lead to a reduced shielding in the tin case, contrary to what is observed. A possible explanation lies in the known variation of diamagnetic susceptibilities for the metal ions. According to the self-consistent set of data compiled by Klemm[6] the following values apply

$$-\chi \times 10^{-6}$$

Al^{III}	2
Sn^{IV}	16
Hg^{II}	37

The possible additional effects arising from structural differences and possible intermolecular effects await further evaluation in the study of these and other related organo-metallic compounds.

Spin-Coupling Values.—The proton–proton J-values observed for the mercury and tin vinyl compounds are consistent with those generally observed for vinyl groups, though some enhancement of all three constants over characteristic values (Pople, Schneider and Bernstein,[4] p. 193) is apparent. In the case of trivinylaluminum etherate the large gem-proton J-value of 6.3 c.p.s. compared with a normal 1–3 c.p.s. strongly suggests a reduction in the terminal H–C–H bond angle.

This conclusion follows from the work of Karplus and Gutowsky,[7,8] who showed the theoretical dependence of the coupling value on the interproton bond angle.

The two sets of metal–proton coupling constants in Table I reveal the expected strength of the $trans$-coupling, but show the cis- and gem-couplings to be nearly equal. An explanation for this anomaly might be found in structural studies of these molecules, although the previously reported behavior of metal alkyl spin-coupling constants indicates the difficulty of attempting to correlate structure with spin-coupling.

(6) P. W. Selwood, "Magnetochemistry," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1956, p. 78.

(7) M. Karplus and H. S. Gutowsky, Abstracts, ACS Meeting, San Francisco, 1958, p. 9-Q.

(8) H. S. Gutowsky, M. Karplus and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).

QUANTUM EFFICIENCIES OF FLUORESCENCE OF ORGANIC SUBSTANCES: EFFECT OF SOLVENT AND CONCENTRATION OF THE FLUORESCENT SOLUTE[1]

By W. H. MELHUISH

Institute of Nuclear Sciences, Department of Scientific and Industrial Research, New Zealand[2]

The absolute quantum efficiencies of fluorescence of deaerated solutions of several organic fluorescent substances in petroleum ether (b.p. 60–80°), ethanol (99%) and benzene at 25° were measured with a rhodamin B quantum counter. An equation was derived for correcting the observed efficiencies of fluorescence for reabsorption of fluorescence and shifts in the region of absorption away from the quantum counter. Absolute quantum efficiencies of fluorescence extrapolated to zero concentration, bimolecular self quenching constants and oxygen-quenching constants were measured at 25°. Quinine bisulfate, $5 \times 10^{-3} M$, in 1 N H_2SO_4, is proposed as a standard with an absolute quantum fluorescence efficiency of 0.51 at 25°.

A knowledge of quantum efficiencies and self-quenching constants of fluorescent organic molecules in solution has many important applications in physical chemistry, for example, in the investigation of internal conversion, intersystem crossing (singlet–triplet transitions), fluorescence quenching and the transfer of electronic excitation energy in solution.

The measurement of quantum fluorescence efficiencies has been reviewed recently by Förster.[3] The method of Bowen[4] has the merit of simplicity and if modified,[5] may be used over a wide range of wave lengths. Since 1951 other methods of measuring fluorescence efficiencies have been published. Bowen[6] integrated the fluorescence intensity (quanta sec.$^{-1}$ mμ^{-1}) emerging from a monochromator, over all wave lengths. The apparatus was calibrated with a sample of known absolute quantum efficiency. Gilmore, *et al.*,[7] used a photocell of known wave length response to view the fluorescence. The fluorescence spectrum was measured in a separate experiment and used to correct the photocell output. Forster and Livingston[8] used an integrating sphere and a thermopile as the detector.

Most authors make no correction for reabsorption of fluorescence which can cause anomalously high efficiencies. Budo, Dombi and Szöllösy[9]

(1) Department of Chemistry, University of California, Los Angeles 24, California.

(2) Contribution No. 83.

(3) Th. Förster, "Fluoreszenz Organischer Verbindungen," Vandenhoeck und Ruprecht, Göttingen, 1951, p. 143.

