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Fluorescence and Internal Rotation: Their Dependence on Viscosity of the Medium[1]

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Substances possessing substituted phenyl groups which are capable of internal rotation increase their fluorescence with increasing viscosity of the medium. The fluorescence yield Φ of auramine O, a substituted diphenylmethane dye, measured in glycerol at various temperatures and in dextrose–glycerol–water mixtures at room temperature followed the relation $\Phi = (\eta/T)/[\alpha + \beta(\eta/T)]$ where α and β are constants, η is the viscosity and T is absolute temperature. The results are interpreted as being due to one of the fluorescence quenching steps passing *via* a rotational diffusion process. We calculate that this step takes place if during the period of excitation of the light-excited dye the groups on the molecule have rotated relative to one another by more than 2°.

Introduction

It has never been entirely clear why certain dyes in solution fluoresce while others do not. However, it has been noted[2] that substituted diphenyl and triphenyl compounds do not fluoresce while their counterparts in which the two rings are joined by a bridge (*e.g.*, as in xanthene dyes) do fluoresce strongly. Apparently, a necessary condition for fluorescence is that the molecule be in a rigid configuration. Stark[3] and Schmidt[4] have noticed that some diphenyl- and triphenylmethane dyes which do not fluoresce in ordinary solvents will, however, fluoresce strongly in highly viscous media such as glycerol at low temperatures.

It is the purpose of the present paper to investigate this phenomenon quantitatively and to suggest a mechanism for the origin of the enhancement of fluorescence with increasing viscosity of the medium. Incidentally, we have found this effect to provide an extremely convenient means for measuring the viscosity of high-viscosity media.

Experimental

A wide variety of normally non-fluorescent aromatic compounds were examined to see whether they reveal fluorescence when present in dilute solution in highly viscous media. The procedure was to compare (by eye) the intensity of fluorescence of these substances in water at room temperature, in glycerol at room temperature, and in glycerol at 0° using for excitation light of wave lengths corresponding to the principal absorption maxima of the compound in question. Of all the substances examined, only the diphenylmethane dyes, the triphenylmethane dyes, substituted aminostilbene derivatives, and substituted benzophenones showed appreciable enhancement of fluorescence in glycerol at 0°. In particular, the effect is very pronounced in the case of auramine O (tetramethyldiaminodiphenylketoimine hydrochloride), in the case of Michler's ketone (tetramethyldiaminodiphenyl ketone) and in the case of Blanchophor BA (a substituted aminostilbenesulfonic acid made by General Dyestuffs Corporation). All three substances also exhibit a striking fluorescence when bound to certain high polymeric substances.[5] Auramine O was chosen for the quantitative studies since its two principal absorption maxima nearly coincide with two spectral lines of the mercury arc and the fluorescence can be isolated easily from the incident light by a filter and is readily detectable using a phototube.

Absorption and Fluorescence Spectra.—The absorption spectra of auramine O (National Aniline, histological grade) in glycerol as measured in a DU Beckman spectrophotometer are illustrated in Fig. 1. When in water the spectra of the dye are identical with that in glycerol except that the maxima are shifted to shorter wave lengths by 5 mμ. The solutions obey Beer's law at least up to 10^{-3} mol.

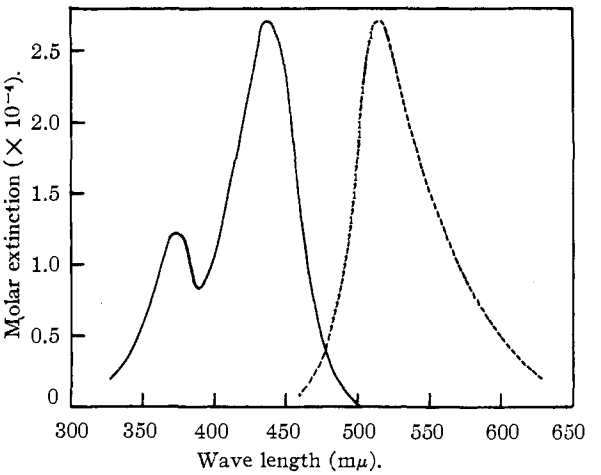

Fig. 1.—Spectra of Auramine O in glycerol at 25°: solid line, absorption spectra; dotted line, fluorescence spectra (relative intensities).

