Excimer Formation of 1-Pyrene-Sodium Sulphonate in Inverse Micelles

Dennis J. Miller, Uwe K. A. Klein, and Manfred Hauser

Institut für Physikalische Chemie der Universität Stuttgart, Germany

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Micelle aggregation numbers may be obtained from the fluorescence time dependence of excimer-forming solubilised substances. The micellar system cetyldimethylbenzylammonium chloride (CDBA)/water/benzene was studied using 1-pyrene sodium sulphonate as the fluorescent probe. Solubilised water increases the CDBA aggregation number. It is shown that the micelles are polydisperse at lower CDBA concentrations. The influence of concentration and aggregation number on the photostationary excimer/monomer fluorescence intensity ratio of the solubilised probe is discussed.

Introduction

A variety of amphiphiles form micelles in non-polar solvents [1, 2]. These are called "inverse" because, unlike micelles in aqueous solution, the polar heads are in the interior, the non-polar tails forming the outer layer. The micelles can solubilise ionic substances and often considerable amounts of water.

Excimer formation of solubilised aromatic molecules has been studied to obtain information on the viscosity in micelles, their size and the distribution of the solubilisate among them [3–8]. Excimers have also been used to study the volume of binding sites in biological membranes [9] and the properties of membrane-like structures in phospholipid solutions [10, 11].

The micelle acts as a cage for the solubilised species during the lifetime of excited singlet states, so that micelles containing only one solubilised aromatic molecule cannot conribute to excimer fluorescence. These investigations have so far been confined to normal micelles in aqueous solutions, but a related method, the examination of the prototropic fluorescence change of oxypyrene trisulphonate, has been used to study the inverse micelles in benzene solutions of cetyldimethylbenzylammoniumchloride (CDBA) and water [12]. The proton and the excited anion are trapped in the same micellar cage, so that the fluorescent substance behaves as if it were in an acid medium. Since only small concentrations of solubilised substance need be used, it should not cause significant changes in the micelle parameters. The polarity of water solubilised in Aerosol OT inverse micelles is known to be lower than that of bulk water [13], so there may also be thermodynamic micellar effects on the equilibrium parameters of fluorescence change which were assumed to be the same as in bulk water. Excimer studies are concerned with kinetic phenomena and kinetic parameters can be measured directly. On the other hand, fairly high concentrations of the solubilised probe substance must be used, and the distribution of its molecules among the micelles must be deduced on the basis of simple assumptions.

In this paper, excimer formation of 1-pyrene sodium suphonate, PS, is studied in the inverse micellar CDBA/water/benzene system. Absorption spectra show that the PS is solubilised in the detergent layer, not in the water pool [14].

Reprint requests to Dr. M. Hauser, Institut für Physikalische Chemie der Universität Stuttgart, Pfaffenwaldring 55, *D-7000 Stuttgart 80*, West-Germany.

Experimental

The substances used have been described previously [14]. Samples were degassed by a freeze-thaw technique. Fluorescence decay curves were obtained using a Single Photon Nanosecond Spectrometer, ORTEC system. (Excitation wavelength 337 nm, temperature 25 °C.) Monomer and excimer fluorescence were isolated by means of filters transparent in the ranges 360 – 420 nm and >515 nm respectively. Fluorescence spectra were measured on a Fluorispec SF-1 Spectrophotometer, the intensities at 397 and 500 nm being taken as proportional to the monomer and excimer intensities respectively.

