Orientational relaxation times of Rhodamine 700 in glycerol-water mixtures
Megens, M.; Sprik, R.; Wegdam, G.H.; Lagendijk, A.

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I. INTRODUCTION

The orientational motion of large molecules in a liquid is usually described in terms of a diffusive process. In this picture, the molecules experience a random force due to collisions with the small molecules of the liquid. The correlations in the motion of the solvent molecules are represented by a friction, which is calculated as if the liquid were a structureless continuum. This yields the well-known Perrin-Stokes-Einstein result for reorientation of spherical probe molecules

\[ r(t) = \frac{1}{5} (3 \cos^2 \theta(t) - 1) = r_0 \exp(-t/t_{ao}), \]

where

\[ \tau_{ao} = \tau_0 + \tau_{PSE}, \]

and

\[ \tau_{PSE} = \frac{\eta V}{kT}. \]

Here \( \theta(t) \) is the angle between the absorption transition dipole at the time of excitation and the emission transition dipole at time \( t \), the brackets denote the average over the ensemble of randomly oriented dye molecules, and \( \tau_{ao} \) is the orientational relaxation time. The orientational relaxation time consists of two parts: an empirical part \( \tau_0 \) that does not depend on viscosity, and the Perrin-Stokes-Einstein part \( \tau_{PSE} \) that depends linearly on the shear viscosity \( \eta \) of the liquid, the temperature \( T \) and the volume \( V \) of the rotating molecule. If the molecule is nonspherical there are up to five different relaxation times, but these are usually too close to be distinguished experimentally, unless the molecule is very elongated.

Experimentally measured orientational relaxation times generally vary linearly with the viscosity of the liquid except at high viscosities. Recent molecular reorientation studies are therefore mostly concerned with the determination of the ratio of the orientational relaxation time \( \tau_{PSE} \) and \( \eta/kT \). This ratio accounts for the shape of the solute molecules and the interaction between a solute molecule and the liquid at their interface. Two limiting conditions for the interactions are “stick,” when there is no velocity difference at the interface between liquid and molecule, and “slip,” where the liquid can move freely but cannot enter the volume occupied by the molecule. Under “slip” conditions, spherical molecules are not hindered by the liquid because they can rotate without having to displace liquid. Usually, the measured orientational relaxation time is somewhere in between stick and slip. However, for some solvents and liquids relaxation times outside the stick or slip bounds are measured. The charge distribution of a solute molecule is suspected to contribute significantly to the friction it experiences in polar solvents. So far there is no single theory which accounts for the wealth of observed phenomena.

Another aspect of previous experimental findings that is not fully understood till now is the dependence of the orientational relaxation time \( \tau_{ao} \) of a dye on the viscosity of the solvent. At low viscosity the orientational relaxation time \( \tau_{ao} \) is linear with the viscosity \( \eta \), but it appears to level off at high viscosities (\( \eta > 20 \) cP) in mixtures of water and viscous liquids like glycerol or ethylene glycol. Mikosch et al. and Rice and Kenney-Wallace proposed that the solvent may form transient cavities due to strong hydrogen bonding interactions. The motion of the probe molecules would not be viscously damped but the probe molecules would rotate through large angular steps determined by interactions with the solvent molecules in the nearest neighbor shell. Hence the hydrodynamic properties of the solvent would be of little importance. Measurements on other dyes in viscous liquids would provide a test of this model.

In this article we present fluorescence depolarization measurements of the orientational relaxation time of rhodamine 700 dye molecules in a series of glycerol-water mixtures. By changing the glycerol content of the glycerol-water mixture we can vary the viscosity over a wide range. This enables us to both verify the value of \( \tau_{PSE}kT/\eta \) at low viscosity and to determine deviations from linear relation between \( \tau_{ao} \) and \( \eta \) at high viscosity. In Sec. II, the theory is presented in some more detail, and in Sec. III we outline the
experimental method and estimate the size of the dye molecules. Results are discussed in Sec. IV.

