February, 1982] ©1982 The Chemical Society of Japan NOTES Bull. Chem. Soc. Jpn., 55, 607—608 (1982)

Dimerization Kinetics of Fluoresceine in Aqueous Solution

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(Received March 30, 1981)

Synopsis. Kinetic studies of the dimerization reaction of fluorescein (doubly charged anion) in aqueous solution were performed by the ultrasonic absorption method. The data obtained could not be interpreted by the rate equation derived with the usual viscosity effect assumed for the diffusion process. Rather, an experimental equation was found, which adequately fits the data. Here, the viscosity contribution is $k_f=k_f^0/\eta_r$, $k_b=k_b^0$, and $K=K^0/\eta_r$.

The aggregation of many dyes have been investigated both spectrophotometrically and kinetically as a simple model system of molecular aggregation. The kinetics of the aggregation of fluorescein, however, has not been investigated because the aggregate is unstable and the rates of the reaction are too rapid to be studied by conventional techniques.

Ultrasonic absorption of fluorescein in aqueous solution was measured in the frequency range 4.5—95 MHz under the condition of pH 12.0, where the dye exists as the doubly charged anion, 25 °C, ionic strength 5 M.† The absorptions could be explained by a single relaxation.

Since fluorescein is known to form a dimer under the present experimental conditions,[1-5] the relaxation absorption is ascribed to the following equilibrium:

$$2\,M^{2-} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} D^{4-}, \qquad (1)$$

where M^{2-} is the monomer and D^{4-} is the dimer. For this reaction, the relaxation frequency f_r is related to the rate constants by the following equation:

$$(2\pi f_r)^2 = k_b^2 + 8k_f k_b C_0, \qquad (2)$$

where C_0 is the total concentration of the dye. This equation predicts that f_r^2 increases monotonously with C_0, which was not satisfied by the experimental data.

The fact that the viscosity of the solution changes greatly with increase of the dye concentration lead us to consider the viscosity effect on the rate constants. Dimerization reaction involves the diffusion process, whose rate constants are theoretically calculated by the Smoluchowski-Debye-Eigen Equations.[6] Combining with the Stokes-Einstein relation, which represents the viscosity dependence of the diffusion coefficients, the diffusion rate constants are shown to have the viscosity dependence $k=k^0/\eta_r$ where η_r is the relative viscosity, and the superscript 0 represents the value at $\eta_r=1$. Following the usual treatment of the kinetics, k_f and k_b have the same viscosity dependence as that of the diffusion rate constants.[7] Accordingly, Eq. 2 becomes

$$(2\pi f_r)^2 = (k_b^{02} + 8k_f^0 k_b^0 C_0)/\eta_r^2. \qquad (3)$$

If this is correct, the $(2\pi f_r)^2\eta_r^2$ *vs.* C_0 plot should be linear. As shown in Fig. 1, however, the plot deviates greatly from a straight line. Further, this idea also fails to give a satisfactory explanation for the concentration dependence of the absorption amplitude. Thus we must look further for a satisfactory explanation.

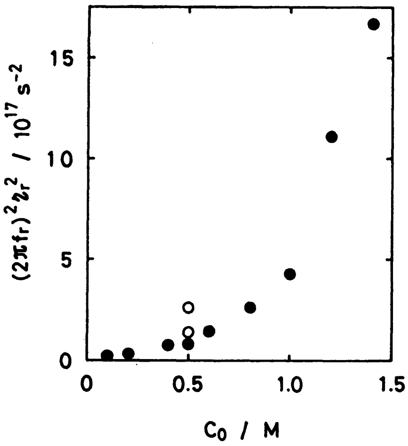

Fig. 1. Plot of $(2\pi f_r)^2\eta_r^2$ *vs.* C_0.
●: Glycerol free, ○: glycerol added.