(4) E. J. Bowen, *Proc. Roy. Soc. (London)*, **154A**, 349 (1936).

(5) W. H. Melhuish, *N. Z. J. Sci. and Tech.*, **37.2B**, 142 (1955).

(6) E. J. Bowen, *Trans. Faraday Soc.*, **50**, 97 (1954).

(7) E. H. Gilmore, G. E. Gibson and D. S. McClure, *J. Chem. Phys.*, **20**, 829 (1952).

(8) L. S. Forster and R. Livingston, *J. Chem. Phys.*, **20**, 1315 (1952).

(9) A. Budo, J. Dombi and L. Szöllösy, *Acta Phys. et Chem. Szeged (N. S.)*, **2**, 18 (1956).
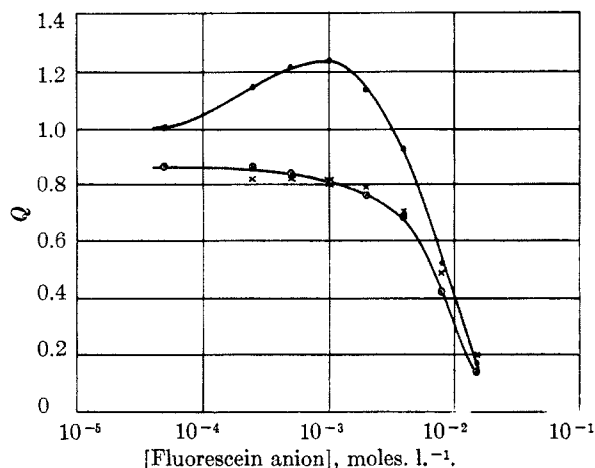
Fig. 1.—Self-quenching of fluorescein in water (pH 12.5): ●, observed (Budó, et al.); ⊙, corrected (Budó, et al.); ✕, corrected (method of this paper).

have shown that reabsorption of fluorescence in solutions of the fluorescein anion is such as to give up to 1.5 times the true value (Fig. 1) when the solution is illuminated and observed from the same side.

In this work, absolute quantum efficiencies of fluorescence were measured using the rhodamin B quantum counter and employing existing equations for calculating absolute quantum fluorescence efficiencies, modified to allow for fluorescence reabsorption and shifts in the region of absorption away from the quantum counter at low solute concentrations. The temperature of the solutions was maintained at 25 ± 0.1°.

Experimental

Fluorescent Substances and Solvents.—Anthracene, anthranilic acid, quinine bisulfate, 2-naphthylamine and acridone were purified from stock chemicals by several recrystallizations. 1,6-Diphenyl-1,3,5-hexatriene was of scintillation grade and was used without further purification. Methyl anthranilate was distilled twice at 15 mm. pressure taking the middle cuts in each case. These various anthracene derivatives were prepared by conventional methods: 9-bromo- (m.p. 99°), 9,10-dibromo- (m.p. 219°), 9,10-diphenyl- (m.p. 246°) and 9,10-di-1-naphthyl- (m.p. 408°). The substances were recrystallized from a suitable solvent, purified on activated alumina columns (eluted with a 50/50 mixture by volume of petrol ether and benzene) and finally fractionally sublimed in a stream of nitrogen.[10] Perylene was prepared from 2-bi-naphthol as outlined by Morgan and Mitchell.[11] A yellow substance (m.p. 155°) fluorescing blue in solution also was isolated. This substance is probably 1,1'-dinaphthyl-2,2'-oxide and must not be confused with perylene. Pure perylene (m.p. 276°) was prepared by chromatography and sublimation as described above. 9-Cyanoanthracene was prepared using the method of Bachmann and Kloetzel.[12] The substance was purified as described above (m.p. 178°).

The purity of the samples was checked by comparing the melting points and absorption spectra with those published (where available).

Benzene (British Drug Houses, A.R.) and ethanol (B.D.H., A.R.) 99/100%, were used without further purification. Petrol ether (B.D.H., 60–80°) and heavy paraffin were purified by shaking with successive quantities of 10% fuming sulfuric acid, washing with aqueous caustic soda and finally with water. After drying with calcium chloride, the solvents were passed down 50 cm. columns of silica gel until the transmission extended to 220 mμ.