II. THEORY

In time-resolved fluorescence depolarization measurements one makes use of the fact that the orientation of an excited molecule affects the polarization of light it will emit.13 In this method, the molecules are excited with a short light pulse of a definite polarization. Molecules with their absorption transition dipole moments along the polarization direction will be predominantly excited by this pulse. After the pulse, the orientational distribution of excited molecules will change in time if the molecules rotate. When the internal excitation of a molecule decays, the molecule emits light polarized along its emission transition dipole moment. Thus, from changes in the polarization of the fluorescent light as a function of time after excitation, one gains information about the rotation of the fluorescent molecules.3–5

The time-resolved fluorescence intensities polarized parallel \((I_\parallel(t))\) and perpendicular \((I_\perp(t))\) to the polarization of the incident beam can be combined to give the decay of fluorescence \(K = I_\parallel + 2I_\perp\), that is independent of the reorientation of the molecules.4,5 The polarization anisotropy \(r(t)\) of Eq. (1) can then be obtained by eliminating the influence of the fluorescence decay:

\[
r(t) = \frac{I_\parallel - I_\perp}{K} = \frac{I_\parallel - I_\perp}{I_\parallel + 2I_\perp}.
\]

The polarization anisotropy changes in time as a result of the rotation of the dye molecules in the liquid. The pertinent model to describe this rotation assumes that the orientations of the transition dipole moments are just diffusing over the unit sphere. If the diffusion is different for different directions, then the polarization anisotropy will be a sum of up to five exponentials.5,14 The number of independent decay constants will be reduced if symmetry is present, e.g., if the diffusion constant \(D\) is isotropic, as for a spherical molecule, then the polarization anisotropy decays as \(\exp(-6Dt)\), so there is only one decay time. For an ellipsoid, there are two diffusion constants, \(D_\parallel\) for rotation around the symmetry axis (i.e., spinning) and \(D_\perp\) for rotation perpendicular to the symmetry axis (tumbling). The two diffusion constants give rise to three decay rates in the polarization anisotropy:

\[
\frac{1}{\tau_i} = \left\{ \begin{array}{ll}
6D_\perp & \text{for } \tau = 2/3 \\
5D_\perp + D_\parallel & \text{for } \tau = 2/5 \\
2D_\perp + 4D_\parallel & \text{for } \tau = 2/7 
\end{array} \right.
\]

The Langevin equation provides a model for the diffusion constants. It describes Brownian motion in a viscous environment. Using Stokes’ law for the frictional torque \(T\) on a sphere of radius \(R\) in a viscous liquid, \(T = 8\pi\eta R^3\), Perrin1 derived that the diffusion constant \(D = 6kT/\eta V\), where \(\eta\) is the shear viscosity of the liquid. Substituting this diffusion constant in the expression for the polarization anisotropy yields the Perrin-Stokes-Einstein result Eq. (3).

To correctly estimate the orientational relaxation time, the shape of the rotating molecule must be taken into account. Perrin already calculated the diffusion constants \(D_\parallel\) and \(D_\perp\) for the case of an ellipsoid using the Langevin approach with an analytic solution to the Navier-Stokes equations for the flow of the liquid and the resulting friction19 in the limit of slow and steady motion. The results of such calculations depend crucially on the type of boundary conditions for the molecule-liquid interface. Perrin assumed stick boundary conditions, i.e., no difference in velocity at the interface between molecule and liquid. For a prolate ellipsoid with axial ratio 2.5, typically expected for a rhodamine 700 dye molecule, this yields diffusion constants1,4,19

\[
D_\parallel = 1.30D_0 \quad \text{and} \quad D_\perp = 0.53D_0,
\]

where \(D_0\) is the diffusion constant for a sphere of equal volume.

