Photochemistry and Photobiology, Vol. 32, pp. 297 to 303
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0031-8655/80/0901-0297$02.00/0

USEFUL PHOTOLUMINESCENCE PROBES OF MICELLAR SYSTEMS—CYCLIC AZOALKANES AS FLUORESCENCE ACCEPTORS AND 1,5-DIMETHYLNAPHTHALENE AS A FLUORESCENCE DONOR

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(*Received* 8 *February* 1980; *accepted* 1 *April* 1980)

Abstract—Certain cyclic azoalkanes are shown to exhibit substantial fluorescence intensity and attractive absorption parameters in detergent solutions. Quenching of the fluorescence of detergent solutions of 1,5-dimethylnaphthalene by these bicyclic azoalkanes has been investigated and is found to provide a useful system to study properties of micellar systems.

INTRODUCTION

During the past several years a number of reports have appeared which describe the utilization of photoluminescence probes for the investigation of the composition, structure and dynamics of micellar systems (Fendler and Fendler, 1975; Mukerjee *et al.*, 1977). Both fluorescence (Thomas, 1977; Turro *et al.*, 1979; Dorrance and Hunter, 1972) and phosphorescence (Turro and Yekta, 1978; Escabi-Perez *et al.*, 1977; Almgren *et al.*, 1979) probes have been effective in the evaluation of micellar parameters.

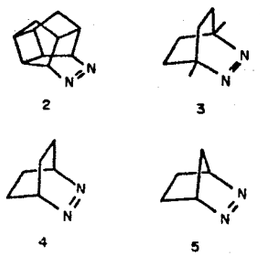
Knowledge of solute binding constants (K) is important for the development of quantitative theory of the solubilizing action of micelles. Knowledge of solute rate constants for entrance (k_i) into and exit (k_s) from micelles is essential for development of a quantitative understanding of the dynamics of solubilization. In most cases studied, the photoluminescent probe, or a quencher, or both have been completely micellized. The use of Poisson statistics has proven to be generally applicable and effective in interpreting the results of such investigations (Almgren *et al.*, 1979; Yekta *et al.*, 1979; Atik *et al.*, 1979; Dederen *et al.*, 1979).

We report here an investigation of the quenching of a completely micellized fluorescent probe (1,5-dimethylnaphthalene, **1**) by a series of cyclic azoalkanes (**2**, **3**, **4**, and **5**) that exist to a significant extent in both aqueous and micellar environments.

MATERIALS AND METHODS

Hexadecyltrimethylammonium bromide (HDTBr) and sodium dodecyl sulfate (SDS) were washed several times with ether and recrystallized from 95% EtOH two times. Compound **1** (Chemical Samples Co.) was purified by sublimation *in vacuo*.

The structures of the azoalkanes used as quenchers in this work are given in Scheme 1. The azoalkanes were prepared as previously described. (Mirbach *et al.*, 1978).



The emission spectra were recorded with a Hitachi Perkin-Elmer MPF-2A spectrofluorometer. Fluorescence quantum yields were determined by comparing the corrected fluorescence spectra with the corrected fluorescence spectrum of quinine sulfate after the method reported in a previous paper (Mirbach *et al.*, 1978). Lifetimes were measured using the time-correlated single-photon counting technique. The design and performance of the instrument have been described in a previous paper (Turro *et al.*, 1979).

RESULTS

Spectroscopic properties

The absorption spectra of 1,5-dimethylnaphthalene and **2**–**5** are shown in Fig. 1 (all were obtained in 0.02 *M* HDTBr solution). Table 1 summarizes the absorption and emission parameters of **2**–**5**. As can be seen from Fig. 1, the absorption spectra of the series of bicyclic azoalkanes **2**–**5** display weak absorption in the region from 280 to 320 nm, thereby providing "optical windows" for selective excitation of donors such as **1** which possesses strong absorption in this region.

