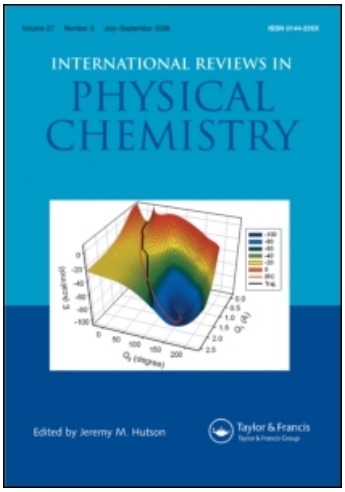

International Reviews in Physical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713724383

Fluorescence probing in aqueous micellar systems: an overview

Angelos Malliaris[a]
[a] National Research Centre, 'Demokritos', Athens, Greece

To cite this Article Malliaris, Angelos(1988) 'Fluorescence probing in aqueous micellar systems: an overview', International Reviews in Physical Chemistry, 7: 2, 95 — 121
To link to this Article: DOI: 10.1080/01442358809353208
URL: http://dx.doi.org/10.1080/01442358809353208

INTERNATIONAL REVIEWS IN PHYSICAL CHEMISTRY, 1988, VOL. 7, NO. 2, 95–121

Fluorescence probing in aqueous micellar systems: an overview

by ANGELOS MALLIARIS
National Research Centre, 'Demokritos', Athens 153 10, Greece

In this review we discuss the most interesting methods of fluorescence probing related to the study of aqueous micellar systems. It is shown that very important information concerning static and dynamical properties of aggregational colloids, can be obtained from fluorescence experiments. The presentation is focused on application of fluorescence methods to micellar media, rather than on interfacial or aggregational problems themselves. This is also true for the selection of the literature cited, which covers only a number of the most essential publications on fluorescence probing methods in micellar systems.

A large part of this article deals with time-resolved fluorescence quenching, since this is the most powerful of all fluorescence probing methods applied in the study of microheterogeneous media. The fluorescence decay of a micelle-bound hydrophobic fluorophor in the presence of different types of quenchers is discussed, and the reliability of the various micellar parameters obtained is evaluated. Systems in which fluorescence quenching must be applied with extreme caution are described and the ambiguities involved are analysed.

Steady-state fluorescence, although simple in its experimental aspects, can give valuable information about microproperties in the interior of micellar aggregates, and occasionally, good estimates of their size. However, steady-state fluorescence probing has found only limited use because its results are very often unreliable, and it cannot give information relating to kinetic parameters.

1. Introduction

Experimental methods based on fluorescence probing have been widely employed in the investigation of a variety of anisotropic physicochemical and biological systems (Edelman and McClure 1968, Brand and Gohlke 1972, Turro *et al.* 1977, Kalyanasundaram 1978, Almgren *et al.* 1979, Thomas 1980, Turro *et al.* 1980, Ghiggino *et al.* 1981, Singer 1982, Thomas 1987, Kalyanasundaram 1987). These methods, classified as either steady state or time-resolved, can provide valuable information about compartmentalized systems of colloid dimensions. Such information concerns size, polarity, fluidity, diffusion, concentration, partitioning between phases, distance between groups, reaction rates etc. Particularly time-resolved fluorescence has found extensive use in the study of microheterogeneous and interfacial systems including, aqueous (Turro *et al.* 1980) and reversed (Luisi and Straub 1984) micelles, vesicles (Morris and Thomas 1977), membranes (Galla and Sackmann 1974), microemulsions (Atik and Thomas 1981), semiconductors (Ramsden *et al.* 1985), clays (Ghosh and Bard 1984), polyelectrolytes (Chu and Thomas 1984), polymerized systems (Atik and Thomas 1982). In the present account we review the main aspects of fluorescence probing and its application to the study of aqueous micellar systems.

Several micellar parameters, of either static or dynamical nature, can be obtained via fluorescence probing. Amongst these, the micellar aggregation number, the intramicellar reaction rate constant, and the rate of exchange of solubilizates between micelles and the aqueous environment, can be cited as some of the most important. By far the most fundamental concept regarding micelles is the mean micellar aggregation

number N_s, i.e. the average number of surfactant ions which, under the appropriate conditions, aggregate to form micelles. Several classical experimental techniques have been employed over the years for the determination of N_s. Methods such as elastic (Anacker 1970) or quasi-elastic light scattering (Mazer *et al.* 1976), ultracentrifugation (Anacker *et al.* 1964), membrane osmometry (Attwood *et al.* 1970, Birdi 1972) and gel-filtration (Coll 1979) have been used for the determination of micellar size in terms of micellar radius, molecular weight, mean aggregation number, etc. However, in the case of ionic micelles all the classical methods which allow determination of N_s have the serious handicap that the measurements must be extrapolated to the critical micelle concentration. This is necessary in order to eliminate the disturbing interference by the strong intermicellar coulombic interactions which exist between ionic micelles. Therefore N_s values can be obtained only at the critical micelle concentration. The problem becomes even more serious when extrapolation is not feasible, e.g. when the micellar composition depends on the surfactant concentration $[C_s]$, as it is the case in mixed micelles (Malliaris *et al.* 1986 a). Moreover, in view of recent findings which have indicated that even in pure micelles N_s is a function (not necessarily linear) of $[C_s]$ (Malliaris *et al.* 1986 b), it turns out that the extrapolation approach can involve large errors. Fluorescence methods however, have conveniently solved the problem of the intermicellar interactions since they are not, in principle, influenced by them. Therefore fluorescence probing can provide reliable information concerning N_s at practically any surfactant concentration of ionic micelles without the need of added electrolytes.

In addition to the determination of N_s, time-resolved fluorescence quenching is a unique method for obtaining information of a kinetic nature relating to intramicellar reaction rates, equilibrium constants of adsorption/desorption of solubilizates to/from micelles, etc. (Van der Auweraer *et al.* 1982, Malliaris *et al.* 1986 c). Such information is of great importance for the kinetics of chemical and biological reactions taking place in organized molecular aggregates, e.g. microemulsions, enzymes, membranes and other similar heterogeneous media. Particularly for micelles, we note that the rate at which a surfactant unit moves from an aggregate into the surrounding aqueous phase is of the order of 10^5–$10^6\ s^{-1}$, depending on the aggregational characteristics of the particular micelle (Lang *et al.* 1975). Small solubilizates exit from their host micelles at similar rates, whereas large and strongly hydrophobic molecules, e.g. pyrene, exit at much slower rates, *c.* 10^4–$10^3\ s^{-1}$. On the other hand, the rate at which a free surfactant monomer combines with a micelle can reach even the rate of a diffusion-controlled reaction, i.e. 10^9–$10^{10}\ M^{-1}\ s^{-1}$. Since the fluorescence time-scale varies between 10^6 and $10^9\ s^{-1}$, fluorescence probing is appropriate for kinetic studies of reactions with rate constants falling in this range. Indeed, this is the range where some important micellar reactions take place. Note, however, that very fast micellar processes, such as the diffusion-controlled ion/micelle association, fall beyond the time scale of fluorescence spectroscopy, whereas slower events are usually studied by relaxation methods (Lang and Zana 1986).

2. Aqueous solutions of surfactant micelles

Surface active ions (surfactants) containing both hydrophobic and hydrophilic groups associate in water above a certain surfactant concentration, the so-called critical micelle concentration (CMC), to form aggregates of colloidal dimensions, the aqueous ionic micelles (Shinoda *et al.* 1963, Elworthy *et al.* 1968, Mukerjee 1979). Note that even at surfactant concentrations lower than the CMC there is some limited surfactant premicellar aggregation. Aqueous micelles offer a simple and convenient

way to aggregate monomers in an organized fashion, but they can also be looked upon as models for more advanced organized molecular assemblies, including the very important biological systems. The structure of a micelle is such that the interior contains the alkyl chains of the aggregated amphiphiles while the hydrophilic groups are located on the surface. There they form a charged electrical double layer, the interface, in contact with bulk water. The shape adopted by a micelle generally depends on the nature and concentration of the particular surfactant. Thus at low amphiphile concentration, just above the CMC, micelles are usually spherical or nearly spherical (Mukerjee 1979), while at higher concentration they assume rod or disc-like shapes (Windsor 1968). Finally, at very high concentrations micelles transform to lyotropic liquid crystalline structures (Gray and Windsor 1967). The detailed structural and dynamical aspects of simple micelles formed at moderate surfactant concentrations have been studied by several physical and chemical methods (Fendler and Fendler 1975, Wennerström and Lindman 1979, Fendler 1982). Also, several models have been proposed to rationalize the wealth of accumulated experimental evidence (Hartley 1936, Harkins and Mittelmann 1949, McBain 1950, Debye and Anacker 1951, Menger 1979, Dill and Flory 1981, Fromherz 1981, Menger and Doll 1984).

3. Fluorescence kinetics

The singlet–singlet absorption of light by molecular species and the ensuing processes leading to dissipation of the excitation energy are depicted in the Jablonski state-diagram of figure 1 (Jablonski 1935). The ground, first, second, etc. singlet excited states are symbolized by G, S_1, S_2, etc., while T_1, T_2, etc, represent the corresponding triplet excited states. Molecules which are capable of de-excitation by a radiative singlet–singlet transition (fluorescence) are called 'fluorophors', whereas molecules which enhance the radiationless de-excitation of an excited fluorophor are called 'quenchers'. Molecules with conjugated double bonds, e.g. aromatic hydrocarbons, can be cited as the most typical class of organic fluorophors. On the other hand, there is a wide variety of substances which act as fluorescence quenchers, most notably molecular oxygen. Other quenchers include paramagnetic ions, heavy atoms, amines, pyridinium salts, etc. (Parker 1968, Turro 1972, Lakowicz 1983). Under conditions of continuous irradiation, equilibrium among the various processes of figure 1 is achieved rapidly, and the system reaches its steady state. However, when instead of being irradiated continuously, the fluorophor is excited by a δ-shape light pulse (time-resolved fluorescence) the intensity of the emission rises sharply to its maximum value and then decays exponentially in the typical fashion of a first order chemical reaction (Demas 1983, O'Connor and Phillips 1984).

Although fluorescence quenching can sometimes occur without the close approach of the reactants (fluorophor and quencher), e.g. the case of energy transfer, in the present account we deal with quenching occurring upon contact of the reactants. Such contact can be realized either by collision of an excited fluorophor with a quencher, or by the formation of a ground-state complex between the two. The former is the case of dynamical and the latter of static fluorescence quenching. These terms however, should not be confused with the time-resolved and steady-state fluorescence quenching, which are also occasionally referred to as dynamic and static respectively. In this review, by fluorescence quenching we shall refer only to the case of dynamical fluorescence quenching which requires collisional encounter of the excited fluorophor with a quencher.

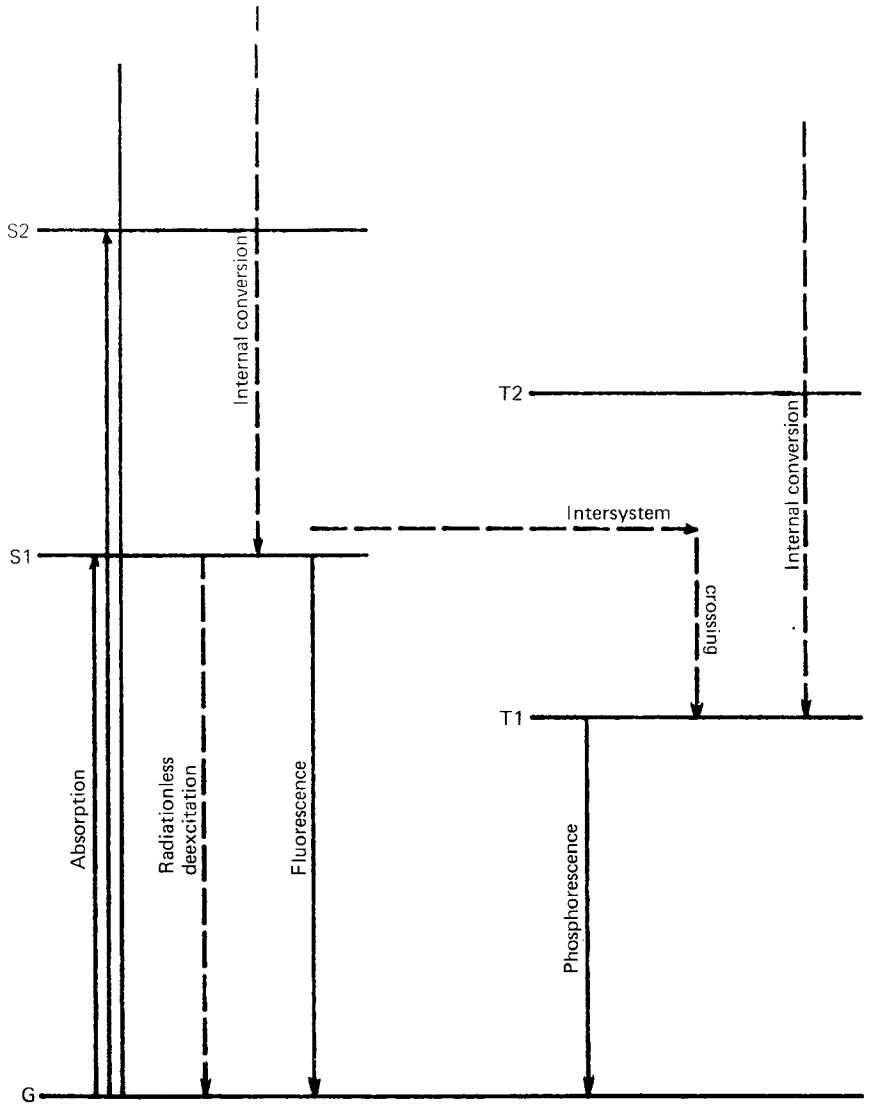

Figure 1. Jablonski state-diagram for a polyatomic molecule.