Irradiation Source.—Solutions were excited with a 125 watt high pressure mercury lamp ("black lamp"). The lamp output was stabilized by connecting it in series with barretters, to a 50 cycle/sec. a.c. supply. The quantum spectrum of the lamp, measured with the spectro-fluorophotometer described below, had a broad band at about 366 mμ (relative quanta = 93%), two lines at 390.6 and 404.6 mμ (each = 1%) and a region between 368 and 385 mμ (= 5%). A 2% correction therefore was made to the quantum efficiencies of those substances which did not absorb in the region of 390 to 405 mμ.

The mean optical density of a solution to heterochromatic radiation may be calculated from an equation given by Kortüm.[13] It was assumed here that the band shape could be approximated by the two lines found at low pressure, 365.0 and 366.3 mμ, the ratios of the intensities being taken as $I(365)/I(366.3) = 0.38$. The mean optical densities of the solutions therefore were calculated from

$$\bar{E} = \epsilon_1 cl + \log 1.38 - \log [1 + 0.38 \times 10^{(\epsilon_1 - \epsilon_2)cl}]$$

where ϵ_1, ϵ_2 are the molecular extinction coefficients of the solute at 366.3 and 365.0 mμ, respectively, c = concentration of the solute (mole/l.) and l = thickness of the solution (cm.). The $\alpha_\lambda x$ term of equation 6 was thus calculated from

$$\alpha_\lambda x = \epsilon_1 c\, 2.303 + 0.323 - 2.303 \log [1 + \\ 0.38 \times 10^{(\epsilon_1 - \epsilon_2)xc}] \quad (1)$$

where

$\alpha_\lambda x$ = the absorption coefficient of the soln. for the exciting radiation

x = 1.14 (45° illumination)

Fluorescence Photometer.—Fluorescence intensities (relative quanta/sec.) were measured with a rhodamin B quantum counter. The fluorescence from the solution fell on the quantum counter and the fluorescence excited in the quantum counter transmitted through a red filter to a photomultiplier. The 100 cycle/sec. component in the fluorescence excited by the mercury lamp was detected by the photomultiplier and the output signal amplified with negative feedback a.c. amplifiers. The signal from the photomultiplier was compared with a standard signal from a photocell, using a difference amplifier and phase detector as a null detector. The photocell was illuminated with light taken from the exciting beam by a plane glass plate. Stability against fluctuations in the intensity of the mercury lamp was good; a 20% change caused less than 0.4% change in the intensity reading. The possibility that photomultiplier noise might affect the linearity of the photometer was tested by injecting noise into the photomultiplier by allowing a small amount of unmodulated light to fall on the photocathode. Deviations were found to be less than 2% with signal to noise ratios down to 0.4.

The cuvette used was disc-shaped (28 mm. diameter and 9.5 mm. thick) whose back and sides were painted with non-reflective black paint to reduce internal reflection. The rhodamin B quantum counter (28 mm. in diameter and 8 mm. thick) was placed 10.5 cm. from the cuvette face. Some tests of the suitability of rhodamin B as a device for measuring quanta has been reported previously.[5,14] The first author (ref. 5) found small deviations at 450 mμ where rhodamin B does not absorb strongly. It was considered that the addition of acriflavin, which absorbs strongly at 450 mμ, might improve the response of the quantum counter since energy absorbed by acriflavin is known to be efficiently transferred to rhodamin B. The quantum counter was a 1 cm. thick solution of rhodamin B (4 g./l.) and acriflavin (1 g./l.) in 90% glycerol and 10% ethanol. The red filter in front of the photomultiplier was a 1 cm. solution of methyl red (indicator solution, 0.75 g./l.) and o-nitrophenol (5 g./l.) in 95% ethanol and 5% 2 N sulfuric acid.

Fluorescence Spectra.—These were determined using a Hilger wave length spectrometer as the monochromator.[15] Intensities were measured with the electronic apparatus described above and were converted to relative quanta sec.$^{-1}$

(10) W. H. Melhuish, Nature, **184**, 1933 (1959).

