Rotational X-ray Microrheology

M. N. Liang[*]

Department of Physics, University of Illinois at Urbana-Champaign,
1110 West Green St, Urbana, IL 61801[†]

R. Harder[‡]

Argonne National Lab, 9700 S. Cass Avenue, Argonne, IL 60439

I. K. Robinson[§]

Department of Physics, University of Illinois at Urbana-Champaign,
1110 West Green St, Urbana, IL 61801 and Centre for Nanotechnology,
University College London, 17-19 Gordon Street, London WC1H 0AH, UK

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Abstract

We demonstrate an experimental method to increase the range of viscosities accessible to passive microrheology by using the X-ray Bragg diffraction of a small tracer crystal to observe rotational motion at the microradian scale. We applied this technique to a system of decanoic acid with 340nm single crystal alumina tracers. The resulting viscosity measurements differ from steady state viscosity literature values and from standard rheological techniques. This difference is attributed to the increased elastic and viscous moduli of a crosslinked fatty acid network and a larger effective particle size due to adsorption and weak flocculation.

There exists an important range of viscoelastic materials, both homogeneous and inhomogeneous, which have properties of both classical elastic solids and viscous liquids[1]. While many examples are man made, such as rubber and coatings, many are biological, such as tissue, collagen, and muscle. The need to understand biomaterials and synthesize artificial counterparts requires understanding their full rheological response. Many of these materials are inaccessible to classical rheology due to their length and time scales, and the desire to understand them has driven the development of new characterization techniques. Traditionally, translational dynamics are more often studied due to the ease of experimental investigation, although it has been appreciated that rotational diffusion would allow for increased sensitivity[2].

A particle embedded in a fluid will undergo fluctuations which are determined by both the energy of the particle, and the dissipative and storage moduli of the surrounding fluid. This idea is the basis of microrheology, which has become an established technique in characterizing viscoelastic fluids[3]. A variety of methods, such as dynamic light scattering and diffusive wave spectroscopy, has been employed to study both the translational and rotational motion of embedded particles for ensemble/bulk characterization[2–4]. Single particle tracking microrheology uses traditional microscopy for transparent and 2 dimensional systems, and confocal microscopy to study 3 dimensional systems.[5, 6] The spatial resolution of single particle tracking is especially important for inhomogeneous fluids and those in which diffusion plays an important role. The most widely used is translational passive microrheology. This type uses the thermal energy of the system as the driving force of a test particle, and utilizes the translational motion of the particle to probe the fluid response to the shear force generated by its motion.

For translational single particle tracking in passive microrheology, the current viscosity limit is 10^4 poise $[g \cdot cm^{-1} sec^{-1}]$ or $10^3 Pa \cdot sec[Nm^{-2} sec]$ for the micron sized particles used as tracers.[7] Higher viscosities are accessible to active microrheology where the particles are driven by external forces such as electric or magnetic fields or mechanical forces.[7] Active microrheology is challenging due to complex sample preparation and experimental procedures, the risk of sample alteration, and the complication of not lying within the linear viscoelastic regime. An idea which has sparked much interest in overcoming the viscosity limitation is rotational single particle tracking. It has been largely unexplored due to the experimental difficulty inherent in determining the orientation of an isotropic particle.

Innovative techniques such as Light Streak Tracking[8], Modulated Optical Nanoprobes[9], and laser trapping of birefringent particles[10] seek to use geometric or optical anisotropy of tracer particles to overcome this difficulty. An important advantage in using rotation in lieu of translation is that the viscosity and complex shear modulus are inversely proportional to the third power of the particle size instead of the first power in the translational regime. This important benefit can extend the range of viscosities accessible to microrheology as the lower limit of particle size is limited by the resolution of optical methods. In this work, we extend the viscosity range by five orders of magnitude using x-ray Bragg diffraction which is highly sensitive to rotational motion.

