

Fluorescence Probes of Viscosity: A Comparative Study of the Fluorescence Anisotropy Decay of Perylene and 3,9-Dibromoperylene in Glycerol

Aleksander Balter^{1,2} and Jacek Szubiakowski¹

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The authors compare the results of fluorescence anisotropy decay measurements for glycerol solutions of perylene with those of 3,9-dibromoperylene (DBP). For both molecules a good linear dependence is observed between the glycerol viscosity (varied by temperature) and the longer rotational correlation time obtained as a result of a global (using data obtained at 256- and 430-nm excitation wavelengths) biexponential analysis of the fluorescence anisotropy decay, at least in the range of 7–60 P for perylene and 4–60 P for DBP. This significantly extends the reported range of 0.5 to 150 cP investigated by Williams and Ben-Amotz [1] with the probe BTBP.

KEY WORDS: Fluorescence anisotropy decay; rotational diffusion; viscosity; perylene; 3,9-dibromoperylene.

INTRODUCTION

Viscosity measurements are usually done with conventional viscometers, determining the mechanical drag on a macroscopic object in the fluid. Since the rotational diffusion of molecular fluorescence probes is viscosity dependent and influences the degree of polarization of emitted light, one may also use this circumstance to monitor the fluid viscosity. Of course, one should be aware that the probe senses the viscosity of the microscopic environment (microviscosity). This may constitute an advantage, particularly in the case of heterogeneous systems, such as micelles or biological membranes. In addition, optical viscosity measurements may be performed in small samples, remotely, and under on-line conditions.

Before a probe can be used routinely as an analytical tool, its properties in well-defined molecular surroundings should be characterized. These properties

include the anisotropy of rotational motion in an isotropic solution. Of particular interest are such molecules which exhibit a high degree of structural symmetry and have electronic transitions with different transition moment directions accessible to excitation. One such probe, which is well-known, is perylene. In this work we would like to compare the results of our own fluorescence anisotropy (FA) decay measurements for glycerol solutions of perylene with those for 3,9-dibromoperylene (DBP).

THEORY

The decay of the FA of ellipsoidal molecules undergoing Brownian rotational motions and excited with an infinitely short pulse of linearly polarized light is the sum of five exponential terms [2]:

$$r(t) = \sum_{i=1}^5 A_i \exp(-t/\tau_i) \quad (1)$$

where amplitudes A_i and correlation times τ_i are func-

¹ Institute of Physics, N. Copernicus University, Grudziadzka 5, 87-100 Torun, Poland.

² To whom correspondence should be addressed.

