

# Shear Rheology of Interfaces: Micro Rheological Methods

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## 1 Introduction

Many of the diverse properties of soft materials (polymer solutions, gels, filamentous proteins in cells, etc.) stem from their complex structures and dynamics that have multiple characteristic length and time scales. A wide variety of technologies, from paints to foods, from oil recovery to processing of plastics, evaporation of complex fluids, design of multiphase chemical reactors, rely heavily on understanding the flow of complex fluids [1, 2]. The viscoelastic responses of complex materials depend on the time scale at which the sample is probed. Measurements of the complex shear modulus,  $G^*(\omega)$ , as a function of frequency are most frequently used for obtaining the elasticity and viscosity. Typical experiments using standard rheometers for bulk systems apply a small oscillatory strain on the sample, and usually probe frequencies from mHz up to tens of Hz.

Although standard rheological measurements have been very useful in characterizing soft materials and complex fluids, they suffer from some drawbacks, e.g. the need of sample volumes larger than one milliliter, which make them unsuitable for rare or precious materials, and for biological samples that are available in minute quantities. Furthermore, only average measurements of the system response are

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obtained using conventional rheometers due to the typical size of their probes, which makes them useless for local measurements in inhomogeneous systems. Microrheological techniques are being used during the last two decades for probing the material response on micrometer length scales, and using microliter sample volumes. The following advantages over macrorheology are worth mentioning: (a) much higher range of frequencies available without using the time-temperature superposition that is valid only for a limited number of systems [2]; (b) the capability of measuring material inhomogeneities that cannot be studied using macrorheological methods, and (c) rapid thermal and chemical homogenization that allow the transient rheology of evolving systems to be studied [3]. Microrheology methods typically use embedded nano- or microparticles to locally deform the samples hence macro- and microrheology probe different aspects of the material. Macrorheology measurements explore extremely long (macroscopic) length scales compared to the characteristic length scales of the system, microrheology effectively measures material properties on the scale of the probe itself (since flow and deformation fields decay on this length scale). Detailed descriptions of the methods and applications of microrheology to the study of bulk systems have been given in review articles published in recent years [4–11]. In the present work we will focus on techniques that use micron-size particles, and their application to the study of the dynamic behavior of fluid interfaces.

Interfaces play a dominant role in the behavior of many complex fluids. Interfacial rheology has been found to be a key factor in the stability of foams and emulsions, compatibilization of polymer blends, flotation technology, fusion of vesicles, mass transport through interfaces, drug delivery from micro- and nanocapsules, etc. [12, 13]. In most cases interfacial rheology has been controlled by a careful selection of surfactants. However, the environmental regulations in the EU are becoming stricter, and conventional synthetic surfactants have to be substituted by environmentally friendly chemicals. One of the most promising possibilities is to stabilize the interfaces using natural or biodegradable particles trapped at the interfaces, due to the high trapping energy of microparticles at fluid interfaces [14].

The dynamics of fluid interfaces is a rather complex problem because, even for the simplest fluid–fluid interface, different dynamic modes have to be taken into account: the capillary (out of plane) mode, and the in-plane mode, which contains dilational (or extensional) and shear contributions. For more complex interfaces, such as thicker ones, other dynamic modes (bending, splaying) have to be considered [15]. Moreover, the coupling of the above mentioned modes with adsorption/desorption kinetics may be very relevant for interfaces that contain soluble or partially soluble surfactants, polymers or proteins [16–18].

Till recently interfacial shear rheology has been studied using macroscopic interfacial rheometers which have a lower sensibility limit of about  $10^{-6} \text{ N s m}^{-1}$  [16, 19–21], however many important systems have surface shear viscosities below this limit, and microrheological techniques have been developed to overcome this limit down to values as low as  $10^{-10} \text{ N s m}^{-1}$ . In spite that the measurement of diffusion coefficients of particles attached to interfaces is relatively straightforward with modern microrheological techniques (see below), one has to rely on

hydrodynamic models of the viscoelastic surroundings probed by the particles in order to obtain variables such as monolayer elasticity or shear viscosity. The more complex the structure of the interface the stronger are the assumptions of the model, which reduces its range of applicability and makes more difficult to test its validity. In the present work we will briefly review two experimental techniques frequently used to study the dynamics of microparticles trapped at interfaces, and the use of microparticles as probes for studying the shear rheology of monolayers at fluid interfaces. We will also summarize two of the available theoretical approaches for calculating the shear microviscosity of fluid monolayers from particle tracking experiments. Finally the relatively few experimental results available for fluid interfaces using the two techniques will be discussed and analyzed using the two theories. The results will show that we are far from understanding microrheology results, and therefore more experimental and theoretical work is necessary.

