

Dipolar Relaxations in Glycerol: A Dynamic Fluorescence Study of 4-2'-(Dimethylamino)-6'-naphthoylecyclohexanecarboxylic Acid (DANCA)

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Abstract: Solvent dipolar reorientations following excitation of DANCA in glycerol at various temperatures were investigated by using multifrequency-phase fluorometry. The time evolution of the emission spectrum was determined by performing lifetime measurements at a number of wavelengths across the emission band and then reconstructing the spectrum at selected times after excitation. At low temperatures, a large displacement of the time-resolved spectrum was apparent. The spectral center of gravity was observed to move faster at short times and then to exhibit a long decay. The spectral width was also observed to increase at short times and then to decrease at much longer times. Several models have been considered to account for the major features of our experimental results. A two-state model agreed well with the data only in the high-temperature regime; at lower temperatures, the data were not consistent with the characteristic features of the two-state model. In the high-temperature regime, we obtained a reorientation rate for the glycerol molecules using the two-state model. Other models which consider a continuous translation of the emission spectra as a function of time also failed to account for the experimental results. Instead, a good qualitative agreement was found with the model proposed by Weber in which the charge separation of the excited fluorophore is followed by reorientation of the solvent molecules around the individual monopoles.

The study of relaxation phenomena which occur upon excitation of certain fluorescence probes in highly viscous polar media, bound to proteins or incorporated into membranes, has attracted considerable interest.²⁻¹⁵ These excited-state processes are accompanied by large shifts in absorption and emission spectra in solvents of different polarity. Furthermore, the position and shape of the emission spectra are dependent upon the excitation wavelength. These phenomena are explained in terms of dipole rearrangements on a nanosecond time scale around the excited state of the fluorophore. Dipolar relaxation phenomena are important in fluorophores which exhibit a large charge separation upon excitation, i.e., a large excited-state dipole. Particularly noteworthy in this regard are a number of naphthalene derivatives which have been extensively studied by G. Weber's group.⁹⁻¹² The excited-state dipole in these fluorophores is much larger than the ground-state dipole, and the orientation of the dipole relative to the nuclear framework may also differ between the ground and excited states. The precise manner in which spectroscopic properties reflect the interactions of excited molecules with surrounding solvent molecules and the correct approach for deriving the characteristic rates of the relaxation process from the time evolution of the fluorescence parameters is, however, still controversial. Several theories have been proposed to explain the modalities of the observed fluorescence decay.^{6-9,11,13} A two-state model has been proposed to explain some of the relevant features of the process, i.e., the movement of the emission maximum and the change of the width of the emission spectrum during the decay.⁸ The two-state model assumes that solvent molecules surrounding the excited fluorophore can exist in only two limiting states, namely, nonrelaxed or relaxed. This simplified model represents a limiting case which is seldom found in real systems. A model based on a continuous displacement of the emission spectrum has been used by Bakhshiev et al.⁷ This model assumes that the emission spectrum, which is described by a Gaussian shape, relaxes exponentially to lower energies but does not explain the observed time evolution of the spectral shape. Furthermore, in most cases

the emission center of gravity does not relax exponentially. In glycerol, more than one relaxation time has been observed, and the original model proposed by Bakhshiev has been modified to account for a continuum of relaxation rates.¹⁶⁻¹⁸ Recently G. Weber has proposed a new model which is based on definite physical assumptions at a molecular level regarding the interactions responsible for the excited-state process and the specific manner in which these interactions determine the spectral properties.^{10,11} Since this model appears more appropriate to describe our experimental results, we will report its basic features in the next subsection.

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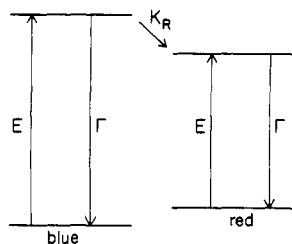


Figure 1. Two-state model depicting interconversion between nonrelaxed (blue) and relaxed (red) states. E is the excitation function, Γ is the decay rate, and K_R is the relaxation rate.