The fluorescence spectra (Fig. 1) were measured using the irradiated sample as the light source, a Bausch and Lomb monochromater as the spectroscope, and an RCA 1P22 photomultiplier (S-8 response) as the detector. The electrical output of the phototube was registered on a Leeds and Northrup Speedomax high impedance recorder whose time axis was synchronized with the movement of the wave length drum on the monochromater.[6] Excitation by the 365 mμ lines of Hg and by the 436 mμ line of Hg gave the same fluorescence spectra.

Intensity of Fluorescence.—The measurement of fluorescence intensities was carried out in an Aminco light scattering photometer.[7] In all these measurements, in order to avoid complicated optical and geometrical corrections due to the penetration of incident light into the cell,[8] the cell was placed so that the front surface was at the center of rotation of the arm supporting the photomultiplier tube as shown in Fig. 2. The blue (436 mμ) and near ultraviolet (365 mμ) lines of the Hg lamp S, isolated with the appropriate Corning glass filter combination F_1, were used to excite the fluorescence. A yellow filter F_2 (Corning 3–70) with cutoff in transmission below 490 mμ was interposed between the sample and the photomultiplier tube P (RCA 1P21 with S-4 response), the latter being at an angle of 30° from the incident beam.

In all cases, the concentration of the dye was sufficiently high (10^{-4} mol.) to absorb nearly all of the light within a

(1) Presented at the Meeting-in-Miniature, American Chemical Society, Brooklyn, N. Y., February 25, 1955.

(2) See, for example, (a) P. Pringsheim, "Fluorescence and Phosphorescence," Interscience Publishers, Inc., New York, N. Y., 1949, Sec. 132, and (b) T. Förster, "Fluoreszenz Organischer Verbindungen," Vandenheock and Ruprecht, Göttingen, 1951, Sec. 20.

(3) J. Stark and P. Lipp, *Z. physik. Chem.*, **86**, 36 (1913).

(4) G. C. Schmidt, *Ann. Physik*, **65**, 247 (1921).

(5) (a) G. Oster, *Compt. rend.*, **232**, 1708 (1951); (b) G. Oster, *J. Polymer Sci.*, **16**, 235 (1955).

(6) This apparatus was designed and constructed by Mr. Neil Wotherspoon of this Laboratory.

(7) G. Oster, *Anal. Chem.*, **25**, 1165 (1953).

(8) See, for example, E. J. Bowen and F. Wokes, "Fluorescence of Solutions," Longmans, Green and Co., New York, N. Y., 1953, Chpt. 4.

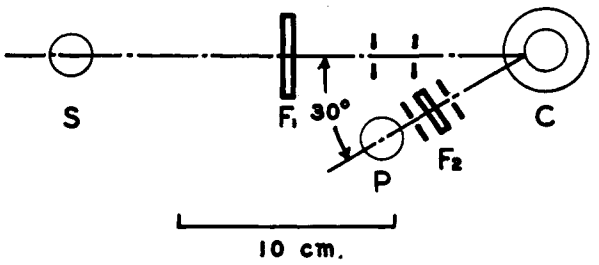

Fig. 2.—Schematic diagram of fluorimeter.

millimeter from the front surface. It was found that even less concentrated solutions (10^{-5} mol.) gave substantially the same result indicating that concentration quenching is not appreciable.