Excimer Kinetics

Excimers are formed according to the following schema [15]

$$\begin{array}{ccc} A^* + A & \underset{k_d}{\overset{k_a \cdot c}{\rightleftharpoons}} & (AA)^* \\ \downarrow k & & \downarrow k' \\ A, h\nu & & A + A, h\nu' \end{array}$$

In homogeneous solution, the time-dependence of excimer fluorescence on excitation by a δ-function flash is given by the biexponential function

$$I' \propto \exp(-\lambda_1 t) - \exp(-\lambda_2 t)$$

where the two time constants obey the equations:

$$\lambda_1 + \lambda_2 = k + k' + k_d + k_a c\,, \qquad (1\,a)$$

$$\lambda_1 \cdot \lambda_2 = k(k' + k_d) + k' k_a c\,. \qquad (1\,b)$$

In micellar solutions, it is convenient to express the concentration as a mole ratio $c' = [\text{solubilisate}]/[\text{detergent}]$; the bimolecular excimer formation rate constant then has units s^{-1} and will be written $\overline{k_a}$.

In Fig. 1 $\lambda_1 + \lambda_2$ and $\lambda_1 \cdot \lambda_2$ are plotted against the solubilised PS concentration for 0.04 M CDBA/0.54 M H_2O/Benzene ($c'_{water} = 13.5$). If Eqs. (1 a, b) are approximately valid for the micellar system, then from the gradients and intercepts of Fig. 1 we obtain

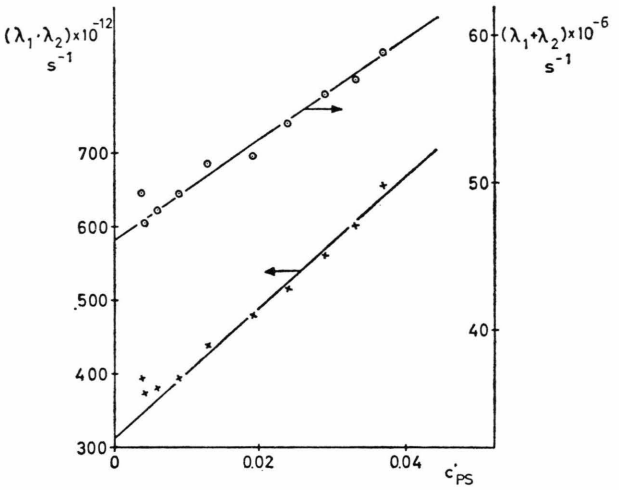

Fig. 1. Time constants of PS excimer fluorescence as a function of c'_{PS}. Solvent: 0.04 M CDBA/0.54 M H_2O/benzene.

all four fluorescence parameters, as shown in Table 1. The mole volume of CDBA, which is needed to find k_a from $\overline{k_a}$, is estimated at 0.5 l mol^{-1}. By measuring the decay function at very low PS concentration, the pure monomer decay rate, k, can be found independently.

Table 1. Fluorescence parameters of PS in 0.04 M CDBA/0.54 M H_2O/Benzene.

Fluorescence decay		Excimer formation/dissociation	
k	$8.7\times10^6\ s^{-1}$	$\overline{k_a}$	$3.4\times10^8\ s^{-1}$
k'	$2.6\times10^7\ s^{-1}$	k_a	$1.7\times10^8\ l\ mol^{-1}\ s^{-1}$
		k_d	$1.1\times10^7\ s^{-1}$

In water excimer formation of PS is much faster, $k_a = 1.6 \times 10^{10}\ l\ mol^{-1}\ s^{-1}$ (see [16]). Excimer studies of pyrene in aqueous cetyltrimethylammonium bromide show the microviscosity in the micelles to be some forty times the viscosity of hexadecane [17]; fluorescence depolarisation experiments suggest the microviscosity is 7 – 10 times that of hexadecane [18]. The mobility of PS in inverse CDBA micelles should be more hindered than that of pyrene in normal micelles. Since the sulphonate group is held in the ionic layer the molecule probably diffuses in two dimensions. The kinetics of diffusion controlled processes do not depend strongly on whether the diffusion is 2- or 3-dimensional. Owens theory [19] of 2-dimensional diffusion controlled reactions, which has been applied to excimer kinetics in phospholipid vesicles [11] could explain a reduction in k_a of about a factor of four. The observed difference of about a hundredfold between the solvents water and CDBA/water/benzene is thus interpreted as chiefly an effect of the high viscosity in the ionic layer.