Recently, a new fluorescent probe, DANCA,¹⁹ has been synthesized in G. Weber's laboratory and characterized.¹⁰ This compound exhibits a large spectral shift upon passing from polar to nonpolar solvents and has a single exponential decay with a relatively short lifetime in nonviscous solvents. DANCA was specifically designed to facilitate the study of dipolar relaxation phenomena, and its spectral properties demonstrate its utility in such investigations. Other naphthalene derivatives frequently used in the study of relaxation processes, such as TNS¹⁹ and ANS,¹⁹ show less pronounced spectral shifts in solvents of differing polarity and have more complex absorption spectra relative to DANCA. The correct interpretation of excited-state processes requires that a pure electronic transition be excited; otherwise, the spectral changes can be complicated by the presence of a heterogeneous population of excited-state molecules. The charges in DANCA, as well as its parent molecule PRODAN,¹⁹ in the excited state are localized on the amino and carboxy groups and hence the excited-state dipole is quite large, on the order of 18–20 D as compared to the ground-state dipole of 2 D.

Recently the application of multifrequency-phase fluorometry to the resolution of complex decays which arise from excited-state processes has been reviewed.²⁰ This frequency domain technique is extremely powerful for the accurate determination of the rate constants involved in dipolar relaxations and can be applied to the study of excited-state processes in the time range from a few picoseconds to several microseconds.

Models of Dipolar Relaxation

Several models have been proposed to interpret the dynamic features of the dipolar relaxation process. A two-state model has been employed by Lakowicz and Balter⁸ for ANS and TNS in viscous solvents. In such a two-state model, as depicted in Figure 1, one assumes that the fluorescence can arise only from one of the two excited states, namely, a nonrelaxed blue-shifted state and a totally relaxed red-shifted state. The rates of relaxation and back-relaxation can also be included. However, due to the typically large energy difference between the relaxed and nonrelaxed species, the rate of back-relaxation may be ignored. Instead, one must take into account the possibility that both the relaxed and nonrelaxed states may be directly excited. One of the salient features of this model is that only two lifetime values will be obtained and that these values will be constant regardless of the emission wavelength. The preexponential values, however, will vary across the emission spectrum in a sigmoidal fashion. Additionally, the two lifetime values cannot be associated with either of the two molecular species; these values are, in fact, a function of both the decay and relaxation rates. Similarly, the preexponential factors do not simply reflect the relative amounts of relaxed and nonrelaxed species but instead are functions of the initial population of the two states upon excitation and the rate processes involved. Indeed, under certain circumstances, one of the preexponential

factors can be negative. Another prediction of the two-state model is that the spectral center-of-gravity will not change exponentially to the relaxed value and that the spectral width will initially increase but will decrease at very long times. This model works relatively well in those cases where the dipolar relaxation rate is fast with respect to the decay rate since in these cases very few fluorophores decay from partially relaxed states. Recently, this simple model was extended by Beechem and Brand¹³ to account for several intermediate states. However, in those cases in which the intermediates cannot be assigned to specific species, this extension of the two-state model will not furnish parameters with a direct physical interpretation. A different model has been developed by Bakhshiev et al.⁷ based on the assumption that a large number of intermediates exist. In this model, the emission center of gravity relaxes exponentially from the initially nonrelaxed state to the final relaxed state, and the spectral shape is Gaussian with constant width. The parameters which are obtained from this simple model relate to the total energy released during the relaxation process and give an estimate of the rate of energy release. A different approach was suggested by Weber¹¹ upon investigation of molecules such as PRODAN. He considered that the excitation process produces a charge separation and that the charges could be localized in specific parts of the molecule. He then considered the reorientation of the surrounding solvent dipoles to minimize the energy of this excited-state charge configuration. Using a numerical approach, Weber calculated the spectral properties as the relaxation process progressed. A characteristic of this model is that the final relaxed state produces a narrow spectral distribution and that the shift of the spectral center of gravity is nonexponential. Unfortunately, this model does not yet provide an analytical function which can be used to directly fit the data. We will therefore make qualitative comparisons of our results with the characteristic features of Weber's model.

Experimental Section

Spectroscopic grade glycerol was purchased from Aldrich. DANCA was obtained from the laboratory of G. Weber. Solutions of DANCA in glycerol were prepared by gentle mixing of the sample at 50 °C to facilitate the solution process. Different samples were used for each measurement to minimize potential photochemical effects.

