FLUORESCENCE POLARIZATION ANISOTROPY AND KINETICS OF MALACHITE GREEN MEASURED AS A FUNCTION OF SOLVENT VISCOSITY

F. PELLEGRINO, A. DAGEN and R.R. ALFANO

Picosecond Laser and Spectroscopy Laboratory,
and Physics Department, The City College of The City University of New York, New York, NY 10031, USA

The fluorescence kinetics and polarization anisotropy of the triphenylmethane dye malachite green were measured as a function of solvent viscosity. The relationship between the relaxation kinetics and the solvent viscosity was investigated in order to obtain information on the effect of environmental changes on the orientational order of dye molecules in solution. It was found that the fluorescence lifetimes follow an $\eta^{2/3}$ dependence for $1 < \eta < 60$ P, $\eta^{1/2}$ dependence for 60 P $< \eta <$ 1000 P and approach a constant for $\eta > 1000$ P. The dependence of the fluorescence decay rates on the solvent viscosity was fit to $k = 5 \times 10^{10} \eta^{-2/3} + 5 \times 10^{8}$ s^{-1}. The fluorescence polarization anisotropy term, $R(0)$, was also measured as a function of solvent viscosity. A marked decrease in $R(0)$ was observed at a viscosity of 1000 P. For $\eta < 1000$ P, $R(0)$ was found to be close to the expected value for a random distribution of molecules, 0.4; and for $\eta > 3000$ P, $R(0)$ was measured to be ≈ 0.11. This small value of $R(0)$ may indicate a nonrandom molecular distribution. The observed change of the polarization anisotropy with increasing viscosity indicates that the dye molecules become ordered at higher viscosities. This may arise through the formation of a long range order due to lack of rotational deexcitation of the malachite green dye molecules at high viscosities.

1. Introduction

Dye molecules in solution provide a particularly interesting means of exploring the energy transfer mechanisms that take place subsequent to photoexcitation. Dye molecules possess optical properties which are known to depend on many environmental factors [1–3]. The fluorescence yield and lifetime, as well as the absorption and emission spectra can be readily affected by various parameters of the solvent such as the polarity, viscosity, *pH*, hydrogen bond donor or acceptor strength and temperature. In particular, the fluorescence yield of malachite green increases by orders of magnitude when the viscosity of the solvent is correspondingly increased [4–6]. This increase of the fluorescence yield and lifetime with increasing viscosity has been attributed to the inhibition of the rotational motion of the phenyl rings of the molecule in viscous media. The molecular structure of the dye malachite green is highlighted by the propeller-like arrangement of its three phenyl rings about the central carbon atom.

Forster and Hoffman [5] measured the quantum yields of various triphenylmethane dyes in different solvents as a function of temperature. They found the quantum yield to vary with viscosity as $\eta^{2/3}$ for viscosity less than 200 poise (P). A model based on the rotational motion of the phenyl rings in solution was proposed to explain the observed $\eta^{2/3}$ relationship. They additionally predicted the time dependence of fluorescence relaxation to be given by $\exp(-bt^3/\eta^2)$. The recovery of the ground state population of malachite green subsequent to excitation has also been measured by the method of time resolved absorption [7]. Ippen et al. [8], have measured the absorption recovery of the ground state using a sub-picosecond dye laser. In their experiment, the absorption recovery for malachite green was observed to behave as a single exponential for $\eta < 1$ P. A second faster exponential component was observed for $\eta > 1$ P. The fast component was found to agree closely with the $\eta^{2/3}$ relationship and was thus attributed to the radiative fluorescence decay process. The longer component was observed to follow more closely to $\eta^{1/2}$, and was

suggested as the time for equilibration within the ground state manifold. Cremers and Windsor [9] measured the time dependence of the absorption recovery of crystal violet in glycerol water mixtures at viscosities of 0.8, 40 and 180 P. They fit their data [9] (figs. 5 and 6) to a series of decaying exponentials. It appears to us, however, that a single exponential recovery time also fits their data.