(11) G. T. Morgan and J. G. Mitchell, J. Chem. Soc., 536 (1934).

(12) W. E. Bachmann and M. C. Kloetzel, J. Org. Chem., **3**, 55 (1938).

(13) G. Kortüm, "Kolorimetrie, Photometrie und Spektrometrie," Springer Verlag, Berlin, 1955, p. 43.

(14) G. Weber and F. J. W. Teale, Trans. Faraday Soc., **53**, 646 (1957).

(15) W. H. Melhuish, J. Phys. Chem., **64**, 762 (1960).

$m\mu^{-1}$. The concentration of the solute ranged from 10^{-2} to 5×10^{-7} M.

Measurement of Absolute Quantum Fluorescence Efficiencies.—A 5×10^{-3} M solution of quinine bisulfate in 1 N H_2SO_4 was used as a standard of fluorescence. The solution showed good stability to radiation, was not quenched by dissolved air and had a very small fluorescence–absorption overlap. However the intensity of fluorescence had a fairly steep temperature dependence, about -0.2 to -0.3% per ° in the range 10 to 40°. The absolute quantum efficiency of fluorescence at 25° was 0.510 (Appendix I). The error in this figure could be as high as 2% as a result of the uncertainty of the reflectivity of magnesium oxide under the conditions of the experiment. Absolute quantum efficiencies of relatively strong air free solutions of some organic substances were determined at 25°, making corrections for stray light scattered from the exciting beam by the cuvette window and the supports and stops in the optical system. A correction also was made for the small amount of stray radiation scattered from the emitted fluorescence by the quantum counter into the photomultiplier.

The effect of the concentration of the solute on its fluorescence efficiency was determined over a wide concentration range using the photocell in place of the quantum counter. The use of a photocell for measuring self-quenching constants did not introduce appreciable errors as little change in the spectral distribution of fluorescence occurred with change in concentration. This was checked for the case of perylene in benzene where overlap between absorption and fluorescence spectra caused a large change in the first maximum of the fluorescence spectrum as the concentration was increased above about 10^{-4} M. Care was taken to minimize the scattering of exciting radiation into the photocell, which was particularly high at low concentrations of solute. Such scattered radiation was reduced further by placing a filter (1 mm. of a solution of ferric alum, 10 g./l. in 2 N H_2SO_4) in front of the photocell. Oxygen-quenching constants were also determined with the photocell by measuring the fluorescence intensities of the solutions before and after de-aeration. Aerated solutions of benzene and ethanol were assumed to contain 1.45×10^{-3} M oxygen and aerated petrol ether (hexane fraction), 2.4×10^{-3} M oxygen. These figures are given by Seidell[16,17] although other values are quoted which differ considerably from these. Consequently there is some uncertainty in the values of the quenching constants given in Table III.

Results

It is first necessary to relate the measured intensity of fluorescence (relative quanta/sec.) to the several variables in the measuring system. Förster (ref. 3, p. 35) has derived an expression giving the intensity of fluorescence $dB'(\lambda')$ in the wave length interval between λ' and $\lambda' + d\lambda'$ falling on a small aperture perpendicular to and at a large distance from a rectangular cuvette, in terms of the absorption coefficients of the solution and the geometry of the cuvette. The equation, however, neglects the possibility of the reabsorption of fluorescence. Errors due to reabsorption followed by re-emission of fluorescence can become high if the exciting radiation is absorbed near the front face of the cuvette. At least 85% of the emitted fluorescence moves back into the solution where a fraction of it may be reabsorbed. The re-emitted radiation adds to the primary fluorescence escaping from the front face of the cuvette. Corrections to Förster's equation for reabsorption of fluorescence have been made by Budo and Ketskeméty[18] and their method with slight modifications was adopted here. Previous workers have also neglected shifts in the region of

(16) A. Seidell, "Solubilities of Inorganic and Metal-organic Compounds," D. Van Nostrand Co., New York, N. Y., Vol. 1, 3rd ed. (1940).

(17) Ref. 10, suppl. (1952).