Steady-state measurements were performed with the microprocessor-controlled photon-counting spectrofluorometer described by Gratton and Limkeman.²¹ Temperature was maintained in the sample compartment to ± 0.2 °C by using a circulating thermostatic bath (Neslab L50). The actual sample temperature was measured in the sample cuvette by using a digital thermometer (Omega Instrument Inc.). Lifetime measurements were obtained by using the laser-based multifrequency-phase and modulation fluorometer described by Gratton and Limkeman.²² An HeCd laser (Liconix Model 4240NB, Sunnyvale, CA) was used for excitation at 325 and 442 nm. The emission was observed by using a set of interference filters from Corion Co. with bandwidths of 10 nm. Phase and modulation data were collected in the frequency range from 2 to 200 MHz at several emission wavelengths spanning the emission spectrum. Both phase and modulation data were analyzed using two or three exponentials by a nonlinear least-square program previously described.^{23,24} Time-resolved spectra were obtained by collecting multifrequency data at several emission wavelengths. At each emission wavelength, a fit of the decay is performed using a sum of exponentials. We note that in this type of analysis there is no simple relation between the number of exponentials and the species present in solution. The multiexponential analysis is used only to generate an analytical function which describes the measured decay. The number of exponentials used varied from two to four depending on the wavelength and temperature. The number of exponentials to be used was determined by increasing the number of components in the fit until the X^2 value was not further improved by addition of one more component. The result of this analysis is a set of values of the preexponential factors $a_i(\lambda)$ and characteristic decay times $\tau_i(\lambda)$ for each emission wavelength which then can be used

(19) Abbreviations: TNS and ANS refer to 2-(*p*-toluidino)naphthalene-6-sulfonate and 1-anilinonaphthalene-8-sulfonate, respectively. PRODAN refers to 6-propionyl-2-(*N,N*-dimethylamino)naphthalene. DANCA refers to 4-2'-(dimethylamino)-6'-naphthoylcyclohexanecarboxylic acid. FWHM stands for full width at half-maximum.

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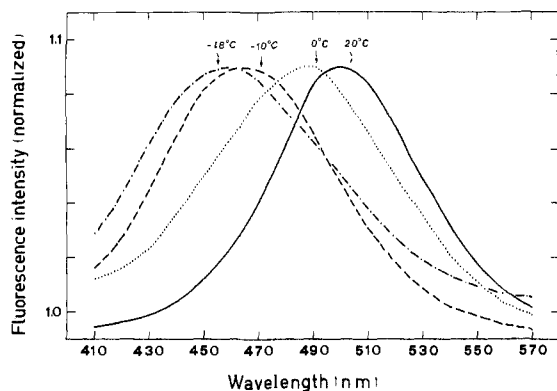


Figure 2. Emission spectra of DANCA in glycerol at different temperatures. The excitation was at 325 nm, and the intensities were normalized.

Table I. Dependence of Emission Maximum and Emission Width on Excitation Wavelength of DANCA in Glycerol at -18°C

λ_{exc} , nm	λ_{em} , nm	FWHM, nm	λ_{exc} , nm	λ_{em} , nm	FWHM, nm
330	459.0	90.8	390	461.5	79.5
340	459.0	84.4	400	463.4	77.9
350	459.0	81.1	410	467.0	77.9
360	460.0	80.6	420	471.0	76.8
370	460.5	80.6	430	475.0	76.8
380	461.0	80.0			

Table II. Phase, Modulation, and Lifetime Values at Different Frequencies for DANCA in Glycerol at 20°C

frequency, MHz	phase, deg	modulation	τ_1^{P} , ns	τ_1^{M} , ns
10.0	16.0	0.967	4.567	4.211
20.0	33.3	0.883	4.654	4.231
30.0	41.8	0.781	4.744	4.242
40.0	50.8	0.683	4.883	4.250
60.0	64.7	0.523	5.622	4.321
90.0	80.2	0.374	10.267	4.390
120.0	88.9	0.273	66.128	4.668
150.0	97.5	0.217	-8.101	4.778

^a τ_1^{P} and τ_1^{M} are the phase and modulation lifetimes,²⁴ respectively.

to reconstruct the emission spectrum at selected times by using the relation

$$I(t, \lambda) = A(\lambda) \sum a_i(\lambda) e^{-t/\tau_i(\lambda)}$$

The normalization factor $A(\lambda)$ is chosen to match the time-averaged, time-resolved spectrum to the steady-state spectrum.

