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Micro-heterogeneity and micro-rheological properties of high-viscosity oat β-glucan solutions ☆

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Abstract

Soluble fibre β-glucan is one of the key dietary materials in the healthy food products known for reducing serum cholesterol levels. The micro-structural heterogeneity and micro-rheology of high-viscosity oat β-glucan solutions were investigated by monitoring the thermally driven displacements of well-dispersed microspheres via video fluorescence microscopy. By comparing the distribution of the time-dependent mean-square displacement (MSD) and ensemble-averaged MSD of polystyrene microspheres imbedded in four concentrations of β-glucan solutions, we found that the solutions exhibited perfectly homogeneous behaviour at $\leqslant 1\%$, but showed a certain degree of heterogeneity at 2%. Micro-rheology investigation revealed that β-glucan solutions displayed nearly perfect viscous behaviour at $\leqslant 1\%$, but the property changed into viscoelastic at 2%. Both micro-structural heterogeneity and micro-rheological property shifts occurred over a small concentration range, between 1% and 2%, of β-glucan.
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Keywords: β-Glucan; Micro-heterogeneity; Micro-rheology; Multiple-particle tracking

1. Introduction

Heart disease is the leading cause of death in the United States (NHLBI, 2000). Compelling evidence suggests that reducing of total and LDL cholesterol concentrations can decrease the risk of heart attack (Downs et al., 1998; Krauss et al., 2000; NCEP, 2001). During recent years, nutrition study has shown that soluble fibres from cereal grains, such as oats and barley, contribute health benefits to food products (Inglett, 1997). Many reports show that soluble fibres can lower serum cholesterol levels (AHA, 1980; FDA, 1997a, 1997b; Malkki, Autio, & Hanninen, 1992; Topping, 1991; Uusitupa, Ruuskanen, & Makinen, 1992; Wood, 1984). Oatrim is one of the products, which has been introduced to the marketplace as a source of soluble fibre β-glucan from oats (Carriere & Inglett, 1998, 1999; Inglett, 1991; Inglett, 1993, 1997; Inglett & Newman, 1994). Nutrition studies indicate that Oatrim-containing foods can address health-related issues, such as insulin response, and weight control (Behall, Schofield, & Clark, 1993; Behall, Schofield, & Hallfrisch, 1997; Hallfrisch, Schofield, & Behall, 1997; Schofield, Behall, & Armero, 1993). Another product, Nutrim, has been developed as an alternative source of soluble fibre β-glucan (Inglett, 1998a, 1998b). Some Nutrim products have higher β-glucan contents than has Oatrim, and could provide higher potential health benefits per unit of material. β-Glucan is one of the major healthy food components in food products. Consumption of β-glucan has been shown to decrease total and LDL cholesterol levels

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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(Davidson et al., 1991; Davidson & McDonald, 1998; Glore, Van Treeck, Knehans, & Guild, 1994; Nicolosi, Bell, & Bistrian, 1999; Ripsin et al., 1992). The mechanism by which β-glucan reduces cholesterol levels might be related to its viscosity, bile salt-binding capacity or fermentability (Davidson & McDonald, 1998; Marlett, Hosig, Vollendorf, & Shinnick, 1994). A 19-clinical trial study concludes that daily consumption of 3 g of β-glucan can lower total cholesterol levels by 0.13–0.15 mmol/l (Ripsin et al., 1992).

Although nutritional functions of food products containing β-glucan are well known, the physical properties of these materials, which are important for developing new products, are very little understood. Previously, we studied Nutrim-10 suspensions' micro-heterogeneities using a technique named as multiple-particle tracking (MPT) (Xu, Chang, Inglett, Carriere, & Tseng, 2006). We found that the degree of heterogeneity of the Nutrim-10 suspensions strongly depended on its concentrations, which might be related to the β-glucan (Xu et al., 2006). In this work, we investigated the micro-structural heterogeneities and micro-rheological properties of β-glucan solutions using MPT.

