significant differences between η^* and η at equivalent rates of deformation, whereas OBG dispersions >1% showed deviations from the rule (i.e., $\eta^* > \eta$) at high shear rates with an increasing difference as the concentration increased. In contrast, H05, H10 and H15 samples at 6% (w/v) concentration all followed the Cox–Merz

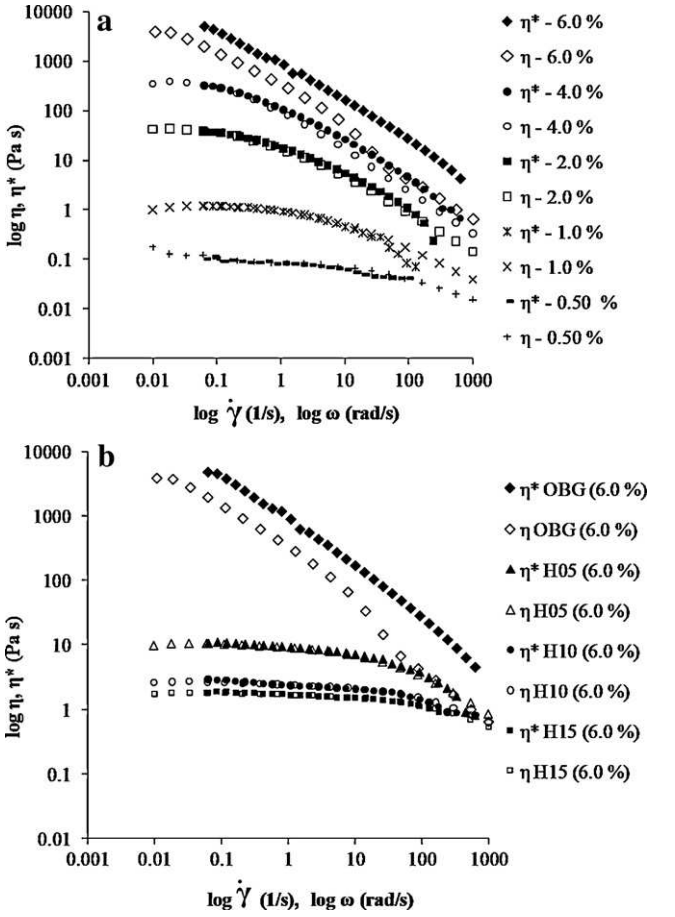

Fig. 6. Cox–Merz rule applicability for oat β-glucan dispersions at (a) different concentrations, OBG (top) and (b) with different molecular weights (bottom) at 6% (w/v).

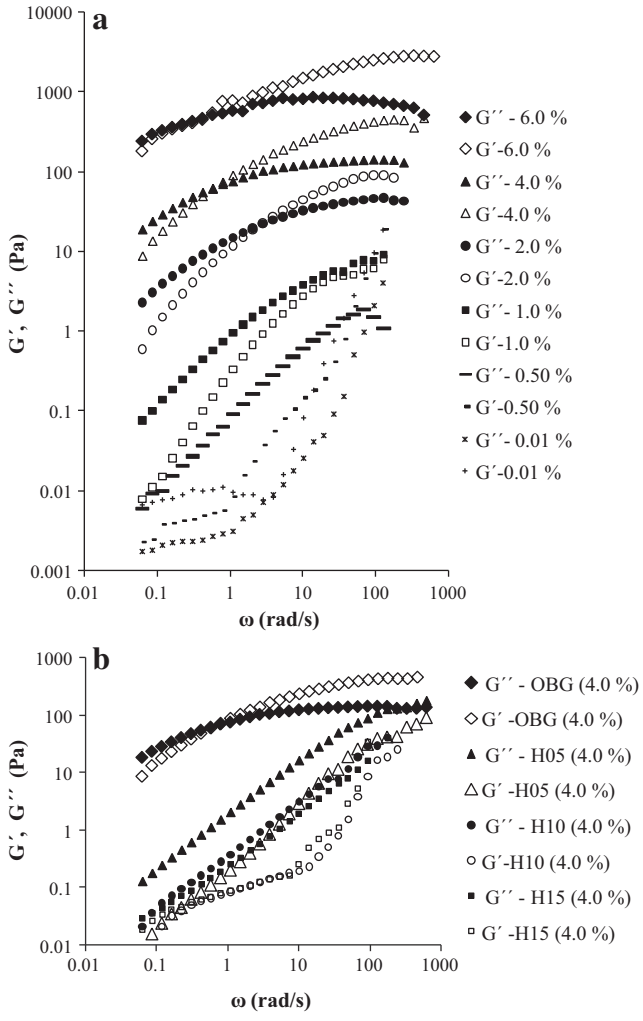

Fig. 7. Frequency dependence of storage (G') and loss (G'') moduli of oat β-glucan dispersions at (a) different concentrations (OBG) (top) and (b) with different molecular weights (bottom) at 4% (w/v).

