

# Quenching of the Fluorescence of Ditolyl Aminoacridine Solutions by Tetrabromomethane

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**Abstract**—It has been shown that fluorescence quenching of 2,7-dimethyl-*N,N*-di-*p*-tolylacridine-9-amine (9-DTAA) solutions in hexane and acetone by tetrabromomethane (TBM) was dynamic in nature with the quenching rate constants of  $1.5 \times 10^{10}$  and  $0.6 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ , respectively. The difference in the constants was explained by the possibility of competition between the processes of the formation of solvation shell around the excited singlet 9-DTAA\* molecule and the formation of the 9-DTAA\*/TBM encounter complex.

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The phenomenon of fluorescence quenching of organic molecules by heavy atoms has been a subject matter of extensive research [1, 2–10]. Fluorescence quenching of the molecules in solutions in the presence of external heavy atoms is associated with an increase in the rate constant for radiationless intersystem crossing between the first singlet and the first or second triplet state of the molecule as a result of enhancement of spin–orbit interaction upon complex formation of the molecule with the quencher [1].

The use of tetrabromomethane as a fluorescence quencher for aromatic molecules, such as anthracene and pyrene, was described in [4, 6, 7]. It has been shown that quenching was considerably efficient in these cases and the bimolecular quenching rate constant was close to the diffusion-controlled value, although the complex formed between the fluorophore and TBM was most likely an encounter complex in nature because of the weak interaction between them.

We have investigated the fluorescent properties of the acridine dye 2,7-dimethyl-*N,N*-di-*p*-tolylacridine-9-amine, which is classified with molecules possessing an extended system of conjugated bonds and displays a distinct solvatochromic effect [11–13]. The maximum of the 9-DTAA fluorescence band is red-shifted and its intensity decreases on passing from nonpolar (hexane, cyclohexane) to polar (THF, acetonitril, acetone) solvents. Solvatochromic shifts are known to be due to significant changes in the intermolecular (dipole–dipole) interactions of the solvent with solute molecule during the transition to the excited state.

The objective of this work was to study the influence of solvent polarity on the fluorescence quenching of the solvatofluorochrome 9-DTAA by tetrabromomethane. Two solvents has been chosen (hexane

and acetone) that had approximately equal viscosities ( $\sim 0.3 \text{ cP}$ ) but significantly differed in polarity. Not only the positions of the excited singlet and triplet levels of 9-DTAA, but also the strength of the interaction of excited singlet 9-DTAA with the surrounding solvent molecules in the solvation shell should change as a result of the solvatochromic effect in polar acetone as compared to nonpolar hexane. It was interesting to find out how significantly this will influence the process of 9-DTAA fluorescence quenching by an external heavy atom, considering that both these factors could in principle considerably affect (probably in opposite directions) the ability of the heavy atom (bromine in this case) to increase the probability of the  $S_1-T_n$  intersystem crossing.

## EXPERIMENTAL

2,7-Dimethyl-*N,N*-di-*p*-tolylacridine-9-amine was synthesized at the Photochemistry Center, Russian Academy of Sciences [11]. To prepare solutions, solvents for UV spectroscopy from Aldrich,  $\text{CBr}_4$  (Acros organics), and polystyrene with an average molecular weight of 80 000 g/mol from Aldrich were used without additional purification.

The fluorescence spectra of the solutions were recorded on a Shimadzu RF-5301PC spectrofluorimeter. To avoid the concentration effects, the solutions contained the dye in a concentration of  $10^{-5} \text{ mol/l}$ . The fluorescence spectra were corrected in accordance with the sensitivity curve of the spectrofluorimeter emission channel that was obtained with the use of a Spectral Calibration Kit from Sigma-Aldrich [14].