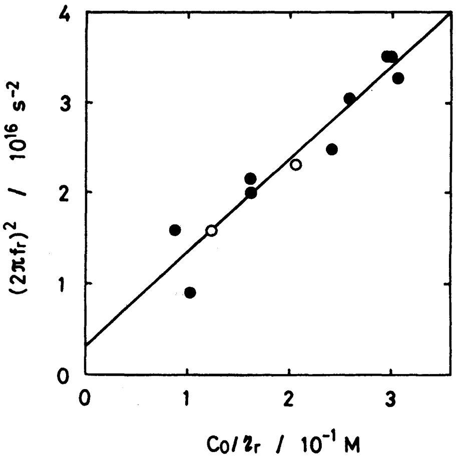

Fig. 2. Plot of $(2\pi f_r)^2$ *vs.* C_0/η_r.
●: Glycerol free, ○: glycerol added.

Petrucci[8] has proposed an empirical formula for the viscosity dependence of the rate constants, *i.e.*, $k_f=k_f^0/\eta_r$ and $k_b=k_b^0$. In this case, Eq. 2 can be rewritten as

$$(2\pi f_r)^2 = k_b^{02} + 8k_f^0 k_b^0 C_0/\eta_r. \qquad (4)$$

Equation 4 predicts a linear relationship for $(2\pi f_r)^2$ *vs.* C_0/η_r plot, which is well satisfied, as shown in Fig. 2. Further, this type of viscosity dependence of f_r was ascertained by changing the viscosity of the solution with the addition of glycerol, *i.e.*, to the 0.5 M dye solution, 8.3 and 16.6 wt% glycerol were added. The results obtained are also plotted in Fig. 2 with the open circles and the tendency displayed is similar to that for dye alone. From the slope and the intercept of the straight

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† In this paper, 1 M=1 mol dm^{-3}.

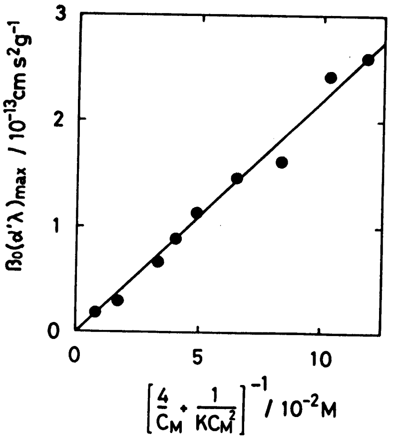

Fig. 3. Plot of $\beta_0(\alpha'\lambda)_{\max}$ *vs.* $(4/C_M+1/KC_M^2)^{-1}$ with $C_M=(-1+(1+8KC_0)^{1/2})/4K$.

line, the rate constants were determined to be $k_f^0=2.3\times10^8$ M^{-1} s^{-1} and $k_b^0=5.6\times10^7$ s^{-1}; the equilibrium constant $K^0(=k_f^0/k_b^0)$ was determined to be 4.1 M^{-1}, which is in good agreement with the literature value determined by the spectrophotometric measurements.[1-5] The value of k_f^0 is in the same order of magnitude as that of other dyes,[9,10] which means that the electric charge effect is relatively small in k_f^0, while the value of k_b^0 is the largest of those dyes studied, which is the reason for the small equilibrium constant. The experimental result that the viscosity dependences of k_f and k_b are different from each other, in conflict with the theoretical prediction described before, is interesting.

The result obtained for the rate constants also means that the equilibrium constant changes with the viscosity, *i.e.*, $K=K^0/\eta_r$. This fact was further ascertained by the result of the absorption amplitude. The relaxation absorption $(\alpha'\lambda)_{\max}$ of reaction 1 is expressed by

$$(\alpha'\lambda)_{\max}=\frac{\pi(\Delta V)^2}{2\beta_0RT}\left(\frac{4}{C_M}+\frac{1}{KC_M^2}\right)^{-1} \qquad (5)$$

with

$$C_M=\{-1+(1+8KC_0)^{1/2}\}/4K,$$

where ΔV is the volume change of the reaction and β_0 is the adiabatic compressibility. If we use the viscosity dependent equilibrium constant $K=K^0/\eta_r$, a good linear relationship is obtained, which is well coincident with that predicted by Eq. 5. The value of ΔV was determined from the slope of the straight line in Fig. 3 to be -6.0 cm^3 mol^{-1}.[11]

In conclusion, the viscosity effect on k_f and the fact that the value of k_f is in the same order of magnitude with those of other dyes, show that the association reaction is diffusion controlled. On the other hand, the viscosity independence of k_b implies that the dissociation reaction is limited by some intramolecular process of the dimer.

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