In homogeneous solution, the limiting value of λ_1 at low concentrations is k, the reciprocal monomer fluorescence lifetime. This does not apply to micellar solutions, for at low concentrations all the excimer fluorescence comes from doubly occupied micelles where the effective concentration for excimer formation is c_m, the reciprocal of the volume of the micelle available to the solubilisate. It is thus convenient to plot $\lambda_1 - k$ against concentration; the general behaviour of such plots is shown in Figure 2. The limiting value of $\lambda_1 - k$ at low concentration, $\Delta\lambda_0$, is a function of the micelle size and the fluorescence parameters k, k', k_d and $\overline{k_a}$. Both the monomer

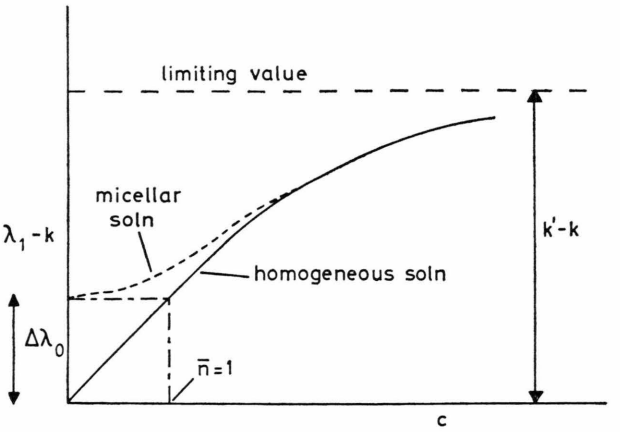

Fig. 2. Slow excimer time constant λ_1 in micellar and homogeneous solution.

and slow excimer fluorescence decay constants increase with concentration, so their difference, $\lambda_1 - \lambda_M$, does not vary strongly with concentration. Measurements of $\lambda_1 - \lambda_M$ are not greatly affected by traces of oxygen or other quenching impurities in the sample, so that the average of several measurements of $\lambda_1 - \lambda_M$ at low PS concentration is a good estimate of $\Delta\lambda_0$. A plot of $\lambda_1 - k$ as a function of c'_{PS} is shown in Fig. 3 for 0.04 M CDBA/0.54 M H_2O/Benzene.

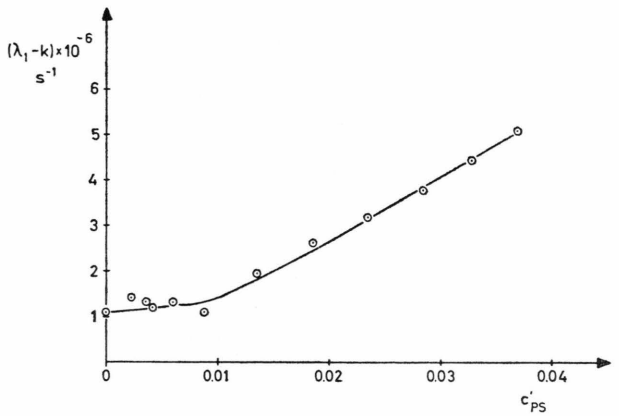

Fig. 3. Slow excimer time constant of PS. Solvent: 0.04 M CDBA/0.54 M H_2O/benzene.

The corresponding curve for 0.02 M CDBA has a similar shape. The large increase in $\lambda_1 - k$ over the c'_{PS} range studied shows that there are high occupation numbers in the most concentrated solutions so they should approximately obey the formulae for homogeneous solutions, Eqs. (1 a, b), and the corresponding formulae [20] for λ_1 and λ_2. To fit the theoretical curve for homogeneous solution to the experimental results, the values of k, k', and k_d in Table 1 were used and $\overline{k_a}$ adjusted to give a fit at high concentration. The result, $\overline{k_a} = 2.6 \times 10^8\ s^{-1}$ is comparable to the value of $3.4 \times 10^8\ s^{-1}$ in Table 1.

