ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 112 (1998) 245–249

Journal of Photochemistry and Photobiology A:Chemistry

Quenching of excited states of pyrene derivatives by amphiphilic nitroxide radicals in cationic micellar solutions. Dynamics and location of the guest molecules in the aggregates

Ewa Szajdzinska-Pietek *, Marian Wolszczak

Institute of Applied Radiation Chemistry, Technical University of Lodz, Wroblewskiego 15, 93-590 Lodz, Poland

Received 16 May 1997; accepted 24 June 1997

Abstract

Steady-state and nanosecond time-resolved studies have been performed on fluorescence quenching of excited pyrene sulfonic acid and pyrene butyric acid (PBA and PSA, respectively, in the form of sodium salts) by *n*-doxylstearic methyl esters (*n*-DSE, $n=5$, 10, 12) in aqueous solution of cationic micelles of hexadecyltrimethylammonium chloride (HTAC, 0.1 M). The results are compared with those reported earlier for pyrene (P) as a luminophore (Szajdzinska-Pietek and Wolszczak, Chem. Phys. Lett., 270 (1997) 527). It is shown that PBA/*n*-DSE and PSA/*n*-DSE luminophore/quencher pairs, unlike P/*n*-DSE pairs, are not suitable for determination of the micellar aggregation number, neither by the 'quasi-static' method of Turro–Yekta nor by the dynamic method of Infelta–Tachiya. It is suggested that the fluorescence decay curves in the examined systems can be adequately described in terms of dispersive kinetics using the time-dependent rate coefficient for intramicellar quenching process. The average location of the luminophore and the quencher molecules in the aggregates is discussed, considering previous findings of electron spin echo modulation studies of *n*-doxylstearic acid spin probes in micellar systems. © 1998 Elsevier Science S.A.

Keywords: Fluorescence quenching; Aggregation number; Hexadecyltrimethylammonium chloride; Pyrene derivatives; *n*-Doxylstearic methyl esters

1. Introduction

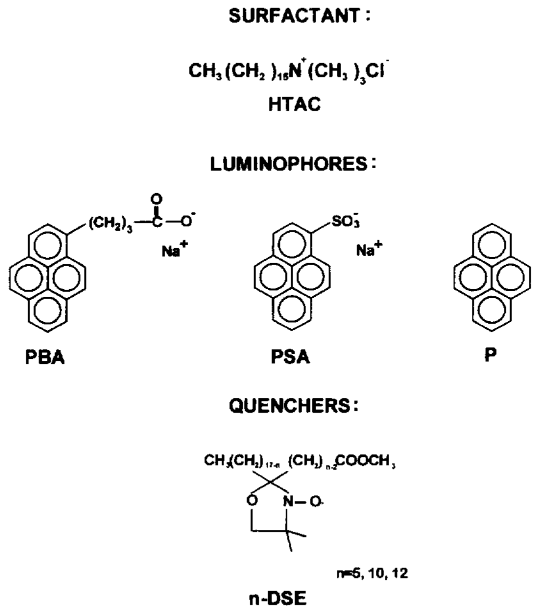
Fluorescence quenching methods are widely used in the studies of self-assembling molecular systems [1]. One of the most exploited applications is to determine aggregation numbers, *N*, in micellar solutions. Using the Infelta–Tachiya model [2,3] one can obtain *N* values from time-resolved fluorescence quenching (TRFQ) experiments, and the simplest procedure applies to systems where both the luminophore and the quencher are immobile, i.e. they do not exit host aggregates during the life-time of the luminophore excited state. In the case of immobile probes, aggregation numbers can be also evaluated from the Stern–Volmer data obtained by the steady-state measurements, using the Turro–Yekta model [4], provided the quenching process is efficient and much faster then the spontaneous fluorescence decay (in the absence of the quencher); otherwise the method leads to underestimated *N* values [5]. Careful inspection of literature data for model micellar systems (cf. for example Ref. [6]) reveals, however, that even TRFQ aggregation numbers are often lower than those determined by other techniques, e.g. light scattering, and that different results are reported for different luminophore/quencher pairs in the same host.

