Scaling form of viscosity at all length-scales in poly(ethylene glycol) solutions studied by fluorescence correlation spectroscopy and capillary electrophoresis

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We measured the viscosity of poly(ethylene glycol) (PEG 6000, 12 000, 20 000) in water using capillary electrophoresis and fluorescence correlation spectroscopy with nanoscopic probes of different diameters (from 1.7 to 114 nm). For a probe of diameter smaller than the radius of gyration of PEG (e.g. rhodamine B or lysozyme) the measured nanoviscosity was orders of magnitude smaller than the macroviscosity. For sizes equal to (or larger than) the polymer radius of gyration, macroscopic value of viscosity was measured. A mathematical relation for macro and nanoviscosity was found as a function of PEG radius of gyration, Rg, correlation length in semi-dilute solution, ξ, and probe size, R. For R < Rg, the nanoviscosity (normalized by water viscosity) is given by \( \exp(b(R/ξ)^a)\), and for R > Rg, both nano and macroviscosity follow the same curve, \( \exp(b(R/ξ)^4)\), where a and b are two constants close to unity. This mathematical relation was shown to equally well describe rhodamine (of size 1.7 nm) in PEG 20 000 and the macroviscosity of PEG 8 000 000, whose radius of gyration exceeds 200 nm. Additionally, for the smallest probes (rhodamine B and lysozyme) we have verified, using capillary electrophoresis and fluorescence correlation spectroscopy, that the Stokes–Einstein (SE) relation holds, providing that we use a size-dependent viscosity in the formula. The SE relation is correct even in PEG solutions of very high viscosity (three orders of magnitude larger than that of water).

Introduction

Water-based polymer solutions appear in a wide variety of systems and industrial processes and products (e.g. biological cells, food production processes, paints, and personal care products). Their viscosity can be larger by several orders of magnitude than that of water. However, because water is the dominant fraction in these solutions, small objects of sub-nanometer size, d, should experience only the viscosity of water while diffusing in the solutions. On the other hand, large objects, much greater than the polymer size, should experience the large macroviscosity of the solution. It follows immediately that the coefficient of viscosity depends on the length-scale at which it is probed: viscosity should change from the value for water, \( \eta_{\text{solvent}} \), at the nano-scale to a large macroviscosity, \( \eta_{\text{macro}} \), at the macroscale. A number of questions are still open in this context: what is the length-scale for which we observe a crossover from \( \eta_{\text{solvent}} \) to \( \eta_{\text{macro}} \)? What is the relation between nanoviscosity determined from the diffusion of nanoprobes and macroviscosity measured by standard rheometers? Is the Stokes–Einstein relation valid for nanoprobe diffusion in polymer solutions? Partial answers, summarized below, have been given to these questions.1–27

The problem of nano and macroviscosity of polymer solutions received a lot of attention in experimental and theoretical studies. In the early fifties, Schachman et al.1 used an ultracentrifuge to study the dependence of viscosity of DNA solutions on size of sedimenting nanoprobes and observed that small and large probes exhibited dramatically different sedimentation coefficients. Later, the dependence of the viscosity on the size of probes and concentration of polymer was found to be a stretched exponential function.2–5 Laurent et al.2 had investigated the sedimentation of bovine serum albumin (BSA—radius 3.55 nm) in hyaluronic acid solution. Rodbard and Chrambach3 applied capillary electrophoresis to the motion of various proteins and dyes of sizes from 0.51 to 6 nm in polyacrylamide gel. A thorough account of all the works in this direction based on capillary electrophoresis,5,6,7 sedimentation experiments in the ultracentrifuge,1,2,5,8 or diffusion9–12 was given by Odijk.13 This exponential dependence of nanoviscosity on probe size and polymer concentration prompted scientists to analyze the problem theoretically.13–21 Theories developed by Odijk,13 following Ogston,5,14 Philles,15–18 Cukier,19 Altenberger,20 Amsden21 and de Gennes22,23 predicted the same exponential dependence of viscosity on...
probe size, but differed in the physical mechanisms which led to such dependence. A more recent theoretical approach which follows Odijk is based on the nonuniform viscosity in polymer solutions around a nanoprobe. All experiments and theoretical calculations showed the same universal stretched exponential dependence of viscosity on the size of nanoprobes and concentration of polymer solutions.

