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Fluorescence Lifetime of Acridine in Water-Organic Solvent Mixtures

Hiroshi KOKUBUN*1

Research Institute of Applied Electricity, Hokkaido University, Sapporo

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Fluorescence lifetime of acridine has been measured in water-ethanol and water-glycerol mixtures of different composition at 25°C. Remarkable decrease in lifetime value was found as the amount of organic component increases. The lifetime of 10.3 ns was obtained for the aqueous solution. The lifetimes in glycerol and in ethanol were determined as 3.2 and 0.7_2 ns, respectively. The observed ratio of the lifetime in the mixture to the lifetime in water, τ/τ_0, has the same functional dependence on solvent composition as the ratio of fluorescence yield, ϕ/ϕ_0. These quenching phenomena by organic molecules have been interpreted as due to the solvent effects upon the radiationless transition rate. Both static and dynamic mechanisms of quenching are not applicable in the present cases of solvent quenching.

Fluorescence properties of organic compounds in solution are frequently affected by a number of factors depending on the interaction with the solvent molecule. The interaction may be hydrogen-bonding, dipolar forces and so on. Acridine is one of the compounds whose fluorescence quantum yield is strongly dependent on the nature of the solvent. At room temperature acridine emits fluorescence only in the presence of hydroxylic molecule such as water, ethanol or acetic acid.[1] However, when the hydroxylic molecule is such that, like phenol or aniline, the proton donating group X–H is conjugated with an aromatic part, the activation of fluorescence does not occur.[2] The quantum yield of the fluorescence differs considerably from solvent to solvent even when active hydroxylic solvent is employed.

Bowen *et al.*[3,4] found a remarkable reduction of the fluorescence yield of acridine with increasing amount of organic component in water-organic solvent mixtures. For example, in the ethanol solution the quantum yield of the fluorescence is less than 1/10 of that in the aqueous solution. The organic solvent mixed with water shows an effective quenching upon the fluorescence. According to them, the enhanced fluorescence in water-containing solvents is due to the occurrence of the rapid hydrogen-bonding formation between the excited acridine and water, while in the ground state the hydrogen-bonding is negligible. However, this view can be easily ruled out, since the spectroscopic study shows that acridine in the ground state forms the hydrogen-bonded complex with ethanol and other protic molecule substances at rather low concentration of donor molecule.[1,2] In protic solvent acridine may be completely hydrogen-bonded with solvent molecule.

On the other hand, Porter and Vander Donckt[5] explained the low fluorescence yield in ethanol as due to a participation of photoreduction processes in the first singlet excited state. Our recent investigation shows that the contribution of photochemical reaction to the radiationless processes of excited acridine is of minor importance.[6]

For the purpose of establishing the mechanism for this interesting behavior of the fluorescence, measurements have been made of the fluorescence lifetime of acridine dissolved in water-glycerol and water-ethanol mixtures of various compositions. Comparative study of lifetime with spectroscopic and or fluorophotometric study can afford the decisive clue to resolve the mechanisms involved. For example, Schmillen[7] has successfully discriminated the static and dynamic mechanisms of the fluorescence quenching for several dyes.

Experimental

Absorption spectra were measured with a Hitachi EPS-II spectrophotometer. Fluorescence spectra were recorded on a modified Hitachi EPU spectrophotometer. Excitation light source was Ushio USH-100 high pressure mercury lamp combined with Toshiba UV-D1A and UV-35 filters. The spectral calibration factors of this photometer were obtained by the method of

*1 Present address: Department of Chemistry, Faculty of Science, Tohoku University, Sendai.

1) N. Mataga and S. Tsuno, This Bulletin, **30**, 368 (1957).

2) N. Mataga and S. Tsuno, *ibid.*, **30**, 711 (1957).

3) E. J. Bowen and J. Sahu, *J. Chem. Soc.*, **1958**, 3716.

4) E. J. Bowen, N. J. Holder and G. B. Woodger, *J. Phys. Chem.*, **66**, 2491 (1962).

5) E. Vander Donckt and G. Porter, *J. Chem. Phys.*, **46**, 1173 (1967).

6) K. Nakamaru, H. Kokubun and M. Koizumi, to be published.

7) A. Schmillen, *Z. Angew. Phys.*, **6**, 260 (1954).

Lippert *et al.*[8] and were applied to the raw data. Lifetime measurements were made with a phasefluorometer modulated at 10.7 MHz. This device is similar to that reported by Müller *et al.*[9] except for slight simplification and working frequency. The excitation light source was the same as employed in spectral measurements. The delaylines were calibrated with the aid of a standard signal generater and a frequency counter.