Since 1,5-dimethylnaphthalene possesses a very low solubility in water, its fluorescence is detectable only in the micellar phase. Compounds **2**–**4** exhibit relatively strong fluorescence in micellar solution ($\Phi_f \simeq 0.3$–0.5), while **5** shows only very weak

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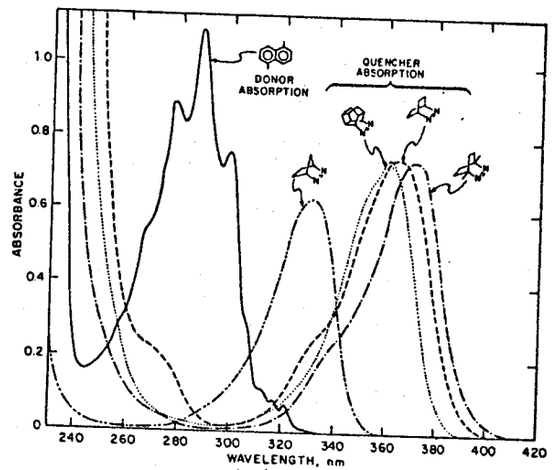


Figure 1. Absorption spectra of **1** and azoalkanes **2**–**5** in 0.02 *M* HDTBr solution.

emission. As was shown in a previous paper (Mirbach *et al.*, 1979), the lowest $n - \pi^*$ absorption band of these azocompounds shows vibration in structure in nonpolar organic solvents but is broad and poorly resolved in aqueous solution. A comparison of the spectral characteristics in micellar solution with those observed in water and hydrocarbon solvents given by Table 1 indicates that, on the average, the "organic" bicyclic azoalkanes in micellar solution are in a water-like environment rather than a hydrocarbon-like environment.

Table 1. Absorption and emission properties of cyclic azoalkanes in micelles‡ and fluid solutions

Compound	Solvent	Absorption§		Fluorescence		
		λ_{max}	ϵ_{max}	λ_{max}	τ_F (nsec)	ϕ_F
2	SDS	363	129	394	110	0.36
	HDTBr	361	112	393	100	0.32
	H_2O	358†	141	395†	275†	0.63†
	Hexane	373†	250†	378†	70†	0.13†
3	SDS	373	65	422	360	0.3
	HDTBr	373	65	422	320	0.25
	H_2O	371†	—	425†	335†	0.3†
	Hexane	383	192†	387†	420†	0.2†
4	SDS	365	69	418	288	0.46
	HDTBr	365	65	418	268	0.43
	H_2O	364†	66	416†	306(410†)	0.5†
	Hexane	377†	193†	405†	330†	0.20†
5	SDS	331	96			
	HDTBr	331	107	Too weak to measure accurately		
	H_2O	331	88			
	Hexane	341†	420†			

†Reference (Mirbach *et al.*, 1978).
‡The concentrations of detergents (SDS and HDTBr) are 0.020 *M*.
§λ_{max} and ϵ_{max} of the absorption spectra are for the lowest excited singlet state ($n\pi^*$) of each compound.

Typical results of quenching of the fluorescence intensity of **1** by azoalkanes in micellar solution are given in Figs 2 and 3. Figure 2 shows the change of the fluorescence intensities of donor *D* and quencher *Q* as a function of quencher concentration [*Q*] at fixed donor and detergent concentration [*Det*]. The sensitized fluorescence of **3** is seen to increase as a function of increasing the concentration of **3**. An isoemissive point near 387 nm is apparent.

Figure 3 shows the change of the fluorescence intensity as a function of [*Det*] at fixed donor and quencher concentration, when **2** is employed as a quencher. Decreasing of the detergent concentration gives the same results as increasing the concentration of quencher. An isoemissive point near 370 nm is observed.