An important breakthrough in the study of the relation between nanoviscosity, probe size and polymer concentration came in 1978 with the experiments of Langevin and Rondelez. They showed experimentally that, instead of two variables (probe size and concentration of a polymer), the nanoviscosity felt by a nanoprobe is an exponential function of only a single variable, $R/\xi$, where $R$ is the size of a nanoprobe and $\xi$ is the correlation length (distance between entanglement points of polymer chains), which is dependent on the concentration, in the semi-dilute polymer solution. This important experimental work followed the suggestion of de Gennes who developed a theoretical approach to the viscosity of polymer solutions. In his approach, a dense polymer solution can be viewed as a transient statistical network of mesh size (correlation length), $\xi$, in a solvent. This size also corresponds to the size of a “blob” inside which all monomers belong to the same polymer chain. De Gennes postulated that the viscosity should depend on $R$ as $n(R/\xi)$, as verified by Langevin and Rondelez. Moreover, he suggested that the crossover from nanoviscosity to macroviscosity occurs when the size of a nanoprobe exceeds the blob size. Thus, in this approach, the viscosity experienced by an object of size $R > \xi$ should have a constant value equal to the macroscopic viscosity $\eta_{macro}$, while for $R < \xi$, the viscosity should depend on $R$ as $n(R/\xi)$. Thus, in this model, the crossover length scale, $L_c$, is equal to $\xi$. Since $\xi$ decreases with polymer concentration, for any size, $R$, there is a well defined concentration, $x(\xi)$, for which a crossover occurs to the macroscopic viscosity. The latter prediction was not confirmed by Langevin and Rondelez, who concluded their paper with the following remark “Also the experimental observation that at high polymer concentration the sedimentation coefficients do not converge towards a constant value proportional to the macroscopic viscosity, is still unclear”. Interestingly, de Gennes’ prediction concerning the crossover length scale was confirmed in sedimentation experiments in polyethylene-propylene in decane (as a solvent) for calcium carbonate core-shell nano-particles of size $R = 4$ nm. Clearly, these two experiments gave conflicting results. Further studies in this direction have been performed by Michelman et al. for probes from 2 to 44 nm in poly(vinyl alcohol) solutions using fluorescence correlation spectroscopy. They noted that 44 nm spheres move in a macroviscosity environment, and that smaller probes feel nano-viscosity, but they did not correlate their results with the blob size in PVA and, consequently, did not obtain a clear-cut crossover between nano and macroviscosity. Finally, in recent computer simulations, Liu et al. found that when the size of a probe is larger than the gyration radius of polymer chain, probes experience macroviscosity. They also observed that the crossover is not sharp and occurs when the radius of gyration of a polymer is between the diameter and radius of a nanoprobe. Summarizing, they found a crossover at a size comparable not to the correlation length, $\xi$, but to the much larger radius of gyration, $R_g$. They also found that the Stokes–Einstein relation is valid only for particles larger than the radius of gyration. In view of these conflicting results, the problem of crossover between nano and macroviscosity as well as the test of the Stokes–Einstein relation is still an open problem, requiring further experimental tests.

The Stokes–Einstein (SE) equation belongs to the larger class of relations known under the common name fluctuation–dissipation relations. The SE relation was partially addressed by Ogston, but there is still a lot of controversy as to how to approach the SE relation at the nanoscale. Liu et al. and also Tuteja et al. concluded that the Stokes–Einstein relation holds only when the size of a particle is larger than the radius of gyration of a polymer. In both cases, the diffusion of particles was compared to the macroviscosity of a polymer solution. Also, Michelman–Ribeiro et al. verified the SE relation for large probes (44 nm) in poly(vinyl alcohol) solutions using fluorescence correlation spectroscopy. Similar conclusions were reached by Zanten et al. using diffusive wave spectroscopy. The list of papers validating the SE relation for large probe sizes using diffusion measurements in polymer solutions is long. A similar problem of the relation between macroviscosity and diffusion exists in the dynamics of concentrated colloidal solutions. Poon et al. using two-color dynamic light scattering (to avoid multiple scattering) showed that collective long-time diffusion coefficients measured for the $q$ vector corresponding to the maximum in the structure factor satisfies the generalized Stokes–Einstein relation. The viscosity of colloidal systems was investigated by Brady and co-workers. The question which still remains is as follows: can we still use the SE relation at the nanoscale? In this case, a typical test of the SE relation is not a simple comparison of diffusion with the macroviscosity, but rather a comparison of hydrodynamic drag exerted on a moving object (measured by $q$ vector corresponding to the maximum in the structure factor) to its diffusion coefficient (measured by dynamic light scattering at vanishing concentration or by fluorescence correlation spectroscopy).