2. Material and methods

2.1. Material and sample preparation

High-viscosity oat β-glucan was obtained from Megazyme Co. (Wicklow, Ireland). The sample contains >97% β-glucan, <0.1% starch, 0.3% proteins, <0.5% arabinoxylan, 1.7% ash, and 1.2% moisture. The sample powder was dissolved in de-ionized water at the desired concentration and was shaken in a water-bath at 37 °C. The β-glucan

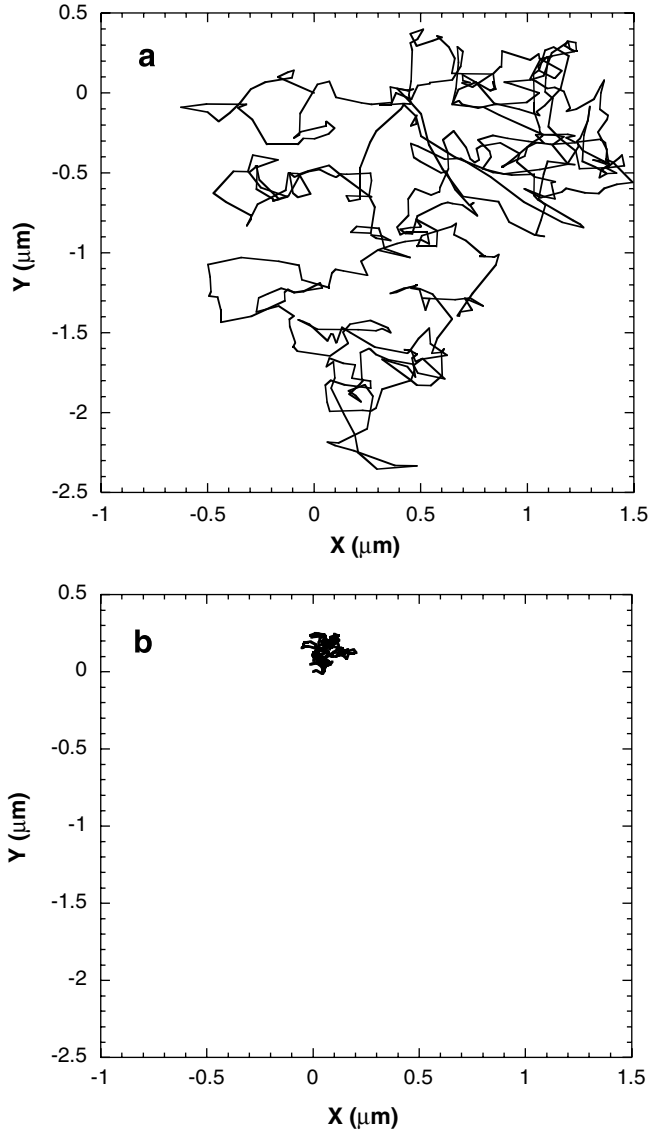

Fig. 1. Typical two-dimensional trajectories of a polystyrene fluorescent bead imbedded in a β-glucan solution. The trajectories are shown for 20 s time span. (a) 0.25% β-glucan solution. (b) 2% β-glucan solution.