The mean occupancy number of quencher $\langle q \rangle$ bound per micelle *M* is expressed using the equilibrium constant *K* between micelle and quencher molecule as follows (Almgren *et al.*, 1979; Yekta *et al.*, 1979)

$$\langle q \rangle = K[Q]/(1 + K[M]) \qquad (1)$$

The probability distribution of quencher P_q among micelles is assumed to be based on Poisson statistics.

$$P_q = (\langle q \rangle^q/q!)e^{-\langle q \rangle} \qquad (2)$$

Equation 1 shows that the decrease of [*M*] ($= [Det]/\bar{n}$; $\bar{n}$ is the mean aggregation number per micelle) as well as the increase of [*Q*] results in the increase of $\langle q \rangle$ and consequently, increases the prob-

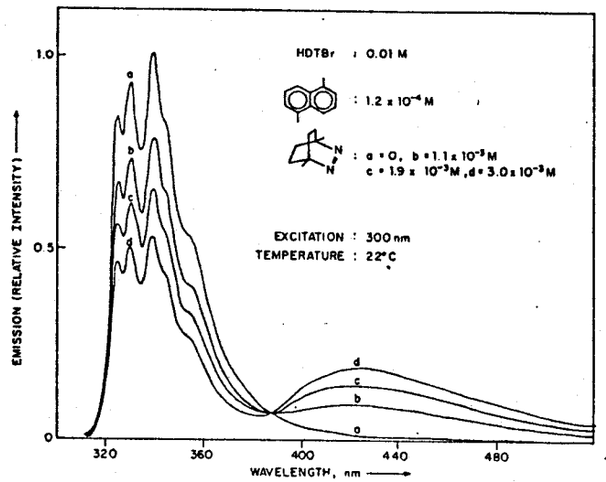


Figure 2. Fluorescence spectra (uncorrected for instrument response) of 1-azocompound 3 system obtained by excitation at 300 nm in 0.01 *M* HDTBr solution at 22°C. The concentration of 3: (a) 0; (b) 1.1 *M*; (c) 1.9 m*M*; (d) 3.0 m*M*.

ability of finding a quencher in a given micelle. Thus, the observed fluorescence intensity changes as a function of [*Q*] (Fig. 2) and of [*Det*] (Fig. 3) correspond well to the behavior of partitioning for quenchers, which is predicted by Eq. 1.

The *D*–*Q* systems using cyclic azoalkanes as quenchers of 1 provide a representative example for testing the partitioning of substrates among micelles.

Fluorescence lifetime studies

Typical results of quenching of the fluorescence lifetime of 1 by azoalkanes in micellar solution are given in Fig. 4, where 4 is employed as a quencher. For comparison, data for the quenching of the fluorescence lifetime of 1 by 4 in isooctane solvent are given in Fig. 5. For this fluid solution, the plots of the

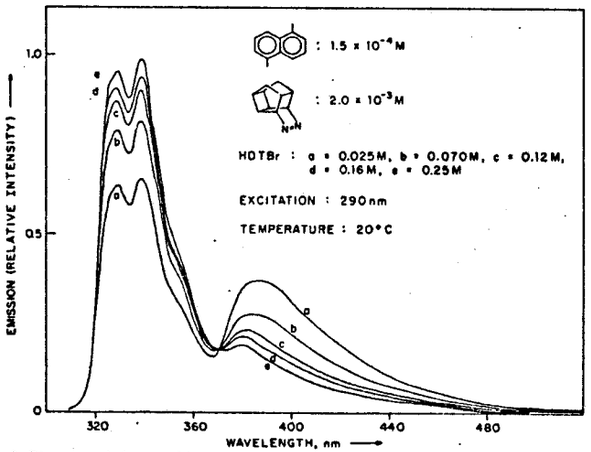


Figure 3. The spectral change of 1-azocompound 2 system as a function of HDTBr concentration. The concentration of HDTBr: (a) 0.025 *M*; (b) 0.07 *M*; (c) 0.12 *M*; (d) 0.16 *M*; (e) 0.25 *M*.

