

FLUORESCENCE QUENCHING IN DODECYLAMMONIUM PROPIONATE REVERSED MICELLES

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Summary

Depending on the kind of quencher (capacity and concentration), multi-exponential decay curves are obtained which can be analysed by a four-parameter equation, on the assumption that ion exchange between micelles is possible.

The occurrence of probe exchange by intermicellar collisions is also suggested.

1. Introduction

Until recently [1, 2], all the decay curves of fluorescence probes quenched by ions in reversed micelles have been reported to be mono-exponential [3 - 6]. Rodgers and Becker [1] first obtained complex decay curves which were analysed as a sum of two exponentials. Atik and Thomas [2] fitted the non-exponential decay curves to a four-parameter equation. In this paper we independently used a four-parameter equation which was based on the kinetic scheme used for quenching in aqueous micelles [7 - 9]. Depending on the water concentration, this scheme enabled us to determine experimentally the mean aggregation number of dodecylammonium propionate (DAP) micelles and the rate constants of quenching and ion exchange.

Deviations from exponentiality were not observed if the quencher capacity or the quencher concentration was too low. Only the apparent quenching constant can then be calculated from the Stern-Volmer equation. For this reason the better quencher I^- was chosen instead of Br^- .

2. Experimental details

DAP was prepared by the method of Kitahara [10]. The solvents *n*-hexane (Merck Uvasol) and cyclohexane (cyclo- C_6H_{12}) (Riedel-de Haen AG. Spectranal) were distilled from sodium wires and stored over Linde-type

4 Å molecular sieves. KI (UCB, p.a.) and NaNO₃ (Merck, p.a.) were used as received.

1-methylnaphthalene (1-MeN) was purchased from Aldrich and purified by vacuum distillation from CaH₂. 2-(1-naphthyl)acetic acid (NAA) (Aldrich) was used after repeated sublimation. The purity of NAA was checked by reversed-phase high pressure liquid chromatography. Solubilization of water (with or without solubilized quenchers) and of the probes ((1 - 4) × 10⁻⁵ M) in the micellar systems was effected by sonication. All solutions were degassed by repeated freeze-pump-thaw cycles.

Fluorescence lifetime measurements were made by the single-photon counting method. The non-exponential decay curves were analysed by a four-parameter non-linear least-squares program with convolution.

3. Results and discussion

In this work the quenching of 1-MeN and NAA by KI or NaNO₃ has been studied in reversed micellar systems of DAP (0.04 - 0.20 M) and water (0.055 - 1.10 M) in cyclo-C₆H₁₂.

When the total quencher concentration is very low (less than 10⁻⁴ M), the decay curves are in all cases mono-exponential. Above a total quencher concentration of 5 × 10⁻⁴ M, there are two possibilities.

(1) If the probe is not associated with the micelle, as in the system 1-MeN-cyclo-C₆H₁₂-DAP(0.08 M)-H₂O(0.11 - 0.55 M)-KI, the decay curves are mono-exponential, and the apparent quenching constant k_{qm} can be obtained from the Stern-Volmer equation: the probe 1-MeN "sees" a time-averaged quencher concentration. The rate constant k_{qm} decreases with the water concentration (Table 1). This is in agreement with the experiments

TABLE 1

The influence of [H₂O] on the value of k_{qm} obtained for the quenching of 1-methylnaphthalene by KI ((0.25 - 2) × 10⁻³ M) in the system 0.08 M DAP-cyclo-C₆H₁₂

[H ₂ O] (M)	0.11	0.22	0.33	0.44	0.55
k_{qm} (× 10 ¹⁰ M ⁻¹ s ⁻¹)	1.28	0.83	0.43	0.43	0.22

of Wong and Thomas [4] for the quenching of pyrene with Cu²⁺ and I⁻ in the micellar system aerosol OT (AOT)-H₂O-heptane. If the water concentration increases, the aggregation number N_{agg} also increases, the micelles become more structured and the apolar chains become closer to each other. Hence the possibility that the probe in the cyclo-C₆H₁₂ phase can approach a quencher in the water phase diminishes, resulting in an initial decrease in k_{qm} . From the moment of maximal hydration ($R = [H_2O]/[DAP] = 4$ [6]), we may assume that the distance between two polar head groups and thus between two apolar chains becomes constant [11]. This can explain why the decrease

in k_{qm} is less if $[H_2O] > 0.32$ M. From this concentration, the decrease in k_{qm} is probably mainly due to a higher quencher mobility (quenchers are less strongly adsorbed on the micellar surface) and an increase in the hydrophilic nature of the micellar core (1-MeN is hydrophobic) [3, 4].