The quantum yield was determined by the Parker–Rees method. A fluorescein (Aldrich) solution in 0.1 M NaOH with a quantum yield of  $\phi_f = 0.9$  [15] was used as a standard. The following equation was

used for calculation of the quantum yield:

$\varphi_c = \varphi_s \frac{A_s F_c (n_c)^2}{F_s A_c (n_s)^2}$ , where  $\varphi_s$  is the fluorescence quantum yield of the standard,  $A$  is the absorbance,  $F$  is the integral under the corrected fluorescence spectrum curve,  $n$  is index of refraction of the solution. The subscripts "s" and "c" refer to the standard and the test compounds, respectively.

Fluorescence lifetimes were measured using two methods, the phase modulation method and from the curves of fluorescence intensity decay after excitation of the sample with the femtosecond laser.

The measurements by the phase modulation technique were performed on a Horiba-Jobin-Yvon Fluorog Tau 3 system. An aqueous dispersion of amorphous silica LUDOX was used as a standard.

The system based on a Tsunami titanium–sapphire laser and a Spitfire regenerative amplifier was used as an excitation source in the second method. The second harmonic of the amplifier emission with a wavelength of 400 nm, a pulse duration of 130 fs, a pulse repetition rate of 5 kHz, and an average power of 50 mW was used for excitation. The system included an Acton Research SpectraPro 2300i automated grid monochromator, an Optoscope SCMU-ST streak camera with a time resolution 2 ps, and a Spec-10:256E CCD camera. The fluorescence lifetimes were determined from the decay curves. The decay curves were analyzed using the software based on the Levenberg–Marquardt algorithm.

## RESULTS AND DISCUSSION

Taking into consideration that oxygen along with TBM is an efficient dye fluorescence quencher [3], the effect of oxygen on the 9-DTAA fluorescence intensity in hexane and acetone solutions was studied first. The removal of oxygen by bubbling argon for 3 min in both cases resulted in an increase of the fluorescence intensity by factors of  $\sim 2.5$  and  $\sim 1.5$ , respectively. Since oxygen quenching is a diffusion-controlled process, such a significant intensity rise means that 9-DTAA fluorescence lifetimes in these solutions are sufficiently long for the molecule to participate in other processes controlled by diffusion.

To preclude the influence of oxygen on the fluorescence quenching process, all further measurements were performed in oxygen-free solutions.

The fluorescence spectra of 9-DTAA in hexane and acetone are presented in Fig. 1. The maximum of the fluorescence band shifts by 100 nm towards longer wavelengths (approximately  $4000 \text{ cm}^{-1}$ ) on passing from hexane to acetone. Obviously, such a decrease in the energy gap between the ground and first singlet excited state should have a substantial effect on the rate constants of all radiative and radiationless processes.

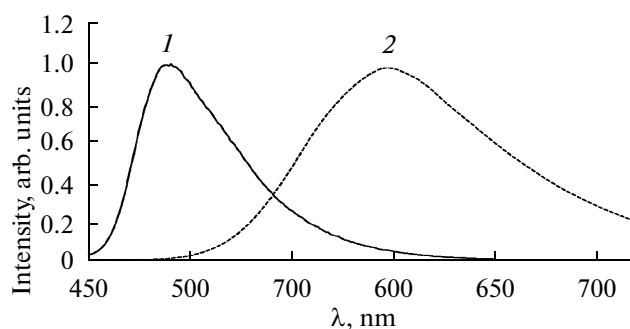


Fig. 1. Normalized fluorescence spectra of 9-DTAA in (1) hexane and (2) acetone ( $c = 10^{-5} \text{ mol/l}$ ).

The 9-DTAA fluorescence lifetimes and quantum yields were measured for the sake of comparison in acetone and cyclohexane, in which the fluorescence spectrum is practically identical to that in hexane.

The fluorescence lifetime measured in the cyclohexane solution by the phase-modulation method was  $\tau_0 = 31.5 \text{ ns}$ , and the quantum yield after oxygen removal was  $\varphi = 0.59$ . The respective values for acetone were  $\tau_0 = 11.9 \text{ ns}$  and  $\varphi = 0.09$ .