(18) A. Budo and I. Ketskeméty, J. Chem. Phys., **25**, 595 (1956).

absorption away from the front face when the solution is diluted. This effect is important if the photocell is placed close to the cuvette. The equation of Budo and Ketskeméty therefore was modified to allow for such shifts

$$dB'(\lambda') = AQf(\lambda')d\lambda'\alpha\lambda x \int_{z=0}^{l} \frac{d^2}{(d+mz)^2} \Big/$$
$$[\exp -(\alpha\lambda x + \alpha\lambda')z][1 + QK' + Q^2K'K'' + \ldots]dz$$

(2)

where

A = $\rho E_0/4\pi n^2$ (see Förster[1])

Q = absolute quantum efficiency of fluorescence

α = absorption coefficient (= k in Förster[1])

= 2.303 ϵc where ϵ = molecular extinction coefficient and c = concentration, mole/l. Thus $\alpha\lambda$ = absorption coefficient at the wave length of the exciting radiation λ; $\alpha\lambda'$ = absorption coefficient at the wave length λ'

l = thickness of the liquid in the cuvette (cm.)

x = $\sec\left[\sin^{-1}\left(\dfrac{\sin\theta}{n}\right)\right]$ where θ = angle of incidence of the exciting beam on the front face of the cuvette

d = distance from the face of the cuvette to the observing aperture (cm.)

m = 0.65 to 0.75 for the solvents used here (see below and ref. 19)

$K', K'', \ldots = \int_0^\infty f(\lambda')M(\lambda',z,c)d\lambda', \int_0^\infty f(\lambda'')M(\lambda'',z,c)-d\lambda'', \ldots$, where $M(\lambda',z,c)$ = fraction of fluorescence reabsorbed at the wave length λ' (secondary fluorescence); $M(\lambda'',z,c)$ = fraction of the secondary fluorescence reabsorbed at λ'' (tertiary fluorescence) .... and so on. (See Budo and Ketskeméty[18]). Note, $\int_0^\infty f(\lambda')d\lambda' = \int_0^\infty f(\lambda'')d\lambda''$ = ... = 1

z = distance from the front face (the glass/liquid interface) into the solution (cm.)

Errors caused by shifts in the region of absorption are corrected by the term $d^2/(d + mz)^2$. In the absence of refraction effects, $m = 1$, but with a liquid in the cuvette $m = 0.7$ (for $n = 1.45$). This foreshortening effect has been discussed in ref. 19. Assuming $m = 0$ can give an error up to 10% if the observing aperture is placed 10 cm. from a cuvette 1 cm. thick. The complete neglect of the reabsorption term can result in an error of up to 50% if about 30 to 50% of the absorption spectrum overlaps the fluorescence spectrum and the fluorescence efficiency is high.

The following assumptions have been made: the fluorescence is assumed to be emitted isotropically; the efficiency of fluorescence is assumed to be independent of wave length; the exciting radiation is assumed to fall on a small area of the cuvette, making virtually a point source.
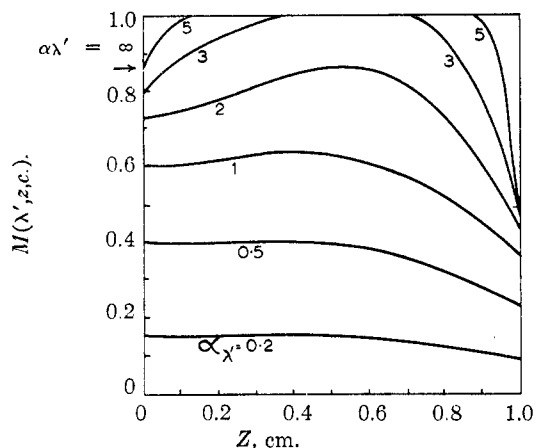
The reabsorption factors $M(\lambda',z,c)$, $M(\lambda'',z,c)\ldots$ depend on the wave length λ', $\lambda''\ldots$, the shape of the cuvette, the concentration of the solute, the direction of the exciting beam and the position of this beam on the cuvette face. Fluorescence measurements were made with disc-shaped cuvettes, focussing the exciting radiation at an angle o-45° on the center of the flat front face. The calsculation of the reabsorption of fluorescence for thif case is outlined in Appendix II. The calculation of fluorescence reabsorption also has been made by