Our new X-ray diffraction microrheology technique does not depend on a known anisotropy to determine particle orientation, but relies on the orientational marker inherent to a crystal which is its lattice. Bragg's Law, $2dsin\theta = \lambda$, where d is the lattice spacing, θ is the Bragg angle, and λ is the wavelength, gives the diffraction condition for a crystal. The diffracted intensity is precisely sensitive to the direction of the lattice planes and by observing the position of the Bragg reflection, the orientation about 2 rotational axes can be determined. Any change in the position of the diffracted intensity will indicate a rotation of the embedded crystal about an axis determined by the scattering geometry. Using this method, the rotational motion of an arbitrarily shaped crystal can be determined. Due to the flux of modern x-ray sources and flexible scattering geometries, we can track the motions of particles as small as 100nm with an angular resolution of $8\mu rad$ at 20ms time resolution, determined by the capabilities of our detector. We use these trajectories to study the rotational diffusion of alumina nanocrystals suspended in decanoic acid to determine the storage and loss modulus of this viscoelastic liquid.

The complex shear modulus $G(\omega) = G'(\omega) + iG''(\omega)$ describes a viscoelastic liquid with a real, storage component $G'(\omega)$, which is the elastic modulus, and an imaginary, dissipative component $G''(\omega)$, which is the viscous modulus. $G(\omega)$ can be experimentally determined from the mean squared displacement (MSD) of the motion of an embedded tracer particle driven by thermal fluctuations, ie passive microrheology. The particle motion is described by a generalized Langevin equation with a time dependent memory function $\eta(t)$. $\eta(t)$ is related to the MSD via the velocity autocorrelation function using the Green Kubo formula, and also to the viscosity and $G(\omega)$. The relation is given by the angular Generalized Stokes

Einstein relation (GSER).

$$\tilde{G}(s) = \frac{k_B T}{4\pi a^3 s < \Delta\tilde{\theta}^2(s) >} \tag{1}$$

where k_B is the Boltzmann constant, T is the temperature, a is the particle radius, s is the Laplace frequency and $< \Delta\tilde{\theta}^2(s) >$ is the angular MSD. Once the MSD is obtained, an inverse numerical Laplace Transform is performed to obtain $G'(\omega)$ and $G''(\omega)$. Mason et al developed an alternative method using a local power law expansion to minimize truncation errors due to the finite extent of the data.[11]

Roughly spherical 340nm alumina tracer crystals (Bueler Inc) were suspended in decanoic acid ($C_{10}H_{20}O_2$) at an 8% volume fraction. The solution was sonicated in a warm water bath for a minimum of 3 hours above the melting point of decanoic acid at 31^oC. Previous studies showed that fatty acids adsorb onto the surface of alumina.[12] We can thus assume that the alumina particles are sterically stabilized and interface effects and particle aggregations are minimized. A $3\mu L$ droplet was deposited on a hydrophobic octadecyltriethoxysiloxane (OTE) coated silicon wafer and placed on a variable temperature sample stage. The sample was measured with a 5 circle diffractometer at sector 34ID-C at the Advanced Photon Source. A focused 9keV beam with a flux on the order of 10^9 photons/sec in a focal spot size of approximately $2\mu m \times 2\mu m$ was incident on the droplet in transmission geometry. Diffraction of the (104) Bragg reflection of single alumina crystals was measured with a charge coupled device (CCD) camera at temperatures above and below the glass transition temperature of decanoic acid at 31^oC. Time series were taken at time intervals of 0.02 sec and 0.1 sec to obtain the trajectory of the orientation. By integrating in the radial direction, we isolate the motion of the diffracted intensity around the powder ring which corresponds to the rotation of the crystal about the axis bisecting the incoming and scattered wavevectors, k_i and k_f. The angular resolution of $82\mu rad$ is determined by the 2x2 binning of $20.5\mu m^2$ pixels resolution of the CCD detector and the sample detector distance of $0.5m$. Higher angular resolution can be obtained by unbinning the detector and increasing the sample to detector distance, but this was not required for our sample.