Using both an optical Kerr gate and streak camera, Yu et al. [6], measured the fluorescence kinetics of malachite green over the viscosity range from 1 to 60 P, and obtained a dependence of the fluorescence lifetime on the solvent viscosity proportional to $\eta^{2/3}$. Both the Kerr gate data and the streak camera results were found to be in good agreement in that experiment, even though the Kerr gate measurements required more extensive normalization. Using fluorescence up-conversion Hirsch and Mahr [10] and Beddard et al. [11], have observed a double exponential decay in the viscosity region $\eta < 50$ P. Their faster component agrees with the fluorescence decay measured by Yu et al. [6] as well as the slow component of the absorption recovery measured by Ippen et al. [8]. It is possible that the non-exponential behavior observed by Hirsch and Mahr [10] in the fluorescence may arise from the sensitivity of the fluorescence up-conversion technique to the polarization states of the fluorescence and pump beams which are mixed in the nonlinear crystal [12].

In the present study, the fluorescence kinetics and polarization anisotropy of malachite green were measured as a function of solvent viscosity in order to investigate the effect of environmental changes on the orientational order of dye molecules in solution. The measurements reported here are an extension of the work of Yu et al. [6], and cover a sufficiently large viscosity range to enable the observation of the overall viscosity dependence of the polarization anisotropy and the fluorescence decay time. The lifetime of the first excited singlet state as well as the polarization effects have been studied as a function of the solvent viscosity from 6 to 60000 P. The measured fluorescence kinetics were found to behave as a single exponential varying with viscosity as $\eta^{2/3}$ for $1 < \eta < 60$ P, $\eta^{1/2}$ for 60 P $< \eta < 1000$ P, and asymptotically approaching a constant value for $\eta > 1000$ P. Our use of single exponential curves to fit our data rather than the use of multi-exponential curves preferred by some

workers is not crucial to the salient results of this paper. The polarization kinetics were also investigated as a function of the solvent viscosity in order to obtain information on the relationship between local molecular order and environmental changes. A discontinuity in polarization anisotropy measured at $t = 0$ was observed near 1000 P.

2. Experimental methods

The fluorescence kinetics were measured with a Hamamatsu streak camera and PAR optical multichannel analyzer (OMA-I) detection system [6]. The fluorescence temporal profiles were transferred from the PAR to a DEC PDP 11/03 minicomputer. Each profile was then corrected for streak rate and analyzed for a single or double exponential fit. For viscosities greater than 350 P, both the simultaneously obtained decays of $I_\parallel$ and $I_\perp$ as well as independent decay curves were obtained in order to reduce the errors in the lifetime determinations at high viscosities. Analyses of log plots of the data were used to determine the feasibility of fitting to an exponential decay behaviour. The data obtained in the viscosity range from 6 to 100 P confirmed the results obtained by Yu et al. [6]. The range of photon flux at the sample site was about 10^{14} photons/cm^2 per pulse. The fluorescence from a thin sample cell was collected at 30° from the front surface. A steady state fluorometer was used to measure the fluorescence spectra from the dye solution.