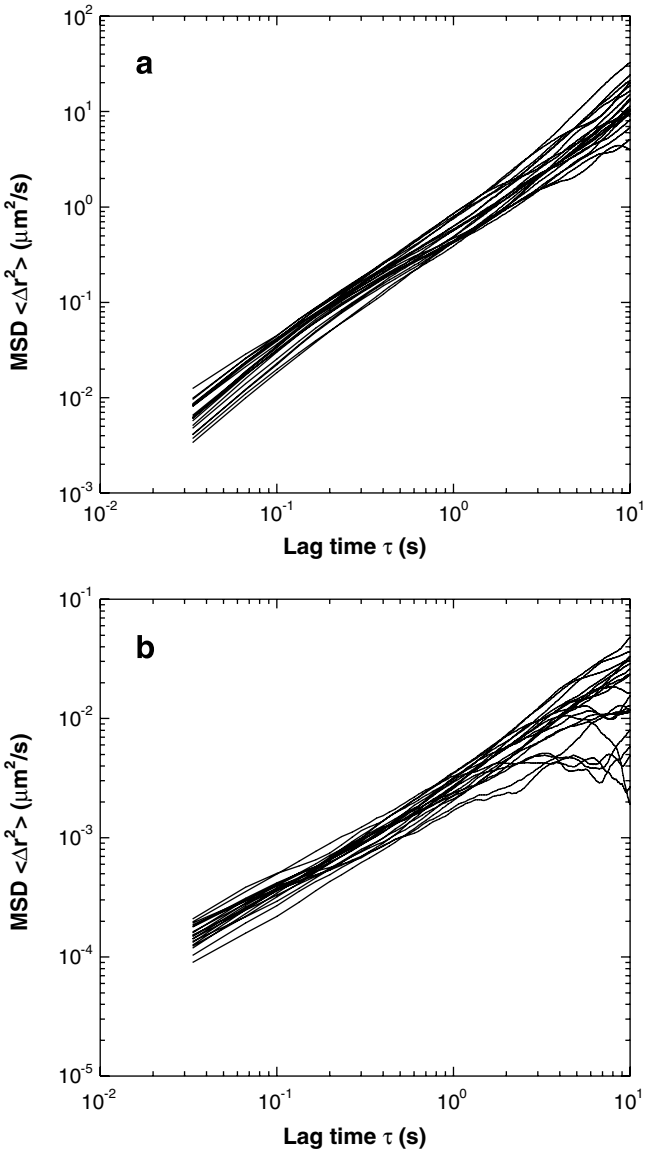

Fig. 2. Typical MSD profiles of microspheres imbedded in a β-glucan solution. (a) 0.25% β-glucan solution. (b) 2% β-glucan solution.

solution was clear and no precipitation was observed within two weeks. Samples were stored at 4 °C and used within two days after preparation to avoid sample degradation. At least two samples were examined for each β-glucan concentration.

2.2. Measurements

The multiple-particle tracking (MPT) method, originally described by Apgar et al. (2000), was used in this study. The principle of this technique is to monitor the thermally driven motion of inert microspheres, which are evenly distributed within the samples, and to statistically analyze their displacement distributions. From these data, information about the extent of heterogeneity can be extracted. For each experiment, a dilute suspension of 0.97 μm diameter, fluorescent, polystyrene microspheres (0.1 vol%) was gently mixed with the β-glucan solution. The sample containing the β-solution mixed with the probe microspheres (total volume ~0.1 ml) was deposited into a PC20 CoverWell cell (Grace Bio-Lab, Eugene, OR), which was placed on the stage of a microscope and allowed to equilibrate for at least two hours at room temperature ($T \sim 295$ K). Images of the fluorescent beads were recorded onto the (large) random-access memory of a PC computer via a SIT camera (VE-100 Dage-MTI, Michigan City, IN) mounted on an inverted epifluorescence microscope (Eclipse TE300, Nikon, Melville, NY). A 100X, 1.3 numerical aperture, oil-immersion lens was used for the measurements, which permitted a 5–10 nm spatial resolution, as assessed by monitoring the apparent displacement of the microspheres firmly attached (i.e. glued) to a glass coverslip with the same microscope and camera settings. Movies were analyzed by a custom MPT routine incorporated into the software Metamorph (Universal Imaging Corp., West Chester, PA) as described by Apgar et al. (2000) and Tseng and Wirtz (2001). The displacements of the particles' centeroids were simultaneously monitored in the focal plane of the microscope for 120 s at a rate of 30 Hz. We tracked about 10–30 individual particles, a number that is limited by potential particle–particle interactions at high particle density and tracking a sufficiently large number of beads per movie, not limited by the tracking capabilities of our microscope software computer system. For each sample of β-glucan solution, we tracked a total of ~240 microspheres. Individual time-averaged mean squared displacements (MSD),

$$\langle \Delta r^2(\tau) \rangle = \langle [x(t+\tau) - x(t)]^2 + [y(t+\tau) - y(t)]^2 \rangle,$$

where τ is the lag time and t is the elapsed time, were calculated from the two-dimensional trajectories (Tseng, Fedorov, McCaffery, Almo, & Wirtz, 2001). From $\langle \Delta r^2(\tau) \rangle$, lag-time-dependent ensemble-averaged MSD, $\langle\langle \Delta r^2(\tau) \rangle\rangle$, and MSD distributions were computed. The ensemble-averaged diffusion coefficient of the microspheres can be calculated as $D(\tau) = \langle\langle \Delta r^2(\tau) \rangle\rangle / 4\tau$ (Xu, Tseng, Carriere, & Wirtz, 2002).