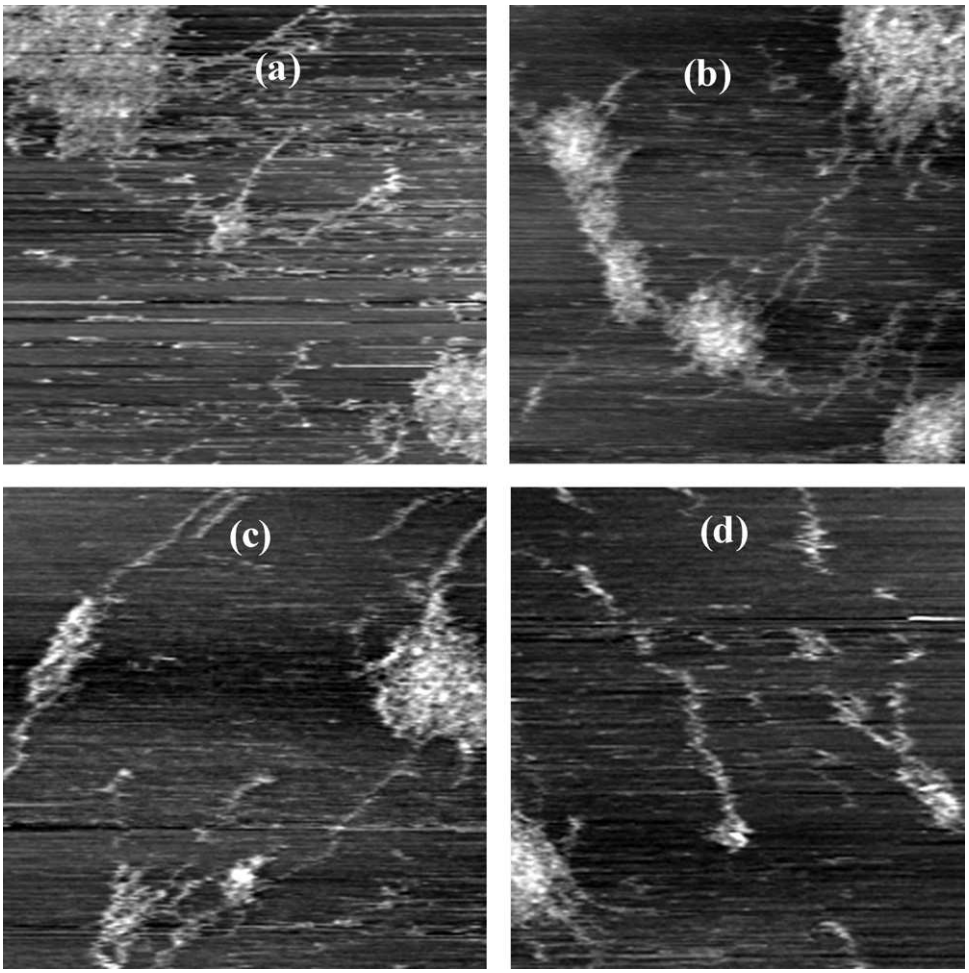

Fig. 8. Topographical atomic force microscopy images of oat β-glucan isolates (a) OBG, (b) H05, (c) H10, and (d) H15 at 2 μg/ml concentration. The scan sizes are 1 μm × 1 μm for all the images.

rule (Fig. 6) whereas H10 and H15 exhibited applicability of the rule even at 8% concentration. Notice that OBG at 6% concentration showed deviations with $\eta^* > \eta$ at all shear rates. The deviations may be due to the high density of entanglements and/or intermolecular chain aggregation of the system. These aggregates are more sensitive to shear forces than to the oscillatory perturbations where they have more time to reform in every cycle of oscillation. These aggregates exist and were visualized by AFM, as will be described in the next section. The results therefore indicate that Cox–Merz rule applicability for the oat β-glucan isolates is dependent on both MW and concentration of the polysaccharides in solution.

Oscillatory measurements may provide indirect information on the conformation of polysaccharides in solution [47] and therefore dynamic measurements were performed for all the isolated samples. Fig. 7 depicts the dependence of the mechanical spectra on concentration and MW for various β-glucan solutions. At low concentrations (≤1.0%) the loss modulus (G'') was greater than storage modulus (G') at all frequencies for the OBG dispersions, with the response corresponding to that of a non-entangled solution (Fig. 7a). As the concentration increased (>2%) the $G' - G''$ crossover, a characteristic of solutions with entangled polymeric chains, became evident. However the entanglement plateau was more evident at high concentrations for the high molecular weight sample (Fig. 7a). On the other hand, the $G' - G''$ crossover was not observed for the low molecular weight solutions even at high concentrations. This behaviour reflects the flexibility of the polysaccharide chain that is attributed to the β-(1→3) glycosidic linkage interrupting the continuity of the stiff cellulosic regions, thus imparting random coil conformation to the polysaccharide. Similar behaviour has been observed in previous studies for other polysaccharides such as locust bean gum [48], levan [49], wheat arabinoxylans [50] and cereal β-glucans [12,14,31].