The solution under examination is contained in a cylindrical glass cell (18 mm. in diameter and 80 mm. in height) which was placed at the center of another cylindrical glass cell (35 mm. in diameter and 70 mm. in height) to make a water jacket around the sample. In order to control the temperature, water from a thermostated bath was circulated through the water jacket. The temperature of the solution at the front surface of the inner cell was measured by a copper–constantan thermocouple placed at this point. The temperature of the sample was varied at a rate of less than 1° per 30 min. in order to better approximate thermal equilibrium in the cell.

The fluorescence intensity of auramine O in glycerol in the neighborhood of room temperature is so low that an accurate determination of the absolute value of the quantum yield of fluorescence is difficult to carry out. Fortunately, acriflavine hydrochloride whose absolute quantum yield is known, namely, 0.40,[9] has a fluorescence spectrum practically identical with that of auramine O in glycerol. The quantum yields of the fluorescence of auramine O in glycerol at various temperatures (Fig. 3) were determined by comparing the photocurrent with that for acriflavine (using blue incident light) when the latter was at concentrations (in water) sufficiently high for total absorption but not in the range where self-quenching occurs. By knowing the relative intensities of the blue and of the ultraviolet incident beams (using the known spectral sensitivity of the photomultiplier tube) we can calculate the absolute quantum yield of auramine O in glycerol when ultraviolet light was used for excitation.

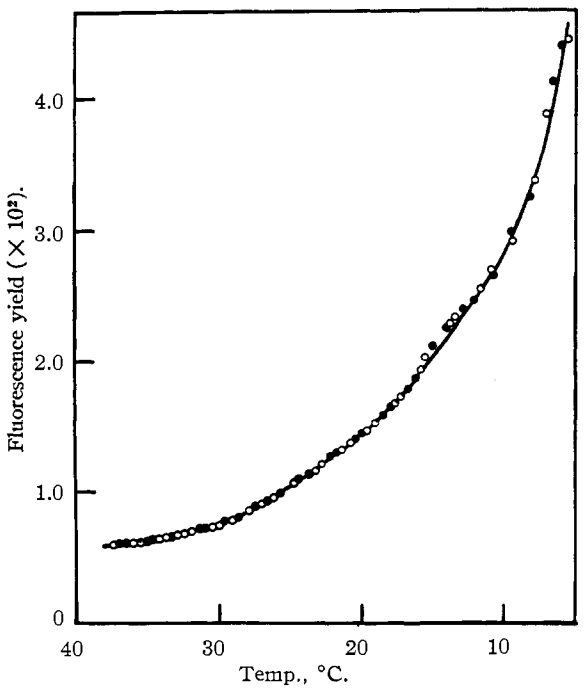

Fig. 3.—Quantum yield of fluorescence of Auramine O in glycerol as a function of temperature: solid circles, excitation by 436 mμ; open circles, excitation by 365 mμ.

(9) S. B. Sengupta, *J. Indian Chem. Soc.*, **15**, 263 (1938). See also G. Oster and A. D. McLaren, *J. Gen. Physiol.*, **33**, 215 (1950).

Measurement of Viscosity.—The viscosities of the various solutions employed were measured in a Brookfield viscometer (Model LVF using spindle no. 2). Even for the most viscous media the viscous behavior was found to be Newtonian. A 400-ml. beaker containing the sample was placed in a thermostated bath and the temperature of the sample was measured with a copper–constantan thermocouple attached to the guard legs of the viscometer which was immersed at the center of the beaker.

The viscosity of the medium was varied in two ways; (1) by changing the temperature of solution of the dye in pure glycerol, and (2) by using as the solvent mixtures of glycerol, dextrose and water at room temperature. The viscosity of the glycerol solution as a function of temperature (Fig. 4) was obtained by measuring the viscosity while the temperature of the bath was varied at the very slow rate of less than 1° per hour. As seen in Fig. 4, the activation energy is about 15 kcal. per mole. Incidentally, we found that higher activation energies are obtained if small amounts of water are present in the glycerol. The mixtures having various viscosities at 25° were prepared in the following manner. Two hundred grams of dextrose was melted at 200° in 500 ml. of glycerol to give a highly viscous glass (medium A). Water was mixed with glycerol in equal volume ratio (medium B). Now auramine O was dissolved in media A and B to give the same concentration of the dye (10^{-4} mol.) for both solutions and then these solutions were intermixed at various proportions to give varying viscosities covering the range of 3000 centipoise to 10 centipoise at room temperature.