As shown schematically in Fig. 2, the aggregation number g may be determined from the concentration at which the homogeneous curve has the value $\Delta\lambda_0$. This concentration corresponds to an average occupation number $\bar{n} = 1$. The aggregation number thus obtained was $g = 150$ for the region 0.02 – 0.04 M CDBA with $c'_{water} = 13.5$. Though excimer kinetics may not give very accurate absolute aggregation numbers, changes in $\Delta\lambda_0$ are useful showing changes in aggregation number with the concentration of detergent or solubilised water. If $\Delta\lambda_0$ is small compared with $k' - k$, it is almost proportional to the intramicellar concentration of a doubly occupied micelle, and so inversely proportional to the aggregation number. The variation of aggregation number with concentration of CDBA at $c'_{water} = 13.5$ is shown in Figure 4. There appears to be a critical concentration in the region of $6 - 8 \times 10^{-3}$ M with the aggregation number levelling off at about 25 for low concentration. This limiting value of 25 probably represents the smallest micelle that can accomodate two PS molecules, rather than an average aggregation number.

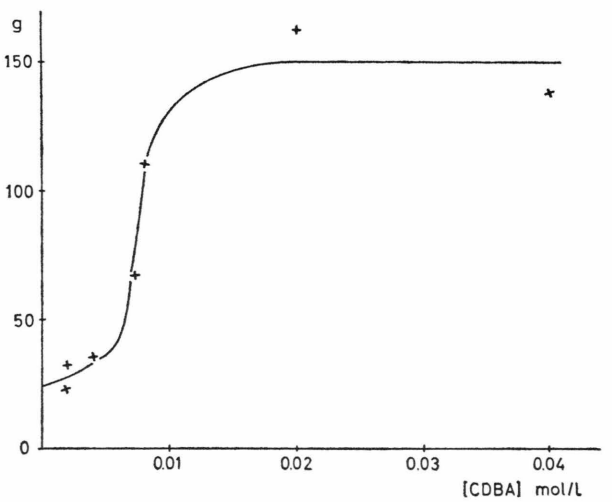

Fig. 4. Aggregation number as a function of [CDBA]; $c'_{water} = 13.5$.

Studies of the hydrolysis equilibrium of solubilised tetrachlorocuprate (II) show a well defined critical concentration at $2 - 3 \times 10^{-3}$ M CDBA for high water concentrations, but not at $c'_{water} = 13.5$ (see [21]). In view of the essential difference between kinetic and thermodynamic measurements, this discrepancy is not surprising.

Solubilised water increases the aggregation number as shown in Figure 5. Light scattering measurements confirm this but indicate that g is strongly dependent on the amount of water present, even at low water concentrations [22]. The aggregation number of Aerosol OT solutions in hydrocarbons is also increased by solubilised water [23]. This effect may be considered in terms of the curvature of the detergent layer, the so-called "R-theory" [2]. On the basis of the mass-action law, an increase in the amount of solubilised water present should make the detergent layer less

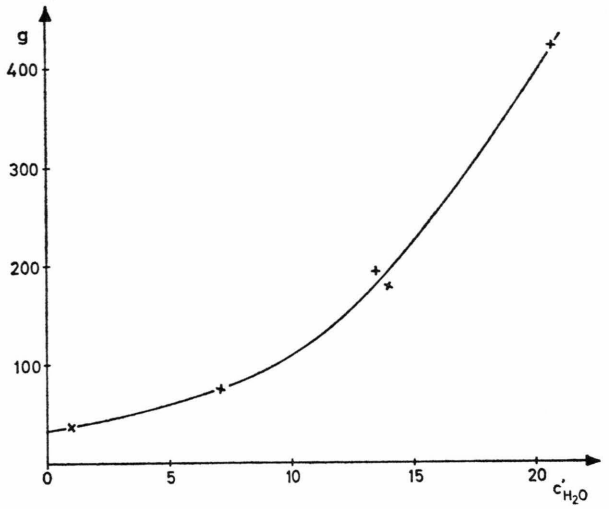

Fig. 5. Aggregation number in 0.02 M benzolic CDBA as a function of c'_{water} (from excimer kinetics).