On the basis of the values obtained for the lifetimes and quantum yields, it is possible to calculate the radiative-transition rate constants and the sum of all constants. For cyclohexane,  $k_r = \varphi/\tau_0 = 0.59/31.5 \times 10^{-9} = 1.8 \times 10^7 \text{ s}^{-1}$ ,  $(k_r + k_{nr} + k_{isc}) = 1/\tau_0 = 1/31.5 \times 10^{-9} = 3.2 \times 10^7 \text{ s}^{-1}$ . For acetone,  $k_r = \varphi/\tau_0 = 0.09/11.9 \times 10^{-9} = 0.8 \times 10^7 \text{ s}^{-1}$ ,  $(k_r + k_{nr} + k_{isc}) = 1/\tau_0 = 1/11.9 \times 10^{-9} = 8.4 \times 10^7 \text{ s}^{-1}$ . Hence, the transfer to the polar solvent is accompanied by a decline in the radiative-transition rate constant and an increase in the sum of radiationless-transition constants.

In the first approximation, the presence of a heavy atom should vary the intersystem crossing rate only and it is its change that should be matched with the measured bimolecular quenching constant [5].

The Stern–Volmer plot for the 9-DTAA solution in hexane is presented in Fig. 2. From this figure we notice that an increase in the TBM concentration from 0.01 to 0.12 mol/l results in an almost 50-fold decrease in the fluorescence intensity. The Stern–Volmer constant calculated from the data presented in Fig. 2 is  $408 \pm 41 \text{ mol}^{-1}$ .

The fluorescence lifetime in hexane is 27.1 ns as measured by the phase-modulation method. The dependence of the  $\tau/\tau_0$  ratio on the concentration of the added TBM (at TBM concentrations 0.001–0.007 mol/l) for the oxygen-free 9-DTAA solution in hexane is presented in Fig. 3. As can be seen, the fluorescence lifetime in hexane is changed significantly (by a factor of 3.7 fold) from 27.1 to 7.3 ns by the addition of  $\text{CBr}_4$ . The calculated Stern–Volmer constant for the dependence of  $\tau/\tau_0$  on the TBM concentration is  $419 \pm 51 \text{ mol}^{-1}$ .

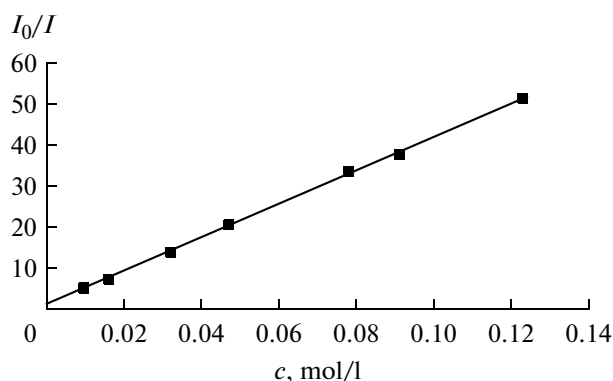


Fig. 2. Dependence of 9-DTAA fluorescence in hexane ( $c = 10^{-5}$  mol/l) upon the tetrabromomethane concentration.

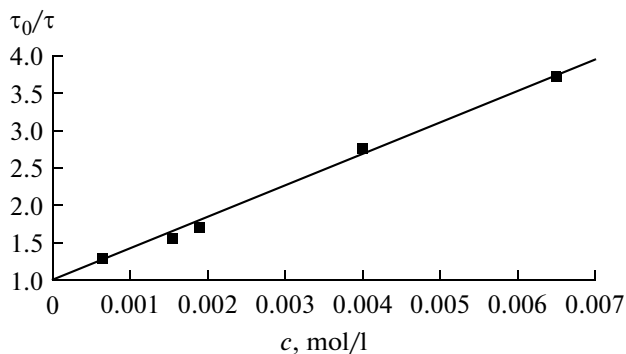


Fig. 3. Stern–Volmer plot for the 9-DTAA fluorescence lifetime in hexane upon quenching ( $c = 10^{-5}$  mol/l).