4. Kinetics in isotropic versus micellar media

Important differences in the fluorescence kinetics are observed when the reactants are not dissolved in isotropic media, but instead are distributed among the aggregates of a surfactant solution. These differences are the result of the different distribution statistics of the reactants in the two cases. Thus, while in homogeneous solutions the reactants are uniformly distributed throughout the volume of the solution, in micellar media their distribution is determined by their association with the micellar species.

The simple case of fluorescence kinetics in isotropic media is described in Scheme A, where electromagnetic irradiation $h\nu$ excites a fluorophor F to F*, in the presence of a quencher Q. The term fluorescence stands for the overall unquenched de-excitation of

F* by both radiative and radiationless processes, therefore the radiationless de-excitation, F*– – →F+heat, is not shown in Scheme A. k_0 (s^{-1}) is the unquenched fluorescence rate constant, equal to $1/\tau_0$, where τ_0 is the measured fluorescence life-

Scheme A

Process	Rate	
$F + h\nu \longrightarrow F^*$	I	Absorption
$F^* \longrightarrow F + h\nu$	$k_0[F^*]$	Fluorescence
$F^* + Q \longrightarrow F + Q$	$k_q[F^*][Q]$	Fluorescence quenching

time. On the other hand, k_q which is the fluorescence quenching rate constant, is expressed in units of $M^{-1}s^{-1}$, since fluorescence quenching is a bimolecular reaction between an excited fluorophor and a quencher molecule. The decrease of the fluorescence intensity due to Q depends on the effective quencher concentration [Q], its quenching efficiency for the particular fluorophor, the viscosity of the medium, the temperature, etc.

In micellar media the reactants are not isotropically dispersed throughout the solution. Instead they are associated with the micellar units either by hydrophobic (e.g. the case of aromatic molecules) or by coulombic (e.g. the case of small ions) interactions. The distribution of reactants among micellar units has been generally assumed to follow standard Poisson statistics (Infelta *et al.* 1974, Turro *et al.* 1980), although other distribution laws have also been proposed (Dorrance and Hunter 1972, 1974, Hunter 1980). Scheme B shows all the processes, except thermal dissipation, following the

Scheme B

Process	Rate	
$F_n + h\nu \longrightarrow F_n^*$	I	Absorption
$F_n^* \longrightarrow F_n + h\nu$	$k_0[F^*]$	Fluorescence
$F_n^* \longrightarrow F_n$	$nk_q[F^*]$	Fluorescence quenching
$F_n^* + [Q_w] \longrightarrow F_{n+1}^*$	$k^+[F^*][Q_w]$	Quencher adsorption
$F_n^* \longrightarrow F_{n-1}^* + Q$	$nk^-[F^*]$	Quencher desorption
$F_n^* + M_j \longrightarrow F_{n+1}^* + M_{j-1}$	$jk_E[F^*][M_j]$	Quencher exchange (addition)
$F_n^* + M_j \longrightarrow F_{n-1}^* + M_{j+1}$	$nk_E[F^*][M_j]$	Quencher exchange (removal)

irradiation with u.v. light, of a typical ionic micellar solution in which fluorophor and quencher molecules have been solubilized. Fluorophor concentration in such solutions is kept very low so that there are practically no micelles containing more than one F molecule. It is always assumed that all the processes of Scheme B occur much faster than the exchange of the fluorophor between the aqueous and the micellar phase (see below). Therefore, the micelle/water exchange of F is ignored. On the contrary, the micelle/water exchange of Q is taken into account in the formulation of the kinetics. F_n symbolizes a micelle containing one fluorophor and n quenchers whereas F_n^* indicates excitation of F (Dederen *et al.* 1981). M_m stands for a micelle containing m quenchers and no F molecules. $[Q_w]$ and $[Q_m]$ are the quencher concentrations in the water and the micellar phase respectively, and $[Q]=[Q_m]+[Q_w]$ is the total quencher concentration. It must be pointed out that k_q is linearly dependent on the number of quenchers per micelle, i.e. $k_q(n)=nk_q(1)$. Similarly, for the quencher desorption rate constant k^- (s^{-1}) we have $k^-(n)=nk^-(1)$. On the contrary, because of the Poisson

statistics, the rate constant of quencher adsorption to a micelle, k^+ ($M^{-1}s^{-1}$), is independent of the number of quenchers per micelle. Finally, the rate constant of the intermicellar quencher exchange between micelles is k_E ($M^{-1}s^{-1}$), and $k_E(n) = nk_E(1)$. It is important to emphasize that, contrary to the case of homogeneous media, in surfactant solutions the intramicellar fluorescence quenching is not a second-order process but instead follows pseudo-first-order kinetics and therefore k_q is expressed in units of s^{-1}. This is a consequence of the compartmentalization and the Poissonian distribution of the reactants in the micelles (Webber 1983). Indeed, it has been demonstrated by the stochastic approach to the theory of intramicellar kinetics (Hatlee and Kozak 1981 a, b), that under the above conditions a reversible photo-induced and diffusion-controlled reaction proceeds in a pseudo-first order fashion (Hatlee *et al.* 1980). The equilibrium constant of the quencher association with a micelle is, by definition, $K = k^+/k^- = [Q_m]/[Q_w][M]$ (Dederen *et al.* 1979 b, Van der Auweraer *et al.* 1981, Malliaris *et al.* 1986 c). A final point which must be made here is that unquenched fluorescence, steady-state and time-resolved, follows the same kinetics in either isotropic or heterogeneous environment. The important differences between the two media arise in the case of fluorescence quenching, where there is interaction between fluorophor and quencher and therefore their distribution becomes the controlling factor of the fluorescence kinetics.

With respect to their association to micelles, reactants can be classified as either 'mobile' or 'immobile' (Infelta 1979). This classification is based on the relationship between the unquenched fluorescence life-time τ_0, of the fluorophor solubilized in a micelle, and the association time of the reactants with that particular micelle. Thus, a reactant is considered to be immobile if its association with a micelle is much longer than τ_0, whereas it is viewed as mobile if it is exchanged between the water and the micellar pseudo-phase at a rate comparable to $1/\tau_0$. Mobile reactants can be further classified as 'mobile hydrophobic' and 'mobile ionic', according to the nature of their association with the micelle. Since a typical hydrophobic fluorophor, e.g. an aromatic molecule, remains associated with its host micelle for *c.* 10^{-3} to 10^{-4} s (Thomas *et al.* 1978), whereas the life-time of molecular fluorescence ranges between 10^{-6} and 10^{-9} s, it is evident that fluorophors can always be treated as immobile solubilizates. On the contrary, quenchers exhibit very different rates of adsorption/desorption to/from host micelles according to the nature of the binding interactions. It is therefore convenient always to assume the fluorescent probe as immobile in order to simplify the formulation of micellar kinetics. It is worth mentioning that pyrene, due to its strong hydrophobicity and its unusually long fluorescence life-time ($\tau_0 = 300$–400 ns), has been used extensively as the fluorescent probe in aqueous micelles. Effective quenchers of the fluorescence of micelle-bound pyrene include among others, alkylpyridinium salts, Cu^{2+}, I^-, etc. Note that pyrene can quench its own fluorescence through excimer formation (Förster and Selinger 1964, Birks 1970, Infelta and Grätzel 1979, Malliaris *et al.* 1985).

5. Steady-state fluorescence

Steady-state experiments, although simple in instrumentation, operation and interpretation of the data (Parker 1968, Turro 1972, Lakowicz 1983), can occasionally provide important information concerning the behaviour and properties of the system under study (Turro and Yekta 1978, Atik and Singer 1978 a, b, Aikawa *et al.* 1979, Atik and Singer 1979 a, b, Thomas 1980, Lissi *et al.* 1980, Encinas *et al.* 1983, Paleos *et al.*

1984). It is therefore interesting to discuss in some detail the main aspects of steady-state experiments from both the unquenched and the quenched fluorescence point of view.

5.1. *Unquenched fluorescence*

Most information obtained from unquenched (i.e. [Q]=0) steady-state experiments derives primarily from spectral patterns and fluorescence polarization. It relates to the static properties of the immediate environment of the probe, particularly microfluidity and micropolarity. As pointed out earlier, there is no fundamental difference in the kinetics of the unquenched fluorescence between homogeneous and heterogeneous media.

The well-known solvent effect on the fluorescence spectra of pyrene (Nakajima 1971) provides good evidence on the polarity of the environment of this fluorophor (Dong and Winnik 1984). Thus, the ratio I_1/I_3 of the first to the third fluorescence peak is an excellent index of the micropolarity at the site of the residence of micelle-bound pyrene (Kalyanasundaram and Thomas 1977 a, Paleos *et al.* 1983). Figure 2 shows fluorescence spectra of pyrene in isotropic (water, hexane) and micellar (sodium dodecyl sulphate) media, where the dependence of the I_1/I_3 ratio on the polarity of the environment is clearly demonstrated. Similarly, the fluorescence maximum of pyrene-3-carboxaldehyde has been used for the determination of the microdielectric constant ε of the micelle–water interface (Kalyanasundaram and Thomas 1977 b, Turro and Okubo 1982). Figure 3 shows a reported linear relationship between ε (10–80) and the

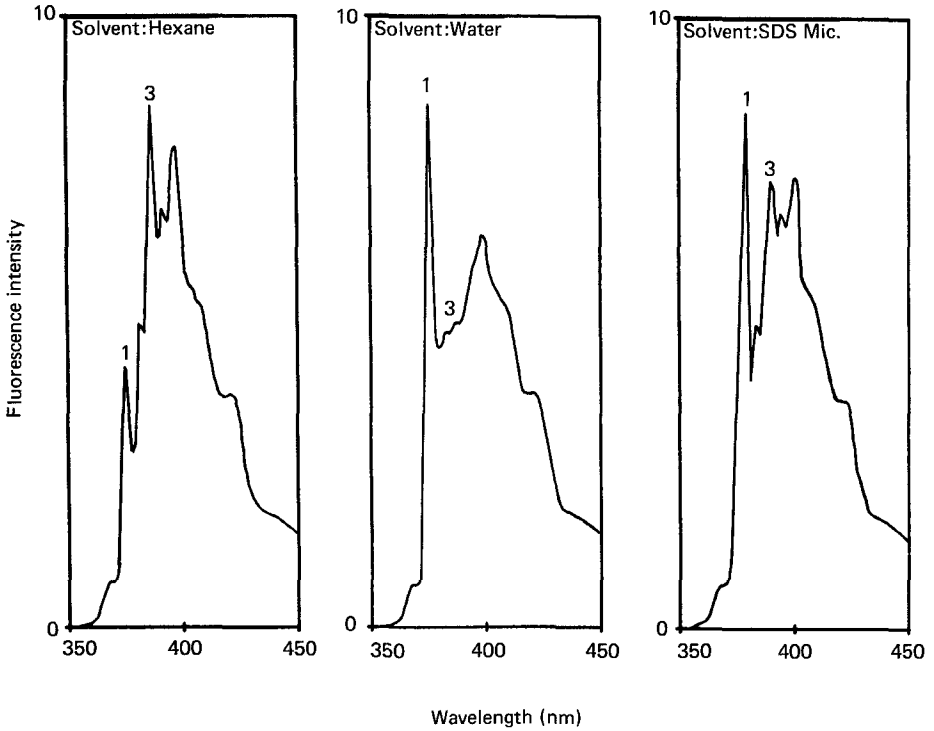

Figure 2. Fluorescence spectrum of pyrene in various media.