Results

Figure 2 shows the temperature dependence of the steady-state emission spectrum of DANCA in glycerol. A large red shift (≥ 40 nm) and a noticeable sharpening of the emission band were observed upon changing the temperature from -18 to 20°C . This result suggests that the fluorescence emission at higher temperatures arises preferentially from relaxed states. A large red shift of the steady-state emission spectrum was observed when the excitation wavelength varied from 325 to 430 nm. The FWHM¹⁹ of the emission band decreased with increasing excitation wavelengths. Table I shows the fluorescence emission maxima (uncorrected spectra) and the FWHM of the emission band of DANCA in glycerol at -18°C at different excitation wavelengths. Upon excitation in the blue side of the absorption spectrum, a selective excitation of nonrelaxed as well as partially relaxed molecules takes place. However, the distribution is less pronounced when the excitation is at longer wavelength. The lifetime data are reported in Table II. The phase lifetime is generally larger than the modulation lifetime as expected for excited-state processes. For the low-temperature experiments, at high modulation frequencies, the absolute value of the phase shift is larger than 90° in the red part of the emission spectrum, indicating that this

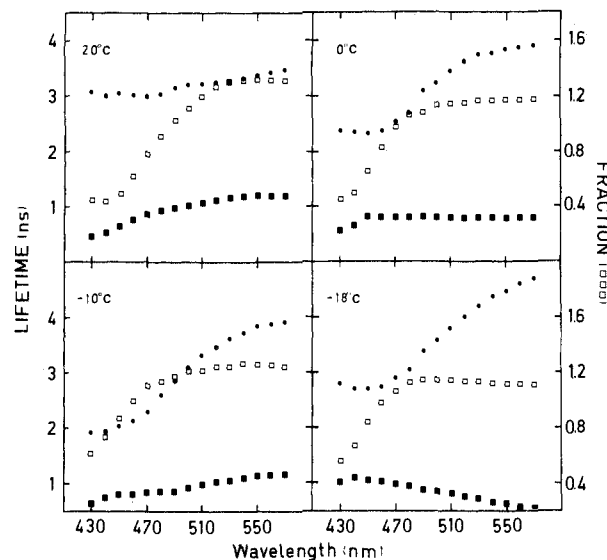


Figure 3. dependence of the emission wavelength on temperature of the two lifetimes τ_1 (\bullet) and τ_2 (\blacksquare) and of the fraction relative of the longer component f_1 (\square) as obtained by the nonlinear least-squares fit using two exponential components of DANCA in glycerol solutions at 325-nm excitation.

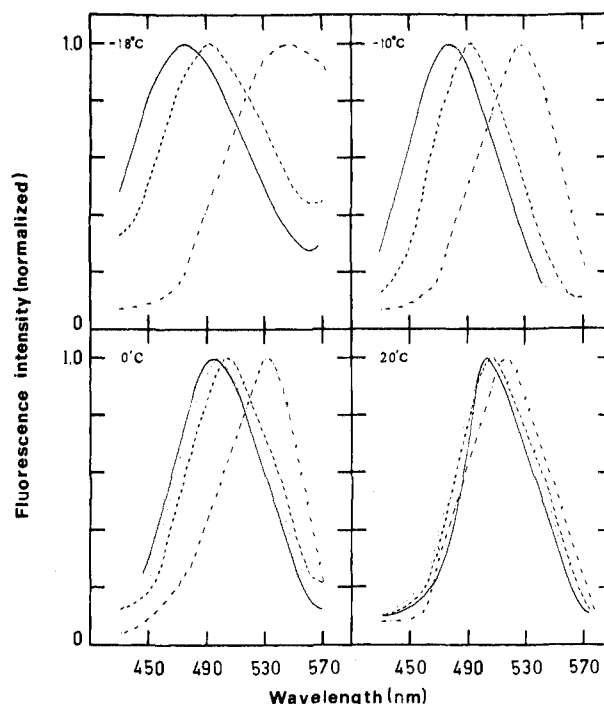


Figure 4. Time-resolved spectra of DANCA in glycerol solution at different temperatures after 0.1 (\rightarrow), 1 (\cdots), 5 ($---$), and 25 ns ($-\cdot-\cdot-$).

part of the spectrum is formed at a later time after excitation. The phase and modulation data were first analyzed using two exponential components to test the applicability of the two-state model. Figure 3 shows the dependence of the two lifetime components on the emission wavelength. On the red side of the emission band, in all cases investigated, the fractional intensity of the longer component became larger than one. At 20°C the two lifetime components were not strongly dependent upon the emission wavelength and the fractional intensity curve was sigmoidal. Thus, at this temperature the relaxation process had the characteristics of the two-state model. The same set of phase and modulation data was also analyzed using a larger number of exponential components to generate time-resolved spectra as described in the Experimental Section. Figure 4 shows the time-resolved emission spectra of DANCA in glycerol at different temperatures obtained using 325-nm excitation. At low tem-

