

Multiple-Particle Tracking Study of Microheterogeneity of Nutrim-10 Suspensions

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ABSTRACT

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Nutrim is a newly developed food product containing dietary soluble fiber β -glucan. The microstructural heterogeneities of Nutrim-10 suspensions were investigated by monitoring the thermally driven displacements of well-dispersed microspheres through video fluorescence microscopy. By comparing the distribution of the time-dependent mean-square displacement (MSD) of polystyrene microspheres embedded in three concentrations of Nutrim-10 suspensions, we found that the degree of heterogeneity of suspensions increased dramatically within a narrow range of Nutrim-

10 concentrations. The ensemble-averaged MSD of 5.5% Nutrim-10 suspension exhibited a power-law behavior scaling linearly with time, which was similar to the behavior for a homogeneous aqueous glycerol solution. But the MSD distribution was wider and more asymmetric than for glycerol. Increasing Nutrim-10 concentration rendered the MSD distribution much more asymmetric and skewed. This study provided a quantitative method to characterize the organization of Nutrim-10 in suspension.

Over the past years, nutrition research has shown that soluble fibers from cereal grains such as oats and barley contribute health benefits to food products (Inglett 1997). Many studies reported that soluble fibers could lower serum cholesterol levels (AHA 1980; Wood 1984; Topping 1991; Uusitupa et al 1992; Malkki et al 1992; FDA 1997a,b). One product, named Oatrim, was introduced to the marketplace as a source of soluble fiber β -glucan from oats (Inglett 1991, 1993, 1997; Inglett and Newman 1994; Carriere and Inglett 1998, 1999). Nutrition studies indicated that Oatrim-containing foods can address health-related issue such as insulin response and weight control (Behall et al 1993, 1997; Scholfield et al 1993; Hallfrisch et al 1997).

Recently, a newly dietary fiber food product, Nutrim, has been developed as an alternative source of soluble fiber β -glucan (Inglett 1998a,b). Nutrim is a hydrocolloidal extract that functions as cream and fat replacers, nutrifiers, and texturizers. Some Nutrim products have higher β -glucan content than Oatrim. So the Nutrim could provide higher potential health benefits per unit of material. The basic physical properties of Nutrim have rarely been reported except for some rheological property studies (Carriere and Inglett 2000). Carriere and Inglett reported that Nutrim suspensions exhibited shear-thinning behavior and the rheological properties of Nutrim suspensions were not affected by shearing the material through steam jet cooker (Carriere and Inglett 2000). In this work, a previously developed technique denoted as multiple-particle tracking (MPT) was used to investigate the microstructural and microstructural heterogeneities of suspensions of Nutrim-10, which contains 10% by weight β -glucan.

MATERIALS AND METHODS

Materials and Sample Preparation

The preparation of Nutrim-10 was described by Inglett (1998a,b). In general, Nutrim-10 hydrocolloid was prepared by adding 900 g of oat bran concentrate (Quaker Oats Company) to 5,100 mL of deionized water in a 5-gal (19L) container and mixing with a dis-

persator (PMC model 90 with a high viscosity head and $\approx 10,000$ rpm, Premier Mill Corp., Reading, PA) to generate a temperature of 80–95°C. Continuous shear force was applied to maintain this temperature for 30 min before adding 6L of boiling water. The slurry was steam jet-cooked at 138–141°C and 40–45 psi with 1.2L/min flow rate and 5 sec of residence time. The hot slurry from the cooker was immediately passed into a separator (Sweco Inc., Florence, KY) with 50 and 80 steel mesh sieves to recover the hydrocolloid liquid. The wet fiber solids from the sieves were collected, reslurried with boiling water, and recollected on the sieves. The liquid wash is combined with the hydrocolloid liquid before drum drying the liquid to give 536 g of oat bran hydrocolloid. The combined wet fiber solids were oven dried to give 175 g. The values of Nutrim-10 hydrocolloid composition are 6.7% moisture, 2.2% ash, 1.1% fat (ether extraction), 9.7% protein ($N \times 6.25$), 0.25% crude fiber, and 10% β -glucan. A 10% slurry has pH 5.5–6.5. The Nutrim-10 powder was suspended in deionized water to a desired concentration using a Polytron PT10-35 homogenizer with a low-foam mixing head (PTA 20TS, Kinematica AG, Switzerland). Nutrim-10 was well dispersed and no sedimentation was observed for four weeks after sample preparation. Samples were stored at 4°C and used within three days after preparation to avoid sample degradation. At least two suspension samples were made for each tested Nutrim-10 concentration.