In order to track examples of motion with sufficient statistics, we selected data for particles whose diffraction stays in the field of view of the detector, and on its rocking curve, for at least 500 time steps. The particle diffraction must also remain sufficiently isolated from the diffraction of other particles during this time. The rotational trajectory is tracked by finding the intensity maxima of a grouping of 5 adjacent pixels for each time step, and the relative angular positions are used to calculate the angular MSD $\Delta\theta(t)^2$. Figure 1 illustrates the procedure to extract the motion of a single particle. Frames of raw data 1(a) are binned to an angle vs time map 1(b) of the whole detector region for the entire measurement time. A single particle trajectory is then isolated 1(c)and tracked 1(d) using the algorithm described above.

Decanoic acid has a steady state kinematic viscosity of $\nu = 6.9018cSt$[13] at 40^oC measured by glass capillary kinematic viscometry, which corresponds to a dynamic viscosity of $\eta = 6.161 \times 10^{-3} Pa \cdot sec$. For standard Newtonian rotational diffusion, the angular MSD $\Delta < \theta(t)^2 >= 2D_r t$ where the rotational diffusion coefficient is given by $D_r = \frac{k_B}{f_r}$ and f_r is the rotational drag coefficient of a sphere given by $f_r = 8\pi\eta R^3$. For our particles and the corresponding viscosity at 40^oC, we would expect the MSD of an alumina sphere in 0.1sec to be $1.66rad^2$. However, in our experiment, the majority of particles in diffraction had a step size of a few pixels per time step, corresponding to a few hundred microradians. Figure 2(a) shows the MSD vs time average of 29 particles at 38^oC. For those particles, the average shows subdiffusive behavior, defined as having a power law exponent of less than 1 on a $log_{10} - log_{10}$ plot. The power law exponent is 0.4 at timescales less than 30-50 sec, which transitions to more diffusive behavior with a slope of 0.79 at approximately 100 sec for the majority of particles.

The most straightforward and well-established analysis assumes that the microenvironment of the particle reflects that of the bulk fluid and thus the bulk viscosity can be obtained from particle motion. From the MSD vs time graph, we determined the storage and dissipative modulus of the decanoic acid using Mason et al's local power law expansion method.[10] The $\omega \rightarrow 0$ limit of the dissipative modulus is directly related to the steady state dynamic viscosity η of the fluid via $\eta = G''(\omega)/\omega$. Our experiment thus estimates the steady state viscosity to be $8.1 \times 10^6 Pa \cdot sec$, nine orders of magnitude higher than the steady state literature value. This large discrepancy in our results from steady state numbers measurements was further investigated by rheological measurements taken with a rotating plate rheometer. From these frequency dependent measurements, it was found that the steady state viscosity was $10.4 Pa \cdot sec$ at the low frequency limit of the measurement of $1.5Hz$. This is 4 orders of magnitude higher than the viscometry measurements, but still 6 orders of magnitude different from our microrheology results.

An alternative interpretation of the data is to use the measured steady state viscosity to determine the effective particle size. This calculation yields approximately $10\mu m$ instead of the 340nm particles used. Previous research has examined similar systems that have exhibited deviations from expected behavior. Bell et al. found that for comparable, but higher particle density samples, flocculation of alumina was observed.[12] Flocculation was found to occur in specific cases at particle concentrations even less than the 8% used in our current study.[14, 15] In Bell et al's bulk rheological study, they also observed a higher $G'(\omega)$ and $G''(\omega)$ than expected, even under the assumption that their system exhibited flocculation. The discrepancy was attributed to greater interparticle attraction than expected, and increased fatty acid chain attraction forces that would increase the stiffness of the network.[12] Our system uses a different fatty acid, particle size and concentration, but also shows a larger viscous and storage modulus, and we can assume some of their arguments also apply to our system. Unlike [12], we did not use a decalin solvent, which has been observed to inhibit gelation in fatty acid systems [16]. The lack of decalin in our two component system is likely to have increased the crosslinking which would lead to higher moduli. It is also known that decanoic acid/water mixtures can form self assembled vesicles of several microns in diameter which have a potential use of encapsulation[17]. The samples in [17] were carefully tailored to create hollow, permeable vesicles, but demonstrate that structures of micron scale can be formed from decanoic acid. Based on the studies from [12, 17], we conclude that the high moduli in our experiment are most likely a combination of the increased stiffness and viscosity due to a network of fatty acids, and a larger effective particle size due to adsorption, self assembly, and weak flocculation, even at the dilute particle limit.