Acridine was recrystallized twice from 1 : 1 ethanol-water mixture. G. R. grade glycerol was distilled under reduced pressure prior to use. G. R. grade 99.5% ethanol was used without further purification. In order to avoid the protonation in the excited state,[10] the mixtures were prepared from the aqueous 0.02 N alkaline solution and the organic solution. The concentration of acridine was kept less than 10^{-5} mol/*l* for all solutions prepared, so that the system is free from the undesired concentration quenching. The effect of dissolved oxygen was examined for several solutions, and it was found that the oxygen quenching is negligible. The present results are those for the aerated solutions.

Results and Discussion

Results of lifetime measurements are presented in Fig. 1. As the amount of ethanol or glycerol increases, the lifetime decreases rapidly and then approaches a limiting value for both mixtures. The lifetime in the aqueous solution is 10.3 ns. The limiting values of 3.2 and 0.7_2 ns were obtained respectively for glycerol- and for ethanol-water mixtures. The value for the aqueous solution is close to the value of 13.8 ns obtained by Schmillen,[11] but is not in good agreement. The value of 2.3 ns for 50% ethanol solution is in complete agreement with that obtained by Birks and Dyson.[12] Berlman's value of 0.9 ns[13] for ethanol solution agrees well with the present result. No other literature value which can be compared with the results of the present work is vavailable.

The most striking feature of the present results for ethanol-water mixtures is that the ratio in lifetime, τ/τ_0, as a function of solvent composition is in good agreement with that of quantum yield, ϕ/ϕ_0, obtained by Bowen *et al.*[4] at 18°C except for the small deviation due to the different working temperature and experimental errors. The subscript 0 stands for the values in the aqueous solution. For glycerol-water mixtures the fit of τ/τ_0 curve to that of ϕ/ϕ_0 obtained by Bowen and Sahu[3] is less satisfactory. However, since they did not take into account the effect due to the protonation in the excited state, their results can not be compared strictly with the present results. Hence, it may be considered that the relation, $\tau/\tau_0=\phi/\phi_0$, holds also for glycerol-water mixtures.

In the usual way of interpreting the quenching mechanism, it is a reliable criterion for dynamic quenching that the ratios of quantum yield fit those of lifetime especially for low quecnher concentration. If this is the case, quenching process can be described by the Stern-Volmer equation

$$\phi_0/\phi = \tau_0/\tau = 1 + k\tau_0[Q], \qquad (1)$$

where k and [Q] are the rate constant of quenching process and the quencher concentration, respectively. For both mixtures studied remarkable deviations from the above equation were found as

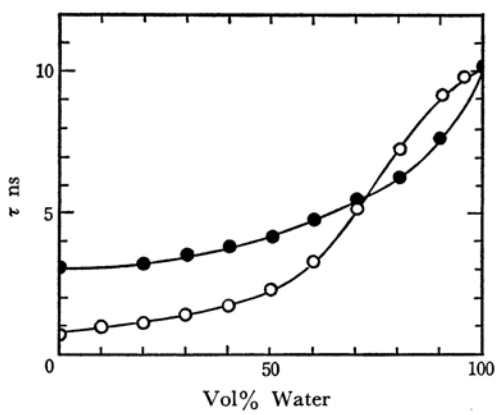

Fig. 1. Fluorescence lifetime of acridine in alcohol-water mixtures at 25°C.
—○—: Ethanol —●—: Glycerol

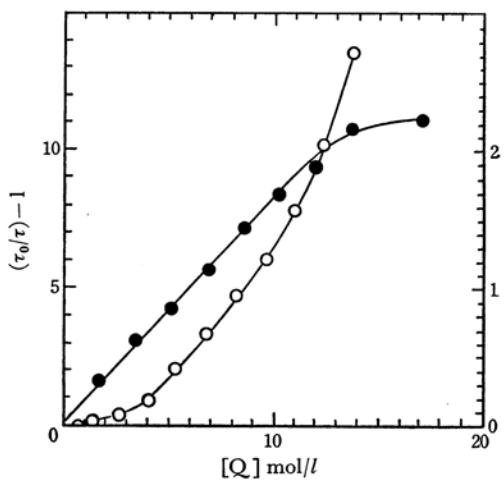

Fig. 2. Stern-Volmer plots.
—○—: Ethanol (left-hand scale)
—●—: Glycerol (right-hand scale)

8) E. Lippert, W. Nägele, I. Seibold-Blankenstein, W. Staiger and W. Voss, *Z. Anal. Chem.*, **170**, 1 (1959).
9) A. Müller, R. Lumry and H. Kokubun, *Rev. Sci. Instr.*, **36**, 1214 (1965).
10) A. Weller, *Z. Elektrochem.*, **61**, 956 (1957).
11) A. Schmillen, in Ref. 10.
12) J. B. Birks and D. J. Dyson, *Proc. Roy. Soc.*, **A275**, 135 (1963).
13) I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic Press, New York (1965).