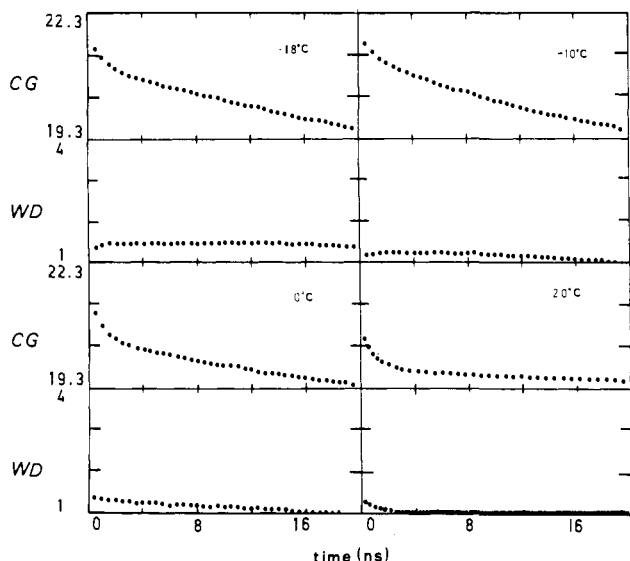


Figure 5. Time dependence of the spectral center of gravity (CG) and width (WD) of the emission band at different temperatures. The units on the vertical scales are in 10^{-3} cm^{-1} .

perature, a large shift in the time-resolved spectra takes place, whereas at high temperature, i.e., 20°C , the shift was strongly reduced. This effect is further demonstrated in Figure 5 which shows the time dependence of the center of gravity of the spectrum and the width of the time-resolved emission bands at several temperatures. Two different slopes of the time evolution of the center of gravity are evident. The initial slope increased with temperature, whereas the slope at longer times approached zero at higher temperatures. Also the spectral width first increased and then slowly decreased with increasing temperature and with time.

Discussion

The two-state model predicts that the emission rates from relaxed and nonrelaxed states are not dependent upon the emission wavelength.⁸ Thus, the lifetime values obtained by decomposing the decay using two exponential components should be independent of the emission wavelength. The large variation of the decay rate across the emission band, especially at lower temperatures, points

out the inadequacy of the two-state model. Also the large values of X^2 obtained by using the two-component analysis (data not shown), particularly at low temperatures, show that two components do not adequately describe the observed decay.

Despite the inadequacy of the two-state model at low temperature, one can use the model to obtain a rough estimate of the relaxation rate at 20°C . If exclusively nonrelaxed molecules are excited at 325 nm , the larger of the two lifetime components obtained with the double-exponential decay analysis should correspond to $1/\Gamma$ and the shorter to $1/(\Gamma + K_r)$, where Γ is the decay rate and K_r is the relaxation rate.⁸ On the basis of these assumptions, we found $K_r = 6.0 \times 10^8 \text{ s}^{-1}$. Also the fraction of the longer component in the red part of the spectrum should be $1 + \Gamma/K_r$. This calculated value corresponds roughly to the measured value of 1.35.

The time-resolved spectra analysis show that at low temperature the emission center of gravity shifts about 2500 cm^{-1} and that the shift follows nonexponential kinetics (Figure 5). Also at low temperature the spectral width first increased and then at longer times decreased. The nonexponential relaxation of the center of gravity and the change of the spectral shape demonstrated the inadequacy of the original model proposed by Bakhshiev et al.⁷ The nonexponential relaxation of the spectral center of mass has previously been observed for 4-aminophthalimide in glycerol, and it was attributed to a continuum of relaxation times as observed in dielectric relaxation experiments on glycerol. The continuous relaxation model was then modified by using an empirical relation for the distribution of relaxation times.¹⁸ Most of the characteristic features of our results can be qualitatively accounted for using the recent description of dipolar relaxations proposed by Weber. One characteristic feature of this model is the existence of many different orientations of solvent molecules at the instant of excitation rather than a distribution of relaxation times as previously proposed.¹⁸ By selecting different excitation wavelengths, it is thus possible to select probe molecules with different energies with the result that the emission spectrum will depend upon the excitation wavelength. Since the excited-state dipole is generally much larger than the ground-state dipole moment, as the time evolves, the emission spectra should narrow due to the increased interaction with the solvent molecules in the excited state as shown in our experiments. In summary, our results are most consistent with dipolar reorientation model proposed by Weber.¹¹ Quantitative conclusions regarding the microscopic details of the relevant rate processes require further development of this model.