## 2 Experimental Techniques

For studying the viscoelasticity of the probe environment there are two broad types of experimental methods: active methods, which involve probe manipulation, and passive methods, that rely on thermal fluctuations. Passive techniques are typically more useful for measuring low values of predominantly viscous moduli, whereas active techniques can extend the measurable range to samples with significant elasticity modulus. In this work we will focus on one passive technique: particle tracking by video microscopy, and one active technique: optical tweezers.

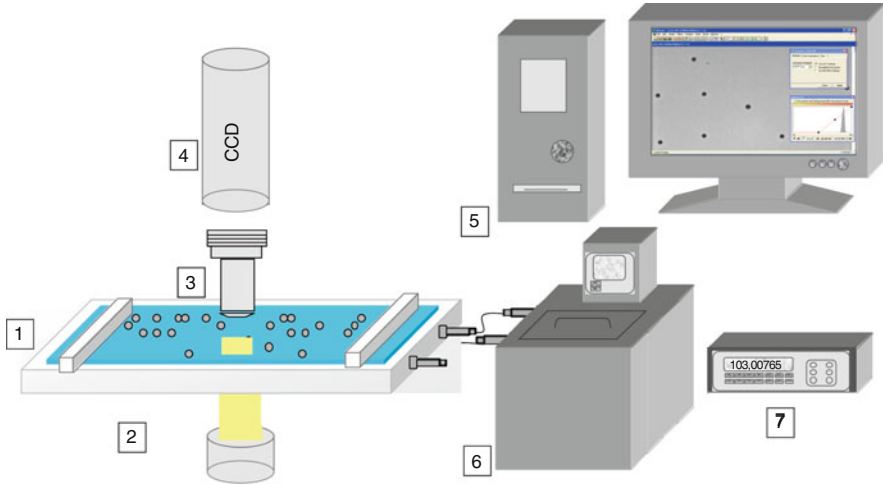
### 2.1 *Fundamentals of Video Microscopy Particle Tracking*

The main idea in particle tracking is to follow the trajectories of probes introduced into (onto) the system by video microscopy. The trajectories of the particles, either in bulk or on surfaces, allow one to calculate the mean square displacement, MSD, which is related to the diffusion coefficient,  $D$ , and the dimensions in which the translational motion takes place,  $d$ , by

$$\langle \Delta r^2(\tau) \rangle = 2dD\tau^\alpha, \quad (1)$$

where the brackets indicate the average over all the particles.

In case of diffusion in a purely viscous material (or interface),  $\alpha$  is equal to 1, and the usual linear relation is obtained between the MSD and the lag time  $\tau$ . For highly viscous materials or interfaces (like condensed surfactant or lipid monolayers and dense polymer monolayers), or when the system is dominated by the probe particles interactions (being this particularly important at high particle surface coverage) (1) does not fully apply. The movement of nano- and micro-particles in these solid-like



**Fig. 1** Typical particle tracking setup for 2D microrheology experiments: (1) Langmuir trough; (2) illumination; (3) microscope objective; (4) CCD camera; (5) computer; (6) thermostat; (7) electronics for measuring the surface pressure

interfaces cannot simply be interpreted assuming sub-diffusivity  $\alpha < 1$ . In fact if we consider a Maxwell viscoelasticity model the mean square displacement adopts the form of

$$\langle \Delta r^2(\tau) \rangle = \sigma/E + \sigma\tau/\eta, \quad (2)$$

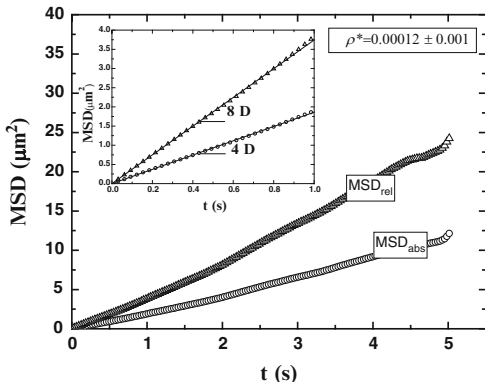
where  $\sigma$  is the stress,  $E$  is the elasticity modulus and  $\eta$  the viscosity coefficient and all of them refer to pure shear deformations. The characteristic Maxwell time is given by  $\tau_c = \eta/E$ . Anomalous diffusion  $\alpha < 1$  has been invoked in many systems of biological interest where the Brownian motion of the particles is hindered by obstacles, or even constrained to defined regions (corralled motion) [22]. The diffusion coefficient is related to the friction coefficient,  $f$ , by the Einstein relation