(2) If the probe is associated with the micelle, the decay curves become more exponential. On the assumption that the probe cannot leave the micelle during its lifetime, the different processes which can contribute to such a decay may be the following: (a) the transition of a quencher from the free water volume (unbound state) to the micellar head groups (bound state), described by k^+ ; (b) the transition of a bound quencher to the free water volume, described by k^- ; (c) the exchange of quenchers between water pools by intermicellar collisions, described by k_e (such a process has already been observed by Eicke *et al.* [12], Robinson *et al.* [13] and Atik and Thomas [2]); (d) fluorescence quenching, described by the unimolecular quenching constant k_{qm} .

Since these processes are exactly the same as those considered in the most general scheme for quenching in aqueous micelles [7], the data should be fitted to the same trial function:

$$i(t) = A_1 \exp[-A_2 t - A_3 \{1 - \exp(-A_4 t)\}] \quad (1)$$

On the assumption that $k_{qm} \gg k^- + k_e[M]$, the parameters have the following meanings:

$$A_1 = F_0 \quad (2)$$

where F_0 is the fluorescence intensity at $t = 0$,

$$\begin{aligned} A_2 &= k_0 + \frac{k^+ + k_e K[M]}{1 + K[M]} [Q]_t \\ &= k_0 + S_2 [Q]_t \end{aligned} \quad (3)$$

$$A_3 = [Q]_t \left(\frac{1}{K} + [M] \right)^{-1} = S_3 [Q]_t \quad (4)$$

$$A_4 = k_{qm} \quad (5)$$

$$\frac{1}{S_2} = \frac{1/k^+ + (1/k^-)[M]}{1 + (k_e/k^-)[M]} \quad (6)$$

$$\begin{aligned} \frac{1}{S_3} &= \frac{1}{K} + [M] \\ &= \frac{1}{K} + \frac{1}{N_{agg}} ([DAP] - CMC) \end{aligned} \quad (7)$$

$$\frac{S_2}{S_3} = k^- + k_e [M] \quad (8)$$

where $k_0 = 1/\tau_0$ (τ_0 is the fluorescence lifetime in the absence of quenchers), $K = k^+/k^-$, N_{agg} is the mean aggregation number, CMC is the critical micellar concentration and $[Q]_t$ is the total quencher concentration. It is obvious that the first two processes (k^+ and k^-) can only be meaningful if the water concentration is higher than the amount of water needed to hydrate the polar head groups. For DAP this means that, as long as $R < 4$, eqns. (3) - (8) can be reduced to

$$A_2 = k_0 + k_e[Q]_t = k_0 + S_2[Q]_t \quad (9)$$

$$A_3 = \bar{n} = [Q]_t/[M] = S_3[Q]_t \quad (10)$$

$$A_4 = k_{qm} \quad (11)$$

$$S_2 = k_e \quad (12)$$

$$\frac{1}{S_3} = [M] = \frac{1}{N_{agg}} ([DAP] - CMC) \quad (13)$$

Similar equations were very recently used by Atik and Thomas [2] to explain the quenching in AOT inverted micelles and to determine k_e .

If $k_{qm} < (k^- +)k_e[M]$ (e.g. quenching by Br^- [5, 6]) or if $[Q]_t$ is very low (as described above), A_3 can be neglected with respect to A_2 [9]; this results in mono-exponential decay curves. For NAA quenched by KI ($[I^-] > 5 \times 10^{-4}$ M) or $NaNO_3$ ($[NO_3^-] > 3 \times 10^{-4}$ M), the decay curves are multi-exponential and can be fitted very well to eqn. (1) as long as $R < 4$ (Fig. 1).