From each MSD trace, a local frequency-dependent elastic modulus G' and loss modulus G'' were computed that has been described elsewhere (Mason, Ganesan, van Zanten, Wirtz, & Kuo, 1997; Xu, Palmer, & Wirtz, 1998). Assuming that the fluid surrounding the probe particle is incompressible, the viscoelastic spectrum $G(s)$, which is derived from a generalized Langevin equation for the motion of the microsphere, is

$$G(s) = k_B T / \pi a s \langle \Delta r^2(s) \rangle.$$

Here, s is the Laplace frequency, $\langle \Delta r^2(s) \rangle$ is the unilateral Laplace transform of $\langle \Delta r^2(\tau) \rangle$, k_B is the Boltzmann con-

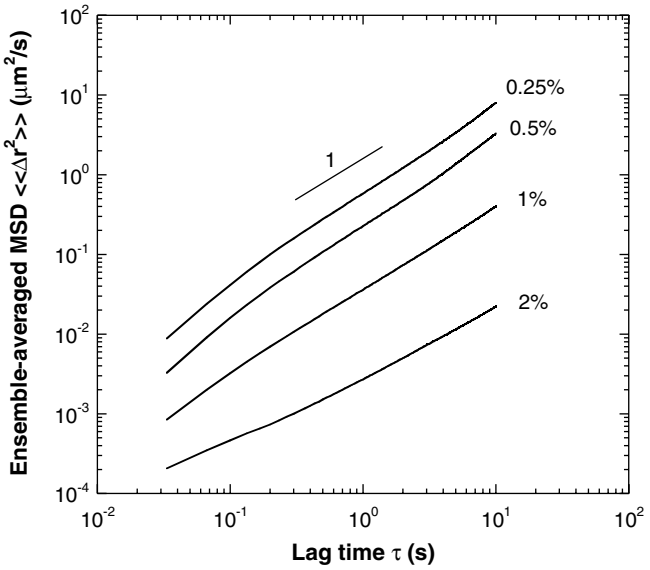

Fig. 3. Time-dependent ensemble-averaged mean squared displacement (MSD) of beads imbedded in four concentrations of β-glucan solutions.

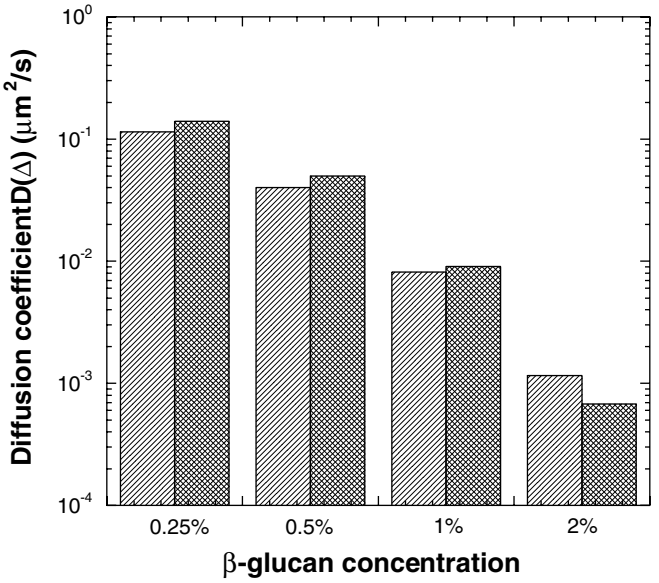

Fig. 4. Diffusion coefficient of beads imbedded in four concentrations of β-glucan solutions at lag time of 0.1 s (first column) and 1 s (second column).

stant, and a is the radius of the probe particle. The viscoelastic moduli, G' and G'', are the real and imaginary parts, respectively, of the complex modulus G^*, which is the projection of $G(s)$ in Fourier space.