$$D = \frac{k_B T}{f}. \quad (3)$$

In 3D  $f$  is given by Stokes law,  $f = 6\pi\eta$ , and for pure viscous fluids the shear viscosity can be directly obtained from the diffusion coefficient. However, as we will discuss below, Stokes law does not apply to interfaces.

Figure 1 shows a sketch of a typical setup for interface particle tracking experiments. A CCD camera (typically 30 fps) is connected to a microscope that permits to image the interface prepared onto a Langmuir trough. The series of images are transferred to a computer to be analyzed and to extract the trajectories of a set of particles. A common problem is that the Brownian motion of the particles is often superimposed to a collective motion of the fluid arising from thermal

**Fig. 2** Mean square displacements and relative square displacement for latex particles at the water/n-octane interface. Experimental details: set of 300 latex particles of 1  $\mu\text{m}$  of diameter, surface charge density:  $-5.8 \mu\text{C cm}^{-2}$ , and reduced surface density,  $\rho^* = 1.2 \cdot 10^{-3}$  ( $\rho^* = \rho a^2$ ),  $25^\circ\text{C}$



gradients, and then it is useful the use of the relative mean square displacement of pairs of particles defined by

$$MSD_{rel} \equiv \langle \Delta r_{rel}^2(\tau) \rangle = \left\langle (\Delta \mathbf{r}_{ij}(t + \tau) - \Delta \mathbf{r}_{ij}(t))^2 \right\rangle = 4dD\tau. \quad (4)$$

The above averages are taken over all the pairs of particles and initial times,  $t$ , of the system. In this way any collective motion is eliminated or reduced.

Figure 2 shows a typical set of results for the MSD of a system of latex particles (1  $\mu\text{m}$  of radius) spread at the water/n-octane interface at low particle surface densities (gas-like phase) [23]. The analysis of MSD and  $MSD_{rel}$  in terms of (1) and (4) and in the linear range allows one to obtain  $D$ . However, it must be taken into account that for laden interfaces, even below the threshold of aggregation or fluid–solid phase transitions, the MSD shows a sub-diffusive behavior ( $\alpha < 1$  in (1)). Therefore, only physically meaningful values of  $D$  can be obtained in the limit of short times, and this should be taken into account when extracting the surface microrheology parameters from  $D$ .

When the samples are heterogeneous at the scale of particle size (a situation rather frequent, specially in biological systems [22, 24–26]), single particle tracking gives erroneous results and the so-called “two-point” correlation method is recommended [27]. In this method the fluctuations of pairs of particles at a distance  $R_{ij}$  are measured for all the possible values of  $R_{ij}$  within the system. Vector displacements of individual particles are calculated as a function of lag time,  $\tau$ , and initial absolute time,  $t$ : Then the ensemble averaged tensor product of the vector displacements is calculated [9]:

$$D_{\alpha,\beta}(r, \tau) = \left\langle \Delta r_{\alpha}^i(r, \tau) \Delta r_{\beta}^j(r, \tau) \delta [r - R_{ij}(t)] \right\rangle_{i \neq j, t}$$

$$\Delta r_{\alpha,\beta}^{i,j}(r, \tau) = r_{\alpha,\beta}^{i,j}(t + \tau) - r_{\alpha,\beta}^{i,j}(t), \quad (5)$$

where  $i$  and  $j$  label two particles,  $\alpha$  and  $\beta$  are coordinate axes and  $R_{ij}$  is the distance between particles  $i$  and  $j$ . The average corresponding to  $i = j$  represents

the one-particle mean-squared displacement. Two-point microrheology probes dynamics at different lengths from distances much larger than the particle radius down to the particle size which reflects extrapolation of long-wavelength thermal fluctuations of the medium to the particle size [28].