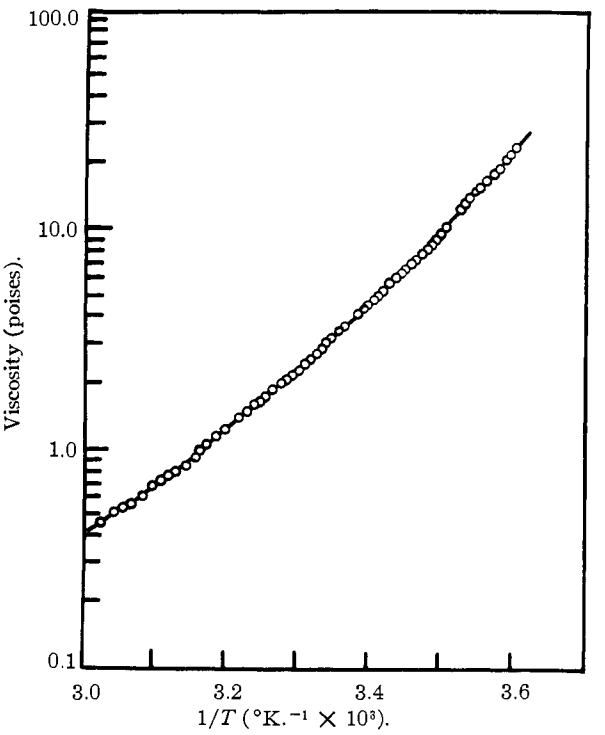

Fig. 4.—Viscosity of glycerol (on logarithmic scale) as a function of reciprocal absolute temperature.

Discussion

As seen in Fig. 1, the fluorescence spectrum of auramine O in glycerol is not a mirror image of the entire absorption spectrum but rather only of the longer wave length peak. This fact, together with the fact that the fluorescence spectrum is identical

whether near ultraviolet or blue light is used for excitation, indicates that the shorter wave length peak corresponds to the transition from the ground state to the second excited electronic state. The transition from the second to the first excited state is a radiationless process and is followed by a transition to the ground state with emission of fluorescence. The quantum efficiency of fluorescence was found to be independent of the wave length of the exciting light, showing that transition from the second excited electronic state directly to the ground state is excluded.

A molecule in the first excited electronic state can fall to the ground state with the emission of fluorescence or fall to the ground state by some radiationless processes with the donation of heat to the surroundings. A still further possibility of transition is a fall *via* a metastable intermediate state but this is not important over most of the viscosity–temperature ranges covered by the present work (see below). Radiationless transitions from the first excited electronic state to the ground state are generally ascribed to the transfer of electronic excitation energy to the vibrational energy of the ground state which is then dissipated as heat to the surroundings.[10] However, for molecules such as the diphenylmethanes where there exists the possibility of internal rotation, another route for energy dissipation should be considered. The mobility of large groups (*e.g.*, substituted phenyl groups) in such molecules would be affected by the surroundings. Thus, we would expect that the extent of this energy dissipation *via* rotational diffusion and hence the fluorescence intensity to be a function of the viscosity of the solvent. More generally, the fluorescence should depend on the rotational diffusion constant of rotating groups of the molecule.

Our fluorescence intensity data for auramine O, excepting that for very low values of T/η (see below), can be represented by the empirical formula

$$\text{intensity} = \frac{(\eta/T)}{\alpha + \beta(\eta/T)} \qquad (1)$$

where α and β are constants and η and T are the viscosity and the absolute temperature, respectively, of the media. Equation 1 is obeyed regardless of whether the medium is glycerol at different temperatures or is one of the glycerol–dextrose–water mixtures at room temperature.