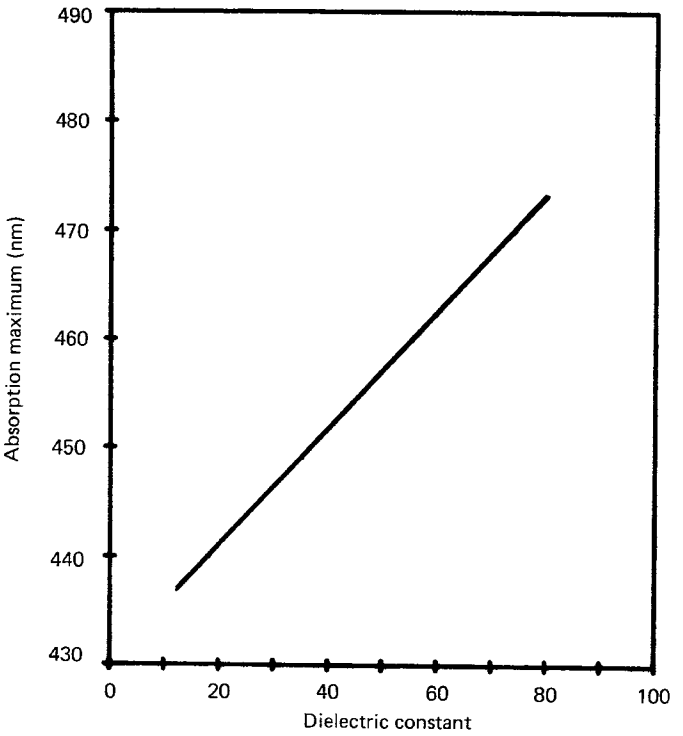

Figure 3. Dependence of the fluorescence maximum of pyrene-3-carboxaldehyde on the microdielectric constant ε of the medium. Figure drawn from the data of Kalyanasundaram and Thomas (1977 b).

fluorescence maximum of pyrene-3-carboxaldehyde (Kalyanasundaram and Thomas 1977 b). However, other solvent parameters, in addition to ε, are probably responsible for changes in the fluorescence spectrum (Dederen *et al.* 1979 a).

Important information concerning the fluidity of a medium can be obtained via intramolecular excimer formation which is sensitive to the viscosity of the environment (Förster and Seidel 1965). For the determination of the microviscosity in the interior of organizates, diarylalkanes have been employed successfully (Zachariasse and Kühnle 1976, Zachariasse 1978, Emert *et al.* 1979, Zachariasse *et al.* 1983). The ratio I_E/I_M of the intramolecular excimer to the monomer fluorescence intensity of micelle-bound dipyrenylpropane, turns out to be a very good index of the microfluidity at the side of the solubilization of the probe (Lianos *et al.* 1982, Zachariasse *et al.* 1983). Figure 4 shows the fluorescence spectrum of 2×10^{-6} M dipyrenylpropane solubized in a 0·2 M aqueous solution of sodium dodecyl sulphate, as well as the calibration curve of I_E/I_M versus viscosity, obtained using various hexadecane/paraffin oil mixtures (Viriot *et al.* 1982). Note that the magnitude of the pyrene intermolecular excimer to monomer intensity ratio can also be used as a measure of the microfluidity in micelles (Pownall and Smith 1973). Moreover, viscosity data can be obtained from fluorescence depolarization measurements based on the competing rates of rotational diffusion and fluorescence decay of the fluorophor (Shinitzky *et al.* 1971). Molecules appropriate for fluorescence depolarization studies must have a short fluorescence lifetime (1–10 ns), a rigid structure to avoid depolarization arising from side group rotation, and a high

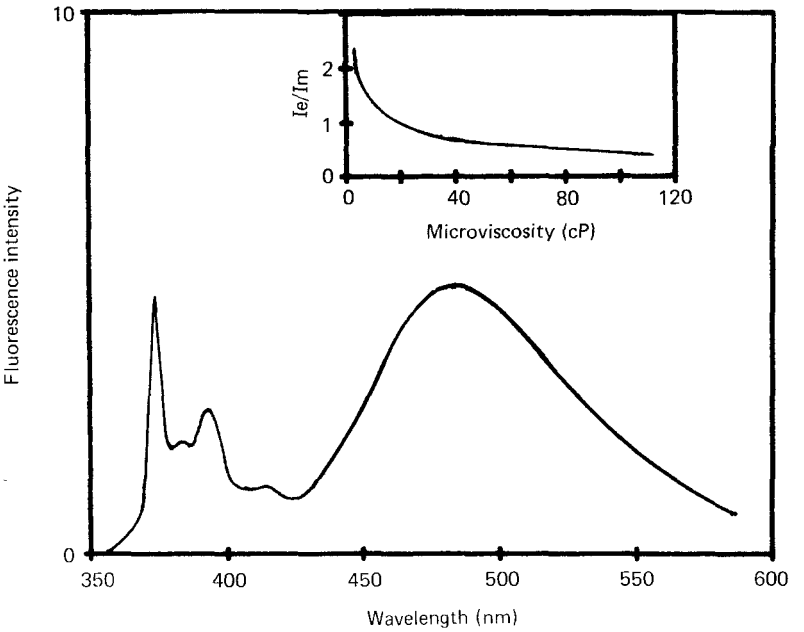

Figure 4. Fluorescence spectrum of 2×10^{-6} M dipyrenylpropane solubilized in a 0·2 M aqueous solution of sodium dodecyl sulphate. Insert: plot of the I_E/I_M ratio versus solvent viscosity (Lianos *et al.* 1982).

fluorescence quantum yield. Perylene, 2-methylanthracene, and 1,6-diphenyl-1,3,5-hexatriene are some of the fluorophors usually employed in fluorescence depolarization studies (Shinitzky *et al.* 1971, Grätzel and Thomas 1973, Humphry-Baker *et al.* 1980).

5.2. *Fluorescence quenching*

Steady-state fluorescence quenching in homogeneous solutions is described by the well-known Stern–Volmer relationship of equation (1) (Parker 1968, Turro 1972, Lakowicz 1983)

$$F_0/F = \tau_0/\tau = 1 + k_q\tau_0[Q] \quad (1)$$

where F_0, F, τ_0 and τ are the unquenched and quenched fluorescence intensities and life-times respectively. It should be pointed out that equation (1) is valid only for the case of dynamic collisional quenching, whereas if the quenching is due to ground-state complex formation then $\tau_0 = \tau$. Also recall that k_q in isotropic media is a second-order rate constant expressed in $M^{-1}\,s^{-1}$. From equation (1), it becomes evident that long τ_0 favours the quenching experiment by improving the experimental accuracy.

In micellar media, steady-state fluorescence quenching has been studied by several investigators (Henglein and Scheerer 1978, Infelta 1979, Atik *et al.* 1979 a). When both the fluorophore and the quencher are immobile, the situation can be described by equation (2) (Infelta 1979, Malliaris 1987)

$$F_0/F = \sum\{[Q]/[M])^{-1}/(R+i)i!\}\exp([Q]/[M])/R \quad (2)$$

where R is the ratio of the rate constant of the unquenched deactivation k_0, divided by the quenching rate constant k_q. [M] is the micellar concentration, defined by equation

(3), and i expresses the number of quenchers residing in a micelle

$$[M]=([C_s]-[CMC])/N_s \tag{3}$$

Figure 5 shows plots of $\ln(F_0/F)$ versus [Q]/[M] according to equation (2), for various values of R.

It is obvious that in order to obtain any information from the fluorescence quenching experiment it is necessary that the fluorescence quenching rate constant k_q is larger, or at least comparable to the fluoresce rate constant k_0. If this is not the case then the system will fluoresce before the quencher has enough time to modify the characteristics of the emission, and therefore very little information will be extracted from fluorescence quenching. When $R=0$, i.e. in the ideal case of a fluorophor with very long fluorescence life-time (k_0 is very small) in conjunction with a very efficient quencher (k_q is very large), equation (2) can be approximated by equation (4) (Turro and Yekta 1978)

$$\ln(F_0/F)=[Q]/[M] \tag{4}$$

The slope of the line obtained by plotting $\ln(F_0/F)$ versus [Q] according to equation (4) (figure 6), is equal to 1/[M]. Therefore when $R=0$ the micellar concentration [M] can

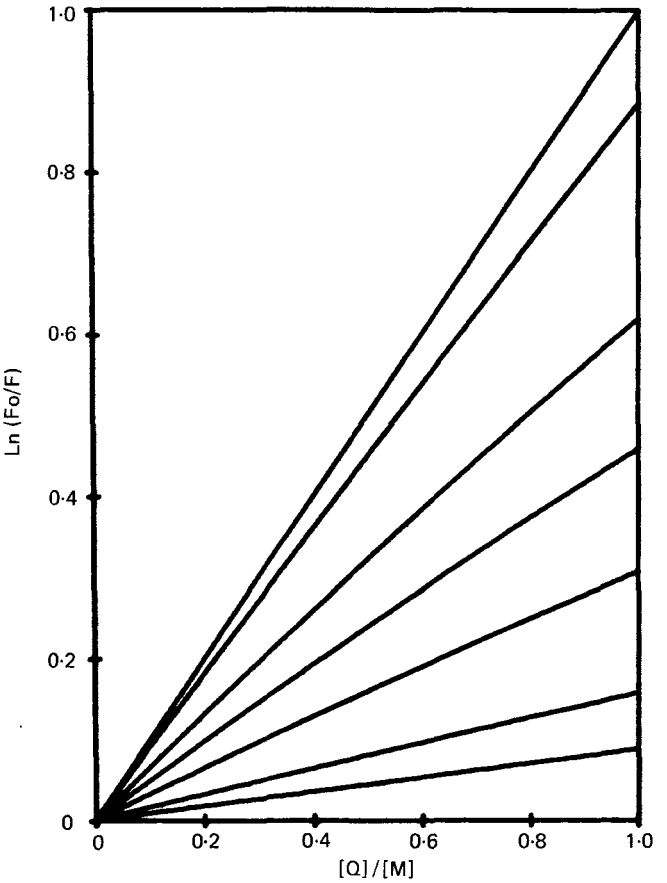

Figure 5. Computer-simulated plots of $\ln(F_0/F)$ versus [Q]/[M] from equation (2). $R=0$, 0·1, 0·5, 1, 2, 5, 10 (from top to bottom).

be obtained from steady-state fluorescence quenching experiments (Atik and Singer 1978 a, b, Aikawa *et al.* 1979, Lianos and Zana 1980, Almgren and Swarup 1982, Almgren and Swarup 1983 a, b, Paleos *et al.* 1984). In practice, when R is small, equation (4) can be applied with confidence as far as the reliability of the N_s numbers obtained is concerned (Malliaris 1987).

Since k_0 has a more or less fixed value in a certain environment, it is k_q which must become large in order to obtain small R values. Note that large k_q values are obtained when: (*a*) an efficient quencher/fluorophor pair is used: (*b*) [Q] is high; (*c*) the viscosity of the medium is low; and (*d*) when there are no quencher or fluorophor interactions with the environment which would impair the mutual approach of the reactants, necessary for the quenching process. Typical example of fluorophor/environment interaction is the case of pyrene solubilized in micelles of quaternary ammonium surfactants (Almgren *et al.* 1979, Lianos *et al.* 1984, Malliaris and Paleos 1984). Appropriate conditions for the determination of N_s by steady-state fluorescence quenching materialize for instance in the case of small sodium dodecyl sulphate micelles which are formed at low surfactant concentration. Figure 7 shows the dependence of N_s on $[C_s]$ reported in the literature. It is clear that for $[C_s]$ below *c.* 0·1 M the aggregation numbers of sodium dodecyl sulphate micelles found by steady-state fluorescence quenching are in excellent aggrement with values obtained by more sophisticated methods.

6. Time-resolved fluorescence

Time-resolved fluorescence allows detailed investigation of important structural and dynamical aspects in the immediate environment of the fluorophor. The theory and experimental details of the methods used for recording fluorescence decay have

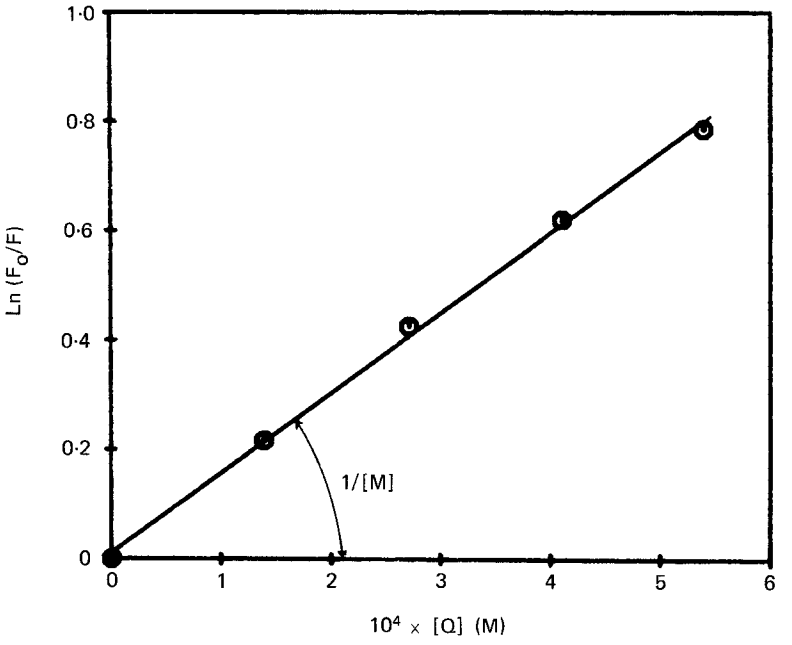

Figure 6. Plot of $\ln(F_0/F)$ of the fluorescence of $7{\cdot}2 \times 10^{-5}$ M $Ru(bipy)_3^{2+}$ versus the concentration of the quencher 9-methylanthracene, in a 0·045 M aqueous solution of sodium dodecyl sulphate. Figure redrawn from the data of Turro and Yekta (1978).