For the case in which the particles are embedded in a viscoelastic fluid, particle tracking experiments allow one to obtain the viscoelastic moduli of the fluids. Manson and Weitz first in an ad-hoc way, and later Levine and Lubensky in a more rigorous way, proposed a generalization of the Stokes–Einstein equation (GSE) [29, 30]:

$$\langle \Delta \tilde{r}^2(s) \rangle = \frac{2k_B T}{3\pi a s \tilde{G}(s)}, \quad (6)$$

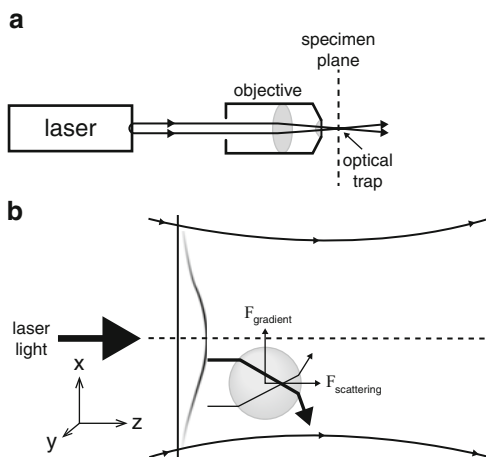
where  $\tilde{G}(s)$  is the Laplace transform of the stress relaxation modulus,  $s$  is the Laplace frequency, and  $a$  is the radius of the particles. An alternative expression for the GSE equation can be written in the Fourier domain [31]. Different methods have been devised to obtain  $\tilde{G}(s)$  from the experimental MSD [31–35]. The GSE equation is valid under the following approximations: (a) the medium around the sphere may be treated as a continuum material, which requires that the size of the particle be larger than any structural length scale of the material; (b) no slip boundary conditions; (c) the fluid surrounding the sphere is incompressible; and (d) there are no inertial effects. Very recently, Felderhof has presented an alternative method for calculating the shear complex modulus from the velocity autocorrelation function, that can be calculated from the particle trajectories [36].

For interfaces the situation is more complex, and the calculation of the surface shear viscosity has relied on the use of hydrodynamic models of the interface (see below). Only very recently Song et al. [37] have performed computer simulations that indicate that the GSE can be applied to fluid interfaces. Furthermore, the same group has applied the GSE to the study of interfaces in oil–water emulsions [37–39]. So far, no comparison has been made between the surface shear viscosity calculated by hydrodynamic models and the GSE.

## 2.2 *Fundamentals of the Optical Tweezers Technique*

This technique uses a highly focused laser beam to trap a colloidal particle, as a consequence of the momentum transfer associated with bending light. The most basic design of an optical tweezers is shown in Fig. 3a: A laser beam (usually in the  $IR$  range) is focused by a high-quality microscope (high numerical aperture objective) to a spot in a plane in the fluid. Figure 3b shows a detailed scheme of how an optical trap is created. Light carries a momentum in the direction of propagation that is proportional to its energy, and any change in the direction of light, by reflection or refraction, will result in a change of the momentum. If an object bends the light, conservation momentum requires that the object must undergo an equal and opposite momentum change, which gives rise to a force acting on the subject. When the light interacts with a bead, the sum of the forces acting on the particle

**Fig. 3** (a) Basic design of an optical tweezers instrument; (b) Details of the physical principles leading to the optical trap



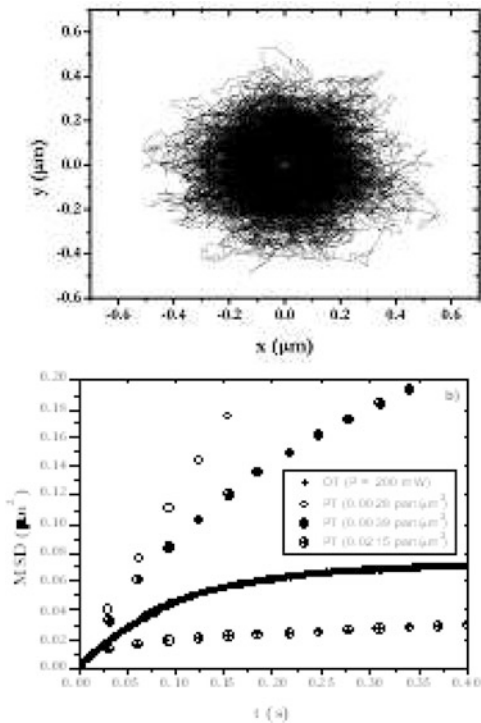
can be split into two components:  $F_{sc}$ , the scattering force, pointing in the direction of the incident beam, and  $F_g$ , the gradient force, arising from the gradient of the Gaussian intensity profile and pointing in the plane perpendicular to the incident beam towards the center of the beam.  $F_g$  is a restoring force that pulls the bead into the center of the beam. If the contribution to  $F_{sc}$  of the refracted rays is larger than that of the reflected rays then a restoring force is also created along the beam direction and a stable trap exists. A detailed description of the theoretical basis and of modern experimental setups has been given in [40–42] that also include a review of applications of optical tweezers to problems of biophysical interest: ligand–receptor interactions, mechanical response of single chains of biopolymers, force spectroscopy of enzymes and membranes, molecular motors, and cell manipulation. A recent application of optical tweezers to study the non-linear mechanical response of red-blood cells is given by Yoon et al. [43]. Finally, optical tweezers are also suitable for the study of interfacial rheology [44].