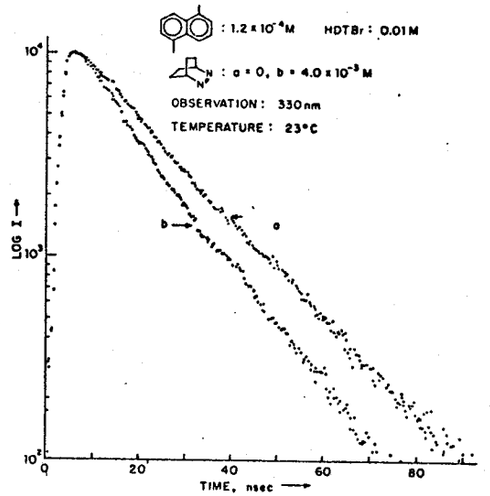


Figure 4. The changes in the lifetime of 1 as a function of 4 in 0.01 *M* HDTBr solution at 23°C. (Peak counts are normalized).

ratio of fluorescence intensity (I_0/I) and lifetime (τ_0/τ) in the absence and presence of quencher vs the concentration yield the same slopes, from which a quenching rate constant of $1.0 \times 10^{10}\ M^{-1}s^{-1}$ is derived. This value is close to the diffusion controlled rate constant in isooctane solution.

On the other hand, the results in micellar solution are different from those found in ordinary fluid solution. Plots of I_0/I do not yield straight lines but deviate upwards for each quencher studied. At low detergent concentration, the measured lifetimes decrease slightly with increasing the quencher concentration (Fig. 4). The plots of τ_0/τ as a function of quenchers, however, give straight lines. However, the change of the lifetime is very small compared with the change of fluorescence intensity (Fig. 7a). The lifetime

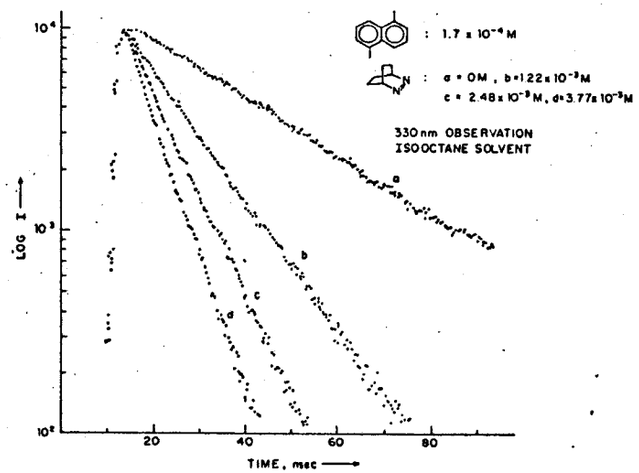


Figure 5. The changes in the lifetime of 1 as a function of 4 in isooctane solution at 23°C.

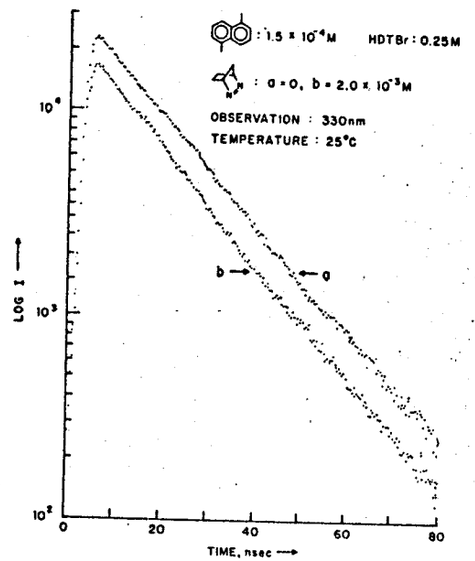


Figure 6. Variation in the lifetime of **1** as a function of **5** in 0.25 *M* HDTBr solution at 25°C. (Calculated lifetimes are (a) 16.4 ns and (b) 15.6 ns, respectively.) Accumulation times of both decay curves are the same.

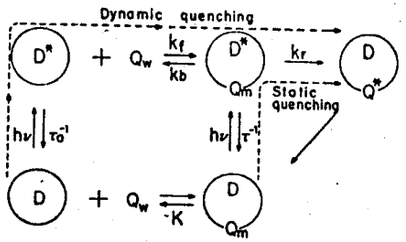
change is also smaller when detergent concentration increases.