Fluorescence polarization kinetics were measured as a function of solvent viscosity using the experimental setup [13] shown in fig. 1. Excitation was provided by two orthogonally polarized 530 nm, 6 ps laser pulses obtained from a single pulse at 530 nm by use of a beam splitter optical delay technique [13]. A half wave plate at 530 nm was used to rotate the polarization direction of the delayed pulse by 90°. The two orthogonally polarized 530 nm excitation pulses were adjusted to have approximately equal intensity and were separated by a 1 ns delay. An analyzer (Polaroid HN 38) was placed between the sample and the streak camera, thus allowing for the simultaneous measurement of the fluorescence signal both parallel to and perpendicular to the incident polarization direction. The system was calibrated by equalizing the fluorescence emission from a 2×10^{-4} M
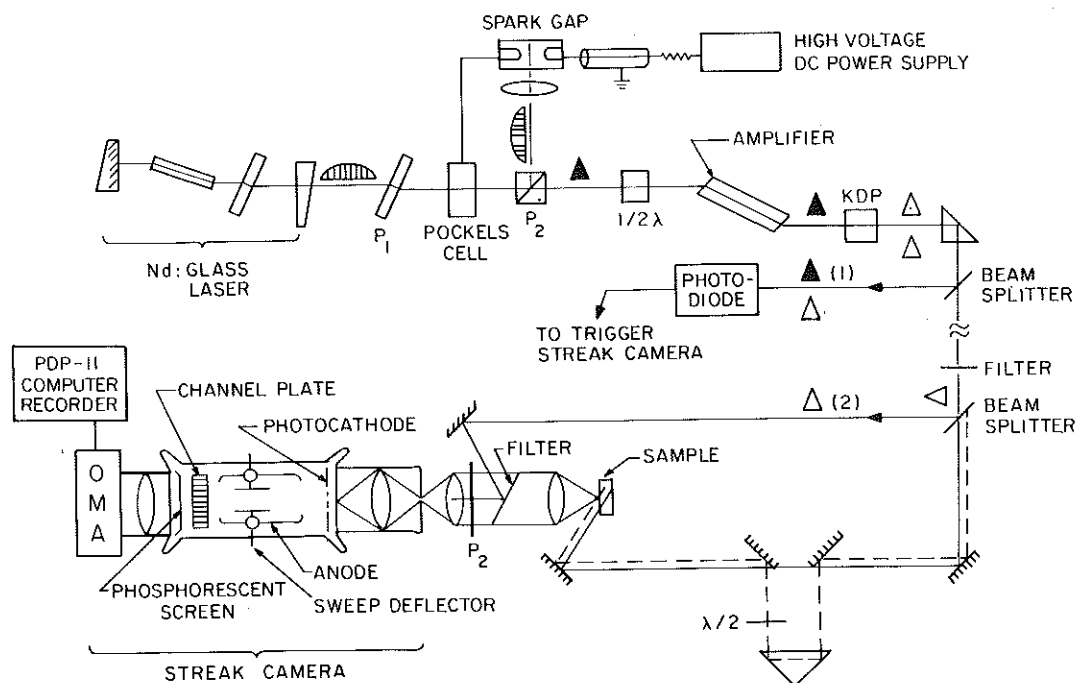
Fig. 1. Optical delay technique for fluorescence polarization measurements.

solution of malachite green in glycerol excited by the two pulses with the analyzer removed. A viscosity range from 6.29 to 60000 P was covered in the polarization experiments, thereby providing overlap with the previous investigation of the fluorescence decay kinetics [6]. The chemicals malachite green (99% certified, Eastman Kodak) and anhydrous glycerol (Baker) were used without further purification. The concentration of malachite green was 5×10^{-4} M in a sample length of 5 or 2 mm. The viscosity values for the glycerol solutions at various temperatures were obtained from standard tables [14]. A viscosity range of 6 to 60000 P was obtained by changing the temperature by flowing cooled nitrogen gas over the samples placed in an optical Dewar. The temperature range used to obtain the 10^4 fold change in viscosity was from 2°C to −43°C. The glycerol used in these studies was checked for the presence of possible strain birefringence by visually inspecting a sample cell placed between two crossed polarizers (Polaroid HN 38). No leakage of the light collimated through the sample region could be detected over the viscosity region from 6 to 10^5 P.