### 3 Dynamics of Particles at Interfaces

#### 3.1 Diffusion Coefficient of Particles Adsorbed at Fluid Interfaces

A problem that appears to be ignored in the analysis of particle dynamics at interfaces is the strong influence of the interactions with other particles that leads to a decrease of the apparent  $D$ , so in what follows we will only refer to the infinite dilution diffusion coefficient. We have already mentioned that quite frequently one finds a subdiffusive behavior of the MSD of particles trapped at fluid interfaces. Figure 4 shows an example of the results obtained by tracking the trajectories of a single particle at an octane/water interfaces in an optical trap. Since no linear range

**Fig. 4** *Up figure*: Typical trajectory observed for a latex macroparticle (radius  $1\ \mu\text{m}$ ) in an optical trap at the octane/water interface and  $25^\circ\text{C}$ ;  $x$  and  $y$  are the displacements from the center of the trap in the  $x$  and  $y$  axis. *Down figure*: Example of subdiffusive dynamics of latex microparticles of  $2.9\ \mu\text{m}$  of radius obtained by video microscopy particle tracking, PT, at different particle surface densities, and for a single particle in an optical trap. The continuous lines are the fits to the Langevin equation (8). All the results were taken at the octane/water interface at  $25^\circ\text{C}$



is observed,  $D$  has been obtained by fitting the MSD results to the solution of the Langevin equation including an elastic force:

$$m \frac{dv}{dt} = -\xi v + f(t) - kx, \quad (7)$$

where  $m$  is the mass of the particle,  $v$  its velocity,  $\xi$  the friction coefficient,  $k$  the characteristic force constant of the elastic force acting on the particle, and  $f(t)$  is the random force, so that time average  $\langle f(t) \rangle = 0$ . Even though the potential well is not strictly parabolic, it is a very good approximation for laser intensities such that the particles are trapped relatively deep inside the potential well. The solution of (7) was given by Chandrasekar [45], and fits very well the data shown in Fig. 4. An important point is that the fits allow one to obtain the diffusion coefficient at infinite dilution,  $D_0$ . For diluted particle monolayers in which a linear dependence of the MSD is observed in the particle tracking experiments, the agreement with the values of  $D_0$  obtained using the optical tweezers technique agree within the experimental error. This is a very important result because, as discussed below, the interfacial shear viscosity is calculated, using hydrodynamic theories, from  $D_0$ . Therefore, the fact that two different microrheological techniques lead to the same values of the diffusion coefficient, ensures that the viscosity obtained will also be the same.



### 3.2 Fischer's Theory for the Shear Micro-Rheology of Monolayers at Fluid Interfaces

For particles trapped at interfaces Einstein's equation, (3), is still valid. Nevertheless, Stokes equation for  $f$  is no longer valid because at interfaces  $f$  is a function of the viscosities of the phases ( $\eta/s$ ), the geometry of the particle (e.g., the radius "a" for spheres), the contact angle between the probe particle and the interface ( $\theta$ ), etc. There is no rigorous solution for the slow viscous flow equations for steady translational motion of a sphere in an ideal 2D fluid, e.g. a monolayer (Stokes paradox), hence we will briefly describe one of the most recent theoretical approaches for describing  $f$ .