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 Nutrim-10 suspension. The sample containing the Nutrim-10 suspension mixed with the probe microspheres (total volume ≈ 0.1 mL) was deposited into a PC20 CoverWell cell (Grace Bio-Lab, Eugene, OR) that was placed on the stage of a microscope and allowed to equilibrate for at least 2 hr at room temperature ($\approx 295\text{K}$). Then images of the fluorescent beads were recorded onto the (large) random-access memory of a PC computer through a SIT camera (VE-100 Dage-MTI, Michigan City, IN) mounted on an inverted epifluorescence microscope (Eclipse TE300, Nikon, Melville, NY). A 100 \times , 1.3 numerical aperture, oil-immersion lens was used for the measurements, which permitted a 5–15 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.

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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' centroids were simultaneously monitored in the focal plane of the microscope for 120 sec at a rate of 30 Hz. We tracked ≈ 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 Nutrim-10 suspension, 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 calculated from two-dimensional trajectories (Tseng et al 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 at fixed lag times were computed. The lag-time-dependent ensemble-averaged diffusion coefficient of the microspheres can be calculated as (Xu et al 2002)

$$D(\tau) = \langle\langle \Delta r^2(\tau) \rangle\rangle / 4\tau$$

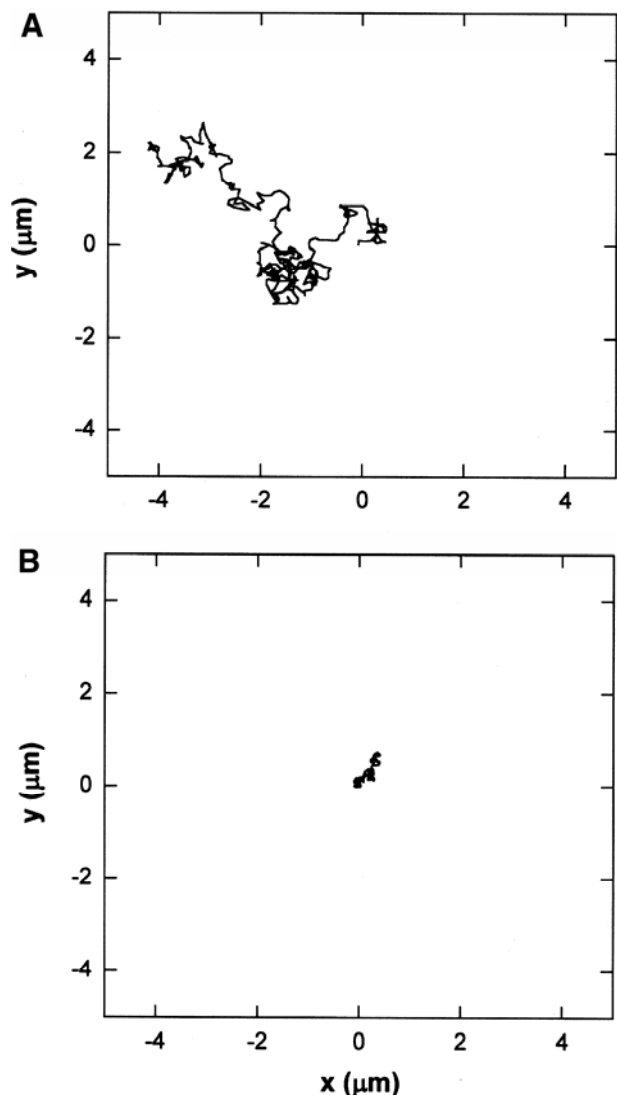


Fig. 1. Typical trajectories (30 sec) of a polystyrene fluorescent bead embedded in a Nutrim-10 suspension. **A**, 5.5% Nutrim-10 suspension. **B**, 7.5% Nutrim-10 suspension.