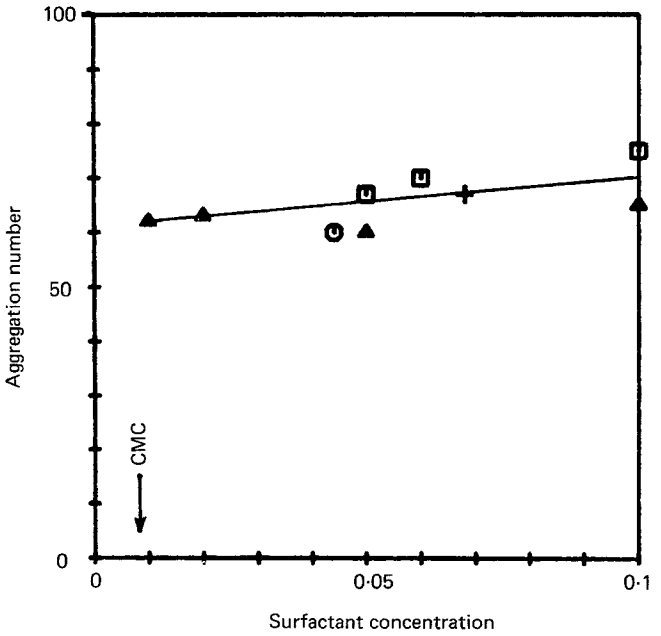

Figure 7. Plot of the aggregation number versus concentration for sodium dodecyl sulphate (Malliaris 1987).

been described in several places (Ghiggino *et al.* 1981, Demas 1983, O'Connor and Phillips 1984). Particularly for micellar systems, the single photon counting technique has been employed in the majority of the reported studies. The experimental and computational aspects of this method, which was first applied in the early 1960s (Koechlin 1961), have been presented in articles, reviews and monographs over the last twenty years (Birks and Munro 1967, Ware 1971, Yguerabide 1972, Poultney 1972, Knight and Selinger 1973, Isenberg 1975, Badea and Brand 1979, O'Connor and Phillips 1984, James and Ware 1985, James *et al.* 1985).

6.1. *Unquenched fluorescence*

The main information obtained from unquenched fluorescence, in either isotropic or microheterogeneous media, is τ_0 which relates primarily to the polarity of the environment of the fluorophor. Thus, pyrene has a life-time of *c.* 400 ns when dissolved in hydrocarbons, and only 250 ns when the solvent is water (Birks 1970). It is possible therefore to determine whether a fluorophor solubilized in a micellar solution, resides in the bulk aqueous phase, or in the micellar pseudo-phase, by simply measuring its life-time. For example, when naphthalene is added to a micellar solution of hexadecyl trimethylammonium bromide it is distributed between the aqueous and the micellar site. This was proved from the unquenched fluoroscence decay of naphthalene which consisted of two different components, the one with $\tau_0 = 10$ ns corresponding to naphthalene dissolved in water, and the other with $\tau_0 = 34$ ns corresponding to micelle-bound naphthalene, as shown in figure 8 (Turro *et al.* 1977).

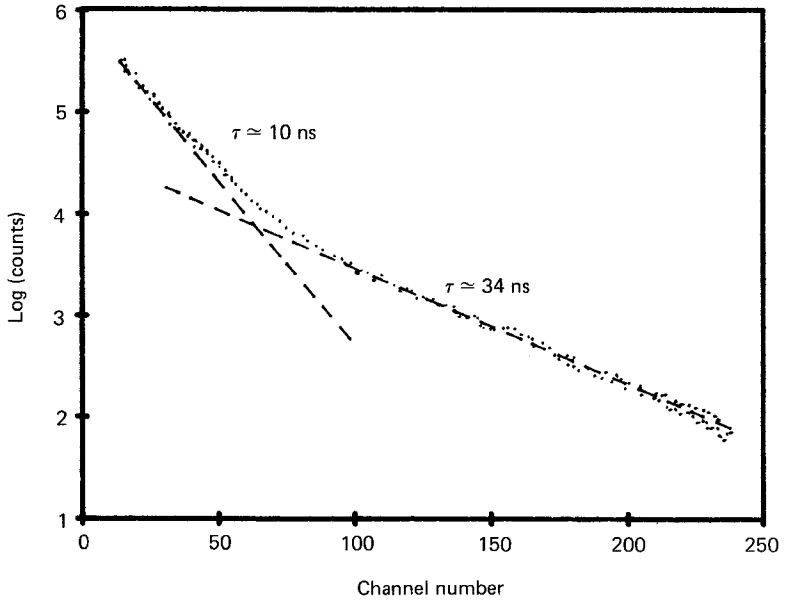

Figure 8. Fluorescence decay of naphthalene (2×10^{-4} M) solubilized in a 2×10^{-2} M aqueous solution of cetyltrimethyl ammonium bromide (Turro *et al.* 1977).

6.2. *Fluorescence quenching*

The most interesting fluorescence probing method is undoubtedly time-resolved fluorescence quenching which, when properly used, can give important information about both the static and dynamic behaviour of isotropic or heterogeneous media. In homogeneous media the change of the fluorescence life-time due to quenching is given by equation (1) which relates τ_0 and τ to k_q and the quencher concentration. Furthermore, in a diffusion-controlled quenching process the decay of the fluorescence follows first-order kinetics, like the unquenched fluorescence, but with parameters k_q and τ instead of k_0 and τ_0.

As stated earlier, Poisson statistics and the compartmentalization of the reactants lead to quite different fluorescence kinetics in micellar media compared to the relatively simple case of homogeneous solutions. The function which describes the quenched decay of the fluorescence of a micelle-bound fluorophor following a δ-shape light pulse, has been discussed in several places (Infelta *et al.* 1974, Tachiya 1975, Atik *et al.* 1979 b, Grieser 1981, Almgren and Löfroth 1981, Van der Auweraer *et al.* 1982, Löfroth and Almgren 1982, Roelants *et al.* 1983, Hashimoto and Thomas 1984, Almgren *et al.* 1984, Roelants *et al.* 1985, Malliaris *et al.* 1986 c), and it has the biexponential form of equation (5)

$$F_t = F_0 \exp\{-A_2 t - A_3[1 - \exp(-A_4 t)]\} \tag{5}$$

F_0 and F_t represent fluorescence intensities at times zero and t after the exciting pulse, whereas A_2, A_3 and A_4 are time-independent parameters having the expressions of equations (6)–(8).

$$A_2 = k_0 + [Q](k_q/A_4)(k^+ + Kk_E[M])/(1 + K[M]) \tag{6}$$

$$A_3 = [Q](k_q/A_4)^2(K/(1 + K[M])) \tag{7}$$

$$A_4 = k_q + k_E[M] + k^- \tag{8}$$

Evidently, the parameters A_2, A_3 and A_4 can be obtained by computer fitting equation (5) to the experimental fluorescence decay curve, and such a fit is shown in figure 9 with a plot of the residuals. However, they can be also estimated by simple graphical methods (Grieser 1981), or by a combination of the two (Malliaris *et al.* 1987 a). Thus, at large t, equation (5) becomes equation (5′)

$$\ln(F_0) - \ln(F_t) = A_2 t + A_3 \tag{5'}$$

and therefore a plot of $\ln(F_t)$ versus time will give a straight line with slope equal to A_2 and intercept with the ln(F) axis equal to A_3 (figure 10). On the other hand, at small t, and with the approximation $\exp(-A_4 t) = 1 - A_4 t$, equation (5) becomes equation (5″)

$$\ln(F_0) - \ln(F_t) = (A_2 + A_3 A_4) t \tag{5''}$$

Since A_2 and A_3 are known from the case of large t, A_4 can be estimated from the limiting slope of equation (5″) at very short times (figure 10). The shape of the fluorescence decay curve (equation (5)), is shown in figure 11 for various combinations of the parameters A_2, A_3 and A_4.

By rearranging equations (6)–(8), equation (9) is derived.

$$k_q = A_3 A_4^2 / (A_2 - k_0 + A_2 A_4) \tag{9}$$

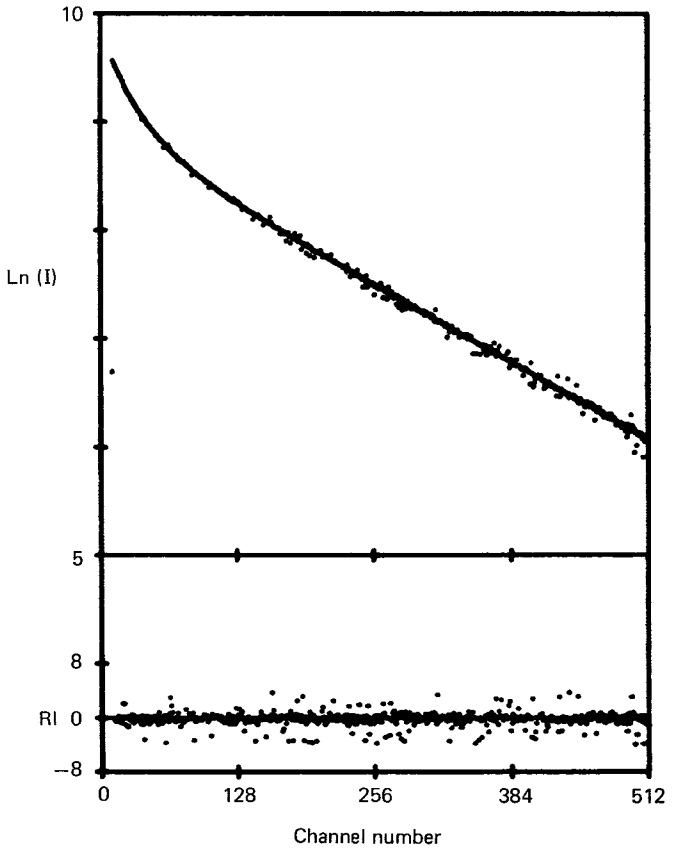

Figure 9. Decay of the fluorescence of pyrene solubilized in an aqueous micellar solution (Malliaris *et al.* 1987 a).

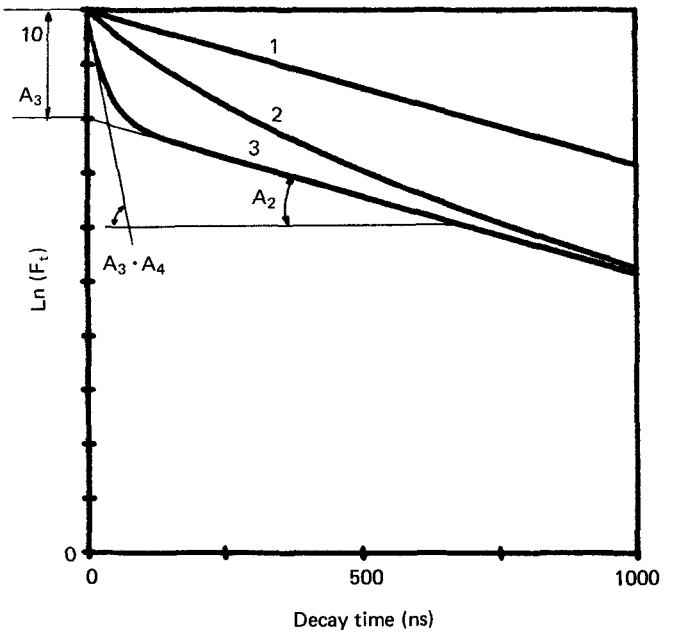

Figure 10. Computer simulated plots of $\ln(F_t)$ versus decay time for immobile reactants, according to equation (5). Curve 1 corresponds to the unquenched decay of pyrene with $\tau_0 = 350$ ns ($A_2 = 2{\cdot}86 \times 10^6$). For curve 2, $A_2 = 2{\cdot}86 \times 10^6$, $A_3 = 2$, and $A_4 = k_q = 2{\cdot}86 \times 10^6$. For curve 3, $A_2 = 2{\cdot}86 \times 10^6$, $A_3 = 2$, $A_4 = 2{\cdot}86 \times 10^7$. The graphic method for obtaining the decay parameters is also indicated.