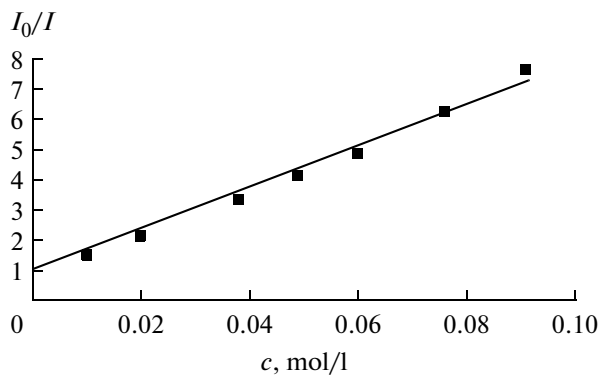


Fig. 4. Stern–Volmer plot for 9-DTAA fluorescence quenching in acetone ( $c = 10^{-5}$  mol/l) by tetrabromomethane.

Thus, the Stern–Volmer constants for  $\tau/\tau_0$  and  $I/I_0$  are almost identical in the case of 9-DTAA solutions in hexane, a fact that is known to be a signature of the dynamic nature of fluorescence quenching.

Based on the 9-DTAA fluorescence lifetime measured in the absence of the quencher, we calculated the value for the bimolecular quenching rate constant as  $1.5 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$ , which is close to the diffusion-controlled rate constant of  $2.3 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$  for hexane.

The change in the intersystem crossing rate constant for the TBM concentration of 0.07 mol/l could be estimated on the basis of lifetimes:  $\Delta k_{\text{isc}} = (13.7 - 3.7) \times 10^7 \text{ s}^{-1} = 10^8 \text{ s}^{-1}$ .

Data on 9-DTAA fluorescence quenching in the oxygen-free acetone solution are presented in Fig. 4. A much less significant effect (intensity declines by a factor of about 8) is observed when the TBM concentration increases over the same range (0.01–0.10 mol/l). The calculated Stern–Volmer constant is  $69 \pm 2 \text{ l mol}^{-1}$ .

As it has been mentioned above, the measured fluorescence lifetime in acetone is 11.7 ns (i.e., is less than that in hexane by a factor of 2.3).

The lifetimes for the 9-DTAA acetone solutions were determined from the fluorescence intensity decay curves using the streak camera with the sample excitation by femtosecond laser, because the fluorescence intensity for these solutions was found to be too low for accurate lifetime measurement in the presence of TBM by the phase modulation method. An example of the 9-DTAA fluorescence decay curve in pure deaerated acetone is given in Fig. 5. The decay curve is well approximated by a single-exponential function with the constant of 11.9 ns. Thus, the 9-DTAA fluorescence lifetimes measured by both methods in pure acetone are in good agreement.

It was established that in contrast to hexane solutions, 9-DTAA fluorescence lifetimes in acetone solutions are changed insignificantly by TBM addition, from 12 to 9.7 ns. The corresponding Stern–Volmer plot for  $\tau/\tau_0$  is presented in Fig. 6. Note that owing to the significant drop of the fluorescence signal in the acetone solutions, the signal-to-noise ratio also significantly decreases, thus resulting in an increase in the lifetime determination error. With allowance for this error, the measured Stern–Volmer constant was estimated at  $46 \pm 9 \text{ l mol}^{-1}$ .

Thus, the Stern–Volmer constant for  $\tau/\tau_0$  measured for 9-DTAA in acetone is less than that for  $I/I_0$ , a fact that suggests a certain contribution of static quenching. However, these differences are relatively small and can be associated with measurement errors. Thus, it can be concluded that 9-DTAA fluorescence quenching by tetrabromomethane is largely dynamic in nature in this case as well.