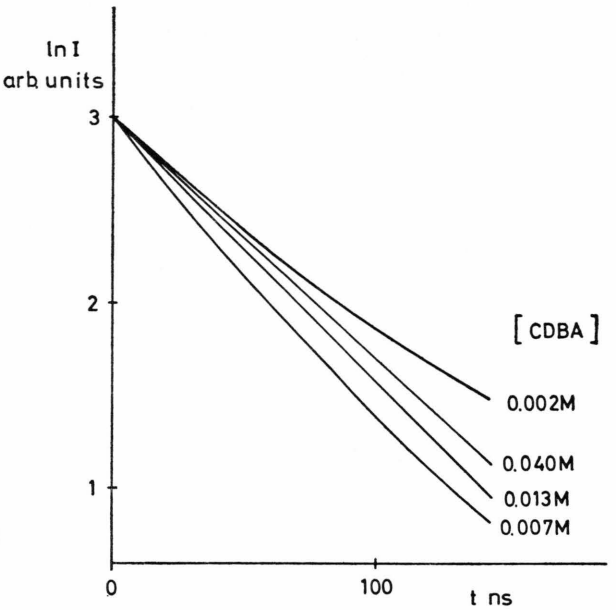

Fig. 6. Monomer fluorescence decay curves for various [CDBA]; $c'_{PS} = 0.047$; $c'_{\text{water}} = 13.5$.

concave towards the water, so that more water can interact with the polar heads. This would increase the aggregation number of the inverse micelles.

Monomer Fluorescence

Whereas excimer fluorescence has no component from singly occupied micelles, monomer fluorescence has components from all occupied micelles, that from singly occupied ones predominating at low average occupation numbers. Dilution of a concentrated micellar PS solution with benzene alters the monomer fluorescence decay curve. Typical curves are shown in Figure 6. At high detergent concentration, the curve can be represented by a simple exponential function. As the CDBA concentration decreases, the fluorescence decay becomes first slightly faster and then markedly biexponential. At low CDBA concentration, the fluorescence lifetime of the slow component is that of the unquenched monomer. The fluorescence can then be fitted to a curve of the type

$$I \propto A \exp\{-\lambda_f t\} + \exp\{-k t\} \qquad (2)$$

where the second term is due to fluorescence from singly occupied micelles and the first due to fluorescence from doubly and multiply occupied ones. This decays faster because excimer formation quenches the monomer fluorescence. (Even if the curve is really multi-exponential, it is not generally possible to resolve it into more than two components in practice.)

The curves in Fig. 6 indicate that on dilution at first the micelle size and/or the number of micelles decrease so that the occupation number increases. On further dilution the critical concentration is reached and there are then many singly occupied micelles. In contrast to the system pyrene/Na-dodecyl sulphate/water [3] there is no precipitation of the solubilisate.

If the occupation numbers of the micelles follow a Poisson distribution, the coefficient A, the ratio of molecules in doubly and multiply occupied micelles to those in singly occupied ones is given by $e^{\bar{n}} - 1$. Since $\bar{n} = g \cdot c'$ the aggregation number may be found. If the fluorescence decay cannot be resolved into components of the form of Eq. (2) a lower limit to A, and hence g, may nevertheless be found. Aggregation numbers calculated from monomer fluorescence curves are shown in Table 2. Only below about 3×10^{-3} M CDBA is the slow time constant near the unquenched monomer decay rate, $8.7 \times 10^6\ s^{-1}$.

The aggregation numbers of Table 2 are smaller than those calculated from excimer fluorescence decay curves (Figure 4). The system is polydisperse at low detergent concentrations. An average aggre-

Table 2. Aggregation numbers of CDBA in benzene calculated from monomer fluorescence. $c'_{water} = 13.5$.