RESULTS AND DISCUSSION

To quantify the microstructural properties, the degree of heterogeneity of Nutrim-10 suspension was measured by tracking the thermally driven motion of a large collection of polystyrene beads $0.97 \mu\text{m}$ in diameter embedded in suspensions of various concentrations. Typical trajectories of microspheres dispersed in suspensions of 5.5 and 7.5% Nutrim-10 suspensions are shown in Fig. 1. The extent of the displacements measured at 30-sec time scales was greatly reduced with increasing Nutrim-10 concentrations, a result that parallels the fact that Nutrim-10 suspensions with higher concentrations exhibit higher viscosity (Carriere and Inglett 2000). It is also possible that the microspheres were trapped within an elastic mesh. The ensemble-averaged MSD was calculated from the individual MSD traces of a large ensemble of microspheres ($n = 240$). For a 5.5% (wt%) Nutrim-10 concentration, the ensemble-averaged MSD traces adopted a power law behavior as a function of time scale with a slope close to unity (Fig. 2), which was similar to that of homogeneous aqueous solution of glycerol (Xu et al 2002). A slope equal to one means that the material is a perfect viscous fluid with no elasticity (Xu et al 2002). Accordingly, the ensemble-averaged diffusion coefficient of the micro-

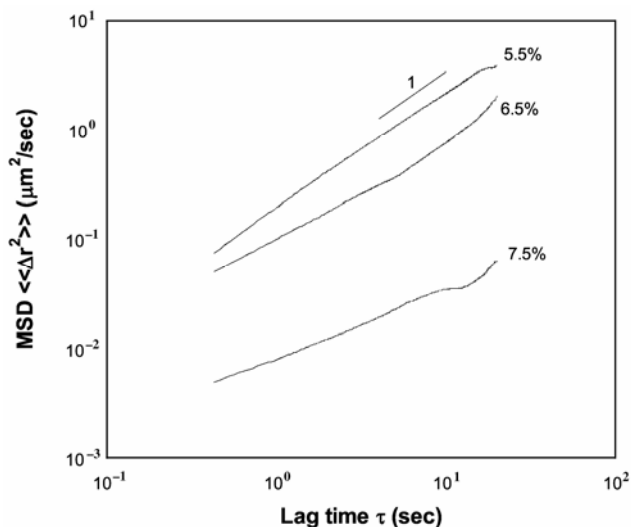


Fig. 2. Time-dependent ensemble-averaged mean squared displacement (MSD) of beads embedded in Nutrim-10 suspensions.

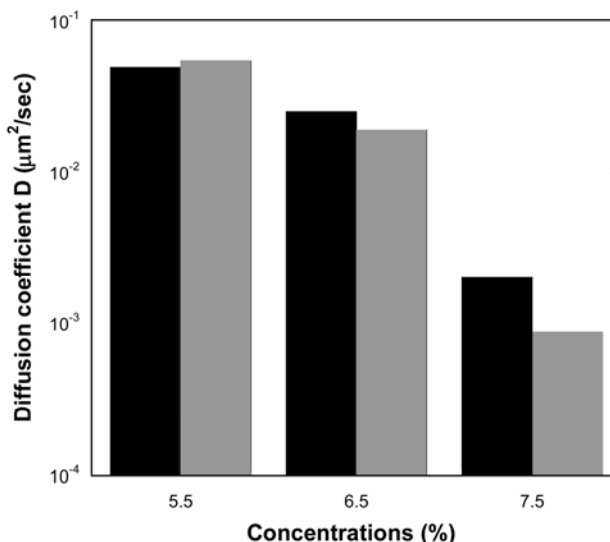


Fig. 3. Diffusion coefficient of beads embedded in Nutrim-10 suspensions at lag time of 1 sec (black bars) and 10 sec (grey bars).