The lifetime changes with and without quencher at high detergent concentration are also shown in Fig. 6, where **5** is employed as a quencher. The plots of log *I* vs *t* give good straight lines. If intramicellar dynamic quenching takes place, one would expect multiexponential decay at shorter times and especially at higher concentration of detergent (Yekta *et al.*, 1979; Turro *et al.*, 1979). Such behavior was not observed for the present donor–acceptor systems; i.e. only clean single exponential decays were found. It is important to note that the lifetime of **1** with and without azoalkane are almost the same at higher detergent concentration (~ 0.25 *M*), even though the emission intensities decrease drastically.

Quenching data based on lifetime and also of fluorescence intensity measurements suggest that only static quenching takes place inside of the micelle and that the quencher is partially micellized. In such a case, a model for the luminescence will be represented as shown in Scheme II. In Scheme II, the circles symbolize micelles, and Q_W and Q_M represent water and micelle solubilized quenchers, respectively. The forward and back rate constants of micellization are k_f and k_b, respectively, and k_r is the unimolecular reactive rate constant of quenching in the micelle.

The change of the lifetime is given by Eq. 3 (Yekta *et al.*, 1979; Aikawa *et al.*, 1979).

$$\tau^{-1} = \tau_0^{-1} + k_f[Q_W] \qquad (3)$$



where

$$[Q_W] = [Q]/(1 + K[M]) \qquad (4)$$

Here $[Q]$ and $[Q_W]$ represent the total quencher concentrations and the quencher concentration in bulk aqueous phase, respectively. The shorter lifetime at low concentration in the water phase is predicted by Eq. 3, and consequently by an increase in the contribution of dynamic quenching.

DISCUSSION

Although quenching data derived from luminescence intensity (static quenching) can be analyzed by a quenching equation based on Scheme II, we shall discuss only the evaluation of micelle parameters from the aspect of dynamic quenching here.

A combination of Eqs 3 and 4 gives eq. 5 which is useful for obtaining some important parameters of micelle aggregates.

$$\frac{1}{\tau^{-1} - \tau_0^{-1}} = \frac{1}{k_f[Q]}(1 + K[M])$$

$$\simeq \frac{1}{k_f[Q]}\left(1 + \frac{K}{\bar{n}}[Det]\right) \qquad (5)$$

The plot of $(\tau^{-1} - \tau_0^{-1})^{-1}$ vs $[Det]$ at fixed quencher concentration yields the values of k_f and K from knowledge of aggregation number $\bar{n}$. The analysis of the data using only dynamic quenching, however, will include errors when static quenching takes place predominantly if the change of lifetime is small.

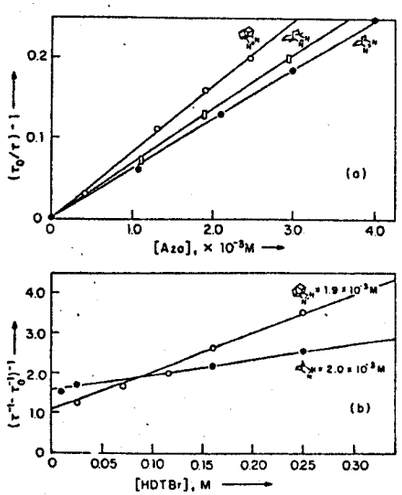


Figure 7. (a) The plots of τ_0/τ against $[Azo]$ in 0.01 *M* HDTBr solution at 22°C. (b) The plots of $(\tau^{-1} - \tau_0^{-1})^{-1}$ against [HDTBr] at fixed donor (~ 0.15 m*M*) and quencher concentrations at 23°C.

Table 2. Micelle parameters obtained for HDTBr solution

Compound	$K(M^{-1})$†	$k_f(M^{-1}s^{-1})$	$k_b(s^{-1})$
2	520	4.9×10^9	1.1×10^7
3	164	3.9×10^9	3.4×10^7
4	63	3.4×10^9	5.4×10^7

†$\bar{n} = 72$ was used for evaluation of the micelle concentration. Temperature ~ 23°C.