Suppose that there are three pathways for the transition of excited auramine O (A*) to the ground state (A), namely

$$A^* \xrightarrow{k_1} A + h\nu_f \quad \text{(fluorescence)}$$

$$A^* \xrightarrow{k_2} A + \text{heat} \quad \text{(usual internal conversion by vibrational processes)}$$

$$A^* \xrightarrow{k_3} A + \text{heat} \quad \text{(internal conversion } \textit{via} \text{ rotational diffusion processes)}$$

The probability for the occurrence of each of these processes is proportional to their rate constants. Hence, the probability for the occurrence of any of these three processes is proportional to the sum of the rate constants, namely

$$k = k_1 + k_2 + k_3 \qquad (2)$$

If no quenching processes had occurred then the rate constant for emission of fluorescence is given by $k_1 = 1/\tau_1$ where τ_1 is the intrinsic lifetime of the fluorescence. The constant k_1 is proportional to the area under the absorption peak corresponding to the state of excitation under question, namely, the longer wave length peak in Fig. 1. Using the well-known Ladenburg formula in the form used by Förster,[11] we obtain from the data in Fig. 1 ($\int \epsilon d(1/\lambda) = 8.4 \times 10^7$ mole^{-1} cm.$^{-2}$) we calculate that $\tau_1 = 4.3 \times 10^{-9}$ sec. Since the fluorescence yield Φ is equal to the mean lifetime $\tau = 1/k$ divided by τ_1, we can then rewrite eq. 2 in the form

$$\frac{1}{\Phi} = 1 + \frac{\tau_1}{\tau_2} + \frac{\tau_1}{\tau_3} \qquad (3)$$

where τ_2 and τ_3 are the reciprocals of the rate constants of steps 2 and 3, respectively. For a diffusion process $1/\tau_3$ must be proportional to the diffusion constant which, in turn, is proportional to T/η. Now eq. 3 becomes

$$\frac{1}{\Phi} = 1 + \frac{\tau_1}{\tau_2} + a\tau_1\left(\frac{T}{\eta}\right) \qquad (4)$$

where a is a proportionality constant. The inverse of eq. 4 has the form of the empirical expression given by eq. 1. The plot of $1/\Phi$ as a function of T/η (Fig. 5) gives a straight line in agreement with eq. 4 except for very low values of T/η. From the intercept of the straight line we obtain the value 0.05 for the quantum yield of normal fluorescence when rotation, theoretically, has ceased. Hence we calculate that $\tau_2 = 2.3 \times 10^{-10}$ sec. From the slope of the line and again using the value of τ_1, obtained previously, we calculate that $a = 2.0 \times 10^8$ g. sec.$^{-2}$ cm.$^{-1}$ degree^{-1}.

We can make a rough estimate of the necessary condition for quenching *via* rotational diffusion processes. The Einstein theory of Brownian movement shows that the average of the square of the angular displacement $\overline{(\Delta\theta)^2}$ during the time Δt is given by $\overline{(\Delta\theta)^2} = 2D\,\Delta t$ where D is the rotational diffusion constant. The Einstein–Stokes relation for a rotating sphere of molar volume V is $D = RT/6V\eta$, where R is the gas constant. In our problem Δt is equal to τ_3, the time during which the excited molecule is quenched *via* the rotational diffusion process, and D is the rotational diffusion constant of a substituted phenyl ring of auramine O. On substituting the expressions for τ_3 and D into the equation for the fluctuation in angle, we obtain $\overline{(\Delta\theta)^2} = R/3aV$. Using our experimental value for a and assuming that $V = 100$ cc. per mole, we obtain for the root mean square angular displacement the value 2.2°. That is, quenching *via* rotational diffusional processes takes place only if the groups of the excited molecule have moved relative to one another by more than 2° during the period of excitation. It is clear, therefore, that any chemical linkages connecting these groups which prevent rotation will not allow quenching of this type to take

(10) Compare, however, ref. 2a, Sec. 83 with ref. 8, Chpt. 5 and M. Kasha, *Disc. Faraday Soc.*, No. **9**, 14 (1950).