As an alternative, Hu and Zwanzig did calculations for slip boundary conditions, i.e., no tangential stress at the interface.20 For a sphere, this implies that there will be no friction at all. If, however, the object is asymmetric, it will have to displace some liquid and there will be viscous dissipation of energy. For an ellipsoid, the friction coefficient \(\xi\) for rotation around the symmetry axis will be zero. Numerical calculations of Hu and Zwanzig20 for a prolate ellipsoid of axial ratio 2.5 yield a friction coefficient for rotation perpendicular to the symmetry axis \(\xi_\perp\) of 0.36 times the corresponding friction coefficient for stick boundary conditions. Knowing that the diffusion constants are inversely proportional to the friction coefficients, \(D_\parallel = kT/\xi_\parallel\), we can calculate from their work the orientational relaxation times \(\tau_{rSE}\) according to Eq. (5). It appears that the free rotation around the symmetry axis causes two out of three relaxation times to be zero.

There are several physical processes that can cause depolarization, apart from reorientation of the molecules. If there is an angle \(\zeta\) between the absorption and emission transition dipole moment, the initial polarization anisotropy is

\[
r_0 = \frac{2}{5} (3\cos^2 \zeta - 1).
\]

If the transition dipole moments are parallel, then \(r_0 = 2/5\) has its maximum value. The depolarization can be total \((r = 0)\) if \(\zeta = 54.37°\) (the “magic angle”). Internal relaxation in the dye molecules can also cause depolarization. Usually this relaxation is very fast (within a few picoseconds18) compared to the orientational relaxation times \((>0.1\ \text{ns})\). Experimentally the relaxation comes into view as a smaller \(r_0\) while the measured orientational relaxation time is not affected.

III. EXPERIMENT

Orientational relaxation times were measured using a time correlated single photon counting technique.21 A cavity dumped Ktont Red dye laser, synchronously pumped by a frequency-doubled pulse-compressed mode-locked Nd^3+:YAG laser (Spectra Physics), excites the dye in the sample. The wavelength of the dye laser is tunable in the 600–660 nm range. Two 10 mm polarizing prisms are used to select a polarization state of the exciting and detected light. The exciting beam has a diameter of about 1 mm at the
position of the sample. The sample is contained in a 1 cm cuvette, at room temperature. The detector is set at right angles with the exciting light. A lens collects the fluorescence on a Hamamatsu R3809U Multi Channel Plate detector (MCP). A Schott RG695 color filter in front of the MCP removes the residual laser light. Two apertures are used to limit the angle of incidence on the polarizing prism in front of the detector. The signal from the MCP is amplified and fed to a constant fraction discriminator (CFD, Tennelec TC454), time to amplitude converter (TAC, Tennelec TC864) and multi-channel analyzer (MCA). A photodiode monitors the incident beam for triggering the TAC.

At a cavity dump rate of 400 kHz, the count rate on the PMT was maintained at about 10 kcounts/s. With this setup we achieve a time resolution of around 60 ps (FWHM of scattered incident light) without further optimization.

For the fluorescent molecule we used rhodamine 700 (LD700 perchlorate) from Radiant Dyes, Inc. The structural formula and typical dimensions are shown in Fig. 1. The molecular weight is 538 g/mol, much more than that of glycerol (92 g/mol) or water. This dye was selected because we can resonantly excite it with our dye laser. (The absorption maximum of LD700 occurs at around 650 nm.23) Thus the dye concentration can be kept low (below 20 \mu mol/l) and reabsorption of the fluorescence in the sample is avoided.

The volume and shape of the dye molecule are important parameters for making an estimate of the orientational relaxation time of the dye in solution. Since the shape of the molecule does not closely resemble an oblate or prolate spheroid, in the description in terms of just a volume and an axial ratio there is inevitably some arbitrariness in choice of parameters.

Because we are unaware of any data on the volume of LD700, we estimate the volume of the van der Waals volumes of benzene (80.3 \AA³) and naphthalene (122.8\AA³).24 From these values, the volume of pentacene is calculated to be 1 \cdot \text{(benzene)} + 4 \cdot \text{(naphthalene\textendash benzene)} = 250 \AA³. If we take the volume of the dye molecule to correspond to 6.5\pm0.5 instead of 5 rings, then the volume is \( V = 310 \pm 20 \text{ \AA}^3 \). The two extra rings do not contribute a full 80 \text{ \AA}^3 because a considerable part of the extra rings is shared with the pentacene structure. The volume per extra ring would then be something like \( 3/4 \) of the volume difference of naphthalene and benzene. Since this does not take into account the volume of the CF₃ group (about 23 Å³), the estimated volume is certainly not too large.