We have recently examined [7] fluorescence quenching of excited pyrene (P), one of the most widely used luminophores, by a series of amphiphilic nitroxide radicals, *n*-doxylstearic methyl esters (*n*-DSE, $n=5$, 10 and 12), in aqueous solution of cationic micelles of hexadecyltrimethylammonium chloride (HTAC, 0.1 M) (see Scheme 1 for the respective chemical formulae). We have shown that the three examined P/*n*-DSE luminophore/quencher pairs are suitable for determination of the micellar aggregation number by time-resolved measurements, while the steady-state data give underestimated *N* values. In the present work it is revealed that even the TRFQ method fails to deliver the proper aggregation number if pyrene butyric acid (PBA, sodium salt) or pyrene sulfonic acid (PSA, sodium salt) are used as luminophores instead of pyrene (cf. Scheme 1). We suggest that, alternatively to Infelta–Tachiya equation, the model of dispersive kinetics, with the time-dependent rate coefficient for intramicellar quenching process, can be used to describe adequately the fluorescence decay curves. The results will be

---

* Corresponding author. E-mail: ESPIETEK@MITR.P.LODZ.PL

1010-6030/98/$19.00 © 1998 Elsevier Science S.A. All rights reserved
PII S1010-6030(97)00204-9



Scheme 1.

discussed in terms of average location of the examined luminophores and quenchers in micellar aggregates, this issue is relevant not only for fluorescence spectroscopic studies, but also for ESR studies with the use of doxylstearic acid derivatives as spin probes.

2. Experimental

HTAC, 25 wt.% solution in water (Aldrich), 5-DSE and 12-DSE (Sigma), and 10-DSE (Molecular Probes, Eugene, OR) were used as received. PSA (Molecular Probes, Eugene, OR) and PBA (Aldrich) were neutralized and kindly supplied to us as sodium salts by P. Uznanski of Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Lodz.

0.1 M HTAC solutions containing luminophores (concentration $\leq 10^{-5}$ M, average occupation of micelles ≤ 0.01) and quenchers (concentrations up to ca. 2 mM for 5-DSE and up to ca. 1 mM for 10- and 12-DSE) were prepared using the previous procedure [7]. The samples were deaerated by careful bubbling with argon for 20 min, and fluorescence spectra and decay curves were recorded at room temperature. The equipment and experimental details have been described elsewhere [7].

3. Results

3.1. Steady-state measurements

Fig. 1 presents Stern–Volmer plots for 5-DSE/PBA and 5-DSE/PSA luminophore/quencher pairs in 0.1 M HTAC solution, together with that obtained earlier [7] using pyrene as a luminophore. Like in the case of pyrene, also in the present systems the results for 10- and 12-DSE quenchers have been practically the same (within ca. 15% experimental uncertainty) as those for 5-DSE. It is clearly seen in Fig. 1 that the quenching efficiency, I_0/I (where I_0 and I stand for fluorescence intensities in the absence and in the presence of the quencher, respectively) decreases in the order: P > PBA > PSA. Thus, taking into account that an underestimated value of HTAC aggregation number was obtained from experiments with pyrene [7], we have not attempted to analyse PBA and PSA data in terms of the Turro–Yekta model [4].

3.2. Time-resolved measurements

3.2.1. Infelta–Tachiya treatment

The fluorescence decay curves $I(t)$ in the presence of quenchers were analyzed by a non-linear least-squares procedure using the equation [2,3]

$$I(t)=A_1\exp\{-A_2t-A_3[1-\exp(-A_4t)]\} \tag{1}$$

where $A_1=I(0)$ is the emission intensity at zero time (the end of the laser pulse), A_2, A_3, and A_4 are parameters. It is assumed in this model that a micelle contains a maximum of one excited probe, which does not exit the micelle during its excited-state lifetime, and the occupation of micelles by the solutes (luminophore and quencher) follows Poisson statistics.

In the case of immobile quenchers, neglecting also their intermicellar exchange directly in micelle collisions and/or via a coalescence–fragmentation process, the parameters A_2–A_4 are given in Eqs. (2)–(4), respectively:

$$A_2=k_0=1/\tau_0 \tag{2}$$

$$A_3=\langle n\rangle=[\mathrm{Q}]/[\mathrm{M}] \tag{3}$$

$$A_4=k_{\mathrm{qm}} \tag{4}$$

where τ_0 is the lifetime of the excited probe in the absence of

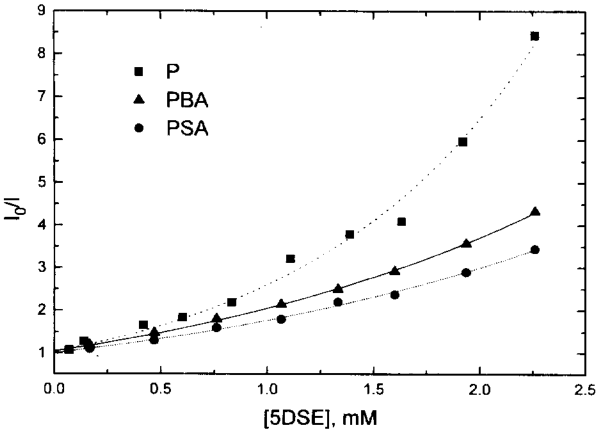


Fig. 1. Stern–Volmer plots obtained from the steady-state measurements of fluorescence quenching of pyrenyl derivatives by 5-DSE in HTAC solutions. See text for details.

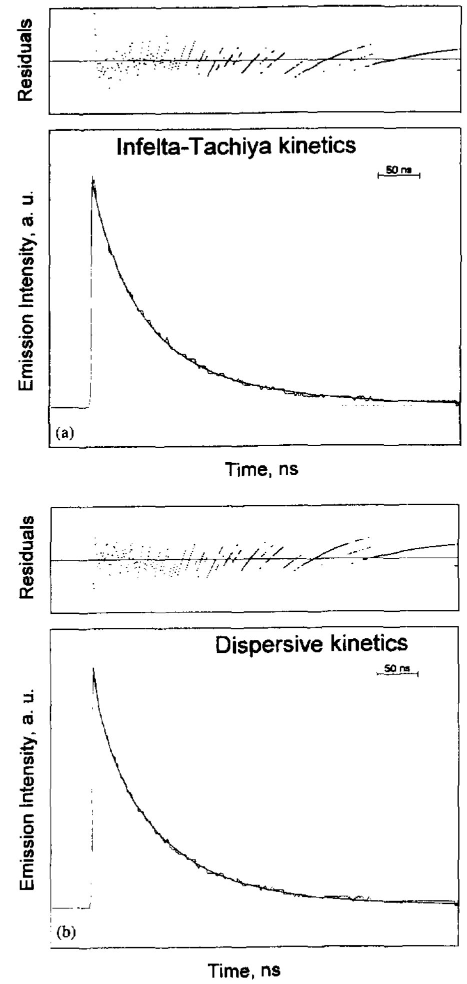


Fig. 2. Fitting of time-resolved data for the HTAC/5-DSE (1.07 mM)/PSA system by the Infelta–Tachiya equation ($A_3 = 0.931$, $A_4 = 1.01 \times 10^7$ s^{-1}) and by dispersive kinetics equation ($\alpha = 0.5$, $\tau_0 = 217$ ns); experimental curve (noisy) supperimposed on the best fitting curve (smooth). See text for details.

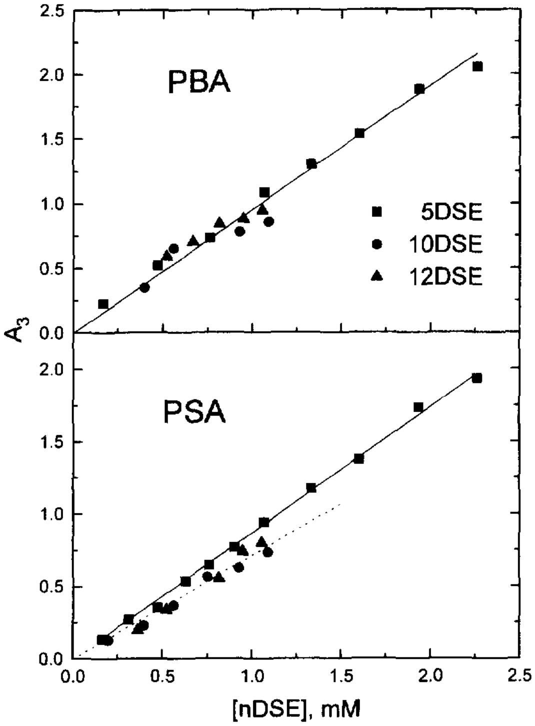


Fig. 3. The dependence of the A_3 parameter (cf. Eq. (1) and Eq. (3)) on concentration of n-DSE quenchers ($n = 5$, 10 and 12 as indicated) for HTAC/PBA and HTAC/PSA systems.