3. Experimental results

The fluorescent kinetics of malachite green in glycerol at room temperature (6.29 P) measured by the streak camera is shown in figs. 2a–2c. The decay of malachite green fluorescence is characterized by a single exponential with a relaxation time of 90 ps. Fig. 2a shows the fluorescence decay of malachite green at room temperature and 6.29 P, measured with the two-pulse-excitation-beam splitter optical delay technique, with the analyzer removed from the front of the entrance slit of the streak camera. Fig. 2b shows the fluorescence polarization observed for malachite green at 6.29 P with the analyzer in place. The first decay profile is due to fluorescence measured parallel to the polarization direction of the incident pulse ($I_{\parallel}(t)$), while the second decay profile measures the fluorescence component with polarization vector perpendicular to the polarization direction of the delayed incident pulse ($I_{\perp}(t)$). By rotating the analyzer 90°, parallel to the polarization direction of the second excitation pulse, the relative signals are reversed as expected (fig. 2c). Fig. 3 shows the fluorescence
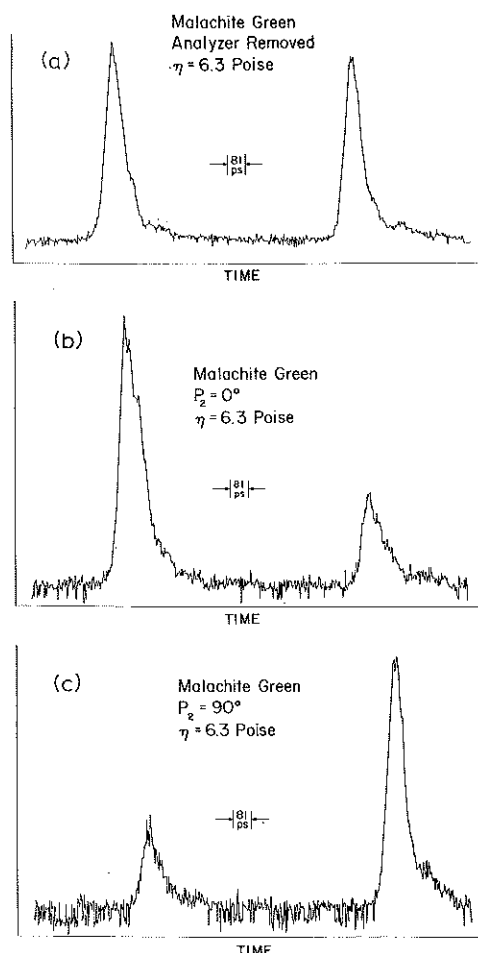
Fig. 2. Fluorescence decay of malachite green at viscosity of 6.29 P: (a) Measured without analyzer. (b) Measured with analyzer in place 0°. (c) Measured with analyzer rotated 90°.

polarization kinetics measured at 121 P. The time $t = 0$, defined as the zero time within the resolution of the apparatus, is located at the peak of each curve. The marked increase of the fluorescence decay time with increasing solvent viscosities clearly shows the effect of the increased solvent viscosity on the local deexcitation of the molecules in solution. For each viscosity region investigated, at least six kinetic measurements were obtained. The measured fluorescence lifetimes from this study are plotted in fig. 4 as a function of solvent viscosity. The errors in the exponential fits to the data were less than 10% over the region from 6 to 355 P and less than 20% over the region from 355 to 60000 P.
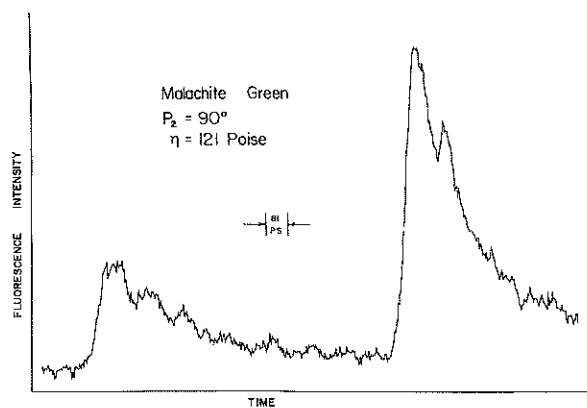

Fig. 3. Fluorescence polarization kinetics of malachite green measured at viscosity of 121 P. First pulse is $I_\perp(t)$ and second pulse is $I_\parallel(t)$.