3. Results and discussion

The degree of heterogeneity of β-glucan solutions was measured by tracking the thermally excited motion of a large collection of 0.97 μm diameter polystyrene beads imbedded in solutions of various concentrations in order to quantify the micro-structural properties. The time-dependent coordinates $[x(t), y(t)]$ of the centroids of the microspheres were recorded (Fig. 1). Typical trajectories of microspheres dispersed in solutions of 0.25% and 2% (wt%) β-glucan solutions are shown in Fig. 1a and b. The extent of the displacements measured at 20 s time scales was greatly reduced with increasing β-glucan concentrations (Fig. 1b), a result that parallels the fact that β-glucan solutions with higher concentrations exhibit higher viscosity. Accordingly, MSD profiles for 0.25% β-glucan solution increased linearly with time (Fig. 2a), which is a behaviour which the micro-environment surrounding the beads responds like a viscous fluid: stresses that are locally applied to the β-glucan solution by the fluctuating beads' movements are relaxed quickly by viscous dissipation (Xu et al., 1998). MSD profiles for 2% β-glucan solution increased most linearly with time at shorter lag time, but deviated a little at longer lag time (Fig. 2b), which suggested that the material was slightly viscoelastic.

In order to derive properties that describe local relatively macroscopic behaviour of β-glucan solutions, the