Fischer et al. [46] assumed that a surfactant monolayer behaves as incompressible because Marangoni forces (forces due to surface tension gradients) strongly suppress any motion at a surface that compress or expands the interface due to any gradient in the surface pressure. Such gradients are instantly compensated by the fast motion of the surfactant at the interface, thus leading to a constant surface pressure, and therefore the monolayer behaves as an incompressible body (Fischer assumes that the velocity of the 2D surfactant diffusion is faster than the motion of the beads). The fact that the drag on a disk in a monolayer is that of an incompressible surface has been verified experimentally [47, 48], although this hypothesis could fail for highly viscous polymer monolayers.

Fischer et al. have numerically solved the problem of a sphere trapped at an interface with a contact angle  $\theta$  moving in an *incompressible* surface [46]. They showed that contributions due to Marangoni forces account for a significant part of the total drag. This effect becomes most pronounced in the limit of vanishing surface compressibility. They solved the fluid dynamics equations for a 3D object moving in a monolayer of surface shear viscosity,  $\eta_s$  between two infinite viscous phases. The monolayer surface is assumed to be flat (no electrocapillary effects). Then the translational drag coefficient,  $k_T$ , was expressed as a series expansion of the Boussinesq number,  $B = \eta_s/((\eta_1 + \eta_2)a)$ ,  $a$  being the radius of spherical particle:

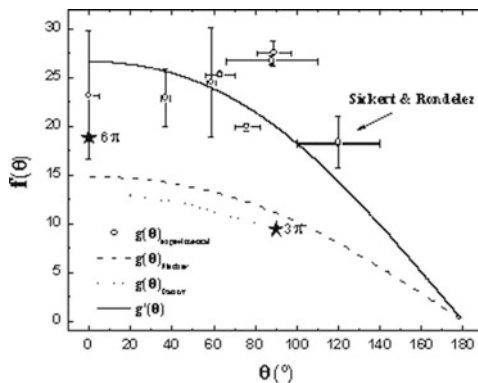
$$k_T = k_T^0 + Bk_T^1 + O(B^2). \quad (8)$$

For  $B = 0$ , and for an air–water interface ( $\eta_1, \eta_2 = 0$ ), the numerical results for  $k_T^{(0)}$  and  $k_T^{(1)}$  are fitted with an accuracy of 3% by the following expressions:

$$k_T^0 \approx 6\pi \sqrt{\tanh\left(32\left(\frac{d}{R} + 2\right) / (9\pi^2)\right)} \quad (9)$$

$$k_T^{(1)} \approx \begin{cases} -4\ln\left(\frac{2}{\pi} \arctan\left(\frac{2}{3}\right)\right) \left(\frac{a^3/2}{(d+3a)^{3/2}}\right) & (d/a > 0) \\ -4\ln\left(\frac{2}{\pi} \arctan\left(\frac{d+2a}{3a}\right)\right) & (d/a < 0) \end{cases} \quad (10)$$

**Fig. 5** Friction coefficients calculated from the experimental diffusion coefficients measured by particle tracking experiments (symbols), by Fischer's theory (dashed line), and by the corrected Fischer's theory (continuous line)



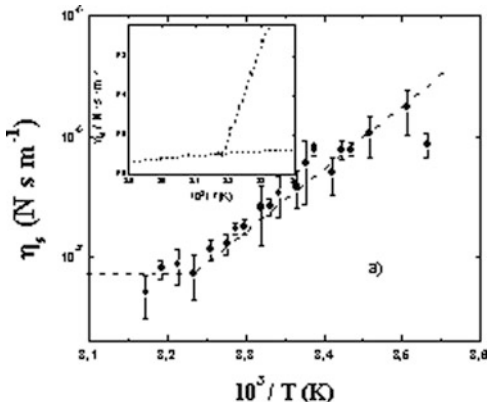
where  $d$  is the distance from the apex of the bead to the plane of the interface (which defines the contact angle). Note that if  $d$  goes to infinity,  $k_T^0 = 6\pi$ , which is the correct theoretical value for a sphere in bulk (Stokes law). They found that, even in the absence of any appreciable surface viscosity, the drag coefficient of an incompressible monolayer is higher than that of a free interface.

Figure 5 shows the friction coefficient for latex particles at the water–air interface obtained from single particle tracking for polystyrene latex particles. It also shows the values calculated from Fischer's theory, pointing out that the theoretical values are smaller than the experimental values over the whole  $\theta$  range. An empirical factor of  $f(\theta)_{exp}/f(\theta)_{Fischer} = 1.8 \pm 0.2$  brings the values calculated with Fischer's theory in good agreement with the experiments at all the contact angle values. A similar situation was found for the water-n-octane interface with a smaller correction factor  $f(\theta)_{exp}/f(\theta)_{Fischer} = 1.2 \pm 0.1$ . So far the physical origin of this correction factor is unknown.