spheres in the 5.5% suspension kept nearly constant of $5.0 \times 10^{-3} \mu\text{m}^2/\text{sec}$ at a lag time of 1 sec and $5.5 \times 10^{-3} \mu\text{m}^2/\text{sec}$ at 10 sec (Fig. 3). For comparison, the same microsphere has constant diffusion coefficients of $0.44 \mu\text{m}^2/\text{sec}$ in water ($= k_B T / 6\pi\eta a$, with viscosity $\eta = 1$ cP and temperature $T = 295\text{K}$) and $4.2 \times 10^{-3} \mu\text{m}^2/\text{sec}$ in glycerol (Xu et al 2002). At higher Nutrim-10 concentrations, however, the suspension showed a clear deviation from the line of power law of a slope of one (Fig. 2). Accordingly, the diffusion coefficient was small and decreased strongly with lag time, from $20 \times 10^{-4} \mu\text{m}^2/\text{sec}$ at 1 sec to $8.9 \times 10^{-4} \mu\text{m}^2/\text{sec}$ at 10 sec for 7.5% Nutrim-10 (Fig. 3). Ensemble-averaged trace of microspheres embedded in suspensions of 6.5% Nutrim-10 was intermediate between 5.5 and 7.5% (Fig. 2). These results suggested that the probe microspheres undergo relatively purely diffusive motion in a material with a viscous liquid character at 5.5% Nutrim-10, but the beads movements were largely confined (Fig. 1) and would be possibly trapped with the mesh of the suspension at higher Nutrim-10 concentrations. This transition occurred within a very narrow concentration range, from 5.5% to 7.5%, and therefore was very significant.

To quantify the level of heterogeneity in Nutrim-10 suspensions, MSD distributions were generated from the MSD traces and statistically analyzed. As a control, we take our previous results of the same analysis applied to an aqueous solution of glycerol,

which is homogenous at length scales at least as small as the bead radius ($\approx 0.5 \mu\text{m}$). In the 5.5% Nutrim-10 suspension, the MSD distribution spread over a wide area and was relatively asymmetric (Fig. 4A,B) compared with the MSD distribution for a solution of glycerol, which has a normal distribution (Xu et al 2002). In addition, the shape of MSD distribution for 5.5% Nutrim-10 was dependent on the lag time scale (Fig. 4A,B), while the shape of MSD distribution for glycerol was independent of the lag time (Xu et al 2002). In the 6.5% suspension, at 0.1 sec of lag time, a vast majority of the MSD values were centered at $\approx 3 \times 10^{-3} \mu\text{m}^2$, but extreme values appeared at more than an order of magnitude of $3 \times 10^{-3} \mu\text{m}^2$ (Fig. 5A). And the shape of MSD distribution was more dependent on the time scale (Fig. 5A,B). In the 7.5% suspension, the shape of MSD became much more asymmetric and skewed (Fig. 6A,B).

To further compare these suspensions, MSD distributions were normalized by the ensemble-averaged MSD (Fig. 7). For the 5.5% suspension, the distribution was wider and more asymmetric than normal distribution; at a lag time of 1 sec, the normalized median, standard deviation, and skewness were 0.97, 0.78, and 0.66, respectively (Fig. 7A). These statistical parameters deviated from those observed with an aqueous solution of glycerol, which is (theoretically) perfectly homogenous and has normal distribution: normalized median, standard deviation, and skewness were 1.02,