In these cases, S_2 is independent of $[M]$, and N_{agg} , k_e and k_{qm} can be determined using eqns. (9) - (13) (Table 2). As would be expected [6, 11, 15], N_{agg} increases with increasing R . From vapour pressure osmometric measurements [16] it is known that, in the absence of water, N_{agg} has a value of about 5. Thus, it seems that the water molecules can hold together several detergent molecules, probably by hydrogen bond formation. However, it must be pointed out that the values of N_{agg} are mean values, because the aggregation mechanism of DAP reversed micelles is described by a multiple self-association [16, 17]. These experimentally determined values of N_{agg} are always larger than the theoretical values determined by Correll *et al.* [6]: at $R = 1.375$, $N_{agg} < 10$ and, at $R = 4$, N_{agg} is only 23. Contrary to the k_{qm} values calculated on the basis of the Stern-Volmer equation (for very small $[Q]_t$), the k_{qm} values in this kinetic scheme decrease with increasing R . k_{qm} decreases for two main reasons: firstly the increase in N_{agg} [18] and secondly the increase in the distance between the naphthalene chromophore and the quencher. Indeed, at small values of R , there is competition between NAA, the propionate anion and H_2O as solvating agents of the dodecylammonium head groups. At these R values, NAA is therefore strongly bound to the micelle. This could explain why the carboxylate group is deeper inside the micelle than the ammonium head group as Lim and Fendler [19] concluded using electron spin resonance measurements. Increasing the water content

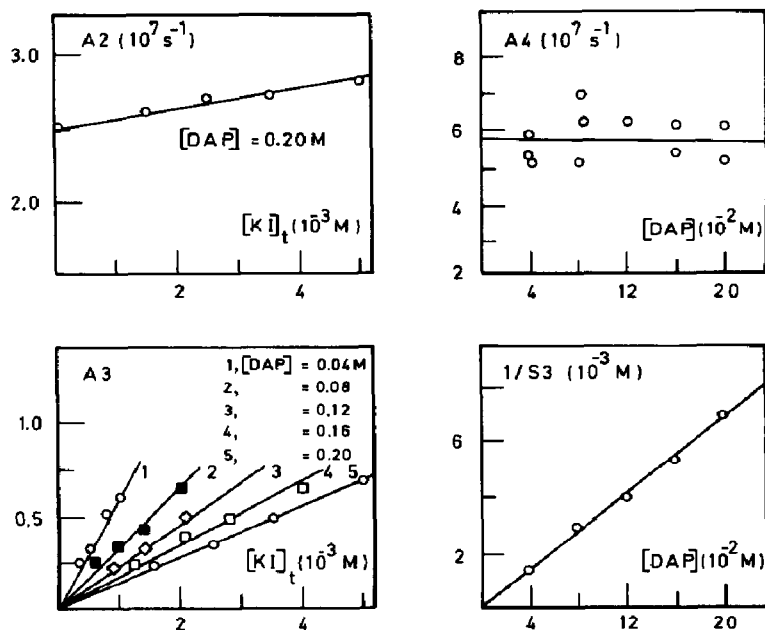


Fig. 1. Illustration of eqns. (3) - (8) for the system NAA-cyclo-C₆H₁₂-DAP-H₂O-KI ($R = [\text{H}_2\text{O}]/[\text{DAP}] = 1.375$); in eqn. (13) the CMC has been neglected; measurements of the lifetime of NAA as a function of the detergent concentration (R kept constant) indicated an operational CMC of 2×10^{-3} M if $R = 1.375$ and of 1.2×10^{-3} M if $R = 2.750$ [14]; the slope S_2 is independent of the micellar concentration and has a value of $8.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$).