It permits determination of k_q from the experimental parameters A_2, A_3 and A_4 without any assumption concerning the nature and mobility of the quencher. Similarly, from equations (8)–(9) the expression $k_E[M] + k^-$ can also be determined independently of the type of quencher used.

The interest in the expression $k_E[M] + k^-$ arises from the fact that it represents the overall rate of migration of a quencher from its host micelle. Generally, such migration can occur via two different mechanisms, the one due to quencher desorption from the micelle (k^-), and the other due to collisions, or close encounters, between micelles (k_E) (Almgren *et al.* 1982, 1984, Malliaris *et al.* 1986 c). In the first mechanism, the migration of the quencher takes place by desorption into the aqueous phase, followed later on by adsorption to a different micelle. Evidently in this case the quencher must always go through the aqueous phase during its intermicellar exchange. Note that this type of exchange can occur with both kinds of mobile quenchers, hydrophobic and ionic, therefore k^- becomes equal to zero only in the case of an immobile quencher. In the second mechanism of quencher migration, the quencher is exchanged between two micelles when they collide, or when they approach each other close enough for the charge of the one micelle to affect the charge distribution on the other. Naturally, collisions between ionic micelles are rather unfavourable on the basis of electrostatic interactions. Therefore, in ionic micelles only micellar close encounters can contribute to k_E. However, quencher exchange resulting from micellar collisions has been observed in water-in-oil microemulsions (Atik and Thomas 1981, 1982). Furthermore, since hydrophobic reactants are associated with their host micelles by non-coulombic

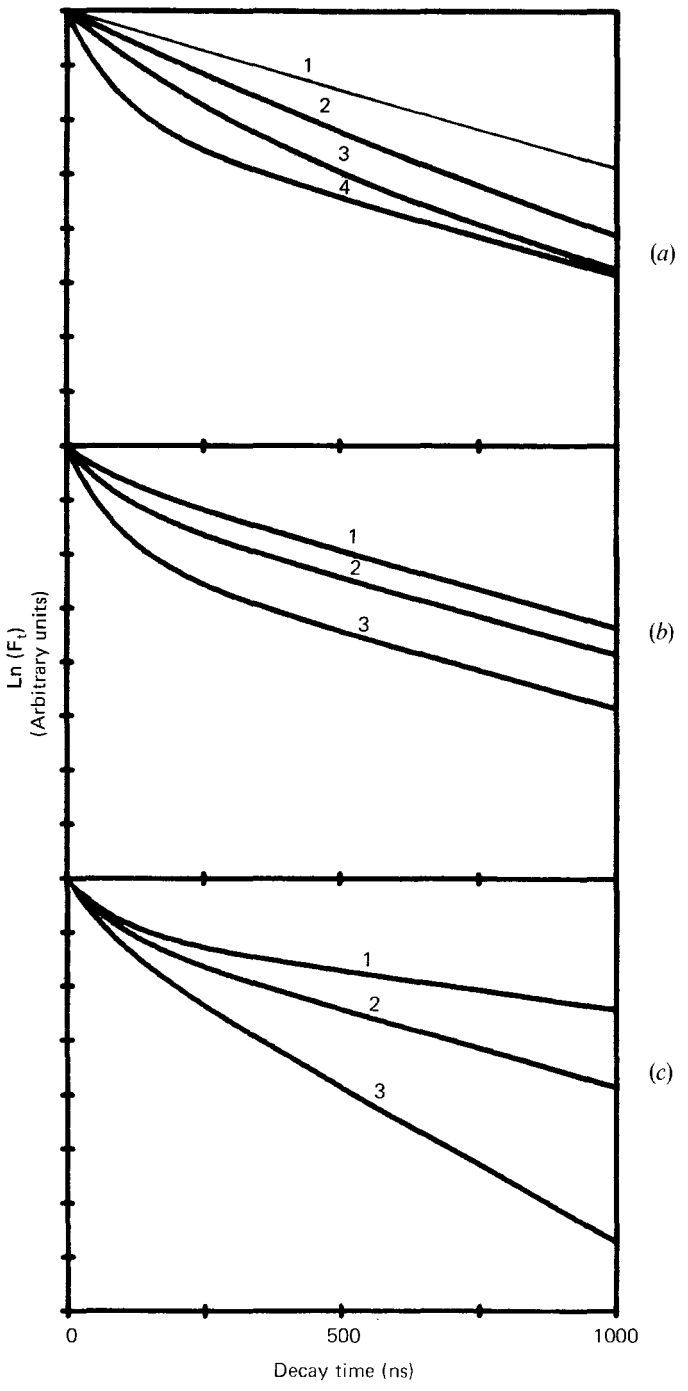

Figure 11. Computer-simulated fluorescence decays for immobile reactants according to equation (5). In each of the three sets of curves, two of the parameters A_2, A_3 and A_4, remain constant while the other changes. Curve (1) of (*a*) corresponds to the unquenched decay for $A_2 = 2{\cdot}86 \times 10^6$. (*a*) Constant parameters, $A_2 = 2{\cdot}86 \times 10^6$, $A_3 = 2$. Variable, $A_4 = 10^6$ in (2), $2{\cdot}86 \times 10^6$ in (3) and $2{\cdot}86 \times 10^7$ in (4). (*b*) $A_2 = 2{\cdot}86 \times 10^6$, $A_4 = 10^7$; $A_3 = 0{\cdot}5$ in (1), 1 in (2) and 2 in (3). (*c*) $A_3 = 1$, $A_4 = 10^7$; $A_2 = 5{\cdot}7 \times 10^6$ in (1), $2{\cdot}86 \times 10^6$ in (2) and $1{\cdot}45 \times 10^6$ in (3).

interactions, it is clear that k_E is equal to zero for this type of quencher. From the above, the conclusion can be drawn that, for an immobile quencher $k_E = k^- = 0$, for a mobile hydrophobic quencher $k_E = 0$, while for a mobile ionic quencher neither k_E nor k^- can be taken *a priori* as equal to zero.

Finally, it should be emphasized that only k_q and $k_E[M] + k^-$ can always be estimated from the experimental parameters A_2, A_3 and A_4 without any reference to the nature of the quencher. All other physical observables involved in the fluorescence decay, i.e. K, [M], k^- and k_E, depend on the type of Q. In the rest of this section, we examine the solution of equations (6)–(8) for the various types of quencher, assuming the fluorophore to be immobile at all times.

6.2.1. *Immobile quencher*

The case of an immobile quencher corresponds to the condition $k_E[M] + k^- = 0$. This means that after the exciting light pulse, there will be adequate time for the fluorophore either to emit or have its excitation quenched, before the quencher leaves the particular micelle. Since for an immobile quencher k^+ is orders of magnitude larger than k^-, the condition $k^+[M] \gg k^-$ (or $K[M] \gg 1$), is always valid and therefore equations (6)–(8) can be approximated by equations (10)–(12).

$$A_2 = k_0 \tag{10}$$

$$A_3 = [Q]/[M] \tag{11}$$

$$A_4 = k_q \tag{12}$$

From these equations k_q and [M] can be obtained, and subsequently the aggregation number N_s can be determined from equation (3). It is evident therefore that in the trivial case of an immobile quencher the micellar parameters [M], N_s and k_q are obtained straightforwardly from the fitting parameters and the quencher concentration [Q]. Obviously, information of a kinetic nature, except k_q, cannot be obtained with immobile reactants.

6.2.2. *Mobile hydrophobic quencher*

When intermicellar collisions are prevented by coulombic repulsions, $k_E = 0$ and equations (6)–(8) transform to equations (13)–(15).

$$A_2 = k_0 + [Q](k_q/A_4)(k^+/(1 + K[M])) \tag{13}$$

$$A_3 = [Q](k_q/A_4)^2(K/(1 + K[M])) \tag{14}$$

$$A_4 = k_q + k^- \tag{15}$$

A characteristic difference between mobile and immobile quenchers immediately becomes evident when equation (10) is compared to equation (13). In the former case A_2 is equal to k_0 and independent of the quencher concentration (equation (10)), while in the latter case A_2 increases as [Q] increases, provided all other factors involved remain constant (equation (13)). This therefore constitutes an easy test to determine if the quencher under investigation is a mobile or an immobile one. In figure 12 the dependence of A_2 on [Q] is shown for the quenching of pyrene solubilized in cetyltrimethyl ammonium chloride micelles, by the immobile quencher cetyl pyridinium chloride and the mobile hydrophobic one dodecyl pyridinium chloride (Malliaris *et al.* 1986 d).

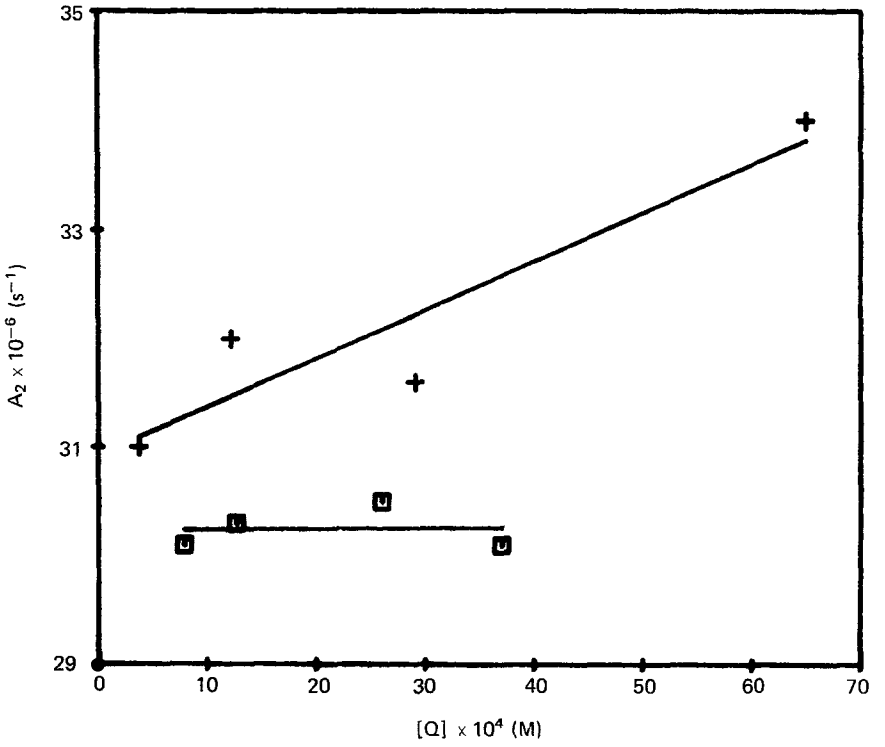

Figure 12. Plot of A_2 versus [Q] for cetyltrimethyl ammonium chloride, and the quenchers: +, the mobile hydrophobic dodecyl pyridinium chloride; and ⊡, the immobile hexadecyl pyridinium chloride.

It is important to emphasize that in the case of a mobile hydrophobic quencher there are four unknowns, k_q, k^+, k^- and [M] and only three equations, equations (13)–(15). Therefore it is impossible to obtain numerical values for all micellar parameters involved. Indeed, although, k_q can always be calculated from equation (9) and then k^- from equation (15), the magnitude of k^+ and [M] cannot be estimated without some additional assumptions. The usual assumption is that N_s is independent of the surfactant concentration $[C_s]$ and therefore [M] is linearly proportional to $[C_s] - [\mathrm{CMC}]$. In this case one could estimate both [M] and k^+ from plots of A_2 versus [Q] according to equation (13) for various $[C_s]$ values. However, this assumption is not always valid as it can be seen from figure 13 where N_s is plotted versus $[C_s]$ for some common surfactants (Malliaris *et al.* 1986 b).