Having the 9-DTAA fluorescence lifetime in pure acetone measured, we calculated the quenching rate

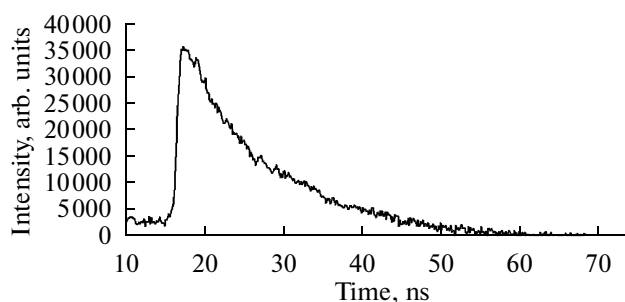


Fig. 5. Decay curve for the 9-DTAA fluorescence in acetone ( $c = 10^{-5}$  mol/l).

constant as  $0.6 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$ , which is less than the this constant in hexane by a factor of 2.5.

On the basis of the measured lifetimes, the change in the intersystem crossing rate constant for the 0.08 M TBM solution can be estimated:  $\Delta k_{\text{isc}} = (10.3 - 8.4) \times 10^7 \text{ s}^{-1} = 1.9 \times 10^7 \text{ s}^{-1}$ . Thus, the calculated change  $\Delta k_{\text{isc}}$  for the same concentration of the quencher correlates with the change in the quenching rate constant upon passing from hexane to acetone.

The obtained value of the bimolecular quenching rate constant for 9-DTAA in hexane is close to the values obtained for pyrene fluorescence quenching by tetrabromomethane in a methylcyclohexane solution under normal conditions [7] and anthracene fluorescence in supercritical  $\text{CO}_2$  [4] ( $1.17 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$  and  $2.17 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$ , respectively). The explanation of the observed relations presented in these studies is based on the concept of the encounter complex formed by the excited molecule and the quencher and the subsequent transformation of the complex to an exciplex. Obviously, a similar model can be accepted as a starting point in the case of 9-DTAA in hexane and acetone.

In terms of this model, the obtained smaller (by a factor of 2.5) value of the bimolecular quenching rate constant in acetone means that the quenching efficiency upon 9-DTAA\*/TBM encounter complex formation between the excited 9-DTAA molecule and the TBM molecule in acetone is significantly less compared with hexane. It can be suggested that this difference is due to the stronger interaction of 9-DTAA\* with the surrounding acetone molecules as compared with the similar interaction of 9-DTAA\* with hexane molecules.

The specific feature of the 9-DTAA molecule is the presence of the nitrogen atom with the lone electron pair in the heterocycle, which leads to the rise of the dipole moment even in the ground electronic state. The magnitude of the dipole moment significantly increases on excitation owing to electron density transfer from the substituent (ditolyl amine) to the nitrogen atom of the heterocycle [11–13]. According to the theory of the solvatochromic effect, the increase of the fluorophore dipole moment in

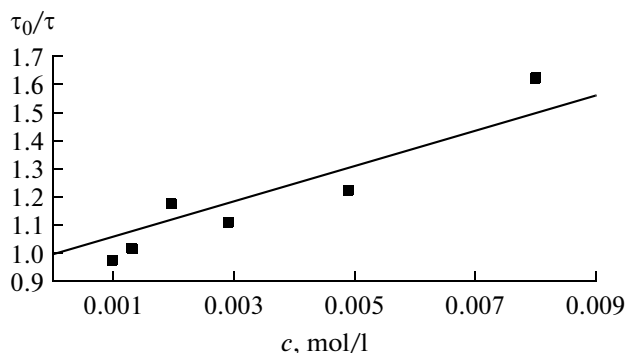


Fig. 6. Stern–Volmer plot for the 9-DTAA fluorescence lifetime in acetone upon quenching ( $c = 10^{-5}$  mol/l).

polar solvents in contrast to the non-polar should result in the rearrangement of the solvation shell made by solvent molecules around the fluorophore molecule in the excited singlet state (acetone molecules surrounding 9-DTAA\* in the given case).

The heavy-atom effect is characterized by the exponential dependence on the distance between the components of the fluorophore/quencher complex, since the intersystem crossing rate constant does not change unless the wave functions of the fluorophore and quencher molecules overlap [9].