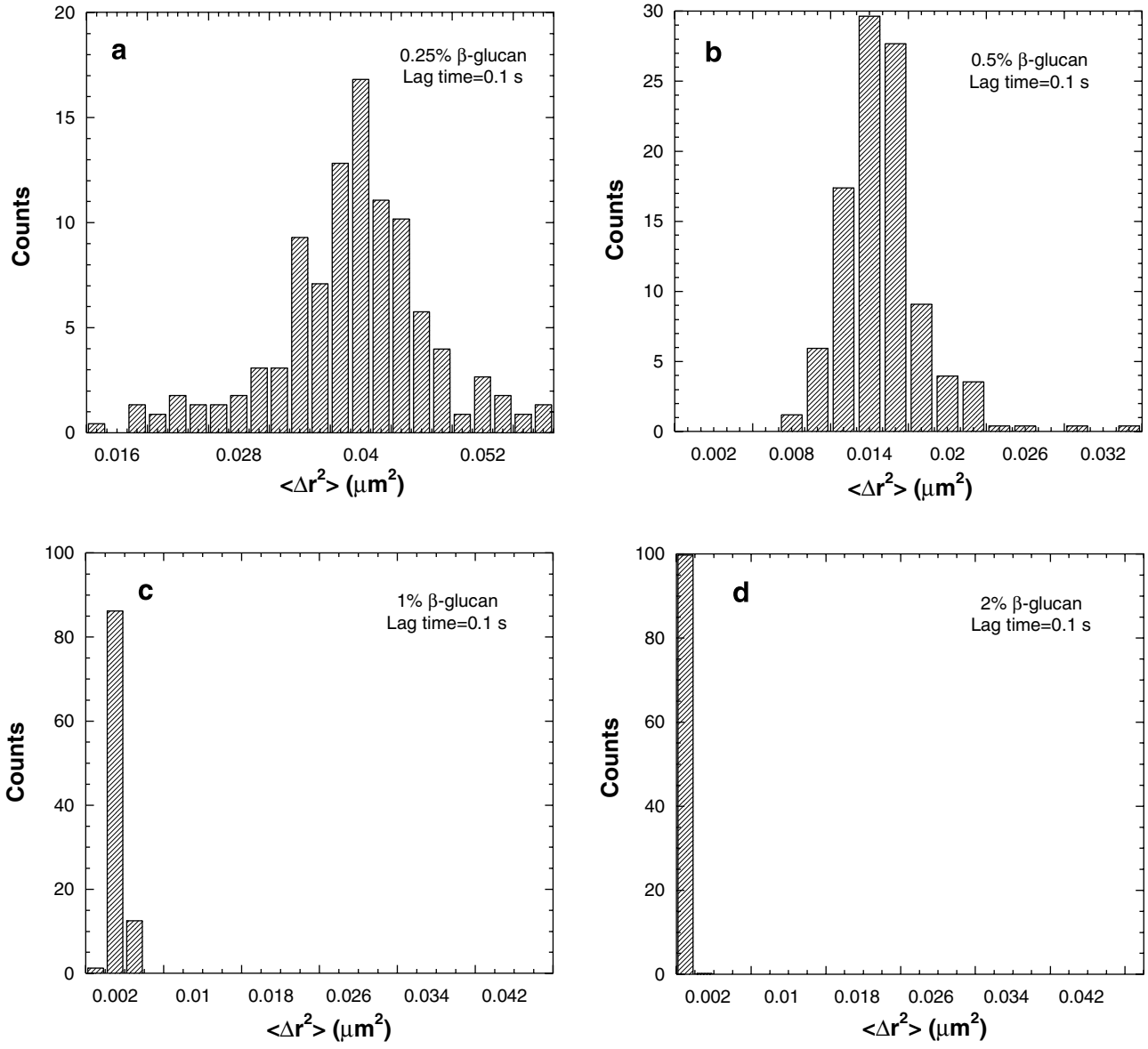

Fig. 5. Mean squared displacement (MSD) distributions of beads imbedded in four concentrations of β-glucan solutions measured at 0.1 s lag time. (a) 0.25% β-glucan solution. (b) 0.5% β-glucan solution. (c) 1% β-glucan solution. (d) 2% β-glucan solution.

ensemble-averaged MSDs were calculated from the individual MSD traces of a large number of particles ($n \approx 240$). For β-glucan concentrations of 0.25%, 0.5% and 1% (wt%), the ensemble-averaged MSD traces showed a power law behaviour as a function of lag time with a slope close to unity (Fig. 3), which was similar to that of a homogeneous aqueous solution of glycerol (Xu et al., 2002). The ensemble-averaged diffusion coefficient of the microspheres imbedded in these β-glucan solutions remained nearly constant with the lag time (Fig. 4). For example, particles imbedded in 0.25% β-glucan solution showed diffusion coefficients of 0.11 $\mu m^2/s$ at 0.1 s and 0.14 $\mu m^2/s$ at 1 s, respectively. For comparison, the same microsphere had constant diffusion coefficients of 0.44 $\mu m^2/s$ in water ($= k_B T/6\pi\eta a$, with viscosity $\eta = 1$ cP and temperature $T =$ 295 K) and 4.2×10^{-3} $\mu m^2/s$ in glycerol (Xu et al., 2002). For 2% β-glucan solution, the ensemble-averaged MSD traces displayed a slight deviation from the line of the power law of a slope of one (Fig. 3). Accordingly, the diffusion coefficient of the microsphere imbedded in 2% β-glucan solution became small and decreased slightly with the lag time, from 1.2×10^{-3} $\mu m^2/s$ at 0.1 s to 6.8×10^{-4} $\mu m^2/s$ at 1 s (Fig. 4). These results indicated that the probe particles undergo relatively purely diffusive motion in a material with a viscous fluid behaviour at lower concentrations of β-glucan solutions (⩽1%). But the beads would be more "trapped" with the mesh of the solution at higher concentrations (2% or higher), which might be a little more elastic. This transition occurred within a very narrow concentration range, from 1% to 2%, and was therefore significant.

In order to further compare these β-glucan solutions, MSD distributions were generated from the MSD traces and were statistically analyzed (Fig. 5). In the 0.25% β-glucan solution, the beads' MSD distribution spread over a wide area and was relatively symmetric, which is similar to the MSD distribution for glycerol (Xu et al., 2002) (Fig. 5a). As expected, MSD distributions shift to the low values with increasing β-glucan concentration (Fig. 5b–d). In 0.5% and 1% β-glucan solutions, the particles' MSD distributions were still relatively symmetric even though they covered a narrower area compared with the 0.25% β-glucan's MSD distribution (Fig. 5b and c). However, in 2% β-glucan solution, the microspheres' MSD distributions became more asymmetric and more skewed (Fig. 5d), which implied a higher degree of heterogeneity. Sometimes, comparing the shape of distributions that encompass different values could be misleading (Goodman, Tseng, & Wirtz, 2002). To further quantify the level of heterogeneity in β-glucan solutions, we used so-called bin-partition analysis (Fig. 6) (Tseng, An, & Wirtz, 2002;