(11) R. Ladenburg, *Z. Physik*, **4**, 451 (1921); for review, see ref. 2b, Sec. 32.

place and, hence, the fluorescence will be independent of the viscosity of the medium.

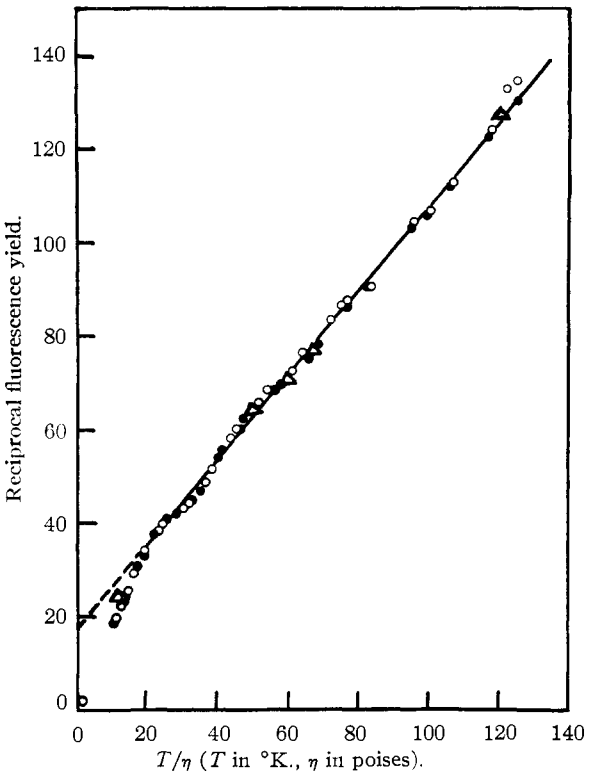

Fig. 5.—Reciprocal of quantum yield of fluorescence *versus* absolute temperature divided by viscosity (in poises): solid circles, excitation by 436 mμ (glycerol as solvent); open circles, excitation by 365 mμ (glycerol as solvent); triangles, excitation by 436 mμ (dextrose–glycerol–water mixtures as solvents at 25°).

The deviations from eq. 4 for low values of T/η as shown in Fig. 5 are due to a change in the nature of the emitting light. In this region of T/η we observed a phosphorescence of the same wave length as that of the fluorescence (α-phosphorescence in the terminology of Lewis and Kasha[12]). The apparent fluorescence is increased by this effect and hence accounts for the apparently low values of $1/\Phi$. We have investigated the emission from auramine O in regions of extremely low values of T/η as well as the solid dye itself and have observed β-phosphorescence and also some new absorption maxima.[13]

The dependence of fluorescence intensity of auramine O on the value of T/η of the medium provides a convenient means for measuring the viscosity of the medium over a wide range of viscosities where measurements by conventional methods become rather difficult to carry out. This method is especially useful when observations on changes in viscosity are desired yet where the system cannot be disturbed as, for example, in the phase transition studies in glasses and plastics now being carried out in our laboratory. The viscosity measured by the fluorescence method is the local viscosity. With high polymer solutions the fluorescence intensity parallels the local viscosity as determined from diffusion studies, rather than the macroscopic viscosity of the solution.[14]

Acknowledgments.—This work was supported in part by the Air Research and Development Command of the Air Corps (Contract No. 18 (600)-1182) and by the Research Corporation.

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(12) G. N. Lewis and M. Kasha, THIS JOURNAL, **66**, 2100 (1944).
(13) G. Oster and Y. Nishijima, to be published.
(14) Y. Nishijima and G. Oster, *J. Polymer Sci.*, **19**, 337 (1956).