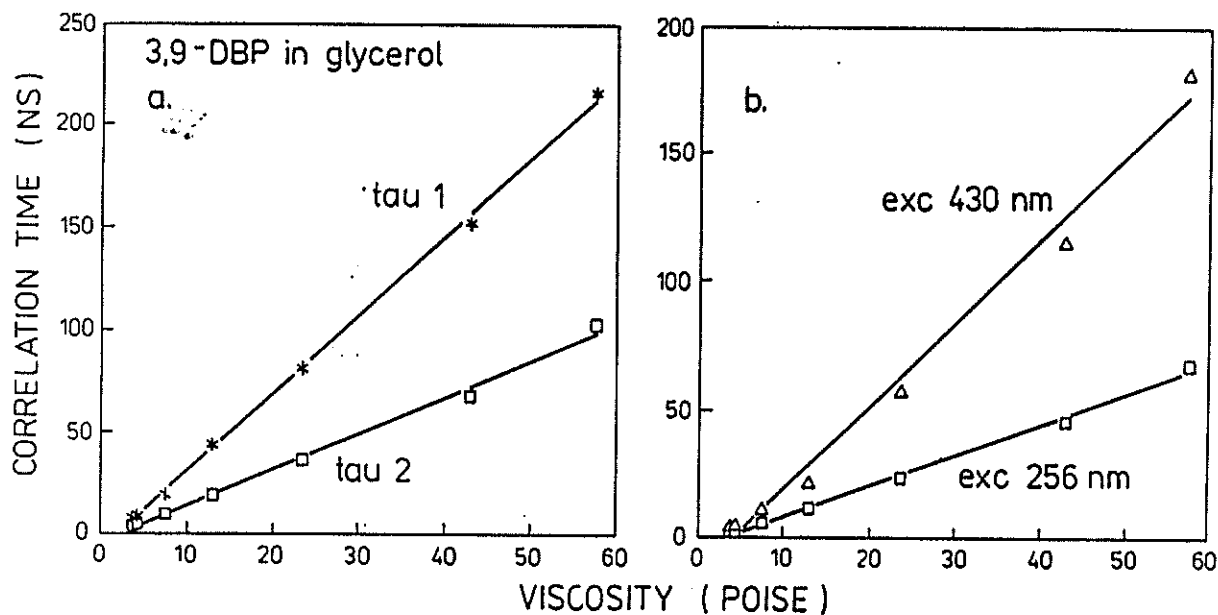


Fig. 1. Rotational correlation times vs glycerol viscosity for perylene recovered from (a) a double-exponential fit and (b) a single-exponential fit of the FA decay.

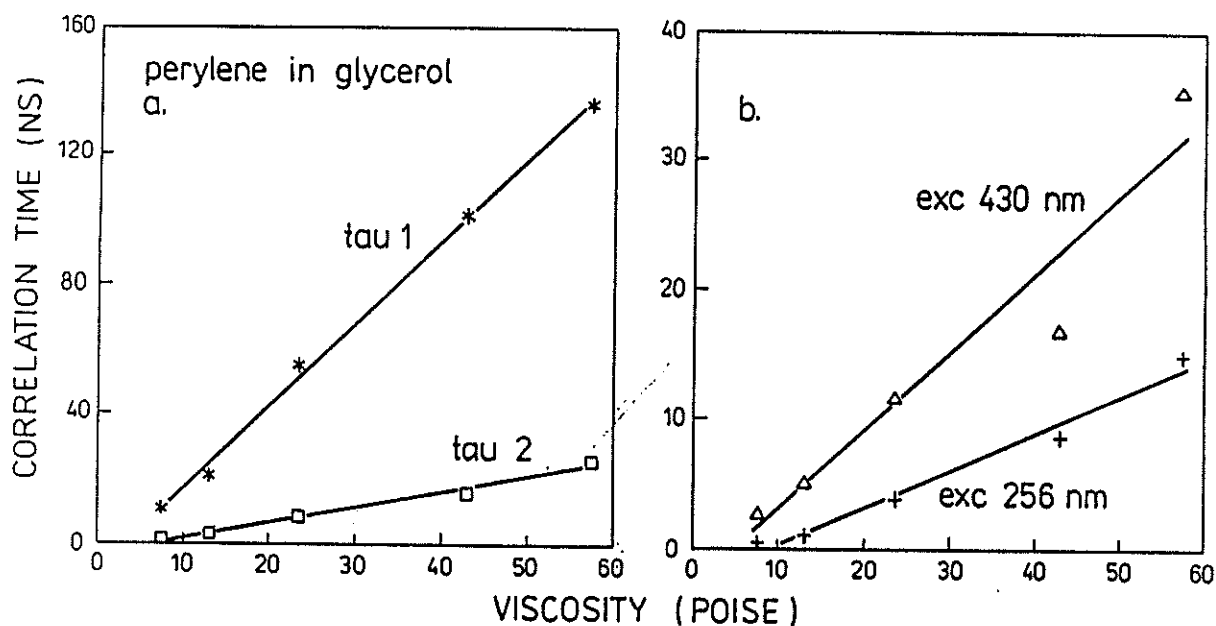


Fig. 2. Rotational correlation times vs glycerol viscosity for DBP recovered from (a) a double-exponential fit and (b) a single-exponential fit of the FA decay.

tions of the angles between the transition moments and the symmetry axes of the ellipsoid as well as functions of the rotational diffusion coefficients D_k ($k=1,2,3$) with

respect to these axes. These coefficients are dependent on the fluorophore size and shape as well as the temperature and viscosity of the solvent.