From the length of an aromatic carbon-carbon bond, 1.395 Å,25 and the van der Waals radius of carbon, 1.77 Å,24 we estimate the length of the long axis of the molecule to be \( a = 10 \cos(30°) \cdot 1.395 \text{ Å} + 2 \cdot 1.77 \text{ Å} = 15.6 \text{ Å} \). The length of the short axes of a prolate ellipsoid with a volume of 310 Å³ is then \( b = 6.2 \text{ Å} \). This corresponds to an axial ratio \( \rho \) of about 2.5.

To determine the orientational relaxation time as a function of viscosity (Fig. 3), we measured the intensities \( I_{\parallel} \) and \( I_{\perp} \) in several glycerol-water mixtures. Since the two curves \( I_{\parallel} \) and \( I_{\perp} \) are measured separately, slight variations in the intensity of the laser pulses affect the relative magnitudes. To correct for this, we multiply \( I_{\perp} \) by a scaling factor, which should ensure that the two curves coincide at long times (\( t \gg \tau_{\alpha} \)). We determined the scaling factor using three different methods. To begin with, we matched the tails of the curves \( I_{\parallel} \) and \( I_{\perp} \). This yields a lower bound for the orientational relaxation time because we cannot be completely sure if the rotation of the dye molecules has created an isotropic distribution at the time \( t \) where we match the tails (i.e., if indeed \( t \gg \tau_{\alpha} \) as required). Therefore, we also tried fitting the curves with longer orientational relaxation times than that determined by the “tail-matching” method. In this case, the true \( I_{\parallel} \) and \( I_{\perp} \) remain separated at the time where we previously did the “tail matching,” and the fit also yields a larger initial polarization anisotropy \( r_0 \), i.e., a larger separation of the curves at \( t = 0 \). However, the assumption of an initially random distribution of orientations of the molecules imposes the constraint \( r_0 < 2/5 \). Thus fitting the curves with fixed \( r_0 = 2/5 \) provides a means to determine an upper bound for the orientational relaxation time. Finally, we also determined the orientational relaxation time by a non-linear least squares fit to both curves \( I_{\parallel} \) and \( I_{\perp} \) at once, with weighting according to the Poisson statistics of a photon counting experiment. The fitting functions for \( I_{\parallel} \) and \( I_{\perp} \) were based on a single exponential fluorescence decay \( K(t) \) and a single exponential decay of the polarization anisotropy \( r(t) \). With both \( r_0 \) and the scaling factor as free parameters the fits yield a reduced \( \chi^2 \) of about 1.2, which indicates a good fit.26 The final values for \( \tau_{\alpha} \) in Fig. 3 are the values determined from the least squares fit. The vertical error bars indicate the relaxation times obtained by the two other methods; the upper value results from requiring \( r_0 = 2/5 \), the lower value from “tail-matching.”

The glycerol-water mixtures were prepared by diluting a known weight of glycerol with known weights of water and dye solution. Values of the viscosities of these mixtures were taken from the CRC handbook.25 A comparison of the specific weight of our glycerol with known values for glycerol-water mixtures indicated that it contained less than 1 mass percent water. The variation in the viscosity, assuming an uncertainty in the mass percentage glycerol of 1%, is indicated by the horizontal error bars in Fig. 3.
dye in solution. Which is of the expected order of magnitude for a fluorescent solution. The life time is around 1.9 ns, fluorescence decay shown. The corresponding polarization anisotropy is adjusted to make the tails overlap. From \( I_0 \) and \( I_L \) we obtain the fluorescence decay \( K(t) \) (B, upper curve) and polarization anisotropy \( r(t) \) (B, lower curve). The straight lines are fits to single exponentials.