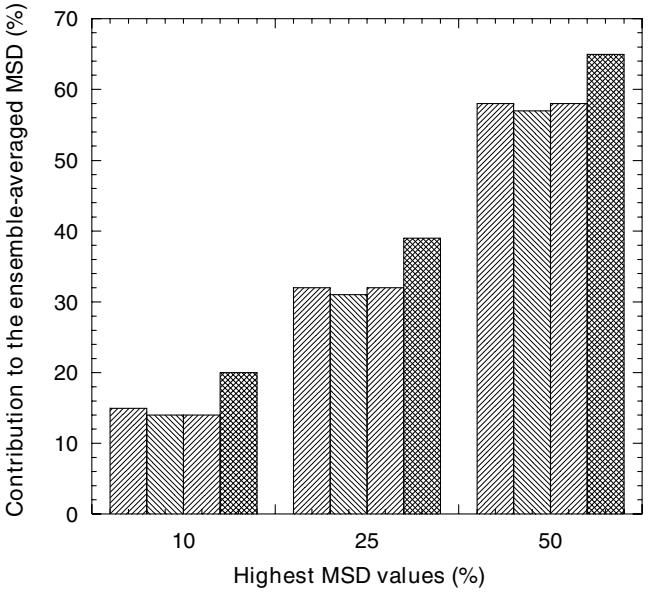

Fig. 6. Time-dependent contributions of the 10%, 25%, and 50% highest MSD values to the ensemble-averaged MSD for four concentrations of β-glucan solutions measured at 0.1 s lag time. First column: 0.25% β-glucan solution; second column: 0.5% β-glucan solution; third column: 1% β-glucan solution; forth column: 2% β-glucan solution.

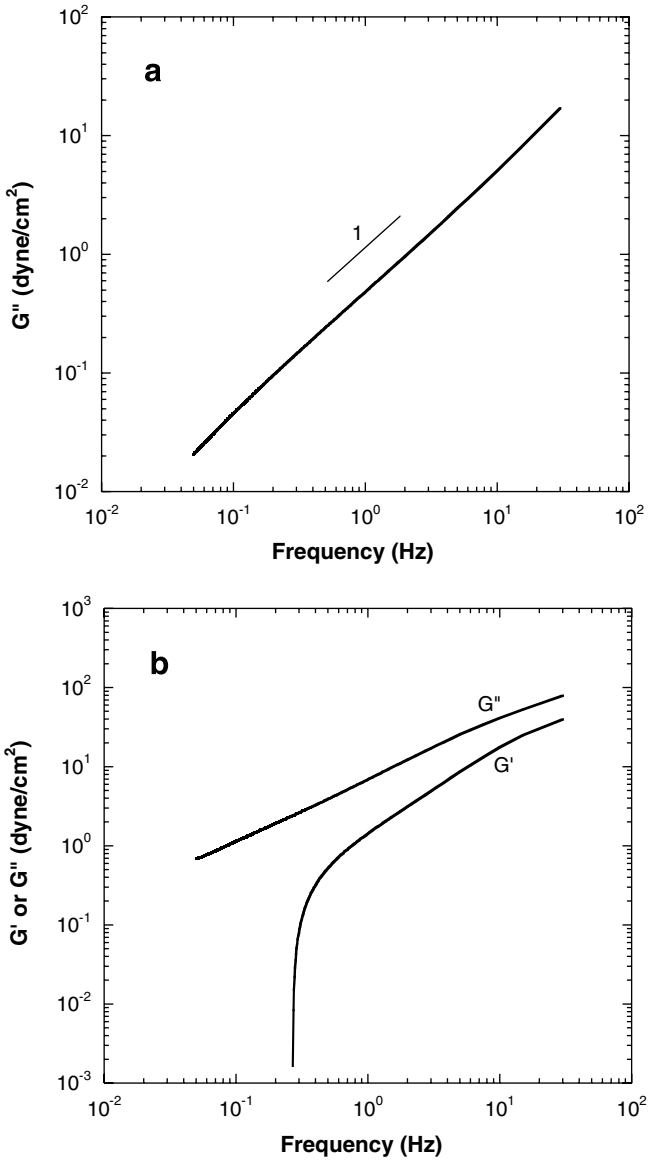

Fig. 7. Micro-rheological properties of β-glucan solutions. (a) 1% β-glucan solution. (b) 2% β-glucan solution.