CDBA mol l^{-1}	c'_{PS}	$\lambda_s \times 10^{-6}\ s^{-1}$	A	g
3.6×10^{-3}	0.029	12.5 [a]	0.90	22
2.6×10^{-3}	0.029	9.2	0.30	9
2.2×10^{-3}	0.047	8.8	0.22	4
1.9×10^{-3}	0.029	9.0	0.14	4

λ_s is slow time constant; [a] decay curve not bi-exponential.

gation number is probably given by the monomer fluorescence method, whereas the excimer method described above gives the aggregation number of micelles that are large enough to contain two pyrene sulphonate molecules.

Photostationary Fluorescence

In homogeneous solution, the photostationary intensities of monomer and excimer fluorescence are given as functions of concentrations by the Stern-Volmer equations:

$$\text{monomer: } I_0/I = 1 + c/c_h\,, \qquad (3\,a)$$

$$\text{excimer: } I'_\infty/I' = 1 + c_h/c\,, \qquad (3\,b)$$

where the half concentration obeys the relation $c_h = k(k' + k_d)/k' k_a$. We may assume that at high occupation numbers, the formulae (3 a, b) for homogeneous solution give an adequatve value of the half concentration in a micellar system. If we work in mole ratios, c', as above, the half concentration denoted c_h' is dimensionless. It is related to the aggregation number, g, and reciprocal micelle volume, c_m, by

$$1/c_h' g = c_m/c_h\,. \qquad (4)$$

In 0.02 – 0.04 M benzolic CBDA, with $c'_{water} = 13.5$ detergent, c_h' for PS was found to be 0.03 from Stern-Volmer plots. Using the measured monomer fluorescence decay constant and the values of k', k_a and k_d obtained from the plots in Fig. 1, c_h' was calculated to be 0.04.

From Eqs. (3 a, b) I'/I is proportional to the concentration in homogeneous solution. This does not necessarily hold for micellar solution. Let us assume that the micelles are monodisperse, that the effective concentration for excimer formation inside an n-fold occupied micelle is $(n-1)c_m$ and that the numbers of n-fold occupied micelles follow a Poisson distribution. Using Eqs. (3 a, b) and (4) and summing the intensities over all occupation numbers it may be shown that

$$\frac{I'}{I}\,\frac{\eta_0}{\eta'_\infty}\,\frac{1}{c'} = \frac{\sum_{i=0}^{\infty}(c'g)^i/[i!\,(1+(i+1)/c_h' g)]}{c_h' \sum_{i=0}^{\infty}(c'g)^i/[i!\,(1+i/c_h' g)]}\,. \qquad (5)$$

Equation (5) predicts that the reduced fluorescence ratio, $I'/(I c')$, which is constant in homogeneous solution, increases with concentration to a limiting value at high occupation numbers. The reduced fluorescence ratio increases with increasing aggregation number but this effect is small if $g \gg 1/c_h'$ or $\bar{n} \gg 1$. At low occupation number, Eq. (5) reduces to

$$\frac{I'}{I}\,\frac{\eta_0}{\eta'_\infty}\,\frac{1}{c'} = \frac{1}{c_h'\,[1 + 1/(c_h' g)]}\,. \qquad (6)$$

At constant PS concentration, the reduced fluorescence ratio decreases with detergent concentration for [CDBA] $\lesssim 8\times10^{-3}$ M (see Fig. 7) showing that in this region the micelles decrease in size. This

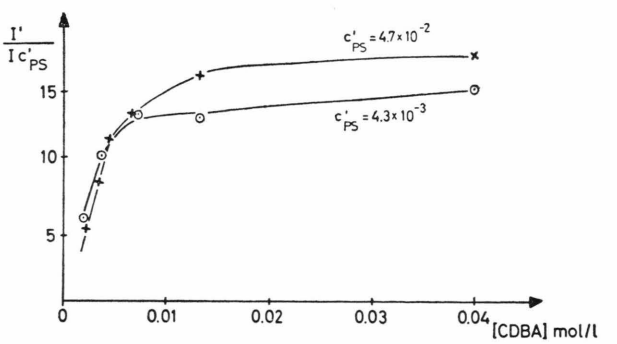

Fig. 7. Reduced fluorescence ratio as a function of [CDBA]; $c'_{water} = 13.5$.