TABLE 2

Rate constants and N_{agg} at various values of R for systems based on NAA-cyclo-C₆H₁₂ where $[Q]_t$, $[\text{DAP}]$ and $[\text{H}_2\text{O}]$ are variable

R	Q	N_{agg}	r^a	$k_e (\times 10^8 \text{ M}^{-1} \text{ s}^{-1})$	$k_{\text{qm}} (\times 10^7 \text{ s}^{-1})$
1.375	NaNO ₃	30	0.99	11.1	3 - 5
	KI	28	0.99	8.0	5 - 7
2.750	KI	40	0.98	13	3 - 5
4.125	KI	67	0.97	—	2 - 4

^aCorrelation coefficient of the linear regression of the plot of $1/S_3$ against $[\text{DAP}]$.

diminishes this competition and decreases the strength of the binding, resulting in a movement of the probe to a more apolar surrounding. From the moment of maximal hydration ($R > 4$), the micelle structure and the position of the naphthalene chromophore will remain constant. Such a movement of the probe can be proved by the lifetime τ_0 of NAA in the absence of quenchers at different R values (Table 3) and can be corroborated by the results of a recent publication about the binding of phenols to inverted micelles [20]. Menger *et al.* [21] mentioned in 1975 that the size of the water pool had an influence on the location of the spin labels they used.

TABLE 3

The lifetime τ_0 of 2-(1-naphthyl)acetic acid in the absence of quenchers in the systems 0.08 M DAP-H₂O-cyclo-C₆H₁₂ with variable [H₂O]

R	0	1.375	2.750	4.125	5.5	6.875	8.250
τ_0	40.5	41.6	42.5	43.4	45.8	45.8	45.8

The difference between the k_{qm} values of KI and NaNO₃ arises because Γ^- is a better quencher than NO₃⁻ in homogeneous solutions.

At $R = 4.125$, A_2 is no longer linearly dependent on $[Q]_t$ but A_3 is still in agreement with eqn. (10). At $R = 5.5$, neither A_2 nor A_3 is linearly dependent on $[Q]_t$. If A_3 (the mean number of quenchers per micelle) becomes larger than unity, deviations arise. Even if at these R values the assumption that $k_{qm} > k_e[M]$ is no longer valid or if eqns. (3) - (8) are used, the parameters A_2 and A_3 must still be linearly dependent on $[Q]_t$. Thus, this cannot explain the results.

A possible explanation could be that the micelle-fixed probe can exchange. During an excimer study of sodium pyrenesulphonate (PSS) molecules in the reversed micellar system didodecyldimethylammonium bromide-H₂O-benzene, Hunter and Younis [22] came to the conclusion that "PSS molecules are capable of intermicellar migration in the monomer excited state lifetime". Indeed, if probes can exchange by intermicellar collisions, this will have no influence on the kinetics, as long as $A_3 < 1$. In this case, there is no difference between the exchange of a quencher and that of a probe. The rate constant k_e , however, is then the mean value of both exchange constants.

The fact that the kinetic scheme is suitable for the quenching of NAA fluorescence, as long as $R < 4$, indicates that this probe will only exchange at higher R values. This is in agreement with the lifetimes of this probe in the absence of quenchers and with the reasoning concerning the decrease in k_{qm} . Because of this exchange process, it was impossible to check whether the additional processes k^+ and k^- must be taken into account if $R > 4$.

4. Conclusions

Above a certain quencher concentration, which depends on the kind of quencher, the decay curves become more exponential and can be fitted to a four-parameter equation. It is possible to calculate the rate constants k_0 , k_{qm} and k_e and the aggregation number N_{agg} at different R values. N_{agg} increases as R increases, resulting in a more structured and dense micelle, which is formed round a water pool. The space for each polar head group remains almost constant as soon as $R > 4$ (maximal hydration).

Depending on the localization of the probe and on the water concentration, the probe can migrate to other micelles by intermicellar collisions. The

localization of NAA changes continuously up to $R > 4$, when the probe is in a more apolar region of the micelle and therefore able to exchange. As a consequence, the kinetic scheme is no longer suitable unless the mean number A_3 of quenchers per micelle is smaller than unity. However, in conditions such that $A_3 \leq 1$, the decay curves are almost mono-exponential and are therefore difficult to analyse.

Only by using a probe which cannot exchange will it be possible to get more information at higher R values.

Preliminary measurements in the micellar system AOT-H₂O-cyclo-C₆H₁₂ have indicated that, in these reversed micelles also, the decay curves of PSS quenched by Cu²⁺ are multi-exponential.

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