In this manuscript, we study the nanoviscosity and macroviscosity of PEG solutions covering a wide range of macroscopic viscosity from $n_{solvent}$ to $n_{macro} \approx 3000 n_{solvent}$ for PEG 20 000 and $n_{macro} \approx 35 000 n_{solvent}$ for PEG 8 000 000. It is worth mentioning that in all previous studies, the solution viscosity rarely exceeded an order of magnitude beyond that of the solvent (with the exception of gels) and here we cover almost 5 orders of magnitude. Our studies are concentrated on establishing a clear relation between nano and macroviscosity and the crossover length scale from nanoviscosity to macroviscosity.

The article is organized as follows: in the next section we will present the materials and describe methods used in experiments. In section 3 we will show and discuss our results. Conclusions are contained in section 4.

### Materials and methods

We studied the viscosity of PEG (molecular mass 6000, 12 000, 20 000) solutions in water using fluorescence correlation
spectroscopy (FCS) (for diffusion), capillary electrophoresis (CE) (for flow) and standard rheology (for macroscopic viscosity) over a wide range of polymer concentration (from 0 to 50% with macroscopic viscosity from \( \eta_{\text{solvent}} \) to \( \eta_{\text{macro}} \approx 3000\eta_{\text{solvent}} \)) and for objects ranging in size from 1.7 nm (rhodamine B) to 114 nm (silica spheres). Fluorescence correlation spectroscopy, although an old technique, was not used on a regular basis for the first 20-30 years after its invention.\(^45\) It became a standard technique for diffusion measurements only 10 years ago, after its combination with confocal microscopy.\(^46\,47\)

**PEG solutions**

We used aqueous solutions (appropriate buffers) of PEG (poly(ethylene glycol)). The molecular weights of PEG, purchased from Fluka, were, on average, 6000, 12 000, 20 000. The measured polydispersity (using mass spectroscopy and size exclusion chromatography GPC) was typically PDI = 1.09 to 1.13 for PEG 20 000 with \( M_n = 16300 \) for PEG 20 000 (average mass between 16 000 and 24 000). For comparison, we have also used a high molecular weight PEG 8 000 000.

**Sample preparation**

We purchased highly purified (by crystallization repeated three times, and by dialysis) lysozyme protein from Sigma-Aldrich. The tertiary structure of lysozyme is a prolate ellipsoid with short and long axes of 30 and 50 Å, respectively (based on the crystallographic structure). For fluorescence correlation spectroscopy, the lysozyme was labeled with the TAMRA (i.e. 5(6)-carboxy-tetramethylrhodamine) fluorescent dye with the absorption and emission in the region of 555 and 580 nm, respectively. We purchased TAMRA from Sigma-Aldrich. TAMRA fluorescent dye can be attached to the amino group, either at the N-terminus of the protein or on the side chain of a lysine amino acid. The amino group has to be in the non-protonated form to react with the fluorescent dye, thus the reaction must be performed at a pH higher than the pK value for the protonation of the amino acid. The protein solution was dialyzed to the 0.1 M phosphate buffer pH 8.4 in value for the protonation of the amino group. The protein reaction must be performed at a pH higher than the pK of a lysine amino acid. The amino group has to be in the either at the N-terminus of the protein or on the side chain of rhodamine. We purchased TAMRA from Sigma-Aldrich.