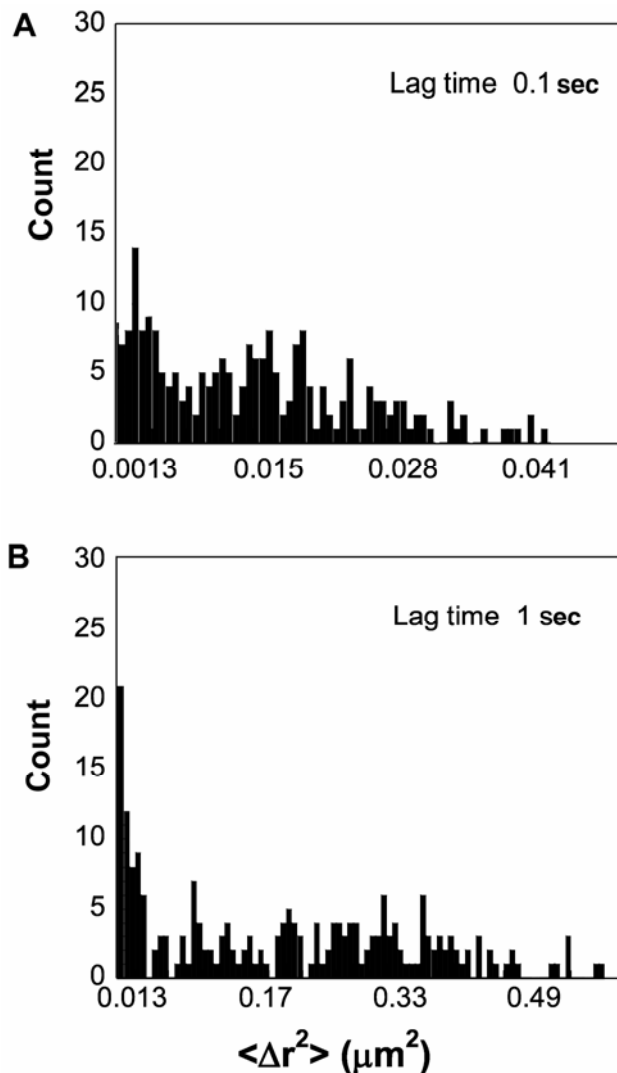


Fig. 4. Mean squared displacement (MSD) distributions for 5.5% Nutrim-10 suspension. **A**, Measured at a lag time of 0.1 sec. **B**, Measured at a lag time of 1 sec.