Proceeding from the fact that the formation of both the solvation shells by solvent molecules around 9-DTAA\* and the encounter complexes with quencher molecules should occur within the time shorter than the fluorescence lifetime, it may be supposed that there is a competition between these processes in the polar solvent, which is absent in the non-polar solvent. The effective average distance in the 9-DTAA/TBM encounter complexes is slightly larger in acetone, as compared with hexane, because of this competition resulting in the observed lower quenching efficiency in acetone relative to hexane.

## CONCLUSIONS

It has been shown that 9-DTAA fluorescence quenching by tetrabromomethane in hexane and acetone solutions is dynamic in nature with the bimolecular quenching rate constants of  $1.5 \times 10^{10}$  and  $0.6 \times 10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$ , respectively. It has been suggested that the diminished quenching efficiency in acetone as compared with hexane is due to the possible competition between the process of solvation shell formation around the 9-DTAA molecule in the singlet excited state and the process of the 9-DTAA\*/TBM encounter complex formation. The obtained results could be used for the interpretation of the heavy atom quenching effects in other polar solvents.

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## REFERENCES

1. McGlynn, S.P., Azumi, T., and Kinoshita, M., *Molecular Spectroscopy of the Triplet State*, New Jersey: Prentice-Hall, 1969.
2. Martinho, J.M.G., *J. Phys. Chem.*, 1989, vol. 93, p. 6687.
3. Tanielian, C. and Wolff, C., *J. Phys. Chem.*, 1995, vol. 99, p. 9831.
4. Zhang, J., Roek, D.P., Chateaneuf, J.E., and Brennecke, J.F., *J. Am. Chem. Soc.*, 1997, vol. 119, p. 9980.
5. Berberan-Santos, M.N., *Phys. Chem. Commun.*, 2000, vol. 3, no. 5, p. 18.
6. Okamoto, M., *J. Phys. Chem. A*, 2000, vol. 104, p. 5029.
7. Okamoto, M., *J. Phys. Chem. A*, 2000, vol. 104, p. 7518.
8. Martinho, J.M.G., Reise Sousa A.T., Oliveira Torres M.E., and Fedorov A., *Chem. Phys.*, 2001, vol. 264, p. 111.
9. Rae, M., Fedorov, A., and Berberan-Santos, M.N., *J. Chem. Phys.*, 2003, vol. 119, no. 4, p. 2223.
10. Chmyrov, A., Sanden, T., and Widengren, J., *J. Phys. Chem.*, 2010, vol. 114, p. 11282.
11. Sazhnikov, V.A., Khlebunov, A.A., and Alfimov, M.V., *Khim. Vys. Energ.*, 2007, vol. 41, no. 1, p. 28 [*High Energy Chem.*, 2007, vol. 41, no. 1, p. 25].
12. Nikitina, E.A., Odinokov, M.A., Grigoriev, F.V., Basilevsky, M.V., Khlebunov, A.A., Sazhnikov, V.A., and Alfimov, M.V., *J. Phys. Chem., B*, 2007, vol. 111, p. 3953.
13. Samsonova, L.G., Selivanov, N.I., Kopylova, T.N., Artyukhov, V.Ya., Maier, G.V., Plotnikov, V.G., Sazhnikov, V.A., Khlebunov, A.A., and Alfimov, M.V., *Khim. Vys. Energ.*, 2009, vol. 43, no. 2, p. 149 [*High Energy Chem.*, 2009, vol. 43, no. 2, p. 105].
14. Galanin, M.D., Kut'enkov, A.A., Smorchkov, V.N., Timofeev, Yu.P., and Chizhikova, Z.A., *Opt. Spektrosk.*, 1982, vol. 53, no. 4, p. 683.
15. Pfeifer, D., Hoffmann, K., Hoffmann, A., Monte, C., and Resch-Genger, U., *J. Fluoresc.*, 2006, vol. 16, p. 581.