Tseng & Wirtz, 2001; Xu et al., 2002). Using this analysis, MSD values at a given lag time are sorted and contributions of the 10%, 25%, and 50% highest MSD values to the ensemble-averaged MSD are evaluated. In theory, these values should remain similar at 10%, 25%, and 50%, respectively, if the material is a perfectly homogeneous liquid (Xu et al., 2002). For 0.25% β-glucan solution, these values were 15%, 32%, and 58%, respectively (Fig. 6). They were 14%, 31%, and 57%, respectively for 0.5% β-glucan, and 14%, 32%, and 58%, respectively for 1% β-glucan (Fig. 6). Therefore, these numbers remained constant when β-glucan was $\leqslant$1%. By comparison, these figures are 11%, 26%, and 52%, respectively, for a perfectly homogeneous viscous fluid, glycerol (Apgar et al., 2000). Thus, for β-glucan solutions of $\leqslant$1%, the materials were very close to a perfectly homogeneous liquid with very little heterogeneity. However, the 10%, 25% and 50% highest MSD values contributing to the ensemble-averaged MSD were 20%, 39%, and 65%, respectively for 2% β-glucan solution (Fig. 6), which indicated that 2% β-glucan solution exhibited a greater degree of heterogeneity than did homogeneous liquid. The transition from nearly almost homogeneous to somewhat heterogeneous, again, occurred between 1% and 2% β-glucan.

The local micro-rheological properties of β-glucan solutions were extracted from MSD measurements by following the method of Mason et al. (1997). After unilateral Laplace transformation of each lag-time-dependent MSD trace, a complex modulus was calculated, from which frequency-dependent elastic and viscous moduli, G' and G'', were extracted (Fig. 7). For concentrations of $\leqslant$1%, β-glucan solutions showed zero elasticity ($G' = 0$), and a nearly straight line of viscous moduli (G'') with a slope of one (Fig. 7a shows the 1% β-glucan data). This result suggested that β-glucan exhibited almost perfectly homogeneous liquid properties at concentrations $\leqslant$1%. However, 2% β-glucan displayed a non-negligible elasticity (G') at high frequencies and a viscous modulus (G'') that also increased almost linearly with frequency (Fig. 7b). This result indicated that the 2% β-glucan solution showed a certain degree of elasticity at high frequencies, but remained a viscoelastic liquid, for which $G'' > G'$ over most of the probed frequency range (0.2 Hz $< f <$ 10 Hz) (Fig. 7b). Here, the micro-rheological study for the β-glucan solutions showed that there was a property shift from viscous fluid to viscoelastic fluid behaviour within a narrow range of concentrations. This concentration range was, once more, between 1% and 2% of β-glucan, which was consistence with the all above results.

4. Conclusions

In this work, we studied the micro-structural micro-rheological properties of high viscosity oat β-glucan solutions using the multiple-particle tracking (MPT) method. We detected a relatively rapid concentration-induced transition of the micro-heterogeneity and micro-rheological properties of the β-glucan solutions. At $\leqslant$1% concentrations, β-glucan exhibited nearly perfect homogeneous and viscous fluid behaviour, which is similar to that of glycerol. However, at 2%, β-glucan solution displayed a certain degree of both heterogeneity and viscoelasticity, which was a clear deviation from homogeneous and viscous behaviour. These property shifts all took place between 1% and 2%, which is a very narrow range and is therefore significant. These behaviour and property alterations should be related to the β-glucan molecules' chain–chain stronger interactions and aggregations at higher concentrations, which will be the focus of our next work.

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