IV. RESULTS AND DISCUSSION

In Fig. 2A a typical pair of curves \( I_0(t) \) and \( I_0(t) \) is shown. The corresponding polarization anisotropy \( r(t) \) and fluorescence decay \( K(t) \) are shown in Fig. 2B. The fluorescence decay \( K(t) \) decays single-exponentially, as expected for a dilute dye solution. The life time is around 1.9 ns, which is of the expected order of magnitude for a fluorescent dye in solution. Since this value is equal to \( \frac{1}{2} \) within experimental error, we conclude that the absorption and emission transition dipole moments are approximately parallel. Using Eq. (6) we estimate an upper bound for the angle \( \zeta \) between the transition dipole moments of 15°.

In Fig. 3 the measured dependence of the orientational relaxation time \( \tau_{\text{or}} \) of the dye on the viscosity of the glycerol-water mixture is plotted. The orientational relaxation time is approximately linear with viscosity \( \eta \) over the whole range of viscosities (1–60 cP). The predictions of the Perrin-Stokes-Einstein model for a prolate spheroid with volume \( V = 310 \ \text{Å}^3 \) and axial ratio \( \rho = 2.5 \) are indicated for both slip (dotted line) and stick (dashed line) boundary conditions. For stick boundary conditions, both the fastest and slowest relaxation times of the ellipsoid are given [cf. Eq. (5)]. For slip boundary conditions the slowest relaxation time is shown. The faster relaxation times are zero because ellipsoids are free to rotate about their symmetry axis. Apparently stick boundary conditions are more appropriate.

\[ \tau_{\text{measured}} / \tau_{\text{sphere}} = 1.27 \pm 0.10. \]
TABLE I. Limiting orientational relaxation time $\tau_0$ (see Fig. 4) for several dyes in glycerol-water (PP2, PTS, LD700) or glycerol-ethylene glycol (R6G) mixtures, together with the effective volumes $V_h$ of the dyes.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Charge</th>
<th>Shape</th>
<th>$\tau_0$ (ns)</th>
<th>$V_h$ (Å³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenyl 2 (PP2)</td>
<td>Anion</td>
<td>Rod</td>
<td>35 ± 5</td>
<td>1650 ± 50</td>
</tr>
<tr>
<td>Pyrene tetrasulfonate</td>
<td>Anion</td>
<td>Plate</td>
<td>45 ± 5</td>
<td>460 ± 10</td>
</tr>
<tr>
<td>Rhodamine 6G (R6G)</td>
<td>Cation</td>
<td>Plate</td>
<td>2.5 ± 1</td>
<td>1200 ± 300</td>
</tr>
<tr>
<td>Rhodamine 700 (LD700)</td>
<td>Cation</td>
<td>Rod</td>
<td>&gt; 6</td>
<td>390 ± 10</td>
</tr>
</tbody>
</table>

(a) Reference 12.
(b) Reference 13.

This should be compared to the values calculated for a prolate ellipsoid of axial ratio $\rho = 2.5$ as discussed in Sec. II. For slip boundary conditions, the fastest and slowest relaxation times are 0.96 and 1.89 times the relaxation time for a sphere of equal volume (dashed lines in Fig. 3), while for stick the slowest relaxation time is 0.68 $\tau_{\text{sphere}}$ (dotted line). Apparently, stick boundary conditions are more appropriate for the rather irregularly shaped LD700 molecules. The fact that the estimated volume per dye molecule is perhaps too small only reinforces this result.

The experimental orientational relaxation time extrapolated to zero viscosity (intercept in Fig. 3) is

$$\tau_0 = 0.18 \pm 0.02 \text{ ns.}$$

(10)

From the molecular weight of the dye one would expect the characteristic time scale for free rotation of a sphere of equal volume to be only

$$\tau = \sqrt{\frac{J}{kT}} = 4 \text{ ps,}$$

(11)

where $J = (2/5)mr^2$ is the moment of inertia, $m$ the mass and $R$ the radius of the sphere. The time scale $\tau$ is nearly 2 orders of magnitude smaller than the value $\tau_0$ from the fit. Apparently the moment of inertia of the dye molecule is not the origin of the experimentally observed large $\tau_0$. Such large experimental values of $\tau_0$ are not uncommon, but what the source of this large $\tau_0$ remains an open question.