The fluorescence polarization anisotropy $R(0)$ at $t = 0$ is plotted as a function of solvent viscosity in fig. 5. The polarization anisotropy at $t = 0$ for viscosities less than 1000 P is measured to be 0.38 ± 0.01 in agreement with the expected theoretical value of 0.4 for a random distribution of molecular dipoles with the emission and absorption dipoles aligned in solution. However, for viscosities > 1000 P, the polarization anisotropy decreases to 0.11 ± 0.01.
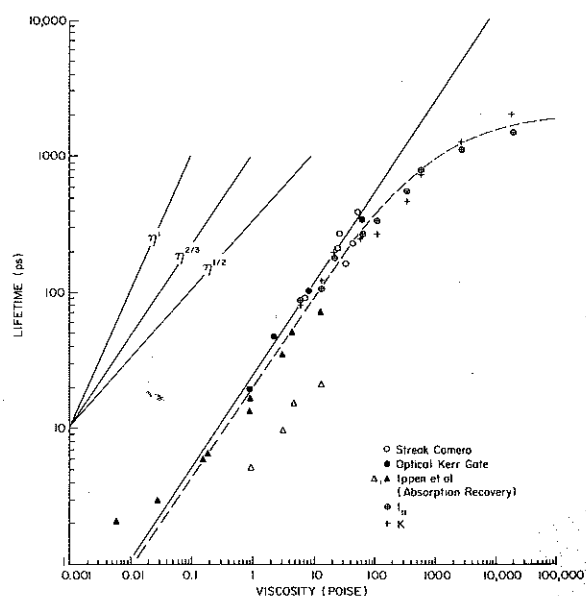

Fig. 4. Dependence of fluorescence decay time of malachite green on solvent viscosity.
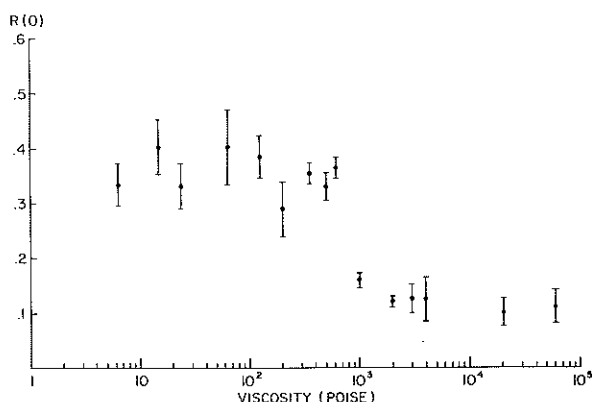
Fig. 5. Polarization anisotropy $R(0)$ of malachite green in glycerol as a function of solvent viscosity.

4. Discussion

The dramatic increase in the fluorescence yield, fluorescence lifetime and rotational decay time with increasing solvent viscosity observed in these measurements is attributed to the inhibition of the rotational motion of the phenyl rings in viscous media. The temporal profile of the fluorescence at any given time measures the relative population of the first excited state at that time. Thus the measured rate of fluorescence decay is made up of the sum of the radiative ($S_1 \to S_0^* + h\nu$), nonradiative internal conversion ($S_1 \to S_0^*$), and inter-system crossing ($S_1 \to T_1$) rates. The radiative lifetimes of the dye molecules can be estimated from the absorption spectra and the 0–0 transition, and are essentially constant for the various solutions studied. In malachite green the intersystem crossing rate is small, as can be inferred from the observation of the fast and complete recovery of the absorption of the ground state in the experiment of Ippen et al. [8]. Therefore the observed variation of fluorescence lifetime in malachite green can be interpreted as the change of the internal conversion rate of the dye molecules for varying viscosities.

The measured viscosity dependence of the fluorescence lifetime of malachite green in this measurement (fig. 4) does not obey a simple viscosity dependence. For $1 < \eta < 60$ P, the fluorescence lifetime can best be described by an $\eta^{2/3}$ dependence, in agreement with the previous work of Yu et al. [6] and with the fluorescence yield measurements of Forster and Hoffman [5], who also theoretically predicted the