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Experimental solutions were made in a following way. First we prepared different PEG solutions: PEG concentrations were 5, 10, 15, 20, 30, 40 and 50 wt%. To avoid aggregation of proteins, and to keep a constant pH, we prepared solutions using a phosphate buffer (pH 7). Buffer solutions were dialyzed. We added about 2 μL of protein/buffer solution to these PEG/buffer solutions. The final protein concentration was about 4 \( \times 10^{-8} \) M. For capillary electrophoresis, the charge ladder of lysozyme was prepared. The preparation of the protein charge ladder is described in detail elsewhere.\(^48\)

**Capillary electrophoresis (CE) set up for determination of hydrodynamic drag**

Electrophoretic experiments were performed on a custom-build apparatus consisting of high-voltage supply model CZE 1000 R (Spellman,. Plainview, NY, USA) and Spectra 100 variable-wavelength UV-Vis CE detector from Thermo Separation Products (Fremont, CA, USA). The capillary was inserted in the plexiglas box with fan. The temperature was controlled inside the box. The fused-silica 75 μm i.d. capillaries with total length of 55 cm and effective length of 43 cm to the detector window for lysozyme and 38–26 cm for rhodamine were used. New capillaries were conditioned by passing a 0.1 M NaOH solution for 20 min and then washing with distilled water for 10 min, and finally equilibrating with an appropriate electrolyte. The electrolyte solutions were prepared by dissolving the appropriate amount of polyethylene glycol (PEG) 6000, 12 000 or 20 000 (concentration range 0–50% v/v) with water for HPLC adjusted to pH = 2.9 with \( \text{H}_3\text{PO}_4 \) for rhodamine or PEG 20 000 with tris-glycine buffer of pH 8.4 (25 mM tris base and 192 mM glycine) for lysozyme. A new capillary was used for each PEG (6000, 12 000 and 20 000) and sample. One capillary was used for all concentrations of each PEG and sample. The capillary was filled with new solution by applied pressure until a drop of the solution appeared on the opposite end of the capillary. The capillary was not washed between runs.

The sample of rhodamine was prepared by dissolving in water adjusted to pH = 2.9 with \( \text{H}_3\text{PO}_4 \). The protein charge ladder was prepared as described elsewhere\(^48\) and the reaction was terminated by the addition of tris-glycine buffer and this solution was introduced into the capillary. The samples were introduced electrokinetically (depending on PEG concentration, for 0.5 to 5 min and at 5 to 15 kV). The applied voltage for analysis was 20 kV. During the experiment, the temperature in the capillary box was maintained at 23 °C. The electrosomotic flow (EOF) was determined using DMSO as the neutral marker.

On electropherograms, for PEG solution of concentration 0–20%, peaks of rhodamine and DMSO (electroosmotic flow marker) were visible. For higher concentrations of PEG, only the rhodamine peak was observed. The electrophoretic mobility of the sample was calculated according to the formula:

\[
\mu_e = \frac{1}{L_T} \left( \frac{1}{\mu_p} - \frac{1}{\mu_{\text{EOF}}} \right) \frac{L_D L_T}{V}
\]

for PEG concentrations 0 to 20% and

\[
\mu_e = \frac{1}{L_T} \frac{L_D L}{V}
\]

for PEG concentrations 20%–42% (for concentrations higher than 20% of PEG the input of electrosomotic mobility was not higher than 5%), where \( L_T, L_D \) are the total length of the capillary and the length of the capillary from the inlet to the
detector, respectively, and \( V \) is the applied voltage during the separation.

**Fluorescence correlation spectroscopy (FCS) setup for diffusion measurements**

The FCS setup used in the experiments was a commercial monochromatic FCS Confocor II (Carl Zeiss, Jena, Germany). The experiments were conducted at 23 °C using a 543 nm He-Ne laser for illumination. The objective used was C-Apochromat 40×/1.2 (N.A. 1.2) with the pinhole diameter set to 78 μm (1 Airy unit for 543 nm laser). An avalanche photo diode was used for detection. The laser intensities were about 40 μW corresponding to the power density at the focal point \( \sim 30 \text{ kW cm}^{-2} \). The accessible time range for the measurements of the autocorrelation function was 1 μs–10 s.