A different assumption which can be made here is $K[\mathrm{M}] \gg 1$. In this case the basic equations (6)–(8) transform to equations (16)–(18).

$$A_2 = k_0 + [\mathrm{Q}](k_q/A_4)(k^-/[\mathrm{M}]) \tag{16}$$

$$A_3 = (k_q/A_4)^2([\mathrm{Q}]/[\mathrm{M}]) \tag{17}$$

$$A_4 = k_q + k^- \tag{18}$$

Since k_q can always be determined from equation (9), k^- can be obtained from equation (18) and [M] from equation (17). Note, however, that when the above made assumption

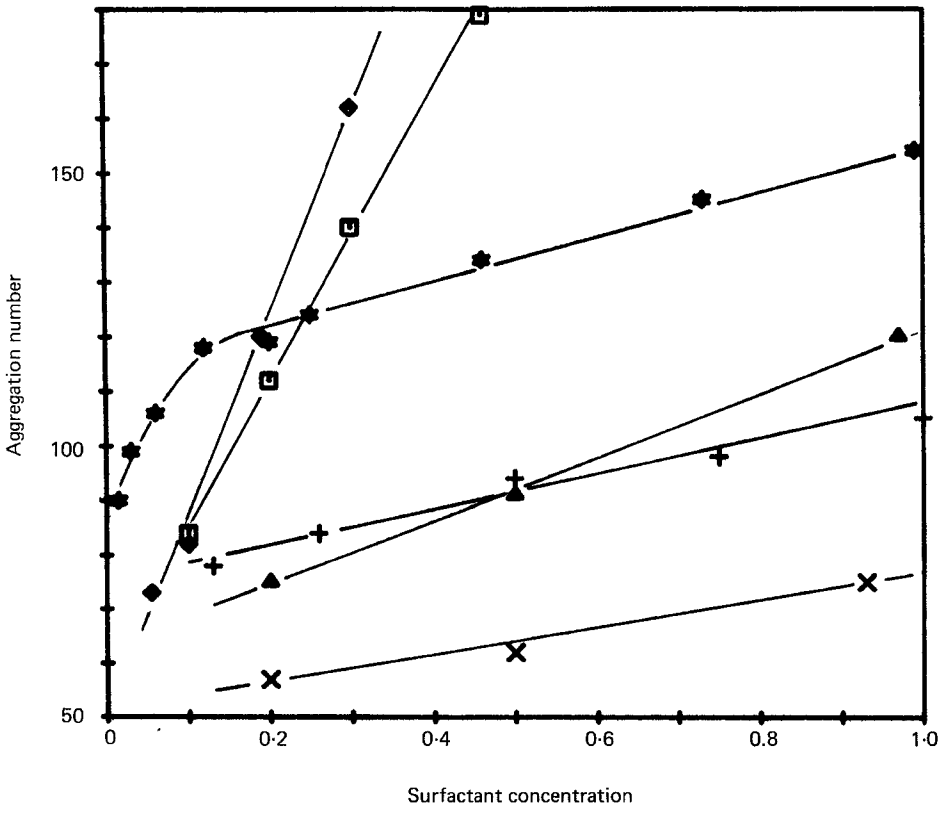

Figure 13. Plots of the aggregation number versus the surfactant concentration (M). ◇, dodecylammonium chloride; ⊡, dodecylmethyl ammonium chloride; ▲, dodecyldimethyl ammonium chloride; ×, dodecyltrimethyl ammonium chloride; +, tetradecyltrimethyl ammonium chloride; ★, hexadecyltrimethyl ammonium chloride.

is valid, i.e. $K[M] \gg 1$, the rate constant k^+ of quencher adsorption to a micelle is expected to be large compared to the rate of fluorescence. Indeed, if the approximation $K[M] \gg 1$ takes the form $K[M] = 100$, and the micellar concentration assumes the typical value of 2×10^{-3} M, K becomes *c.* 5×10^4 M^{-1}. Since, on the other hand, k^- is usually 10^5–10^6 s^{-1}, it turns out that $k^+(=Kk^-)$ is of the order of 10^9–10^{10} s^{-1}, and therefore impossible to measure by fluorescence methods.

Figure 14 shows N_s values for micelles of cetyltrimethyl ammonium chloride, obtained with the hydrophobic mobile quencher dodecyl pyridinium chloride on the assumption $K[M] \gg 1$ (from equations (9), (17) and (3)), as well as the N_s values obtained with the immobile quencher cetylpyridinium chloride (from equations (11) and (3)). It is clear that the two sets of N_s values converge as the surfactant concentration increases. Moreover, using the [M] values obtained with the immobile quencher (equation (11)), the values of the equilibrium constant K and of the product $K[M]$ were calculated from equation (14). These values of the product $K[M]$ are also shown in figure 14, and it becomes evident that the approximation $K[M] \gg 1$ is not always valid. In fact it is a very poor approximation for concentrations of cetyltrimethyl ammonium chloride below *c.* 0·45 M. A consequence of the breakdown of the condition $K[M] \gg 1$ is that the difference between the N_s values measured with the mobile and immobile quencher

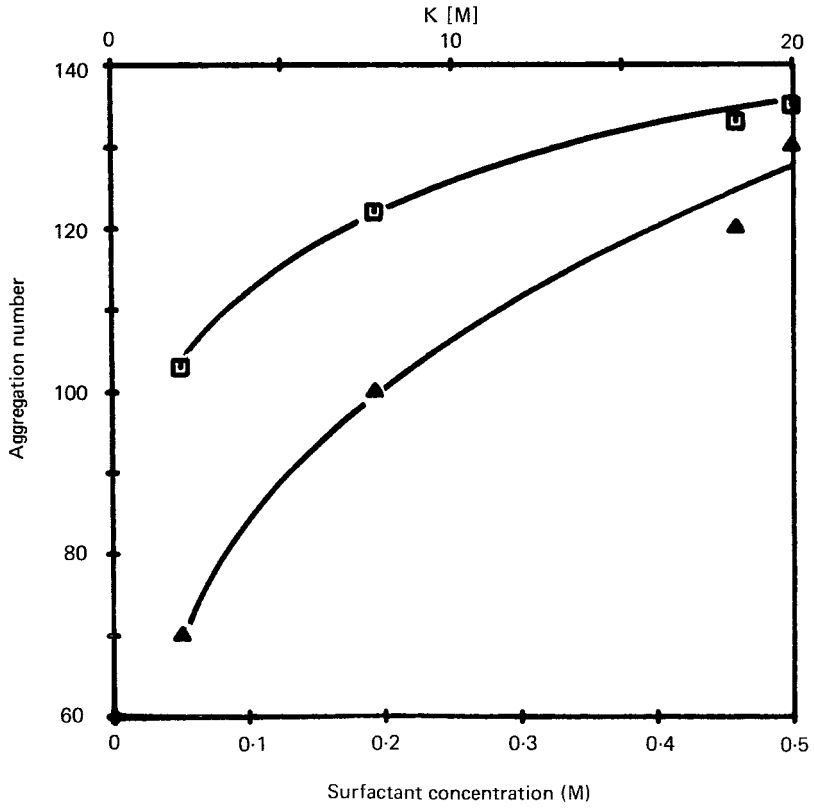

Figure 14. Aggregation number versus surfactant concentration and $K[M]$ for cetyltrimethyl dodecylammonium chloride; ⊡, dodecylmethyl ammonium chloride; ▲, dodecyldimethyl cetylpyridinium chloride on the assumption $K[M] \gg 1$; and ▲, the mobile hydrophobic dodecyl pyridinium chloride. Note that the axis $K[M]$ refers only to the mobile quencher (▲), since in the case of the immobile $K[M]$ is always $\gg 1$.

increases as the approximation $K[M] \gg 1$ becomes less rigorous (see figure 14). Finally, note that k^- which is equal to the difference $A_4 - k_q$ (equation (15)), is independent of [M] for the case of a hydrophobic mobile quencher (figure 15).

6.2.3. *Mobile ionic quencher*

When the quencher is mobile ionic both possibilities of intermicellar quencher exchange can materialize, i.e. by close encounters (k_E), as well as by desorption/adsorption (k^- and k^+). This implies that neither $k_E[M]$ nor k^- can be assumed negligible and therefore the complete set of equations (6)–(8) must be used to describe the situation. In the present case there are five unknowns (k^+, k^-, k_q, k_E and [M]) and only three equations (equations (6)–(8)), therefore a solution is impossible unless some approximations are made. Recall however that k_q is always determinable from equation (9) independently of the nature of the quencher. Following determination of k_q it is possible to estimate k_E and k^- by plotting $A_4 - k_q$ versus [M] according to equation (8), provided [M] is known from measurements with an immobile quencher. In figure 15 such a plot is shown, and the values of k_E and k^- are

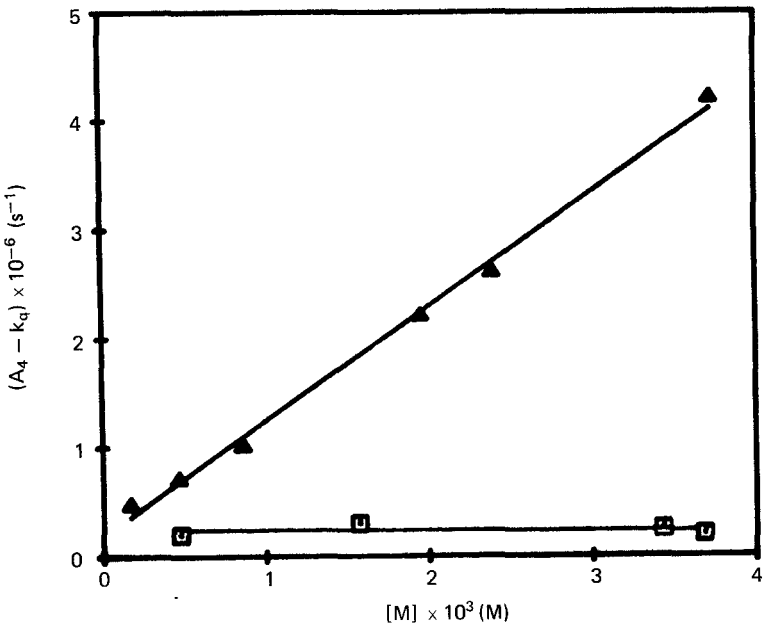

Figure 15. Plot of $A_2 - k_q = k^-$ versus micellar concentration for micelles of cetyltrimethyl ammonium and quencher, the hydrophobic mobile dodecyl pyridinium chloride (⊡), and the ionic mobile I^- (▲).

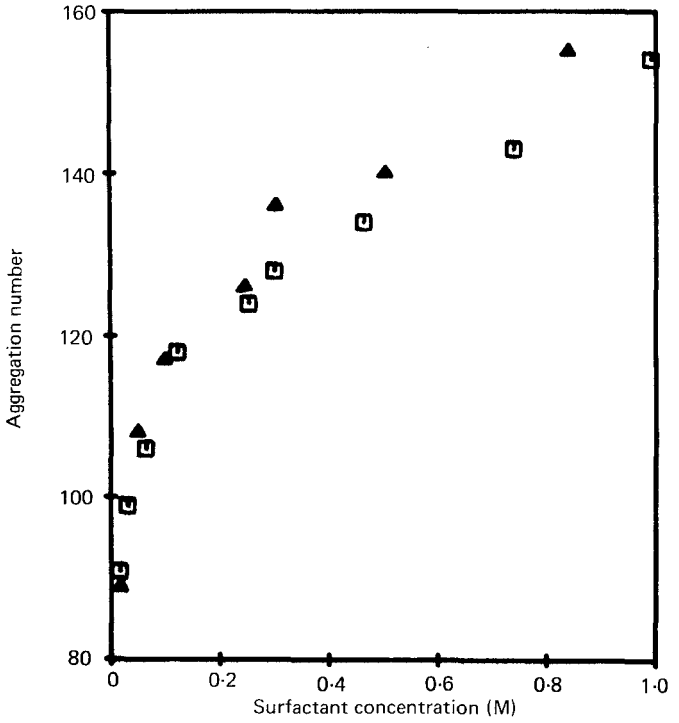

Figure 16. Plot of the aggregation number of micelles of cetyltrimethyl ammonium chloride versus the micellar concentration, for the immobile quencher hexadecylpyridinium chloride (⊡) and the mobile ionic I^- (▲).

indicated for micelles of cetyltrimethyl ammonium chloride with the quencher I^- (Malliaris *et al.* 1986 d). Clearly, in order to obtain values for any other of the two remaining parameters [M] and k^+, it is necessary to simplify equations (6)–(8). If, for this purpose, the usual assumption $K[M] \gg 1$ is made, equations (6)–(8) reduce to equations (19)–(21):

$$A_2 = k_0 + (k^- + k_E[M])(k_q/A_4)([Q]/[M]) \tag{19}$$

$$A_3 = (k_q/A_4)^2([Q]/[M]) \tag{20}$$

$$A_4 = k_q + k_E[M] + k^- \tag{21}$$

Again under this assumption values for k^+ cannot be determined with any accuracy by fluorescence measurements, because k^+ turns out to be large compared to the fluorescence rate. However, using the value of k_q obtained from equation (9), the magnitude of [M] can be found by means of equation (20), and then the aggregation number N_s from equation (3). The thus estimated N_s numbers for micelles of cetyltrimethyl ammonium chloride with the mobile ionic quencher I^-, are plotted in figure 16 along with the corresponding numbers obtained with the immobile quencher cetylpyridinium chloride using equation (11). The two sets of N_s values agree very well indicating that the approximation $K[M] \gg 1$ is valid in this case of an ionic mobile quencher.