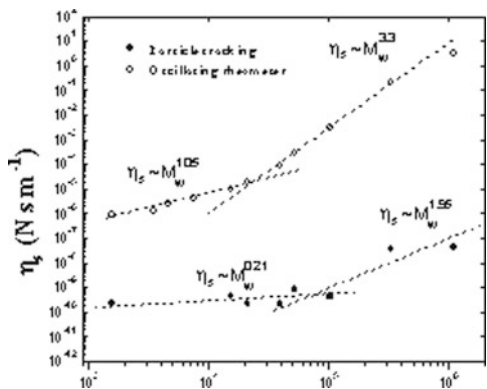
## 4 Particle Tracking Results

Sickert and Rondelez were among the first to calculate the surface shear viscosity of monolayers by tracking the trajectories of spherical particles embedded in Langmuir monolayers at the air/water interface [49]. They have measured the surface viscosity of three monolayers formed by pentadecanoic acid (PDA), L- $\alpha$ -dipalmitoylphosphatidylcholine (DPPC) and N-palmitoyl-6-n-penicillanic acid (PPA) respectively. The values of the shear viscosities for PDA, DPPC and PPA reported were in the range of  $1$  to  $11 \cdot 10^{-10} \text{ N s m}^{-1}$  in the liquid expanded region of the monolayer, that, as already said, are beyond the range of macroscopic mechanical methods. More recently, Bonales et al. have calculated the shear viscosity of two polymer Langmuir films, and compared these values with those obtained by canal viscosimetry [50]. Hilles et al. [51] studied the glass transition in Langmuir films. Figure 6 shows the results obtained for a monolayer of poly(4-hydroxystyrene) at

**Fig. 6** Temperature dependence of the surface shear viscosity of a monolayer of poly(4-hydroxystyrene) at the air–water interface obtained by particle tracking (the insets show the corresponding values measured with a macroscopic canal viscometer. Experiments done at  $\Pi = 8 \text{ mN} \cdot \text{m}^{-1}$



**Fig. 7** Surface shear viscosity for monolayers of poly(t-butyl acrylate) as a function of the molecular weight and for a surface pressure  $\Pi = 16 \text{ mN} \cdot \text{m}^{-1}$ . The lower curve corresponds to data obtained from video microscopy particle tracking. The upper curve was obtained from conventional oscillatory interfacial rheometers



an air–water interface. For all the monolayers reported in Refs. [50, 51] the surface shear viscosity calculated from Fischer’s theory using the  $D$  values obtained from the MSD results was lower than that measured with the macroscopic canal surface viscometer. Similar qualitative conclusions were reached at by Sickert et al. for their monolayers [52]. These authors have later reanalyzed their original data [49] and they found that the relation  $D_0/D_{\rightarrow 0}$  ( $D_0$  being the diffusion coefficient of the beads at a free compressible surface, and  $D_{\rightarrow 0}$  the value of an incompressible monolayer which surface concentration is tending to zero) is, theoretically, not equal to 1 but to about 0.8, which is confirmed by their experiment, and also confirms the observations of Barentin et al. [19] and Lee et al. [3] for different systems. In spite of the apparent success of Fischer’s theory, the surface viscosity values are rather low when compared to the results obtained by macrorheology methods (see below).

As above mentioned there is a quantitative inconsistency between macro and micro-rheology results. Figure 7 shows clearly the large difference found between micro- and macrorheology for monolayers of poly(t-butyl acrylate) near the collapse surface concentration,  $\Gamma^{**}$  [16]. The macrorheology results have been obtained using two different interfacial oscillatory rheometers [21].