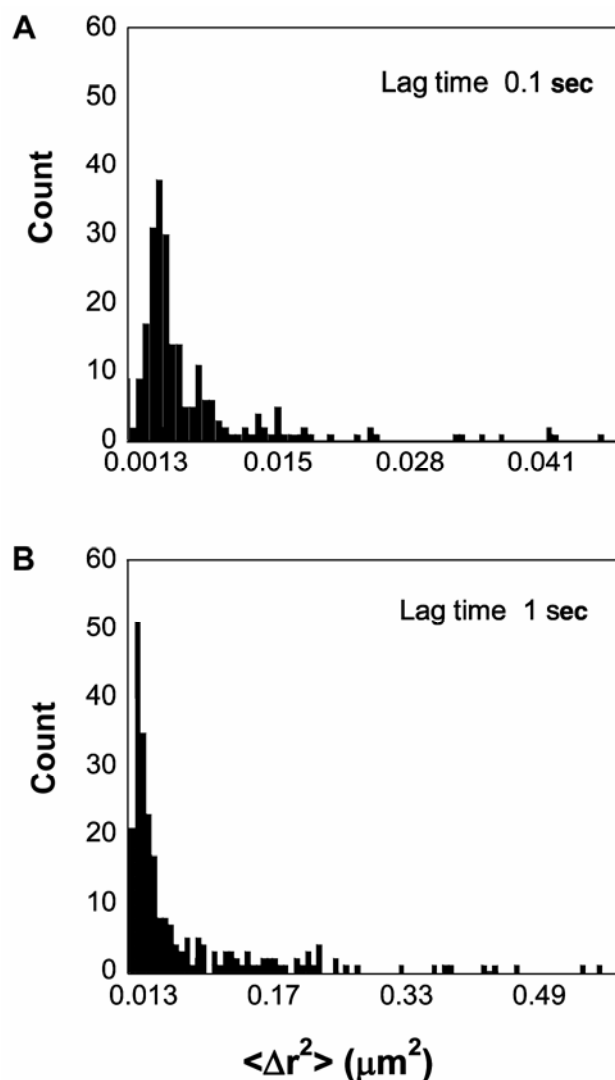


Fig. 5. Mean squared displacement (MSD) distributions for 6.5% Nutrim-10 suspension. **A**, Measured at a lag time of 0.1 sec. **B**, Measured at a lag time of 1 sec.

0.15, and 0.39, respectively (Xu et al 2002). For the 6.5 and 7.5% suspensions, the normalized median, standard deviation, and skewness further deviated from those observed with glycerol and 5.5% suspension. The median decreased rapidly. The normalized median, standard deviation, and skewness for 6.5% suspension at 1 sec were 0.19, 0.95, and 2.66, respectively (Fig. 7B). While the normalized median, standard deviation, and skewness for 7.7% suspension were 0.001, 2.62, and 12.59, respectively (Fig. 7C).

These results indicated that the Nutrim-10 was not homogenous, even at the lower concentration of 5.5% and its heterogeneity and elasticity became stronger with increasing concentrations. Nevertheless, by analyzing the contributions of the 25, 50, and 75% highest MSD values to the ensemble-averaged MSD at a given lag time, we found that the glycerol solution was much more homogenous than the 5.5% Nutrim-10 suspension (Fig. 8) (Xu et al 2002). Indeed, for glycerol, these parameters were close to those expected for a perfectly homogenous liquid (25, 50, and 75%) (Xu et al 2002), for which all MSD values should theoretically be similar. In contrast, for the 5.5% Nutrim-10 suspension, these parameters were typically twice as large as those observed in glycerol over the tested range of time scales (Fig. 8A). The 7.5% suspension displayed behavior similar to the 5.5% suspension for the contributions of the 25, 50, and 75% highest MSD values to the ensemble-averaged MSD (Fig. 8B). Therefore, despite the fact that a 5.5% Nutrim-10 suspension behaved mostly like a liquid from a standpoint of the ensemble-averaged MSD (Fig. 2), the

suspension exhibited a larger degree of heterogeneity than the homogenous glycerol solution. The transition of the properties of Nutrim-10 was strongly dependent of the concentrations, while the concentration range was very small. The very possible reason for this transition is due to the microstructural shift of the Nutrim-10 with the concentration increase. The key content for controlling this transition might be β -glucan, which will be the focus of future investigations.