An important aspect of measurements of orientational relaxation times $\tau_0$ is their viscosity dependence. At low viscosities ($\eta < 20$ cp), the orientational relaxation time is linear with viscosity, but Mikosch, Dorfmüller, and Eimer and Rice and Kenney-Wallace observed that the relaxation time levels off at high viscosities for their three dyes. To explain their observations, Mikosch and Rice suggested that at high viscosities the solvent molecules would form transient cavities through hydrogen bonding. The time scale for motion of the liquid “cage” would be comparable to or even larger than the Perrin-Stokes-Einstein orientational relaxation time, so a dye molecule could rotate in its cage relatively unperturbed by the liquid. As a result, the orientational relaxation time $\tau_0$ at high viscosity would be lower than expected. The results of Mikosch et al. suggest that the shape of $\tau_0$ as a function of $\eta T$ is independent of the kind of dye, i.e., the relaxation times for different dyes can be scaled on a common curve by plotting them as a function of $\eta V_h/kT$, where $V_h$ is the “effective volume” of a dye molecule. The volume $V_h$ is determined from the relaxation times at low viscosity (see Table I). The rescaled data for all the dyes are shown in Fig. 4. The results of Rice et al. do not scale onto the common curve, but in their case the solvent is different as well as the dye. The dye of Rice et al. is a cation, while the two dyes used by Mikosch et al. are anions. We used a cationic dye, but nevertheless our results do not scale onto the curve of Rice and Kenney-Wallace. Our results are not in conflict with Mikosch’s curve. This suggests that the orientational relaxation time $\tau_0$ at the crossover from the regime where $\tau_0$ is linear with viscosity (solid line in Fig. 4) to the regime where $\tau_0$ is less sensitive to $\eta T$ (dashed lines) is a characteristic of the solvent, not of the dye or the interaction. The cause of the crossover is therefore to be sought in a change of the spectral distribution $S(k, \omega)$ of the density and concentration fluctuations of the solvent, with $k$ vectors around the main peak in $S(k, \omega)$. This is an interesting observation, which might be subject of further theoretical investigation.

V. CONCLUSIONS

Time-resolved fluorescence depolarization measurements on rhodamine 700 dye in glycerol-water mixtures show that the orientational relaxation time $\tau_0$ of the solute molecules varies linearly with viscosity $\eta$ of the mixture in the range 1–60 cP, in accordance with the Perrin-Stokes-Einstein model with slip boundary conditions. Previously Mikosch, Dorfmüller, and Eimer and Rice and Kenney-Wallace observed that the orientational relaxation time becomes less sensitive to the viscosity at very high viscosities for two anionic dyes in glycerol-water mixtures.
and a cationic dye in glycerol-ethylene glycol mixtures. In these dyes the orientational relaxation time starts to become independent of the viscosity of the solvent at high viscosities, which was explained by Mikosch and Rice in terms of the formation of a “cage” of solvent molecules in which a dye molecule could rotate relatively unperturbed by the liquid. The influence of dye and solvent on the relation between orientational relaxation time and viscosity suggests that the relaxation times as a function of viscosity of the anionic dyes can be scaled on a common curve. The relaxation times of the cationic dye in the glycerol-ethylene glycol mixture do not scale onto the curve of the anionic dyes. Our experimental findings for the cationic rhodamine 700 dye do not scale onto the curve of the cationic dye that is dissolved in a different mixture, but they are not in conflict with the common curve of the two anionic dyes that are also dissolved in glycerol-water mixtures. This suggests that the scaling constant is a characteristic of the solvent alone, not of the dye or the interaction, so that the leveling off of the relaxation time at high viscosities must be a property of the solvent too.

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