**Fig. 8** Surface concentration dependence of the surface shear rheology for a monolayer of poly(*t*-butyl acrylate). The *symbols* are experimental results obtained by video microscopy particle tracking using particles of different chemical nature and size and using both Fischer's theory and the Generalized Stokes Einstein theory (Weitz method)

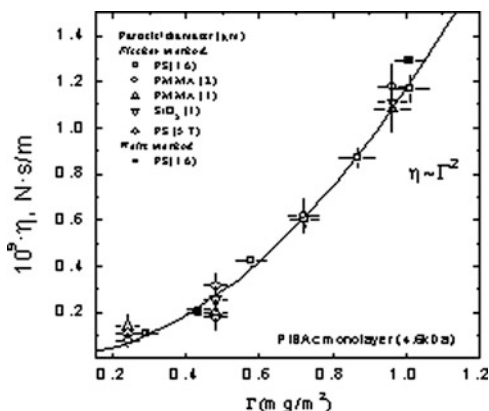


Figure 8 shows that the interfacial shear viscosities obtained with particles of rather different chemical nature and size agree within the experimental uncertainty. This discards that the origin of the discrepancies between macro- and microrheology can stem from the interactions between the particles and the monolayer molecules. Moreover, the values calculated from the modified-Fischer's theory or by direct application of the GSE equation lead to almost indistinguishable surface shear viscosities.

This discrepancy between micro- and macrorheology in the study of monolayers seems to be a rather frequent situation and no clear theoretical answer has been found so far for this fact. This type of disagreement has been also found in 3D systems, where in some cases the origin of the problem has been identified to be the inhomogeneity of the system [25, 26]. In the analysis of the particle tracking at interfaces shown above, it has been assumed that systems are homogeneous, which might not be the case. Prasad and Weeks have applied the two-particle correlation method (5) to the motion of particles trapped to the air–water interface covered with a Langmuir monolayer of human serum albumin (HSA) as a function of surface concentration [53]. They found that for high surface concentrations the one and two particle (correlated) measurements give different values of the viscosity. They explained this by suggesting that the monolayer was inhomogeneous. Both methods agree when the particle size is of the same order than the scale of the inhomogeneities of the system. However, the authors did not compare particle tracking results with macrorheology. In conclusion we need to be very careful when extracting surface viscosities in this kind of systems from single particle tracking, and whenever possible one should use two-particle correlated analysis.

However, the problem might be not only due to the length scale of the rheology but also because the active or passive character of the technique used. In fact, Lee et al. [3] combined active and passive microrheology methods to study protein ( $\beta$ -lactoglobulin) layers at the air–water interface. They used *magnetic nanowire microrheology* and *particle tracking* with correlated analysis as a function of adsorption time, and found that the surface viscosity obtained is about one order

of magnitude larger when measured with the active technique. Both techniques are micro-rheology methods but give quite different values for the surface viscosity. However, as indicated above, in our case both video microscopy and optical tweezers give the same values of  $D_0$  from which the interfacial surface viscosity is calculated, therefore the use of passive or active techniques does not seem to be the source of the discrepancy between macro- and microrheology.

It is also needed to bear in mind that ideal 2D systems do not exist; the interface is a region of certain thickness which makes the interpretation of the results quite slippery. For example, Prasad et al. [54] have measured the surface viscosity of a commercial dishwasher surfactant (soluble) in a soap film by single and correlated particle tracking as a function of the film thickness. They found unphysical values for the surface viscosity for thickness larger than a certain value. Above this critical thickness, single particle tracking gives negative values for the surface viscosity, and two-particle correlated MSD gives large positive values compared to the values found in thin films. It would be possible to extend this idea to thick monolayers (for example, for some polymer monolayers), and consider that the motion of the beads does not take place in a 2D environment but in a quasi-3D one. This would make quite tricky the interpretation of the particle tracking results obtained using the theories outlined in the previous paragraphs.

It has also been shown that sometimes for very dense layers of polymers, the probes move faster than they do in layers formed at lower surface concentrations of the same polymer [3]. In these cases we can imagine that the particle probes could be expelled out of the interface and keep under (onto) the layer giving erroneous values of the diffusion coefficient, and of the surface viscosity calculated from the MSD.

## 5 Conclusions

Microrheology techniques, and specially particle tracking, are probably the only suited techniques for the study of the rheology in many systems of interest, as for example the dynamics inside cell membranes or in the expanded region of monolayers. However one must be very careful in interpreting the results obtained from single particle tracking and the available theories. Whenever possible the correlated two-particle MSD should be used. It is clear from the results that for fluid interfaces much more experimental and theoretical work is needed to explain why the shear surface microviscosity is much smaller than the one measured with conventional surface rheometers. Despite all the problems mentioned, in our opinion it is worth continuing working on this microrheological techniques for its potentiality in the study of the dynamics of systems of biological importance.

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