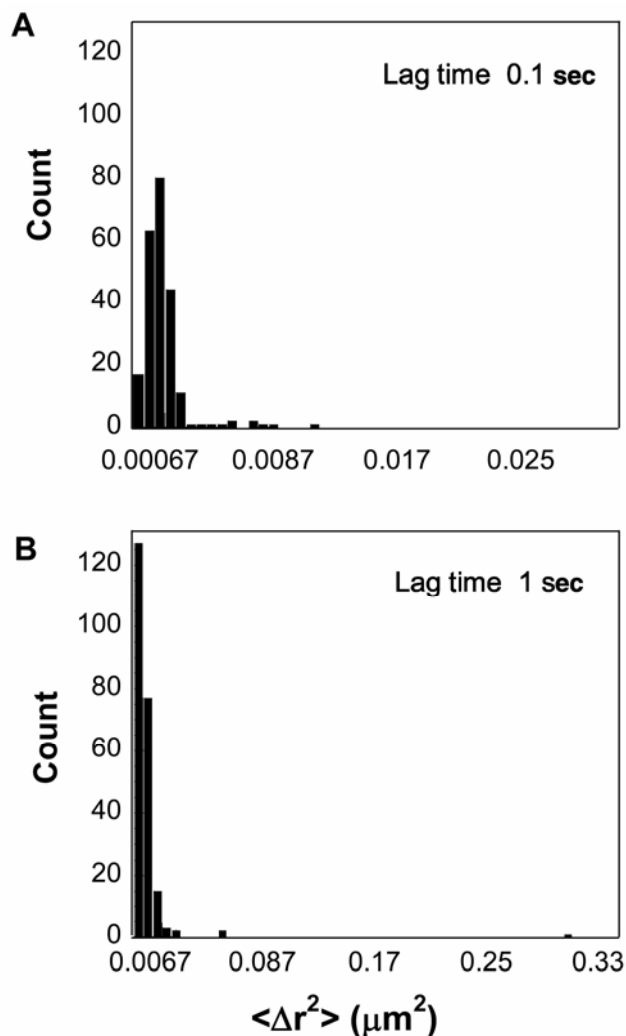


Fig. 6. Mean squared displacement (MSD) distributions for 7.5% Nutrim-10 suspension. **A**, Measured at a lag time of 0.1 sec. **B**, Measured at a lag time of 1 sec.