It is important to study the radiation effects on the motion of the alumina. Radiation effects on particle motion were experimentally investigated by attenuation and shuttering the x-ray beam. In the attenuation study, we observed the motions of a single particle which remained in diffraction for both the high and low flux conditions. A $200\mu m$ Al attenuator was used to decrease the flux to 13.5% of its original value. Figure 3(a) shows the effects of attenuation for a single particle. The shuttering studies, Figure 3(b), are for 9 averaged particles in the shuttered case and 6 particles for the not shuttered case where there was constant illumination. For both studies, the particle was seen to have a higher MSD vs time value for all lag times at the higher flux values, indicating higher mobility of the particles. Depending on the delay time, particles which were not shuttered had between a 40-50% higher MSD, and unattenuated particles had between a 12-86% higher MSD. Such behavior correlates to lower $G(\omega)$ values and thus a lower calculated steady state viscosity. These effects are likely to be due to both radiation pressure and radiative heating. Previous studies [18] have indicated that the radiation pressure on a 50nm gold particle with unfocused white beam synchrotron radiation to be of order $10^{-15}N$. Our comparable experimental setup in terms of flux and particle scattering would indicate comparable forces on the Alumina tracer particles.

The considered radiation forces should affect the particles mainly in translation. Any effect on the rotation is due to a gradient in beam intensity which would cause a torque on the particle. In order to estimate the torque on the alumina tracers, we approximate a focused Gaussian beam profile with a full width at half max (FWHM) of $2\mu m \times 2\mu m$ with a total flux of 3×10^9 photons. Also, we take into account that the attenuation length of alumina at 9keV is $100\mu m$ and that component of the force normal to the lattice planes is $F_{\perp} = Fsin\theta$ where θ is half the scattering angle. Estimating the particle as a highly reflecting surface, the momentum of the photons and the intensity difference at the FWHM of a length 150nm is 0.104 and the torque, τ, on the particle is $\tau = 2.34 \times 10^{-26}N \cdot m$. We assume angular diffusion follows $\Omega = \frac{\tau}{f_r}$ where Ω is the angular drift velocity. With η equal to the dynamic viscosity of decanoic acid, the angle through which a particle rotates due to the radiation pressure in the 0.1sec exposure time is $4.48\mu rad$, undetectable with the angular resolution of our detector in the present geometry. Thus, the torque due to the beam gradient is a minor contribution to the change in MSD observed.

In addition to the effect of radiation pressure on the particle motion, local heating due to the x-ray beam will cause the local environment of the particle to differ from average bulk values. Average bulk temperature was seen to remain constant within the sensitivity of thermocouple readings. In the simplest treatment, we estimate the energy per second delivered by the x-ray beam by $E = hc/\lambda$ and a beam flux of 3×10^9 photons per second in a $2 \times 2\mu m$ focal spot. Decanoic acid has an attenuation length of 3mm at 9keV and a specific heat of 2.09J/gK, and thus a sample 1mm thick would absorb approximately 28% of the flux and we can estimate a heating rate of 16.2^oK per 0.1sec exposure. The viscosity of decanoic acid at 60^oC is $4.2557cSt$[13] compared with $6.9018cSt$ at 40^oC. In the Newtonian rotational diffusion approximation, this would increase the MSD from both an increase in thermal energy k_BT and a decrease in the viscosity and thus rotational drag coefficient. The increased MSD vs time that we observe can therefore be accounted for by a combination of both radiation pressure produced torque, to a lesser extent, and the local heating from the x-ray beam which would decrease the viscosity of the liquid in the vicinity of the tracer.