Table 1
Rate constants of intramicellar quenching, k_{qm}, in the HTAC/PBA and PSA/n-DSE systems (this work), as compared to the HTAC/P/n-DSE system (Ref. [7])

Luminophore	k_{qm} ($\times 10^{-6}$ s^{-1})		
	5-DSE	10-DSE	12-DSE
P	14.0	13.0	10.5
PSA	10.2	10.1	10.0
PBA	8.8	8.7	7.2

the quencher, $\langle n \rangle$ denotes the mean occupancy of micelles (M) by the quencher (Q), and k_{qm} (s^{-1}) is the intramicellar quenching rate constant. For the systems examined in this work the intermicellar exchange of the quenchers can be neglected as they are practically insoluble in water and we are dealing with a diluted solution of ionic micelles which repel each other. The exchange of the luminophores is more probable as both PBA and PSA are soluble in water; it should be noted, however, that hydrophobic and electrostatic interactions promote their binding to HTAC aggregates.

Fig. 2(a) shows an example of the kinetic data fitting with the A_2 parameter fixed and equal to 5.15×10^6 s^{-1} and 8.00×10^6 s^{-1} for PBA and PSA, respectively, as determined from the exponential decay of their excited state fluorescence in the absence of quenchers. In Fig. 3 the values of the A_3 parameter, obtained for the three examined n-DSE derivatives, are plotted versus concentration of the quenchers. In the case of PBA the data for all the quenchers fit reasonably well the same straight line whose slope, according to Eq. (3), corresponds to the reciprocal concentration of micelles. It gives the aggregation number $N = 94 \pm 6$, via the relation

$$[M] = \frac{[HTAC] - CMC}{N} \qquad (5)$$

where CMC = 1.4 mM is the critical micellar concentration for HTAC [8,9]. In the case of PSA, however, the slope is distinctly higher for 5-DSE than for 10- and 12-DSE, and the resulting aggregation numbers are 86 ± 2 and 70 ± 4, respectively. The parameters A_4 have been found independent of the quencher concentration, within the uncertainty of ca. ± 10%; their average values (the rate constants of intramicellar quenching, k_{qm}) are given in Table 1 which includes also our previous results for P as a luminophore [7].

3.2.2. Dispersive kinetics

Alternatively, the data for the 5-DSE/PSA/HTAC system have been treated in terms of dispersive kinetics [10,11] with

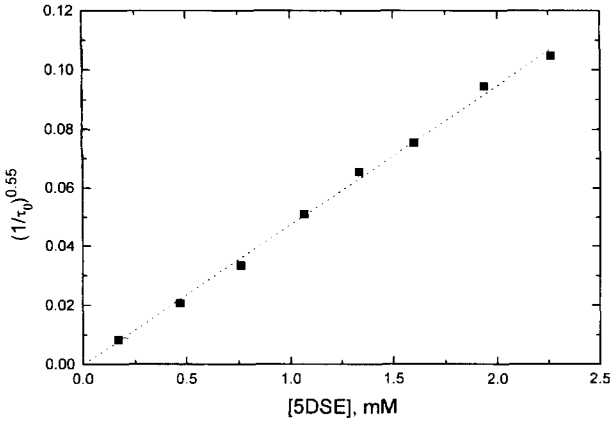


Fig. 4. The dependence of $(1/\tau_0)^\alpha$ on the quencher concentration (cf. Eq. (9)) for the HTAC/PSA/5-DSE system.

the use of a time-dependent rate coefficient for the pseudo-first order process of intramicellar quenching

$$k_{qm}(t)=Bt^{\alpha-1} \tag{6}$$

where B and $0<\alpha\leq 1$ are parameters, the latter characterizing the width of the distribution of the reaction life-times, and the effective life-time, τ_0 is given by

$$\tau_0=(\alpha/B)^{1/\alpha} \tag{7}$$

In such a case the observed fluorescence decay curves are described by the equation

$$I(t)=I(0)\exp\left[-k_0t-\left(\frac{t}{\tau_0}\right)^\alpha\right] \tag{8}$$

As seen in Fig. 2 the above model fits experimental data as well as the Infelta–Tachiya model, and in the examined system $\alpha=0.55\pm0.05$, independent of 5-DSE concentration.