In the FCS experiment, the fluorescence intensity emitted from a small volume element of solution, optically defined as the focal volume of the confocal microscope objective, is recorded as a function of time. The recorded fluorescence fluctuates as the fluorescently labeled proteins diffuse in and out of the focal volume or change their photo-physical properties. The focal volume is of the order of 1 fl (i.e. \( 10^{-15} \) liter) and the concentration of the fluorescent molecules is in the nanomolar range, making FCS an example of a single molecule detection technique. The fluorescence intensity fluctuation time series can be analyzed by means of the autocorrelation function which contains the information about the average number of fluorescent molecules in the focal volume and their average residence time in the focal volume. Distribution of the intensity (\( I \)) of the laser light in the focal volume is often approximated as a three dimensional Gaussian: 

\[
I(x,y,z) = I_0 \exp(-2(x^2 + y^2)/F^2 - z^2/P^2)
\]

where \( F \) is the cross-sectional length in the \( x-y \) plane, and \( P \) is the height of the illuminated element of volume. We recorded the intensity, \( S(t) \), of fluorescence emitted from this volume as a function of time. This intensity fluctuated as single fluorescently labeled molecules diffused in and out of the focal volume. The signal, \( S(t) \), allowed the distribution of residence times (\( \tau\text{res} \)) of the tracers in the focal volume by analyzing the autocorrelation function of \( S(t) \):

\[
g(\Delta t) = \frac{\text{S}^2}{\text{S}^2} S(t) S(t + \Delta t)
\]

The autocorrelation function adopts different analytical forms depending on the assumed physicochemical processes taking place in the studied solution. For three dimensional multicomponent diffusion, with the triplet state correction, the autocorrelation function has the following form:

\[
G(t) = \left(1 + \frac{\text{s}}{1 - \text{s}} e^{-t/\tau}\right) \left(\frac{1}{N}\right) \sum_{i=1}^{n} \frac{A_i}{\left(1 + \left(\frac{t}{\tau_i}\right)\right)^{1/2}}
\]

where \( s \) is the fraction of dye molecules in the triplet state, \( \tau \) is the triplet lifetime (usually in the range of nanoseconds up to microsecond), \( N \) is the average number of molecules in the focal volume (usually of the order of 1, thus we register a signal from a single molecule at a given time), \( \tau_0 \) is the residence time of the molecule in the focal volume, \( \omega = P/F \) is the structure parameter describing the ratio between longitudinal and transverse size of the focal volume, \( A_i \) is the fraction of the \( i \)th component, \( n \) is the number of diffusing species (e.g. a free dye and a protein-bound dye are the two objects (\( n = 2 \)) with different diffusion coefficients).

To exclude the possibility of a non-Gaussian shape of the confocal volume, free diffusion of rhodamine 6G in water was measured prior to each experiment and obtained data fitted well with a single component autocorrelation function for normal diffusion. The value of the fitted structure parameter \( \omega = P/F \) for rhodamine 6G amounted to about 5. Typical residence time for rhodamine was about 31 μs in our FCS setup. It gives the following dimensions of the focal volume:

\[
D = D_{\text{cal}} \frac{\tau_{\text{cal}}}{\tau_D}
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Macroposcosity in PEG solutions

The shear viscosity was measured using a standard TA Instruments AR2000N. The macroscopic values of the viscosity for PEG 6000, 12000, 20000 and 800000 (8M) solutions are shown in Fig. 2.

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Fig. 1 Semi log-plot of the normalized autocorrelation function for rhodamine 6G in PEG 20000. PEG weight concentrations increase from 0 to 50%. The average time that the fluorescent probe spends in the focal volume is given by the characteristic decay time of the autocorrelation function. For FCS, this autocorrelation function is algebraic in time (eqn (1)) and only one mode of diffusion was present.
Using four different polymers, we covered almost 5 orders of magnitude of solution viscosity and two orders of magnitude in concentration.

**Results and discussion**

PEG (or PEO) is a flexible polymer and its radius of gyration in water, as a function of molecular mass, is given \( R_g = 0.02 M_0^{0.58} \) [nm]. We find \( R_g = 3.1 \) nm (for PEG 6000), \( R_g = 4.6 \) nm (for PEG 12 000) and \( R_g = 6.2 \) nm (for PEG 20 000). The size of a “blob” inside which all monomers belong to the same polymer chain, is a function of polymer concentration, \( x, \xi = R_g(x/x^*0.75, \) where \( x^* \) is the polymer concentration at which polymer chains start to overlap. The overlap concentration is given by \( x^* = M_p/(4/3 \pi R_g^3 N_A), \) where \( M_p \) is the molar mass of polymer and \( N_A \) is Avogadro’s number.