shown in Fig. 2. In these mixtures, especially for glycerol-water mixtures, strong variation in viscosity might be a cause of the deviations found, since dynamic quenching is a diffusion controlled process. However, no significant relation was found between the apparent quenching constant obtained by the formal application of the Stern-Volmer equation and the viscosity of the corresponding solution. The transient effect is another conceivable factor which causes deviation from the Stern-Volmer equation. Deviation due to this effect is always upward. For glycerol-water mixtures deviation from linearity is contrary to the expected direction. For ethanol-water mixtures it is as expected. However, the value of the slope extrapolated to pure water is almost zero. This means that the dynamic part scarecely contributes to the entire quenching process. Furthermore, the very fact that τ/τ_0 coincides with ϕ/ϕ_0 even for the mixtures rich in organic component (*i. e.*, large quencher concentration), implies a negation of the participation of the dynamic mechanism in quenching process, since the quantum yield measurement detects both dynamic and transient contributions, while the lifetime measurement by phase shift method detects only dynamic contribution. Thus, in every respect it is conclusive that the dynamic collisional mechanism does not participate in the present quenching phenomena.

An alternative is the static quenching, in which the formation of non-fluorescent associate between fluorescer and quencher is an essential origin of quenching. In this case the fluorescence can be emitted only from the free molecule, and the quencher does not affect the decay process of the emitting molecule. No change in lifetime is expected. In the present case, however, we should consider an associate of lower fluorescence yield, *i. e.*, hydrogen bonded acridine with ethanol or glycerol, instead of completely non-fluorescent associate. The number of emitting species is two at least, if the static mechanism is involved in the present system. Although the lifetime to be observed should vary with quencher concentration, τ/τ_0 can in no way be identical with ϕ/ϕ_0. Furthermore, as shown in Fig. 3, the fluorescence spectrum of acridine shifts merely to blue continuously and becomes better structured gradually with the increasing amount of organic component. There is no evidence that the observed fluorescence spectra consist of two components. Hence the static mechanism in a simple form was also excluded.

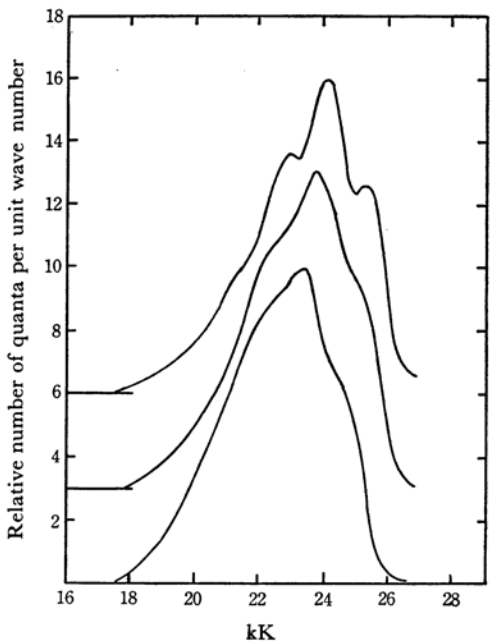

Fig. 3. Fluorescence spectrum of acridine.
Upper: in ethanol
Middle: in 50% ethanol
Lower: in water

Thus it appears that the present result is interpretable only in terms of the solvation in mixed solvents. In the aqueous solution acridine is solvated by water, and all the sites for solvation may be occupied by water molecules. By mixing an organic component into water, the organic molecules replace the water molecules on the solvation sites. This causes the changes in τ and ϕ which are given by

$$\tau = \frac{1}{k_f + k_q} \tag{2}$$

$$\phi = \frac{k_f}{k_f + k_q} \tag{3}$$

respectively. k_f is the rate constant for radiative process, and k_q is that for nonradiative.

Since the absorption intensity of acridine is practically constant in all the solutions studied, k_f can be regarded as constant throughout. Hence, it is clear that the reductions in τ and ϕ are attributed to the increase in k_q. This leads to $\tau/\tau_0 = \phi/\phi_0$ consistent with the experimental finding.