(19) B. A. Brice, M. Halwer and R. Speiser, J. Opt. Soc. Am., 4,0 768 (1950).
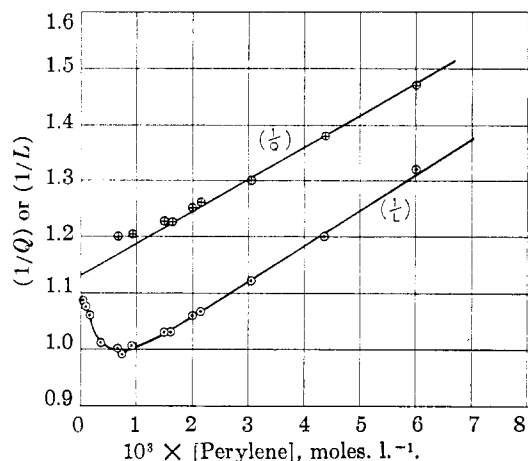
Fig. 2.—Self-quenching of perylene in benzene −25°.
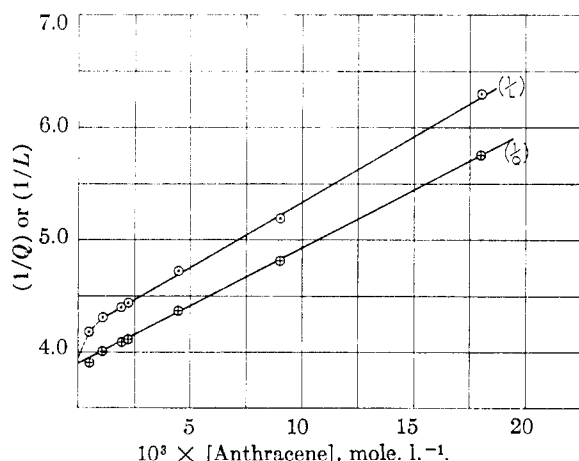
Fig. 3.—Self-quenching of anthracene in benzene −25°.

Fig. 4.—Reabsorption factor $(M(\lambda',z,c)$ for a disc-shaped cuvette (3 cm. diam., 1 cm. thick).

Budó and Ketskeméty[16] but the conditions for which their equation holds were not stated clearly.

The observed absolute efficiency L, is obtained if reabsorption is neglected in equation 2 (i.e., $\alpha_{\lambda'} = \alpha_{\lambda''} = \cdots = 0$)

$$dB'(\lambda') = ALf(\lambda')d\lambda'\alpha_\lambda x \int_{z=0}^{l} \frac{d^2}{(\alpha + mz)^2}(\exp(-\alpha_\lambda x)dz$$

$$(3)$$

The relation between L and Q can be easily determined for two special cases: (a) when absorp-

tion of exciting radiation is virtually complete in the first 1 to 2 mm. Then $M(\lambda',z,c) \approx M(\lambda'',z,c) \approx \cdots \approx 1$ up to a cut-off wave length λ_c, the long wave length edge of the absorption band. Thus $K' \approx K'' \approx \cdots = \int_{\lambda'=0}^{\lambda_c} f(\lambda')d\lambda'$. Then on integrating equation 2

$$\int_{\lambda'=0}^{\infty} dB'(\lambda') = AQ(1 - K'Q)^{-1} \Big/$$

$$\left\{ \int_{\lambda'=0}^{\lambda_c} f(\lambda')[\alpha_\lambda x/(\alpha_\lambda x + \alpha\lambda')]d\lambda' + 1 - K' \right\}$$

Also under the same conditions equation 3 becomes

$$\int_{0=\lambda'}^{\infty} dB'(\lambda') = AL$$

Thus

$$L = Q(1 - K'Q)^{-1} \left\{ \int_{x=0}^{\lambda_c} f(\lambda')[\alpha_\lambda x/(\alpha_\lambda x + \alpha\lambda')]d\lambda' + 1 - K' \right\} \quad (4)$$