The overlap concentration depends on the molecular mass of PEG: \( x^* = 0.08 \) g cm\(^{-3} \) (for PEG 6000), \( x^* = 0.05 \) g cm\(^{-3} \) (for PEG 12 000), \( x^* = 0.03 \) g cm\(^{-3} \) (for PEG 20 000). Thus for a 3% solution of PEG 20 000 in water, the polymer chains start to overlap. The “blob size”, \( \xi \), only weakly depends on the molecular mass (Fig. 3).

According to de Gennes’ theory, \(^{22,23} \) above a certain concentration \( x_{\text{limit}} = x^*(R/R_g)^{-4/3}, \) a small probe (e.g. rhodamine B, \( R = 1.7 \) nm), of size comparable to the “blob” size, \( \xi \), should experience, during its motion, the macroscopic viscosity of the solution. For PEG 6000, 12 000, 20 000 we find that \( x_{\text{limit}} = 0.18 \) g cm\(^{-3} \) (15% w/w) for rhodamine B. Above this concentration of PEG, rhodamine B should diffuse or flow in all PEG–water solutions and experience the macroscopic viscosity of the solution. Fig. 4 contradicts this theory. Rhodamine B diffuses in PEG 6000, 12 000 and 20 000 experiencing a viscosity which is much smaller than the macroscopic viscosity (at high concentrations of PEG 20 000, the nanoviscosity “felt” by rhodamine is almost 100× smaller than the macro-viscosity of the solution). Moreover, this nanoviscosity weakly depends on the molecular mass of PEG. The viscosity was determined from the diffusion coefficient, \( D, \) measured in FCS and from the electrophoretic mobility, \( \mu, \) measured in CE. The diffusion coefficient, \( D, \) was normalized by its value for pure water, \( D_0, \) similarly to the electrophoretic mobility. The analysis of CE and FCS

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**Fig. 2** Macro-viscosity is shown for different molecular weights (PEG 6000, 12 000, 20 000, 8 000 000) versus polymer weight fraction.

**Fig. 3** Calculated (see text) blob size (correlation length) \( \xi \) as a function of the polymer weight fraction for PEG 6000, 12 000, 20 000 and 8M. The blob size does not exceed the radius of gyration. We consider concentrations larger than the overlap concentration.

**Fig. 4** Nano-viscosity, normalized by the water viscosity, as a function of the polymer weight fraction, determined from the flow of rhodamine B under the influence of the electric field measured in capillary electrophoresis (CE) (open circles) and from its diffusion measured in fluorescence correlation spectroscopy (FCS) (filled circles) compared to the macroviscosity (see also Fig. 2) (shown as filled squares) (a) for solution of PEG 6000 (b) 12 000 and (c) 20 000. Please note that rhodamine B, according to current theories, should follow the macroviscosity line for concentrations above 15% w/w.
experiments for rhodamine leads to the following equation (Fig. 4):

\[
\frac{D_0}{D} = \frac{\mu_0}{\mu} = \frac{\eta}{\eta_0}
\]

and therefore we identified \( \eta \) as the nanoviscosity (normalized by the water viscosity, \( \eta_0 \)). Eqn (3) and Fig. 4 give direct experimental proof of the validity of the Stokes–Einstein relation at the nanoscale. Thus, the coefficient of nanoviscosity, \( \eta \), at the nanoscale can be determined using the Stokes–Einstein formula, \( \eta = kT/3\pi DR \), where \( k \) is the Boltzmann constant, \( T \) is the temperature, and \( R \) is the diameter of the probe. In fact, for each system we have to check that the SE relation is valid before we can use it to determine the viscosity.