For ellipsoids of revolution with transition moments lying in the plane perpendicular to the symmetry axis, the decay of the FA reduces to a double exponential. In such a case there are only two diffusion coefficients, D_{\perp} and D_{\parallel} (for rotations perpendicular and parallel to the symmetry axis, respectively). The two correlation times are $\tau_1 = 1/6D_{\perp}$ and $\tau_2 = (2D_{\perp} + 4D_{\parallel})^{-1}$, while the amplitudes are $A_1 = 0.1$ and $A_2 = 0.3 \cos 2\alpha$, where α is the angle between the absorption and the emission transition moments. We analyzed the experimental data for glycerol solutions of perylene and DBP in terms of the above model as proposed for perylene by Barkley *et al.* [3]. A detailed discussion [4] of our results is, however, not intended here. We restrict ourselves to drawing the reader's attention to the possibility of using the two studied fluorophores as viscosity probes.

EXPERIMENTAL

Perylene (Aldrich) and 3,9-DBP (a gift from Dr. H.-G. Löhmannsröben, Technical University, Braunschweig, Germany) were chromatographically checked for purity and dissolved in spectrophotometric grade 99.5 + % glycerol (Aldrich). The solute concentrations were $5 \cdot 10^{-6}$ and $< 10^{-6}$ M, respectively. The solutions were not degassed. Their viscosity was determined as a function of temperature in the 5–55°C range by means of an ultrasonic viscometer (Unipan 505, Poland), based on the principle of damping the vibration of a magnetostrictive alloy by a fluid. It should be stressed here that the viscosity determined in such a way is different from the usual "static" shear viscosity since it also incorporates compressional viscous processes which are of importance in glycerol [5].

A home-built, time-correlated single-photon instrument was used to measure the decay profiles of the FA. The excitation source was a flashlamp filled with either nitrogen or hydrogen and the half-width of the instrumental response function did not exceed 1.5 ns. The data were analyzed by a nonlinear least-squares iterative convolution fitting procedure based on the Marquardt algorithm using a personal computer with a 40-MHz 80386 CPU and 80387 coprocessor.

RESULTS AND DISCUSSION

The FA decays of perylene and DBP in glycerol were analyzed assuming the model outlined above and

therefore by fitting a sum of two exponential functions. The samples were excited at wavelengths of 256 and 430 nm, corresponding to absorption bands with nearly orthogonal polarizations. Figures 1a and 2a present the values of correlation times as a function of the glycerol viscosity. They were obtained as a result of the global analysis of anisotropy decays assuming the same pair of viscosity-dependent correlation times for both excitation wavelengths. For comparison, Figs. 1b and 2b show results of single-exponential fitting for the two excitation wavelengths.

For both molecules we found an excellent linear dependence between the glycerol viscosity and the longer rotational correlation time obtained as the result of a global (using data obtained at 256- and 430-nm excitation wavelengths) biexponential analysis of the FA decay, at least in the range of 7–60 P for perylene and 4–60 P for DBP. This significantly extends the reported viscosity range of 0.5 to 150 cP investigated by Williams and Ben-Amotz [1] with the probe BTBP. In Figs. 1 and 2 the correlation coefficients R for both straight lines corresponding to τ_1 are equal to 1.00. A slightly worse fit was obtained for τ_2 ($R = 0.98$ for both molecules). For DBP a single-exponential fit is also satisfactory ($R = 0.99$ and 1.00 for the 430- and 256-nm excitation, respectively), while in the case of perylene such a fit is evidently worse ($R = 0.92$ and 0.98 for the 430- and 256-nm excitation, respectively). This is not surprising since perylene appears to be a more anisotropic rotor than DBP [4].

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