The 'dispersive' term in Eq. (8) can be written as

$$\left(\frac{1}{\tau_0}\right)^\alpha=k'[\mathrm{Q}] \tag{9}$$

where k' ($\mathrm{s}^{-\alpha}\,\mathrm{M}^{-1}$) is a parameter characterizing the intramicellar quenching process (related to the bulk quencher concentration, not to that in the micellar phase). As seen from Fig. 4, for the 5-DSE/PSA/HTAC system the dependence of $(1/\tau_0)^\alpha$ is indeed linear, and $k'=(4.2\pm0.2)\times10^6\ \mathrm{M}^{-1}\ \mathrm{s}^{-0.55}$ is obtained from the slope of the straight line.

4. Discussion

The present results indicate that PBA/n-DSE and PSA/n-DSE luminophore/quencher pairs are not suitable for determination of micellar aggregation numbers from fluorescence quenching experiments, neither by steady-state nor by time-resolved measurements. That the method of Turro–Yekta fails is not surprising. As discussed in the Ref. [7], the condition $k_{qm}\gg k_0$ is not fulfilled (cf. Table 1 data vs. $k_0=5.15\times10^6\ \mathrm{s}^{-1}$ for PBA and vs. $k_0=8.00\times10^6\ \mathrm{s}^{-1}$ for PSA) and we are not dealing with a so called 'quasi-static' quenching. In derivation of Eq. (1), however, the assumption concerning rate constants was that $k_{qm}\geq k_0$ only, and it does hold. But while the TRFQ aggregation number determined by us with pyrene, $N=114$ [7], may be considered a proper value for 0.1 M HTAC solution ($N=117$ was reported by others [9]), the values obtained using PBA and PSA are too low. The apparent lowering of N value parallels the k_0 decrease in going from P to PBA and to PSA, and in the latter case the result also depends on the kind of the quencher used; $N=70$ from 10- and 12-DSE data and $N=86$ from 5-DSE data. It should be stressed here that the underestimation of aggregation numbers cannot be related to possible exchange of PBA and PSA luminophores between micelles. Gehlen et al. [12] proved that accounting for this effect in the Infelta–Tachiya model one would obtain even lower aggregation numbers, and, at the same time, higher k_{qm} values.

Thus, the Infelta–Tachiya model does not work properly in the PBA/n-DSE/HTAC and PSA/n-DSE/HTAC systems, in spite of acceptable fitting of kinetic curves by Eq. (1). We suggest it reflects a restricted diffusion of the reagents inside micelles; due to specific electrostatic and hydrophobic interactions of the guests with host molecules, not all the nitroxide groups are available for the excited luminophore during its life-time. One can try to rationalize our results considering the most probable locations of the reactive centres inside the aggregates. Let us first recall what was inferred about n-doxylstearic acid (n-DSA) location in cationic tetraalkylammonium micelles from electron spin echo modulation (ESEM) studies. The n-DSA molecule has its carboxylic group anchored in the Stern layer and the stearic chain probably assumes a U-shaped conformation, so that the terminal carbon atom penetrates the regions close to the interface rather than the interior of the aggregate, while the 10th or 12th carbon atoms are most deeply buried in the micellar core [13]. Our A_3 data for PSA and PBA can be well reconciled with such a picture. As the PSA molecule is electrostatically bound to the head group region, 5-DSE nitroxide has more chance of encountering the pyrenyl group than 10- and 12-DSE nitroxides have. In the case of PBA these chances are bigger and equal for all the examined n-DSEs (A_3 values higher and independent of the doxyl group position). This suggests that the amphiphilic PBA molecule is co-assembled with HTAC molecules similarly to the n-DSE molecule, the butyric chain of PBA being probably more extended then the stearic chain of n-DSE (on average). The pyrenyl group would be buried well below the head-group region, at a distance larger than that of 5-DSE nitroxide and smaller then that of 10-DSE nitroxide. This is an important conclusion; some authors argue that, independently of the structure of the guest molecule, its pyrenyl moiety preferentially locates in the Stern layer, due to specific interactions with quaternary ammonium groups of HTAC [14–16] and, in the case of an amphiphilic guest, due self-coiling of its chain in spherical aggregates [17].

In view of the above discussion the k_{qm} values obtained for the PBA/n-DSE/HTAC and PSA/n-DSE/HTAC systems, cf. Table 1, may be interpreted as the rate constants corresponding to a part of the luminophore/quencher population including only the pairs of reactants relatively close each other, so that they are able to meet together during the life-time of the excited state. The k_{qm} values decrease from P to PSA to PBA (at least for 5- and 10-DSE quenchers) and the trend seems to be related with decreasing mobility of the luminophores, as a result of electrostatic interactions of HTAC heads with PSA and PBA anions, and hydrophobic interactions between alkyl chains of HTAC host and PBA guest. It is relevant to note that while k_{qm} values are lower for PBA then for PSA, an opposite trend is observed for I_0/I data from the steady-state experiments. This finding can be explained assuming that, in the case of PBA (unlike PSA), static quenching occurs which involves pyrenyl and nitroxide groups residing in a close neighbourhood, on the same side of the micelle. Such an explanation would be in accordance with the above conclusion concerning PBA location in the aggregates.