The subdiffusive behavior of the alumina tracers in decanoic acid, seen as a change in the power law exponent from 0.4 to 0.79 over the range of figure 2, indicates the existence of a characteristic timescale of the dynamics of the system. This timescale is on the order of 100 sec with little temperature dependence. A similar timescale has been seen in ensemble translational microrheology of wormlike micelles by van Zanten[19] and was interpreted as a caging mechanism at short times. The transition time to purely diffusive behavior gives the timescale of the formation and destruction of the cages in that work. The caging in our system would be rotational caging of tracer particles with an adsorbed layer, as opposed to translational caging in the van Zanten experiment.

In conclusion, we have increased the range of viscosities accessible to single particle tracking microrheology by using x-ray diffraction to track the orientation dependent Bragg diffraction of alumina crystal tracer particles. This increase in range is due to both the viscosity being inversely proportional to the cube of the particle size in rotational motion, and in the capability of using smaller tracer particles due to the flux of modern x-ray sources. In pure decanoic acid with alumina crystals in the dilute limit, we observed subdiffusive behavior indicative of caging at times less than 30 sec, transitioning to more diffusive motion at longer timescales. The observed viscosity is 6 orders of magnitude greater than the viscosity determined from bulk rheological studies. This disparity is likely due to both the increased stiffness and viscosity due to the crosslinking of fatty acids which form a network, and a larger effective particle size due to adsorption of a steric layer, self assembly of fatty acid chains, and weak flocculation, even at the dilute particle limit.

This method has the potential to make single particle tracking passive rheology applicable to high viscosity polymers and other high viscosity liquids which cannot be studied with current techniques. Systems with voids or a network that may hinder translational microrheology would greatly benefit from this technique by allowing one to study the local viscosity within a single void as well as the viscosity of the macroscopic system. In this way, one can use the differences between the microscopic and macroscopic behavior to gain a more complete understanding of the system. In particular, to understand transport and diffusion in biological systems, one must understand the properties at length scale which is relevant to the motions. Tailoring the adsorption characteristics of the tracer/fluid system by using a different species of tracer or altering the surface properties of the current tracer could be used to investigate the effect of surface interactions on rotational viscosity. This work shows the important role of nanoscale restructuring in mesoscopic systems that are highly relevant to biology. A 6 order of magnitude discrepancy has been found in the viscosity experienced by a small object and the bulk value, which is due to a considerable enlargement of its effective size. Our result is an important step towards understanding diffusive motion in structured media.

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[*] mengning.liang@desy.de

[†] Present address: Center for Free-Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany

[‡] rharder@aps.anl.gov

[§] i.robinson@ucl.ac.uk

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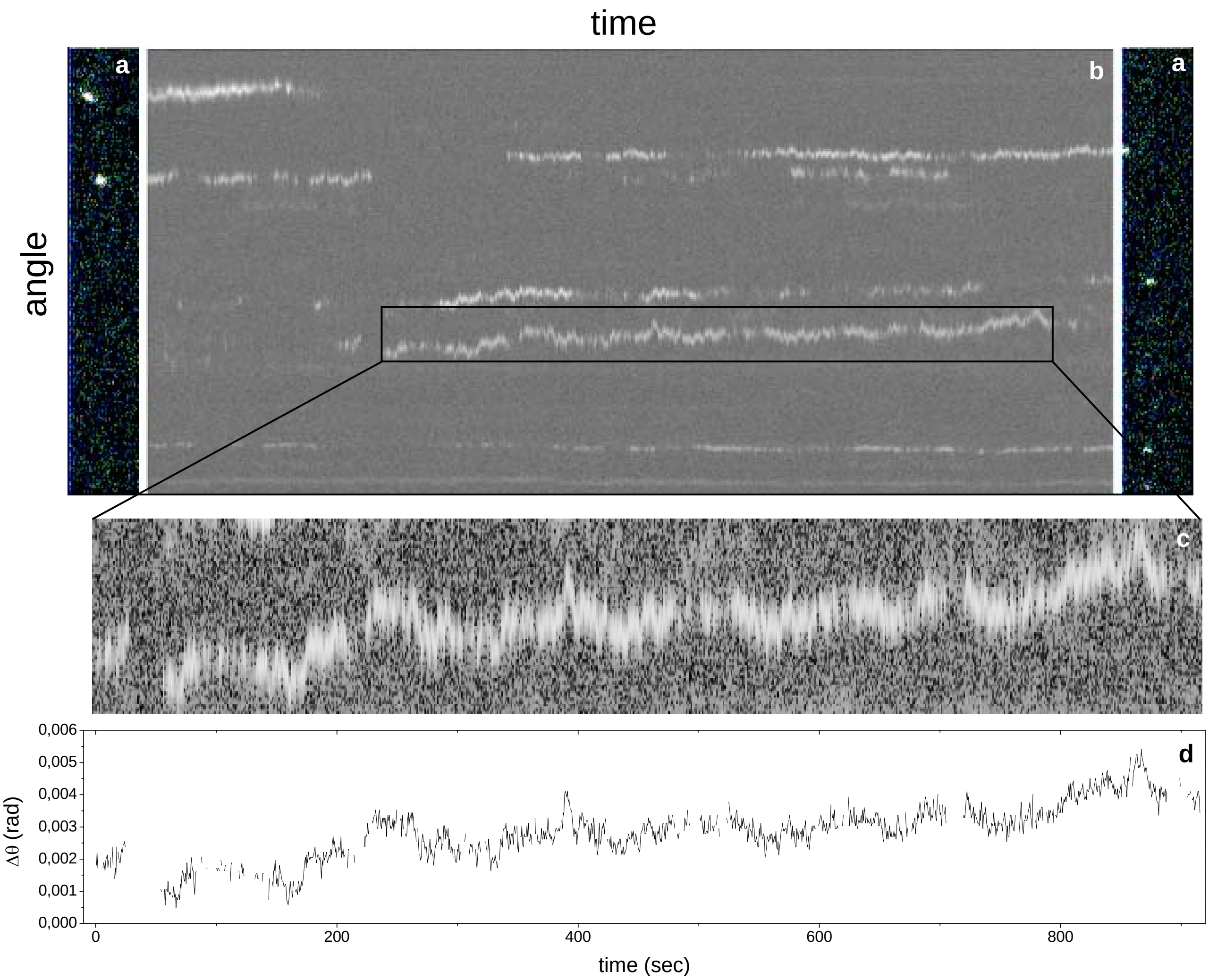

FIG. 1. X-ray diffraction microrheology determines very small angular displacements by using the diffraction intensity of individual crystals. (a) Two CCD frames, corresponding to the first and last frames of the time series, showing the (104) alumina powder ring in the vertical direction. (b) Each of the powder ring intensity frames is radially integrated (horizontally) and plotted vs time. (c) Trajectories which have sufficiently many time points (500 in the present case) are extracted to obtain the angle vs time for a single particle. (d) The trajectory is then tracked to obtain the angular motion of a single particle