The results clearly indicate that the rheological properties of the β-glucans depend on both molecular weight and concentration, which inevitably influence the interactions of the polysaccharide chains in solution. Therefore, the microstructure of β-glucans in solution was investigated by atomic force microscopy to identify any link between the rheological and microstructural properties as a function of molecular weight.

3.4. Microstructure of oat β-glucans by atomic force microscopy (AFM)

Polysaccharides including carrageenan [19], xanthan [21], gellan [17], pectin [20,51], polygalacturonic acid [52], psyllium polysaccharide [53] and β-glucans [54–57] form macromolecular aggregates when dispersed in water due to intermolecular hydrogen bonding.

Fig. 8 depicts the typical microstructure of β-glucans of varying molecular weight indicating the formation of aggregates upon drying from solution. It is probable that as water is evaporated from the sample on the mica, the β-glucan concentration increases which would accelerate aggregation due to possible change in conformation of the glucan molecules. However, the aggregate morphology

was found to be highly dependent on the MW of the sample. Generally, the size of the aggregates formed increased with increasing MW of the sample in solution. Clusters of aggregates are seen with single molecules emerging from them, which are scattered heterogeneously throughout the system. It can be seen from the AFM images that the β-glucan clusters extend with thread-like polymer chains of variable thickness that join neighbouring molecules, short chain fragments or cluster of aggregates. It has been previously observed that oat β-glucan dispersions have a strong tendency to form aggregates even in dilute solutions whereby the shape and size of the aggregates also increase with increasing concentration [27]. In another previous study, aggregates of oat β-glucans in aqueous solutions have been detected using static light scattering but not observed using osmotic pressure measurement for the same concentration range. It has been suggested that only a fraction of the molecules are involved in association to form large stabilized aggregates which has been described by a closed association model [55]. Also, both static and dynamic light scattering techniques have been employed from which a fringed micelle model was proposed for barley β-glucan aggregates in dilute aqueous solution whereby formation of aggregates increased the measured chain stiffness [56]. It can be seen that, the AFM images obtained in the present study are very similar to the suggested fringed micelle in a high aggregated state. It has also been shown that the aggregation behaviour of cereal β-glucans in aqueous solution is a fast dynamic process whereby the molecular association and dissociation are quick phenomenon [57]. A cluster–cluster aggregation was suggested to be dominant whereby the average apparent diameter of β-glucans was also concentration dependent. As the MW increased, the degree of aggregation decreased due to the lower diffusion rate of large molecules. Apparently, the nature of aggregation varies depending on the source and MW of β-glucans, as well as the concentration in solution.

Formation of aggregates, therefore, is believed to have had an important influence on the rheological properties [58]. The polydispersity of the aggregates increases with time [52]. It has been suggested that, when the system is at rest, the aggregates grow in size, possibly by means of diffusion [57]. In contrast, under the force of deformation, aggregate breakdown occurs [17] which also explains why η was generally lower than η^* at higher shear rates or frequencies in the steady shear and oscillatory measurements. Both η^* and η increased with increasing MW at equivalent concentration and shear rate (Fig. 6). The deviation from the Cox–Merz rule at even lower concentration for the high molecular weight sample (in comparison to the lower molecular weight samples) may be due to the presence of high-intensity entanglements or large clusters of aggregates and their associated fragments, which are sensitive to shear forces. It is possible that it is the thread-like structures linking the aggregates which may rupture under shear. The viscoelastic behaviour of β-glucans (as shown by the mechanical spectra in Fig. 7a) was also typical of entangled or aggregated solution for the OBG sample, which correlates well with the AFM images obtained. The beginning of the rubbery zone (i.e. the cross-over point when $G' > G''$) occurred at lower frequencies as the concentration of the polymer increases. This suggests that increased polysaccharide concentration enhances chain/aggregate interactions resulting in increase in the elasticity of the sample [17].

4. Conclusions

In the present investigation the rheological and microstructural properties of oat β-glucan isolates varying in molecular weight were examined by means of rheometry and atomic force microscopy. Results showed that the intrinsic viscosity, critical concentration, the flow and viscoelastic properties were highly dependent on the molecular weight of the samples. Pseudoplastic and Newtonian flow behaviour was exhibited depending on the concentration and molecular weight of the samples. The Cox–Merz rule was found to be applicable for the lower MW samples at higher concentrations, whereas the high MW samples showed a deviation at concentrations greater than 1.0% (w/v), suggesting the presence of aggregates. Atomic force microscopy revealed formation of clusters of aggregates linked via individual molecules scattered heterogeneously throughout the system. The aggregate size and morphology was also dependent on molecular weight of the samples and influences the rheological behaviour of β-glucan solutions.

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