From the data in Table 1 it appears that pyrene itself is the most mobile probe. We have suggested that the lower value of k_{qm} for 12-DSE, in comparison to 5- and 10-DSE, reflects on the one hand that 12-DSE nitroxide penetrates deeper below the Stern layer, and on the other hand that P diffusion from the interface to the centre of the micelle is slower than the lateral diffusion [7]. Interestingly, a lowering of the k_{qm} value for 12-DSE is also observed in PBA system where the pyrenyl group may undergo some radial movement due to flexibility of the terminal segment of the butyric chain.

The finding that the Infelta–Tachiya model does not lead to proper aggregation numbers in the examined systems has prompted us to seek for an alternative description of TRFQ data. Our choice is the dispersive kinetics developed by Plonka [10,11]. By the example of the 5-DSE/PSA/HTAC system one can see that Eq. (8) with $\alpha = 0.55$ fits the experimental decay curves well, cf. Fig. 3. The parameter $\alpha < 1$ indicates that we are dealing with a broad distribution of the reaction life-times, which might be related to a distribution of the luminophore–quencher distances. From the data for different quencher concentrations we are able to evaluate the k' parameter characterizing the rate of the bimolecular intramicellar process. We are currently extending this treatment on the other systems to see if the two kinetic parameters will reflect different locations of the guest molecules in micelles as postulated above. Besides, it is possible to relate dispersive parameters to fractal properties of self-assembled systems, cf. Lianos [18]. The results will be presented in a separate paper.

Acknowledgements

This work has been supported by the Polish Committee of Scientific Research (in part KBN project 3 T09A 034 11). The authors thank Prof. Andrzej Plonka for many helpful discussions.

References

[1] M.H. Gehlen, F.C. De Schryver, Chem. Rev. 93 (1993) 199, and references cited therein.
[2] P.P. Infelta, M. Grätzel, J.K. Thomas, J. Phys. Chem. 78 (1974) 190.
[3] M. Tachiya, Chem. Phys. Lett. 33 (1975) 289.
[4] N.J. Turro, A. Yekta, J. Am. Chem. Soc. 100 (1978) 5951.
[5] A. Malliaris, Progr. Colloid Polymer Sci. 73 (1987) 161.
[6] F. Grieser, C.J. Drummond, J. Phys. Chem. 92 (1988) 5580, and the Supplementary Material.
[7] E. Szajdzinska-Pietek, M. Wolszczak, Chem. Phys. Lett., 270 (1997) 527.
[8] A. Malliaris, W. Binana-Limbelle, R. Zana, J. Colloid Interface Sci. 110 (1986) 114.
[9] A. Malliaris, J. Lang, R. Zana, J. Chem. Soc. Faraday Trans. 1 82 (1986) 109.
[10] A. Plonka, Lecture Notes in Chemistry, Vol. 40, Springer, Berlin, 1986.
[11] A. Plonka, Ann. Rep. Progr. Chem., Section C 89 (1992) 37.
[12] M.H. Gehlen, N. Boens, F.C. De Schryver, M. Van der Auweraer, S. Reekmans, J. Phys. Chem. 96 (1992) 5592.
[13] E. Szajdzinska-Pietek, R. Maldonado, L. Kevan, S.S. Berr, R.R.M. Jones, J. Phys. Chem. 89 (1985) 1547.
[14] M. Almgren, F. Grieser, J.K. Thomas, J. Am. Chem. Soc. 101 (1979) 279.
[15] M. Almgren, B. Medhage, E. Mukhtar, J. Photochem. Photobiol. A 59 (1991) 323.
[16] P. Lianos, M.-L. Viriot, R. Zana, J. Phys. Chem. 88 (1984) 1098.
[17] J. Shobha, V. Srinivas, D. Balasubramanian, J. Phys. Chem. 93 (1989) 17.
[18] P. Lianos, Heterogeneous Chem. Rev. 3 (1996) 53, and references cited therein.