$\eta^{2/3}$ viscosity dependence. However, their predicted $\exp(-bt^3/\eta^2)$ time dependence was not confirmed in this experiment. For the viscosity range $60 < \eta < 1000$ P, the dependence can be best fit to an $\eta^{1/2}$ viscosity dependence. As expected, for viscosities greater than 1000 P the lifetime approaches a constant value. Although attempts have been made to fit the viscosity dependence to $\eta^{2/3}$ and $\eta^{1/2}$, a more complicated viscosity dependence is indicated by the results of these measurements. Physically, a simple dependence of the fluorescence lifetime on the solvent viscosity should not be expected since the reciprocal of the fluorescence lifetime must asymptotically approach the unimolecular decay rate as the rotational non-radiative deexcitations are frozen out. The fluorescence decay rate may be described by the relation:

$$k = k(\eta) + k(\infty), \tag{1}$$

where $k(\eta)$ is a viscosity dependent term which may be taken to be of the form $A\eta^{-2/3}$ for viscosities in the region of 1 to 60 P, and $k(\infty)$ is a viscosity independent term of the form $k(\infty) = k_{ST} + k_{NR} + k_R$. From the data presented in fig. 4 a value of 5×10^8 s^{-1} can be estimated for $k(\infty)$ in the high viscosity region. In addition, $k(\eta)$ can be obtained in the low viscosity range as $5 \times 10^{10}\eta^{-2/3}$. The functional form for k,

$$k = 5 \times 10^{10} \, \eta^{-2/3} + 5 \times 10^8 \, s^{-1} , \tag{2}$$

was fit to the data to within ±10% and is represented as the dashed line in fig. 4.

To this date, no satisfactory microscopic theory has been found to explain the functional form of the decay rate, the time-resolved fluorescence decay profile and steady state fluorescence quantum yield dependence on the viscosity. A detailed theoretical study of these effects is required in order to fully understand the relationship of the structural properties of dye molecules in solution with respect to solvent properties. Such a study is beyond the scope of the present work.

The absorption recovery times measured by Ippen et al. [8] are also plotted in fig. 4 for completeness. It is interesting to note that the absorpion recovery times of the slow component measured by Ippen et al. [8] fall close to the measured fluorescence lifetime and the $\eta^{2/3}$ line. The fast component, however, measured to be less than 5 ps for viscosity < 0.1 P, does

not obey this relationship. Since the values of the slow component of the absorption recovery are found to be close to the values for the measured fluorescence lifetime for $\eta < 20$ P, the observed slow component of the absorption recovery apparently manifests the internal conversion kinetics. The fast component may possibly reflect the time required for non-radiative equilibration in the ground state manifold. In principle, the S_1 equilibration time is reflected in the fluorescence risetime. The risetimes measured in this experiment were resolution limited to $\leqslant 12$ ps. Recently, the S_1 equilibration time for large dye molecules has been reported to be less than 0.2 ps, and independent of solvent properties. Therefore the ground state equilibration time can also be assumed to be of this order. The interpretation of the short component remains in doubt.

As a first approximation, the molecules of malachite green may be considered to be spheroidal. In the analysis of the viscosity dependence of the fluorescent polarization both a random distribution model, for the case of malachite green dye molecules in solution at low viscosity, and an oriented distribution model, for the case of malachite green dye molecules in solution in the high viscosity limit (where the molecular rotations have been essentially frozen) are considered. The fluorescence polarization anisotropy was analyzed in terms of a model describing the rotational motion of a system of dipoles in viscous media.

When a linearly polarized pulse of light excites a distribution of molecules in solution, a non-random distribution of excitations is achieved since molecules having their absorption dipole in the plane of polarization are preferentially excited. If such a molecule rotates or otherwise transfers its energy to another state or molecule prior to emission, the fluorescence will depolarize. If one considers the dye molecule to behave as a spheroid in viscous media, two conditions arise. Either the molecule "sticks" to its viscous surrounding, or it "slips" in that surrounding. More specifically if the velocity vector of the solute and solvent are equal at their surfaces, then the solvent sticks to the solute and rotates with it. If on the other hand, there is no tangential component of the stress tensor at the solute–solvent interface, then the solute molecules can rotate freely and the slipping boundary condition is said to hold. The molecular symmetry as well as the polarity of the molecule with respect to its en-

vironment determines which condition applies. For a randomly oriented system of dipoles, the intensity of fluorescence observed through an analyzer set either parallel to or perpendicular to the incident polarization direction is obtained by applying a Green's function operator to the parallel and perpendicular components of the fluorescence decay relative to an initial random distribution of dipoles described in terms of the second Legendre polynomial. These components are:

$$I_\parallel(t) = (\tfrac{1}{3} + \tfrac{4}{15} \langle P_2(\hat{e}(0)\cdot\hat{e}(t))\rangle) u^2 K(t) ,$$

$$I_\perp(t) = (\tfrac{1}{3} - \tfrac{2}{15} \langle P_2(\hat{e}(0)\cdot\hat{e}(t))\rangle) u^2 K(t) , \qquad (3)$$

where P_2 is the second Legendre polynomial; $\hat{e}(0)$, $\hat{e}(t)$ are the directions of the transition dipole moment u at times 0 and t respectively; and $K(t)$ describes the temporal behavior of the fluorescence decay [15].

An order parameter which characterizes the polarized fluorescence emission in terms of the intensity of fluorescence measured parallel to and perpendicular to the incident polarization direction [$I_\parallel(t)$ and $I_\perp(t)$ respectively], is $R(t)$ the fluorescence polarization anisotropy, defined by the following relation:

$$R(t) = \frac{I_\parallel(t) - I_\perp(t)}{I_\parallel(t) + 2I_\perp(t)} . \qquad (4)$$

For a random distribution of spheroidal molecules in solution, with aligned emission and absorption dipole, $R(0) = 4$. This agrees with the measured value for $R(0)$ for viscosities less than 1000 P. If these molecules are now placed in a matrix or ordered array, the value of $R(0)$ is expected to change as well as the form of $I_\parallel$ and $I_\perp$. The emission anisotropy at $t = 0$ for an oriented molecular distribution, for the case where the absorption dipole is parallel to the unique symmetry axis of the system, is given by [16]:

$$R(0) = [2\langle P_2^2\rangle + \langle P_2\rangle]P_2(\cos\varphi)/(1 + 2\langle P_2\rangle) , \qquad (5)$$

where $\langle P_2\rangle$ is the expectation value of the second order Legendre polynomial for the distribution function $f(\theta)$, and is given by:

$$\langle P_2\rangle \equiv P_2(\cos\theta) = \int\limits_0^\pi \sin\theta \ d\theta \ f(\theta) P_2(\cos\theta) , \qquad (6)$$

and where θ is the angle between the long axis of the molecule and the major symmetry axis of the system, and φ is the angle between the absorption and emission dipole.

For a distribution of molecules described by a distribution function of the form $f(\theta) = \frac{3}{2} \cos^2\theta$, $R(0) = 0.10 \pm 0.01$[13]. For viscosities greater than 1000 P, the values for the polarization anisotropy were measured to be $R(0) = 0.11 \pm 0.01$. Since for $\eta < 1000$ P, $R(0)$ was measured to be 0.38, an ordering of the malachite green molecules from a random distribution to an ordered molecular array appears to have taken place at $\eta \approx 1000$ P. This interpretation, though speculative is consistent with the theory presented here and is also consistent with the observed change in the lifetime dependence as a function of solvent viscosity in the 1000 P viscosity region where the rotational deexcitation mode appears to have been "frozen out".

At low viscosities the molecules are free to rotate in solution, so that no long range order can be established. As the viscosity increases, the rotational degree of freedom gradually decreases to a point where molecules may form local ordered clusters through covalent bonding or ionic interactions. This allows for a long range order to be established which manifests itself in the lower value of $R(0)$ measured at high viscosity. However, the possibility that the decrease in $R(0)$ with increasing viscosity arises solely from the freezing out of the individual molecular rotations and thereby being indicative of the molecular polarization measurements alone (as opposed to a long range structural order effect) cannot be entirely ruled out. The dependence of the fluorescence lifetime and fluorescence polarization anisotropy on the viscosity of the solvent can therefore provide a measure of the orientational order of the molecules in relation to the viscosity of the solvent. Measurements of the $t = 0$ polarization anisotropy from dye molecules can provide a new technique to probe phase changes such as in the "glass transition".

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