For convenience we write k_8 as a sum of two components,

$$k_q = k_{q0} + k_q(\varphi), \tag{4}$$

where k_{q0} is a radiationless rate constant in the aqueous solution and $k_q(\varphi)$ is the increment of radiationless rate constant upon addition of organic component as a function of volume fraction of organic solvent, φ. Assuming that the $k_q(\varphi)$ is proportional to the concentration, a, of the organic molecules on the acridine surface, we get

$$\frac{k_q(\varphi)}{k_{q\infty}} = \frac{a}{a_\infty} \tag{5}$$

where subscript ∞ describes the quantities for the state completely solvated by the organic molecules. For simplicity, only the first solvation sphere is considered. By means of the similar

procedure as in the treatment of monomolecular-layer adsorption isotherm, we get

$$\frac{a}{a_\infty} = \frac{\phi}{B + \varphi} \tag{6}$$

where B is a constant. Combining Eqs. (2), (4), (5) and (6), we get

$$\frac{\tau}{\tau_0 - \tau} = \frac{B}{k_q \tau_0 \varphi} + \frac{1}{k_{q\infty}\tau_0}. \tag{7}$$

This kind of treatment was first applied by Lippert and Moll[14] to the shift of fluorescence maximum of certain stilbene derivatives in organic mixed solvent.

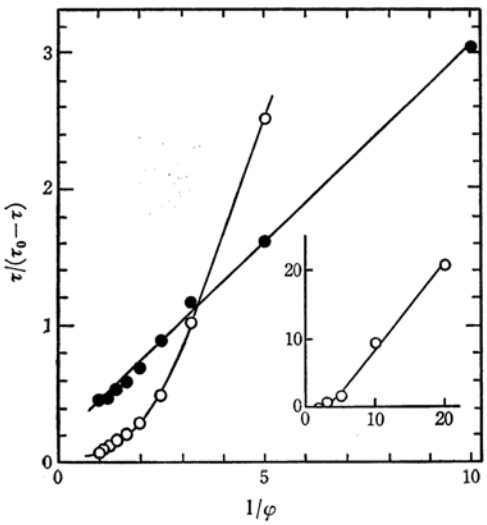

Fig. 4. $\tau/(\tau_0-\tau)$ *vs.* $1/\varphi$ plots.
—○—: Ethanol —●—: Glycerol

Plots of $\tau/(\tau_0-\tau)$ *vs.* $1/\varphi$ are given in Fig. 4. For water-glycerol mixtures the fit to Eq. (7) is quite satisfactory. However, for water-ethanol mixtures the deviation from Eq. (7) is appreciable. What kind of factors are responsible for such a discrepancy between ethanol and glycerol remain inexplicable. Taking the complex nature of these mixtures into account, no further attempt was made to find an equation of better fit.

Thus, the quenching action of organic solvent upon the acridine fluorescence has been interpreted as due to the enhanced radiationless transition produced by solvent effect. The mechanisms based on the simple collision involving two molecules and on the complex formation with definite composition have been completely precluded.

The present experimental data do not discriminate whether intersystem crossing or internal conversion is a dominant cause for the present significant radiationless process. According to Bowen *et al.*[4] the temperature dependence of k_q is well described by an Arrehnius equation for ethanol-water mixtures. The activation energy decreases with increasing ethanol fraction; from 6.59 kcal in 10% ethanol to 4.75 kcal in 50% ethanol solution. That the internal conversion requires activation energy of several kcal is highly improbable, since the density of states is very high. Furthermore, with the above fairly large activation energy the radiationless transition should be inhibited at moderate low temperature. These considerations suggest the minor importance of the internal conversion in the radiationless transitions at the first singlet excited state of acridine.

Although the position of the singlet n–π^* state is not known, it lies above ^1L_a π–π^* state[15] and may be dependent on the solvent composition. It is expected that the organic component lowers the energy of the $(n\text{–}\pi^*)^1$ state. This might be the origin of the solvent dependence of activation energy. The intersystem crossing through the $(n\text{–}\pi^*)^1$ state to the second $(\pi\text{–}\pi^*)^3$ state seems to be most probable.

In connection with present result, the author's attention was called to the work of Medinger and Wilkinson.[16] They found a remarkable deviation from the Stern-Volmer plots in the quenching of 9-phenylanthracene fluorescence in liquid paraffin by bromobenzene. They interpreted this deviation as a result of decreasing activity factor due to very high quencher concentration. However, it seems probable that the quenching by bromobenzene is not a collisional process. High quencher concentration decreases the viscosity of the solution appreciably, nevertheless the deviation from the Stern-Volmer equation is opposite as in the present acridine-glycerol-water system.

Changes in absorption and fluorescence spectra are very small, hence k_f is safely assumed to be practically constant. Equation (7) is easily modified for variables F/F_0 and [B] as follows:

$$\left(\frac{F_0}{F} - 1\right)^{-1} = \frac{C}{k_{q\infty}\tau_0[\mathrm{B}]} + \frac{1}{k_{q\infty}\tau_0} \tag{8}$$

where F/F_0 is the ratio of fluorescence intensity with the presence of bromobenzene to that without quencher, [B] is bromobenzene concentration and C is a constant. The values of F/F_0 found in literature were applied in Eq (8) and a straight line was obtained. Thus, the bromobenzene quenching is explained by the solvent effect upon the radiationless process.

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