An important conclusion drawn from the preceding analysis of the various cases of quenchers is that when the quencher is immobile the parameter A_2 is independent of [Q] and equal to k_0. Also in this case the aggregation number N_s is easily determined from equations (11) and (3). However, further experiments have shown that this is not always correct (Malliaris *et al.* 1986 d). Thus a quencher which behaves as immobile in one micellar solution, i.e. $A_2 = k_0$, can occasionally behave as mobile in a more concentrated solution of the same surfactant, in the sense that A_2 becomes a function of [Q]. This being the case the determination of N_s through equations (11) and (3) leads to serious underestimation of the aggregation number. This important situation of an immobile quencher which under certain conditions becomes mobile and is exchanged among micelles as evidenced by the fact that A_2 becomes a function of [Q], will be discussed in the next section.

7. Intermicellar exchange of 'immobile' quencher

In figure 17 the dependence of A_2 on [Q] is shown for pyrene solubilized in 0·1 M and 0·6 M aqueous solutions of dodecyl ammonium chloride, in the presence of the immobile quencher cetylpyridinium chloride. In the former case of the dilute solution, A_2 is indeed independent of [Q] and equal to k_0, but in the latter case A_2 is a linearly increasing function of the quencher concentration. The same effect was exhibited by aqueous solutions of dodecylmethylammonium chloride at concentrations above *c.* 0·5 M (Malliaris *et al.* 1986 d). This indicates that in the 0·6 M solution of dodecylammonium chloride as well as in solutions of dodecylmethyl ammonium chloride at concentration above 0·5 M, an immobile quencher undergoes intermicellar exchange.

A common feature of the two aqueous surfactant solutions mentioned above is that their micellar size increases steeply as the surfactant concentration increases (see figure 13). Consequently the micelles are expected to be elongated at high $[C_s]$ (Ozeki and Ikeda 1982, Porte and Appell 1984). Moreover, it is known that fast-increasing micellar size and elongated shape are associated with micellar polydispersity (Corkill *et al.* 1969,

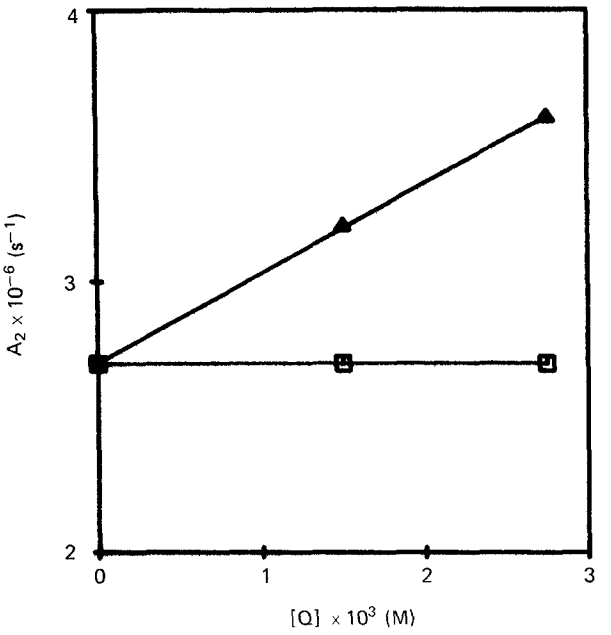

Figure 17. Plot of A_2 versus quencher concentration, for 0·1 M (⊡) and 0·6 M (▲) aqueous solutions of dodecyl ammonium chloride. The quencher in both cases is the immobile hexadecyltrimethyl ammonium chloride.

Mukerjee 1972, Israelachvili *et al.* 1976, Mazer *et al.* 1976). It therefore seems that the phenomenon of an immobile quencher which behaves as a mobile one is observed in micelles which show strong polydispersity. The phenomenon of an immobile quencher, like pyrene (in quenching through excimer formation) or hexadecylpyridinium chloride, behaving like a mobile one when solubilized in certain micellar solutions has been observed not only in solutions of pure micelles (Malliaris *et al.* 1986 d), but also in water–surfactant–alcohol tertiary systems (Malliaris *et al.* 1987 b). Moreover, it is interesting to mention that when alkanes are added to a surfactant–alcohol aqueous solution in which an immobile quencher behaves as a mobile one, the immobility of the quencher is re-established, i.e. A_2 becomes equal to k_0 and independent of [Q] (Malliaris *et al.* 1987 b).

The mechanism which has been proposed for the intermicellar exchange of immobile quenchers is based on a fragmentation–coagulation process (Malliaris *et al.* 1986 d). According to this mechanism, in micellar systems which show strong polydispersity, fragments made of only few surfactant ions, can break away from one micelle and subsequently incorporate into another (Baümuller *et al.* 1978, Lessner *et al.* 1981, Kalhweit 1981, 1982, Lang and Zana 1986). It is possible that a quencher can be carried away in one of those fragments and therefore be exchanged between micelles. In this way a strongly hydrophobic immobile reactant like pyrene undergoes intermicellar exchange without ever coming in contact with the aqueous phase. Note that if the amount of the surfactant involved in the fragments is very small with respect to the total surfactant concentration [C_s], the intermicellar migration of immobile quenchers is quite similar to the previously discussed exchange of mobile hydrophobic quenchers.

Consequently, equations (16)–(18) and the assumption $K[M] \gg 1$ can be used to obtain the relevant micellar parameters in exactly the same way they were used in the case of mobile hydrophobic quencher. However, in the present case k^- and k^+ depend on [M], whereas K is not a true equilibrium constant (Malliaris *et al.* 1986 c). Finally, note that k^- and k^+ correspond to the rate constants of detachment from a micelle and attachment to a micelle, of a micellar fragment which carries a quencher.

8. Conclusions

In concluding, we point out that steady-state and time-resolved fluorescence experiments can give valuable, and occasionally unique, information concerning structure, size and dynamical behaviour of micellar systems. It is, however, essential to emphasize that in either case extreme caution must be exercised in the interpretation of the data to avoid erroneous conclusions.

By steady-state fluorescence methods, which are simple and straightforward, basically, the following limited but valuable information can be obtained.

(*a*) Static properties of the microenvironment of the fluorescent probe, primarily micropolarity and microfluidity.

(*b*) In special cases, the micellar aggregation number N_s can be estimated from equations (3) and (4), provided the fluorophor and the quencher are immobile on the fluorescence time-scale.

Concerning time-resolved fluorescence quenching, the following main conclusions can be drawn, on the assumption that the fluorophor is immobile and has a long fluorescence life-time.

(*a*) k_q and $k_E[M]+k^-$ are always determinable from the fitting parameters and equations (8)–(9), for any type of quencher and without the need of any approximations or assumptions.

(*b*) When the quencher is truly immobile, i.e. $A_2 = k_0$, [M] can be determined from equation (12), and then N_s from equation (3).

(*c*) When $k_E = 0, k^-$ can always be determined for either hydrophobic (equations (9) and (15)), or ionic (equation (9)) mobile quenchers.

(*d*) With mobile hydrophobic quenchers N_s cannot be obtained with accuracy unless $K[M] \gg 1$. If however, [M] is known from measurements with an immobile quencher, then all kinetic parameters, K, k^- and k^+, can be determined.

(*e*) When the condition $K[M] \gg 1$ is valid neither K nor k^+ can be estimated by fluorescence quenching.

(*f*) In the case of mobile ionic quenchers the condition $K[M] \gg 1$ is usually valid and therefore N_s can be determined with accuracy. When k_q and [M] are known, k_E and k^- can be obtained graphically from $A_4 - k_q$ $(= k_E[M]+k^-)$ versus [M] plots.

(*g*) In certain micellar solutions, reactants which are known to be immobile on the time-scale of the fluorescence, behave as mobile ones in the sense that A_2 becomes a function of the quencher concentration.

(*h*) Whenever there is intermicellar migration of an immobile quencher, evidenced by the condition $A_2 > k_0$, N_s can be calculated from equations (16)–(18) and the assumption $K[M] \gg 1$. If this assumption is not valid, the N_s values found will be underestimated.

References

AIKAWA, M., YEKTA, A., and TURRO, N. J., 1979, *Chem. Phys. Lett.*, **68,** 285.

ALMGREN, M., GRIESER, F., and THOMAS, J. K., 1979, *J. Am. Chem. Soc.*, **101,** 279.

ALMGREN, M., GUNNARSSON, G., and LINSE, P., 1982, *Chem. Phys. Lett.*, **85,** 451.

ALMGREN, M., LINSE, P., VAN DER AUWERAER, M., DE SCHRYVER, F. C., GELADE, E., and CROONEN, Y., 1984, *J. Phys. Chem.*, **88,** 289.

ALMGREN, M., and LÖFROTH, J.-E., 1981, *J. Colloid Interface Sci.*, **81,** 486.

ALMGREN, M., and SWARUP, S., 1982, *J. Phys. Chem.*, **86,** 4212.

ALMGREN, M., and SWARUP, S., 1983 a, *J. Colloid Interface Sci.*, **91,** 256.

ALMGREN, M., and SWARUP, S., 1983 b, *J. Phys. Chem.*, **87,** 876.

ANACKER, E. W., 1970, *Cationic Surfactants*, edited by E. Jungermann (New York: Marcel Dekker).

ANACKER, E. W., RUSH, R. M., and JOHNSON, J. S., 1964, *J. Phys. Chem.*, **68,** 81.

ATIK, S. S., KWAN, C. L., and SINGER, L. A., 1979 a, *J. Am. Chem. Soc.*, **101,** 5696.

ATIK, S. S., NAM, M., and SINGER, L. A., 1979 b, *Chem. Phys. Lett.*, **67,** 75.

ATIK, S. S., and SINGER, L. A., 1978 a, *Chem. Phys. Lett.*, **59,** 519.

ATIK, S. S., and SINGER, L. A., 1978 b, *J. Am. Chem. Soc.*, **100,** 3234.

ATIK, S. S., and SINGER, L. A., 1979 a, *Chem. Phys. Lett.*, **66,** 234.

ATIK, S. S., and SINGER, L. A., 1979 b, *J. Am. Chem. Soc.*, **101,** 6759.

ATIK, S. S., and THOMAS, J. K., 1981, *J. Am. Chem. Soc.*, **103,** 4367.

ATIK, S. S., and THOMAS, J. K., 1982, *J. Am. Chem. Soc.*, **102,** 5868.

ATTWOOD, D., ELWORTHY, P. H., and KAYNE, S. B., 1970, *J. Phys. Chem.*, **74,** 3529.

BADEA, M. G., and BRAND, L., 1979, *Meth. Enzymol.*, **61,** 378.

BAÜMULLER, W., HOFFMANN, H., ULBRICHT, W., TONDRE, C., and ZANA, R., 1978, *J. Colloid Interface Sci.*, **64,** 418.

BIRDI, K. S., 1972, *Kolloid-Z. Z. Polym.*, **250,** 731.

BIRKS, J. B., 1970, *Photophysics of Aromatic Molecules* (London: Wiley-Interscience).

BIRKS, J. B., and MUNRO, I. H., 1967, *Prog. Reac. Kinet.*, **4,** 239.

BRAND, L., and GOHLKE, J. R., 1972, *Ann. Rev. Biochem.*, **41,** 843.

CHU, D., and THOMAS, J. K., 1984, *Macromolecules*, **17,** 2142.

COLL, H., 1979, *J. Phys. Chem.*, **74,** 520.

CORKILL, J., GOODMAN, J., WALKER, T., and WYER, J., 1969, *Proc. Roy. Soc. Lond.*, A, **312,** 243.

DEBYE, P., and ANACKER, E. W., 1951, *J. Phys., Colloid Chem.*, **55,** 644.

DEDEREN, J. C., COOSEMANS, L., DE SCHRYVER, F. C., and VAN DORMEAL, A., 1979 a, *Photochem. Photobiol.*, **30,** 443.

DEDEREN, J. C., VAN DER AUWERAER, M., and DE SCHRYVER, F. C., 1979 b, *Chem. Phys. Lett.*, **68,** 451.

DEDEREN, J. C., VAN DER AUWERAER, M., and DE SCHRYVER, F. C., 1981, *J. Phys. Chem.*, **85,** 1198.

DEMAS, J. N., 1983, *Excited States. Lifetime Measurements* (New York: Academic Press).

DILL, K. A., and FLORY, P. J., 1981, *Proc. natn. Acad. Sci., U.S.A.*, **78,** 676.

DONG, D. C., and WINNIK, M. A., 1984, *Can. J. Chem.*, **62,** 2560.

DORRANCE, R. C., and HUNTER, T. F., 1972, *J. Chem. Soc., Faraday I*, **68,** 1312.