One can also look at the SE relation in a slightly different way. For a probe moving in a complex liquid there are two important time scales: a typical time, \( \tau_p \), needed for the probe to change the local structure of the fluid and the time, \( \tau_R \), required for the relaxation of this perturbation. If \( \tau_R \ll \tau_p \), the structured fluid relaxes rapidly during the motion of the object and the SE relation is satisfied. We stress that, in order to have reasonable and comparable results from the flow and diffusion (each provides a drag coefficient proportional to viscosity), the Stokes–Einstein relation must be valid. The same is not true for solutions of stiff polymers, semi-dilute networks of F-actin (with a persistence length 1000× the diameter of the actin fiber), solutions of fd virus \(^{52,53} \) (of size 800 nm and persistence length 2200 nm) or in solutions of supramolecular polymers that are hydrogen bonded (of persistence length well exceeding 100 nm \(^{54,55} \)). In these cases, several characteristics are observed, including: different diffusion coefficients at short and long time scales, anomalous diffusion, and the lack of clear crossover between the nano- and macro-scale. The diffusing tracers do not probe the average structure of the fluid—instead, they probe the local non-equilibrium configurations of the complex liquid. The long-time and short-time diffusion coefficients differ significantly \(^{52,55} \) from each other and the Stokes–Einstein equation is not valid. In such systems, visco-elasticity changes with the scale of motion i.e. at the nanoscale we observe a viscous flow, while at the macroscale we have a fully elastic system (e.g. like in a gel \(^{56} \)). In our system, satisfying the Stokes–Einstein formula, we can use it to determine the nanoviscosity at all length scales. Therefore, in our case, a sphere of diameter \( R \) experiences hydrodynamic drag in the PEG solution of \( 3\pi \eta(R)R \), with a size-dependent viscosity, \( \eta(R) \). In Fig. 5, another test of the Stokes–Einstein relation for a lysozyme protein moving in a concentrated PEG 20000 solution is given.

For the determination of the crossover length scale we used the following probes: rhodamine B (\( R = 1.7 \) nm), lysozyme (and lysozyme charge ladder for electrophoretic mobility) \(^{48} \) (\( R = 3.1 \) nm), apoferritin (\( R = 13.8 \) nm) quantum dots (\( R = 25 \) nm) and silica spheres (\( R = 114 \) nm). The results are summarized in Fig. 6. The rhodamine B and lysozyme experience a viscosity very different from the macro-viscosity, while apoferritin, quantum dots and silica spheres move according to the macro-viscosity in PEG 20000 solution. All data for these nanoprobe, and also for macro-viscosity of PEG 6000, 12000, 20000 and, additionally, for PEG 8000 000, were collected and put on a single plot shown in Fig. 7. From this plot we found the viscosity in the form:

\[
\frac{\eta}{\eta_0} = \exp\left( b \left( \frac{R}{\xi} \right)^a \right)
\]

for the probes of size smaller than the radius of gyration i.e. \( R < R_g \). For \( R > R_g \), the viscosity given by eqn (4) attains its macroscopic value given by:

\[
\frac{\eta_{macro}}{\eta_0} = \exp\left( b \left( \frac{R_g}{\xi} \right)^a \right).
\]

All our results (Fig. 7) follow the master curve given by eqn (4) and (5) with \( a = 0.70 \pm 0.05 \) and \( b = 1.45 \pm 0.15 \). Fig. 3 and eqn (4) and (5) shown in Fig. 7 combine all our results from macrorheology and nanorheology and demonstrate (together with Fig. 6) that the crossover length scale, \( L_c \), is indeed given by the radius of gyration, \( R_g \). These results are consistent with our previous studies where we unambiguously demonstrated \(^{37} \) a sharp crossover from nano- to macro-viscosity at length.
Fig. 7 The normalized plot for macroviscosity of PEG 6000, 12 000, 20 000, 8 000 000 solutions and nanoviscosity of rhodamine B and lysozyme in PEG 20 000 solution. On this plot we present all data using the scaling form given by eqn (4) and (5). We plot ln(n/η0) (viscosity divided by the viscosity of water—see eqn (3)) versus R/ξ, where ξ is the “blob” size in the polymer solution and R is the size of the probe (for macroviscosity we use eqn (5) and instead of R we use the radius of gyration). All data points collapse on a single master curve showing that the crossover length scale is given by the radius of gyration. On this plot, we present data for rhodamine of size 1.7 nm and PEG 8 000 000 of radius of gyration 201 nm—showing an intrinsic connection between nano and macroviscosity. Please note that for the polymer itself R/ξ = (x/x*).75 is related to concentration, x, divided by the overlap concentration, x*.