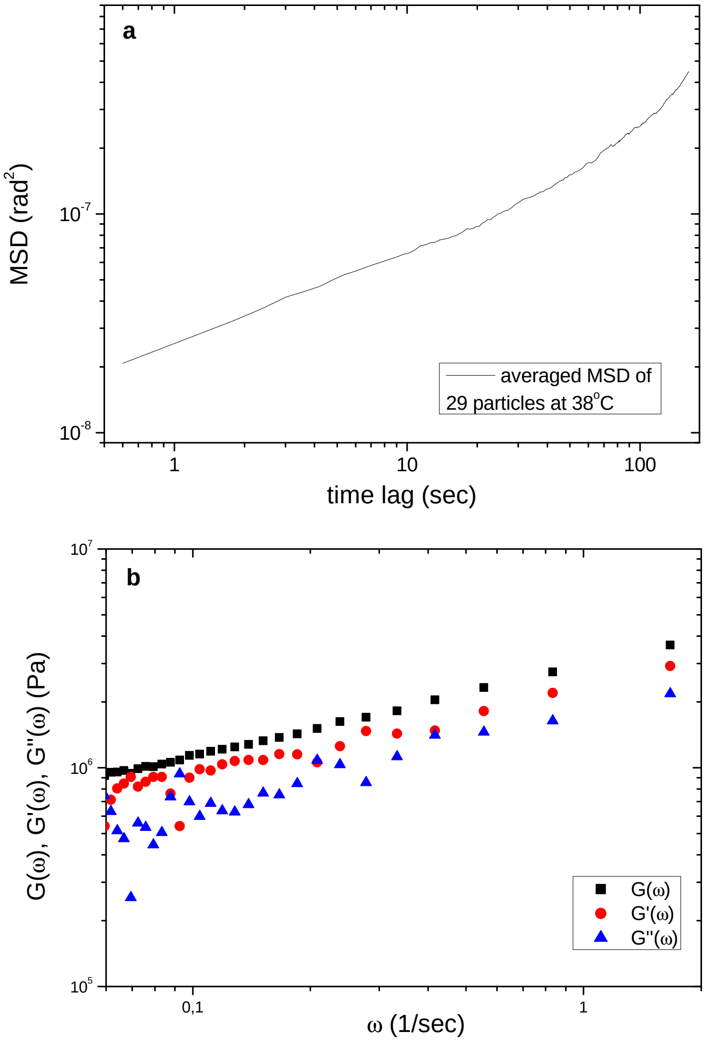

FIG. 2. (a)The MSD of 29 particles at 38^oC and (b) the corresponding $G(\omega)$, $G'(\omega)$, and $G''(\omega)$ obtained by a local power law expansion of the MSD.

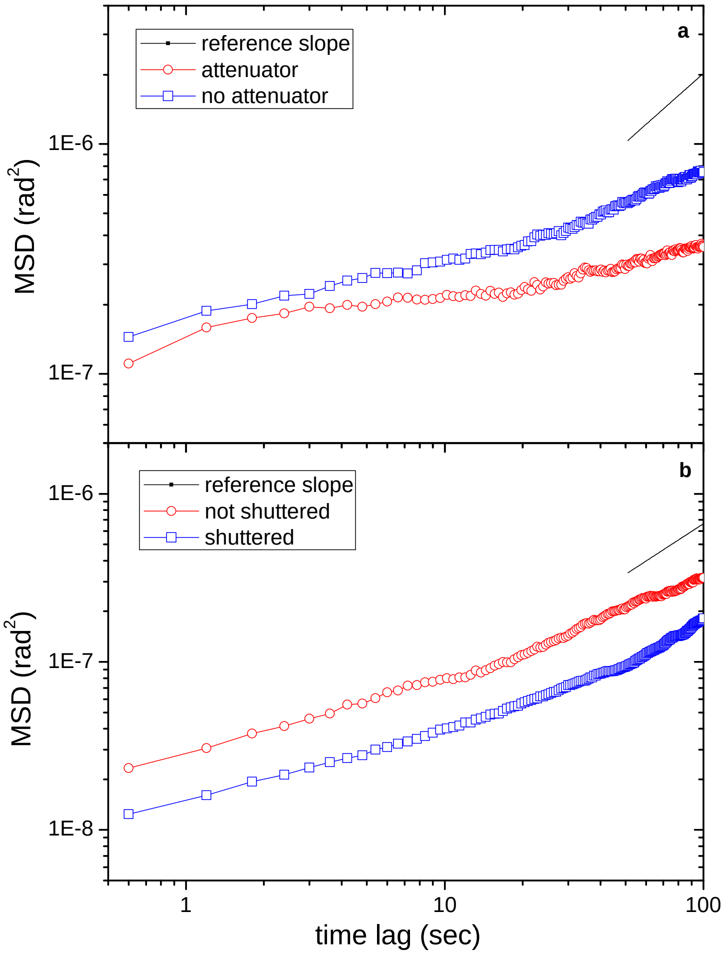

FIG. 3. (a) The MSD of a single particle with and without attenuation and (b) The average of 9 particles with shuttering and 6 particles without shuttering. Both radiation tests show a higher MSD vs. lag time for the higher flux state.