DORRANCE, R. C., and HUNTER, T. F., 1974, *J. Chem. Soc., Faraday I*, **70,** 1572.

EDELMAN, G. M., and MCCLURE, W. O., 1968, *Acc. Chem. Res.*, **1,** 65.

ELWORTHY, P. H., FLORENCE, A. Y., and MACFARLANE, C. B., 1968, *Solubilisation by Surface-active Agents* (London: Chapman and Hall).

EMERT, J., BEHRENS, C., and GOLDENBERG, M., 1979, *J. Am. Chem. Soc.*, **101,** 771.

ENCINAS, M. V., GUZMAN, E., and LISSI, E. A., 1983, *J. Phys. Chem.*, **87,** 4770.

FENDLER, J. H., 1982, *Membrane Mimetic Chemistry* (New York: Wiley).

FENDLER, J. H., and FENDLER, E. J., 1975, *Catalysis in Micellar and Macromolecular Systems* (New York: Academic Press).

FÖRSTER, TH., and SEIDEL, H. P., 1965, *Z. Physik. Chem., NF*, **45,** 58.

FÖRSTER, TH., and SELINGER, B. K., 1964, *Z. Naturforsch.*, **19a,** 38.

FROMHERZ, P., 1981, *Ber. Bunsenges Phys. Chem.*, **85,** 891.

GALLA, H. J., and SACKMANN, E., 1974, *Ber. Bunsen. Phys. Chem.*, **98,** 949.

GHIGGINO, K. P., ROBERTS, A. J., and PHILLIPS, D., 1981, *Adv. Polymer Sci.*, **40,** 69.

GRÄTZEL, M., and THOMAS, J. K., 1973, *J. Am. Chem. Soc.*, **95,** 6885.

GRAY, G. W., and WINDSOR, P. A., 1967, *Advances in Chemistry Series*, edited by R. F. Could (Washington D.C.: American Chemical Society).
GRIESER, F., 1981, *Chem. Phys. Lett.*, **83,** 59.
HARKINS, W. D., and MITTELMANN, R., 1949, *J. Colloid Sci.*, **4,** 367.
HARTLEY, G. S., 1936, *Aqueous Solutions of Paraffinic Salts* (Paris: Hermann et Cie).
HATLEE, M. D., and KOZAK, J. J., 1981 a, *J. Chem. Phys.*, **74,** 1098.
HATLEE, M. D., and KOZAK, J. J., 1981 b, *J. Chem. Phys.*, **74,** 5627.
HATLEE, M. D., KOZAK, J. J., ROTHENBERGER, G., INFELTA, P., and GRATZEL, M., 1980, *J. Phys. Chem.*, **84,** 1508.
HASHIMOTO, S., and THOMAS, J. K., 1984, *J. Colloid Interface Sci.*, **102,** 152.
HENGLEIN, A., and SCHEERER, R., 1978, *Ber. Bunsenges Phys. Chem.*, **82,** 1107.
HUMPHRY-BAKER, R., GRATZEL, M., and STEIGER, R., 1980, *J. Am. Chem. Soc.*, **102,** 847.
HUNTER, T. F., 1980, *Chem. Phys. Lett.*, **75,** 152.
INFELTA, P., 1979, *Chem. Phys. Lett.*, **61,** 88.
INFELTA, P., and GRÄTZEL, M., 1979, *J. Chem. Phys.*, **70,** 179.
INFELTA, P., GRATZEL, M., and THOMAS, J. K., 1974, *J. Phys. Chem.*, **78,** 190.
ISENBERG, J., 1975, *Biochemical Fluorescence*, Vol. 1, edited by R. F. Chen and H. Edelhoch (New York: Marcel Dekker).
ISRAELACHVILI, J., MITCHELL, J., and NINHAM, B., 1976, *J. Chem. Soc., Faraday Trans. 2*, **72,** 1525.
JABLONSKI, I. A., 1935, *Z. Phys.*, **94,** 38.
JAMES, D. R., LIY, Y.-S., DEMAYO, P., and WARE, W. R., 1985, *Chem. Phys. Lett.*, **120,** 460.
JAMES, D. R., and WARE, W. R., 1985, *Chem. Phys. Lett.*, **120,** 455.
KALHWEIT, M., 1981, *Pure Appl. Chem.*, **53,** 2069.
KALHWEIT, M., 1982, *J. Colloid Interface Sci.*, **90,** 92.
KALYANASUNDARAM, K., 1978, *Chem. Soc. Rev.*, **7,** 453.
KALYANASUNDARAM, K., and THOMAS, J. K., 1977 a, *J. Am. Chem. Soc.*, **99,** 2039.
KALYANASUNDARAM, K., and THOMAS, J. K., 1977 b, *J. Phys. Chem.*, **81,** 2176.
KALYANASUNDARAM, K., 1987, *Photochemistry in Microheterogeneous Systems* (New York: Academic Press).
KNIGHT, A. E. W., and SELINGER, B. K., 1973, *Aust. J. Chem.*, **26,** 1.
KOECHLIN, Y., 1961, Thesis, University of Paris.
LAKOWICZ, J. R., 1983, *Principles of Fluorescence Spectroscopy* (New York: Plenum).
LANG, J., and ZANA, R., 1986, *J. Phys. Chem.*, **90,** 5258.
LESSNER, E., TAUBNER, M., and KALHWEIT, M., 1981, *J. Phys. Chem.*, **85,** 3167.
LIANOS, P., LANG, J., STRAZIELLE, C., and ZANA, R., 1982, *J. Phys. Chem.*, **86,** 1019.
LIANOS, P., VIRIOT, M., and ZANA, R., 1984, *J. Phys. Chem.*, **88,** 1098.
LIANOS, P., and ZANA, R., 1980, *Chem. Phys. Lett.*, **72,** 171.
LISSI, E., ABUIN, E., and ROCHA, A. M., 1980, *J. Phys. Chem.*, **84,** 2406.
LÖFROTH, J.-E., and ALMGREN, M., 1982, *J. Phys. Chem.*, **86,** 1636.
LUISI, P. L., and STRAUB, B. E. (editors), 1984, *Reverse Micelles* (New York: Plenum).
MCBAIN, J. W., 1950, *Colloid Science* (Boston: Heath).
MALLIARIS, A., 1987, *Adv. Colloid Interface Sci.*, **27,** 153.
MALLIARIS, A., BINANA-LIMBELE, W., and ZANA, R., 1986 a, *J. Colloid Interface Sci.*, **110,** 114.
MALLIARIS, A., DAIS, P., and PALEOS, C. M., 1987 a, *J. Phys. Chem.*, **91,** 1149.
MALLIARIS, A., LANG, J., STURM, J., and ZANA, R., 1987 b, *J. Phys. Chem.*, **91,** 1475.
MALLIARIS, A., LANG, J., and ZANA, R., 1986 b, *J. Colloid Interface Sci.*, **110,** 237.
MALLIARIS, A., LANG, J., and ZANA, R., 1986 c, *J. Phys. Chem.*, **90,** 655.
MALLIARIS, A., LANG, J., and ZANA, R., 1986 d, *J. Chem. Soc., Faraday Trans. 1*, **I,** 109.
MALLIARIS, A., LE MOIGNE, J., STURM, J., and ZANA, R., 1985, *J. Phys. Chem.*, **89,** 2709.
MALLIARIS, A., and PALEOS, C. M., 1984, *J. Colloid Interface Sci.*, **101,** 364.
MAZER, N. A., BENEDEK, G. B., and CAREY, M. C., 1976, *J. Phys. Chem.*, **80,** 1075.
MENGER, M., 1979, *Acc. Chem. Res.*, **12,** 11.
MENGER, M., and DOLL, D. W., 1984, *J. Am. Chem. Soc.*, **106,** 1109.
MORRIS, D. A., and THOMAS, J. K., 1977, *Micellization, Solubilization, and Microemulsions*, Vol. 2, edited by K. L. Mittal (New York: Plenum).
MUKERJEE, P., 1972, *J. Phys. Chem.*, **76,** 565.
MUKERJEE, P., 1979, *Solution Chemistry of Surfactants*, Vol. 1, edited by K. L. Mittal (New York: Plenum).

NAKAJIMA, A., 1971, *Bull. Chem. Soc. Jpn.*, **44,** 3272.
O'CONNOR, D. V., and PHILLIPS, D., 1984, *Time-correlated Single Photon Counting* (London: Academic Press).
OZEKI, S., and IKEDA, S., 1982, *J. Colloid Interface Sci.*, **87,** 424.
PALEOS, C. M., DAIS, P., and MALLIARIS, A., 1984, *J. Polym. Sci., Polym. Chem. Ed.*, **22,** 3383.
PALEOS, C. M., STASSINOPOULOU, C. I., and MALLIARIS, A., 1983, *J. Phys. Chem.*, **87,** 251.
PARKER, C. A., 1968, *Photoluminescence of Solutions* (New York: Elsevier).
PORTE, G., and APPELL, J., 1984, *Surfactants in Solutions*, Vol. 2, edited by K. L. Mittal and J. Lindman (New York: Plenum).
POULTNEY, S. K., 1972, *Adv. Elect. Elect. Phys.*, **31,** 39.
POWNALL, H. J., and SMITH, L. C., 1973, *J. Am. Chem. Soc.*, **95,** 3136.
RAMSDEN, J. J., WEBBER, S. E., and GRATZEL, M., 1985, *J. Phys. Chem.*, **89,** 1740.
ROELANTS, E., GELADE, E., SMID, J., and DE SCHRYVER, F. C., 1985, *J. Colloid Interface Sci.*, **107,** 337.
ROELANTS, E., GELADE, E., VAN DER AUWERAER, M., CROONEN, Y., and DE SCHRYVER, F. C., 1983, *J. Colloid Interface Sci.*, **96,** 288.
SHINITZKY, M., DIANOUX, A.-C., GITLER, C., and WEBER, G., 1971, *Biochem.*, **10,** 2106.
SHINODA, K., NAKAGAWA, T., TAMAMUSHI, B.-I., and ISEMURA, T., 1963, *Colloidal Surfactants* (New York: Academic Press).
SINGER, L. A., 1982, *Solution Behavior of Surfactants*, edited by K. L. Mittal and E. J. Fendler, Vol. 1 (New York: Plenum), p. 73.
TACHIYA, M., 1975, *Chem. Phys. Lett.*, **33,** 289.
THOMAS, J. K., 1980, *Chem. Rev.*, **80,** 283.
THOMAS, J. K., 1987, *J. Phys. Chem.*, **91,** 267.
THOMAS, J. K., GRIESER, F., and WONG, M., 1978, *Ber. Bunsenges Phys. Chem.*, **82,** 937.
TURRO, N. J., 1972, *Modern Molecular Photochemistry* (New York: Benjamin/Cummings).
TURRO, N. J., GEIGER, M. W., HAUTALA, R. R., and SCHORE, N. E., 1977, *Micellization, Solubilization and Microemulsions*, edited by K. L. Mittal, Vol. 1 (New York: Plenum).
TURRO, N. J., GRATZEL, M., and BRAUN, A. M., 1980, *Angew. Chem. Int. Ed. Engl.*, **19,** 675.
TURRO, N. J., and OKUBO, T., 1982, *J. Phys. Chem.*, **86,** 159.
TURRO, N. J., and YEKTA, A., 1978, *J. Am. Chem. Soc.*, **100,** 5951.
VAN DER AUWERAER, M., DEDEREN, M., GELADE, J. C., and DE SCHRYVER, F. C., 1981, *J. Chem. Phys.*, **74,** 1140.
VAN DER AUWERAER, M., DEDEREN, C., PALMANS-WINDELS, C., and DE SCHRYVER, F. C., 1982, *J. Am. Chem. Soc.*, **104,** 1800.
VIRIOT, M., BOUCHY, M., and DONNER, M., 1982, *J. Chim. Phys. Phys.—Chim. Biol.*, **79,** 525.
WARE, W. R., 1971, *Creation and Detection of Excited States*, Vol. 1A, edited by A. A. Lamola (New York: Marcel Dekker).
WENNERSTRÖM, H., and LINDMAN, B., 1979, *Phys. Repts.*, **86,** 2.
WINDSOR, P. A., 1968, *Chem. Rev.*, **68,** 2.
WEBBER, S. E., 1983, *J. Phys. Chem.*, **87,** 347.
YGUERABIDE, J., 1972, *Meth. Enzymol.*, **26,** 498.
ZACHARIASSE, K., and KUHNLE, W., 1976, *Z. Physik. Chem., NF*, **101,** 267.
ZACHARIASSE, K., 1978, *Chem. Phys. Lett.*, **57,** 429.
ZACHARIASSE, K., KOZANKIEWICZ, B., and KUHNLE, W., 1983, *Photochemistry and Photobiology*, edited by A. H. Zewail, Vol. 2 (London: Harwood).