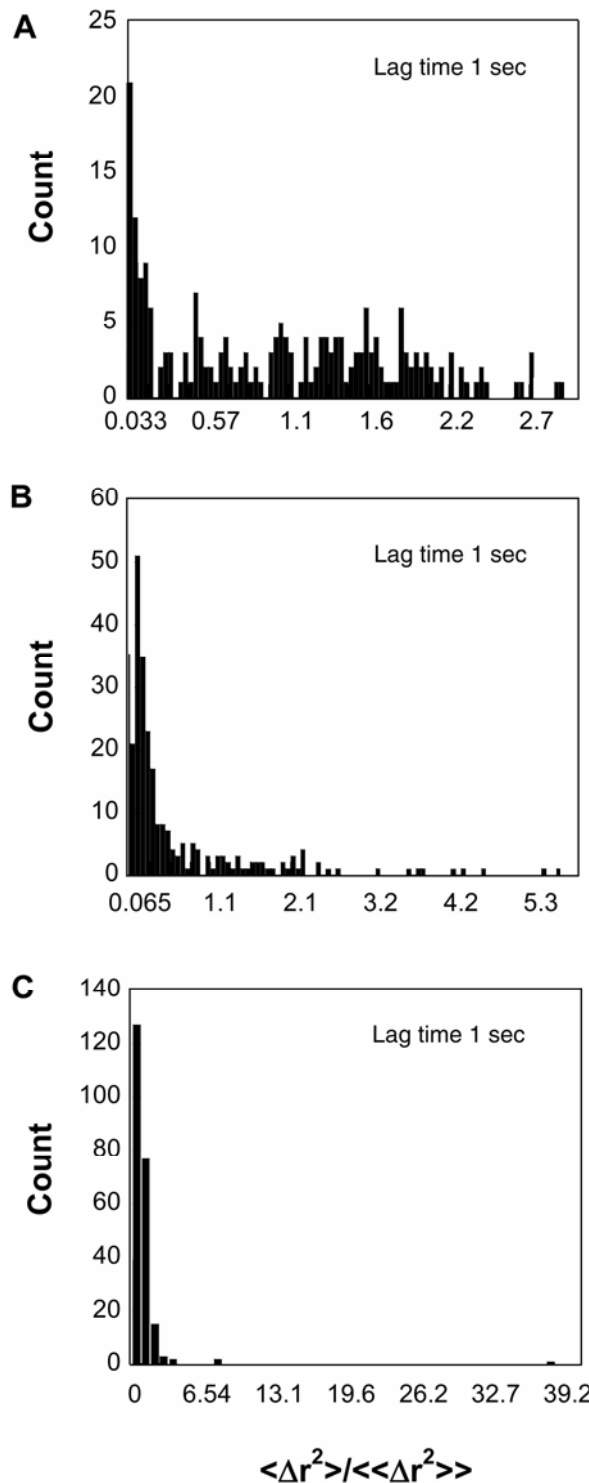


Fig. 7. Mean squared displacement (MSD) distributions normalized by corresponding ensemble-averaged mean (taken from Fig. 2) for Nutrim-10 suspension at a lag time of 1 sec. **A**, 5.5% Nutrim-10 suspension. **B**, 6.5% Nutrim-10 suspension. **C**, 7.5% Nutrim-10 suspension.

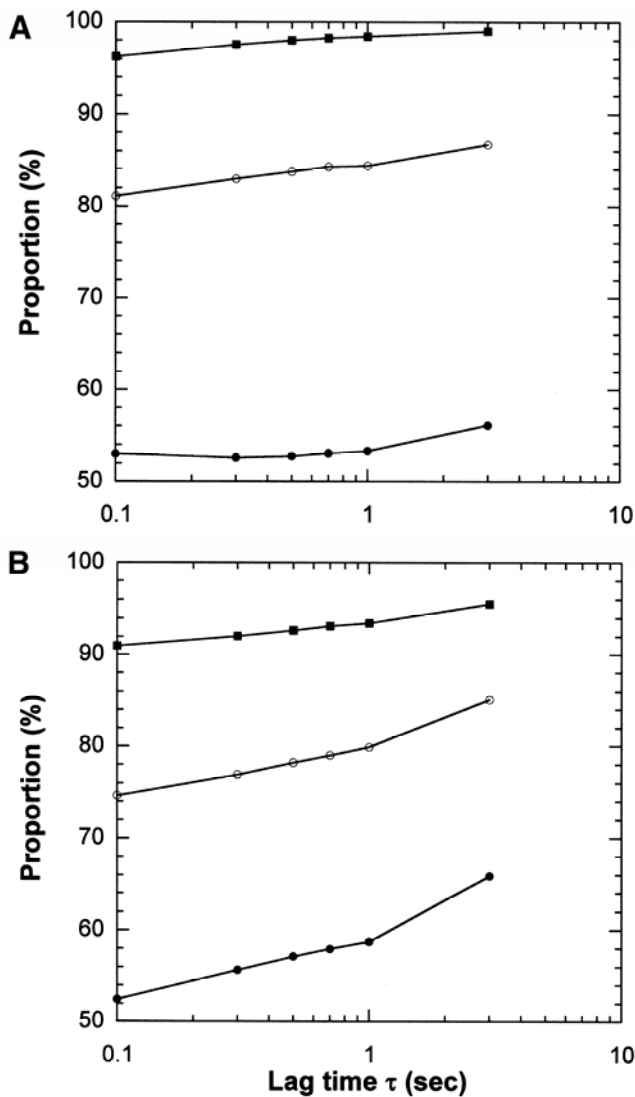


Fig. 8. Contributions (%) of 25% (solid circle), 50% (open circle), and 75% (solid square) highest MSD values to the ensemble-averaged MSD for Nutrim-10 suspensions. **A**, 5.5%. **B**, 7.5%.

In summary, microstructural properties of Nutrim-10 suspensions were investigated by using the Multiple-Particle Tracking (MPT) method. The outcome of this work indicated a relatively rapid concentration-induced transition of the properties of the Nutrim-10 suspensions. Pretransitional effects were apparent at low concentrations as clearly detected by the shape of the MSD distribution of embedded particles. While the overall ensemble-averaged MSD at lower concentration (5.5%) was very similar in shape to that of a viscous liquid, the MSD distribution was wider than that measured in a homogeneous solution of glycerol. At higher concentrations (6.5 and 7.5%), the heterogeneities of the Nutrim-10 suspensions became more evident. This work gave us some insight of the Nutrim properties that would be beneficial to the processing food products with Nutrim.

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