Figure 7a shows that a plot of our data according to Eq. 5 yields a straight line. The change in lifetime as a function of $[Q]$ at fixed detergent concentration is also shown in Fig. 7b. From knowledge of K and k_f, the exit rate of substrate from micelles (k_b) is evaluated. The values of the parameters thus obtained are summarized in Table 2.

The values of k_f do not show a substantial difference with changing of the quencher and are close to those expected for the diffusion rate constant in water phase.

The values of K and k_b of cyclic azoalkanes serve as a measure of the hydrophilic character of these compounds. For example, upon decreasing the number of carbon atoms in the azoalkane molecules from 10 for **2** to 6 for **4**, the value of K decreases from 520 M^{-1} to 63 M^{-1} and the residence time (k_b^{-1}) decreases from 91 ns to 18.5 ns in HDTBr solution (see Table 2). It has been shown that various aromatic compounds show large K values (e.g. benzene; 9.3×10^3 M^{-1}, naphthalene; 9.1×10^4 M^{-1} and pyrene; 1.02×10^7 M^{-1} in HDTBr solution) and small exit rates (on the order of 10^5–10^3 $M^{-1}s^{-1}$ for these aromatics in HDTBr solution) (Almgren *et al.*, 1979). Comparison of K and k_b values of cyclic azoalkanes with those of aromatic compounds yield a general trend of the hydrophilic nature of the former species. The dynamic character of solubilization also provides an explanation for the fact that the spectroscopic properties of the "organic" bicyclic azoalkanes indicate that they reside in a rather water-like environment in micellar solution.

CONCLUSION

The fluorescence of **1** in both anionic and cationic micellar solution is quenched by bicyclic azoalkanes which are distributed both in the micellar phase (static quenching) and in the bulk aqueous phase (dynamic quenching). The model used to analyze the quenching data is based on Poisson statistics of solubilized donor molecules among micellar aggregates. When applied to appropriate *D*–*Q* systems, the model yields dynamic parameters such as binding constants, residence times of the quenchers and aggregation number.

Acknowledgements—The authors thank the National Institutes of Health for its generous support of this work. A. Y. thanks the Arya-Mehr University of Technology and the Iranian Ministry of Sciences for a Visiting Research Fellowship.

REFERENCES

Aikawa, M., A. Yekta and N. J. Turro (1979) *Chem. Phys. Lett.* **68**, 285–290.
Almgren, M., F. Grieser and J. K. Thomas (1979) *J. Am. Chem. Soc.* **101**, 297–291.
Almgren, M., F. Grieser and J. K. Thomas (1979) *J. Am. Chem. Soc.*, **101**, 2021–2026.
Atik, S. S., M. Nam and L. A. Singer (1979) *Chem. Phys. Lett.* **67**, 75–80.
Dederen, J. C., M. van der Auweraer and F. C. deSchryver (1979) *Chem. Phys. Lett.* **68**, 451–454.
Dorrance, R. C. and T. F. Hunter (1972) *J. Chem. Soc., Faraday Trans. I.* **68**, 1312–1321.
Escabi-Perez, J. R., F. Nome and J. H. Fendler (1977) *J. Am. Chem. Soc.* **99**, 7749–7754.
Fendler, J. H. and E. J. Fendler (1975) *Catalysis in Micellar and Macromolecular Systems.* Academic Press, New York.
Mirbach, M. J., K. C. Liu, M. F. Mirbach, W. R. Cherry, N. J. Turro and P. S. Engel (1978) *J. Am. Chem. Soc.* **100**, 5122–5129.
Mukerjee, P., J. R. Cardinal and N. R. Desai (1977) *Micellization, Solubilization and Microemulsions.* (Edited by K. L. Mittal), Vol. I. Plenum Press, New York.
Thomas, J. K. (1977) *Acc. Chem. Res.* **10**, 133–138.
Turro, N. J., M. Aikawa and A. Yekta (1979) *Chem. Phys. Lett.* **64**, 473–478.
Turro, N. J. and A. Yekta (1978) *J. Am. Chem. Soc.* **100**, 5951–5952.
Yekta, A., M. Aikawa and N. J. Turro (1979) *Chem. Phys. Lett.* **63**, 543–548.