scale L ≈ 17 nm in aqueous surfactant solutions (hexaethylene glycol monododecyl ether , C12E6). This length-scale did not depend on surfactant concentration. In this experiment,57 the concentration of surfactant varied over two orders of magnitude (from 0.1 to 35% by weight in water, w/w) and the diameters, R, of the probes varied over three orders of magnitude (ranging from 0.28 nm for heavy water to 190 nm for fluorescent polystyrene spheres). We have recently performed SANS scattering studies and identified 17 nm in our previous study57 with the length of semi-flexible (practically rigid) micelles of C12E6. Our results for the macroviscosity (eqn (5) and Fig. 3) are also consistent with the macroviscosity measurements performed for linear polymers over a wide range of concentrations by Takahashi et al.58 In previous works,1–12 summarized by Odijk,13 the following form of the macroviscosity was proposed:

\[
\frac{\eta}{\eta_0} = \exp(KR^a x^\nu) .
\]  

(6)

In many experimental works,2–12 values of two exponents µ and ν were determined. For different systems, they varied from 0.69 to 1.0 and 0.5 to 1.1, respectively. If we transform our eqn (4) to the form of eqn (6), we obtain the following relation between the parameters of K, µ and ν and our parameters:

\[
K = b \left( \frac{M_p}{4/3\pi N_A} \right)^\frac{3/4}{a} R_g^{\frac{5/4}{a}} , \mu = a, \text{and} \nu = 0.75 a .
\]

Conclusions

Nanorheology is an emerging field of physical chemistry. Our present studies using CE and FCS for bulk aqueous polymer solutions are complementary to those performed for confined fluids,59 where the confining surfaces at the nanoscale very strongly affect rheological behavior. It is also complementary to the bulk rheology of polymers.58 We found a connection between the motion of nanoscopic probes (nanoviscosity) in polymer solutions and their macroviscosity (Fig. 7 and eqn (4) and (5)). Our results for aqueous PEG solutions demonstrated that the crossover length scale, L, in polymer solutions is not related to the “blob” size, ξ, contrary to the theoretical assumption.8,22,23 We found experimentally that the crossover length scale, L, is given by R_g, the radius of gyration (in accordance with recent computer simulations of Liu et al.28).

Our results explain the lack of a crossover in the experiments of Langevin and Rondelez8 (their probes BSA and Ludox spheres were smaller than the radius of gyration of PEG in the experiment). Finally, we observed that de Gennes’ scaling form, η(R/ξ), is obeyed for all probes and even for macroviscosity, if we identify R with the radius of gyration, R_g. In this approach we identified R as the diameter of a probe (and not its radius). In fact, from computer simulations, we have learned that the crossover is not28 sharp (see Fig. 1 of ref. 28) but occurs for R between R_g and 2R_g. We may expect some correction to our results which comes from the specific structure of a polymer solution near the surface of a nano-object.60 At the crossover length scale, i.e. R comparable to R_g, the distribution of polymer chains near the surface is very different from the bulk i.e. a depletion layer forms around the object in the non-adsorbing case. Therefore, the local viscosity is smaller near the surface than in the bulk.24,25,60

Thus, the local motion of an object in the depletion layer is faster (larger diffusion coefficient) than the long time motion. In our case, at the crossover, the depletion layer is, at most, of nanometer size, whereas the size of the focal volume in FCS confocal microscopy is around 1000 nm i.e. three orders of magnitude larger. Therefore FCS determines the long time and large scale motion and the depletion layer does not influence our results. In FCS, we are not able to observe the local motion of a protein at the nanometer scale.

The results of the present paper, together with the results of our previous study,57 give a clear picture of the crossover length scale in complex fluids. This length scale is related to the size of the object which forms a complex liquid (apart from the solvent). A consequence of this observation is the hypothesis that a polymer chain in a polymer solution should move experiencing the macroviscosity of the solution and a micelle from a micellar solution should also experience the macroviscosity of the solution during its motion. Using FCS we can trace the motion of a single object (which forms the complex liquid) in this complex liquid and determine the diffusion coefficient D_macro. Additionally, we can easily measure the macroviscosity of such a solution, η_macro. Using the Stokes–Einstein formula, D_macro = kT/3πη_macroR (or its analog for elongated molecules) allows the determination of the hydrodynamic diameter of the object, R.

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Notes and references