phenomenon parallels the effect of detergent concentration on the ratio of acid to basic form fluorescence of oxypyrene trisulphonate in CDBA/water/benzene [12]. On substituting aggregation numbers from excimer kinetics (Fig. 4) into Eq. (6) a reduced fluorescence ratio curve is obtained which is in poor agreement with the measured one below the critical concentration, 8×10^{-3} M. In particular, the photostationary measurements show no levelling off of aggregation number at low detergent concentrations, probably because the system is polydisperse here: the reduced fluorescence ratio depends on the average micelle size, but excimer kinetics at low

CDBA concentration are related to the size of micelles containing two PS molecules. As the CDBA concentration tends to zero the number of such micelles (and hence the excimer fluorescence intensity) tends to zero, but their limiting aggregation number may be much greater than one.

Figure 8 shows that at 0.02 – 0.04 M CDBA the reduced fluorescence ratio is almost independent of c'_{PS}. On the basis of Eq. (5) this is reasonable since g is about five times $1/c_h'$. At low detergent concentrations, when the micelles are small, the reduced fluorescence ratio should increase with c'_{PS}. If this were so, the curves in Fig. 7 would not cross. Equation (5) may fail at low detergent concentrations because the system is then polydisperse.

Solubilised water increases the reduced fluorescence ratio (Fig. 9), as is to be expected from the results in Figure 5. At high water concentrations the reduced fluorescence ratio levels off in spite of increasing micelle size, because the micelles are so large that $g \gg 1/c_h'$. The fact that the reduced fluorescence ratio is almost constant at high water concentration supports the view that PS is solubilised in the detergent layer rather than in the water [14].

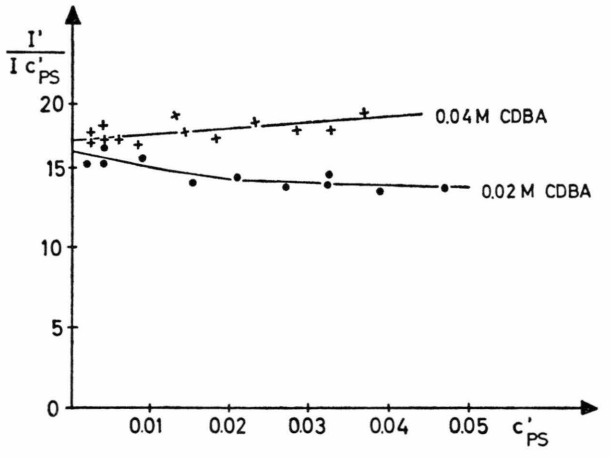

Fig. 8. Reduced fluorescence ratio as a function of c'_{PS}; $c'_{water} = 13.5$.

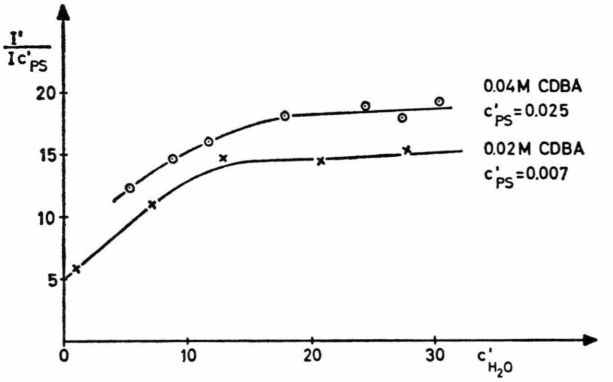

Fig. 9. Reduced fluorescence ratio as a function of c'_{water}.

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