(b) When the absorption of exciting radiation is small. Then $M(\lambda', z, c) = M(\lambda', 0, c)$ since $M(\lambda', z, c)$ is nearly independent of z when α is small (Fig. 4). Also assuming $M(\lambda', 0, c) \approx M(\lambda', 0, c) \approx \ldots$, etc., and since $K'(0, c) = \int_{\lambda'=0}^{\infty} \Big/ f(\lambda')M(\lambda', 0, c)d\lambda'$, then following the same proceedure as above

$$L = Q(1 + K'(0,c)Q + K'(0,c)^2Q^2 + \ldots)\Big/$$

$$\left[\alpha_\lambda x \int_{z=0}^{l} \frac{\alpha^2}{(\alpha + 0.7z)^2}(\exp(-\alpha_\lambda x)dz \right]$$

$$= Q(1 - K'(0,c)Q)S(\alpha_\lambda x) \quad (5)$$

The exciting radiation was assumed to be absorbed more strongly than any of the fluorescence radiation (i.e., $\alpha_\lambda > \alpha_{\lambda'}$). The integral in brackets

$$\alpha_\lambda x \int_{z=0}^{l} \frac{d^2}{(d + 0.7z)^2}(\exp(-\alpha_\lambda x))dz = S(\alpha_\lambda x) \quad (6)$$

has been evaluated for $d = 10.5$ cm. and $b = 1.0$ cm. and is compared with the expression $1 - \exp(-\alpha_\lambda x)$ in Table I.

TABLE I

VALUES OF THE INTEGRAL $S(\alpha_\lambda x)$ (EQUATION 6)

$\alpha_\lambda x$	$S(\alpha_\lambda x)$	$1 - \exp(-\alpha_\lambda x)$
0.02	0.0185	0.0198
.06	.0556	.0582
.10	.0890	.0952
.4	.314	.330
.8	.520	.551
1.0	.595	.632
4	.932	.982
8	.977	1.000
10	.985	1.000
15	.994	1.000

Absolute quantum efficiencies of fluorescence were calculated from equation 4 since the concentrations of the solutions were such as to absorb greater than 99% of the exciting radiation in 10 mm. The fluorescence efficiency of 9,10-diphenylanthracene was, however, determined over a wide concentration range and the measurements corrected by both equations 4 and 5. The corrected efficiencies shown in Table II are remarkably constant over the concentration range 10^{-4} to 10^{-2} M. Self-quench-

TABLE II

CORRECTION OF OBSERVED FLUORESCENCE EFFICIENCIES OF 9,10-DIPHENYLANTHRACENE (25°)

$10^3 \times$ concn. (mole/l.)	Obsd. intensity	$S(\alpha_\lambda x)$	L	K'	Q (cor.) Eq. 4	Eq. 5
10.0	1.000					
7.8	1.003					
5.0	1.000	1.000	1.000	0.35	0.84	..
2.0	1.005					
1.5	1.000					
1.11	0.970	0.995	0.075	..	..	..
0.71	.935	.985	.950	..	..	..
.50	.925	.972	.950	..	..	..
.220	.835	.908	.920	0.14	..	0.81
.130	.728	.820	.89	.09	..	.82
.090	.610	.690	.88	.07	..	.83
.050	.418	.480	.87	.05	..	.83
.030	.280	.338	.84	.03	..	.83
.0153	.152	.185	.82	.01	..	.82

ing constants were determined by plotting $1/Q$ against concentration. The deviation from the Stern–Volmer law (equation 7) if reabsorption was neglected was particularly noticeable for perylene where a plot of $1/L$ against concentration actually went through a minimum (Fig. 2). At low concentrations, there was usually a deviation from the simple law which probably was due to the inadequacy of equation 4 at these concentrations. The effect of concentration on the efficiency of fluorescence of anthracene in benzene is shown in Fig. 3 where both $1/L$ and $1/Q$ are plotted against concentration. It is evident that neglect of reabsorption would have led to an error in both k_s and Q_0. The results of similar measurements on several organic substances are given in Table III.

The self-quenching of fluorescein in water (pH 12.5) has been measured by Budó, Dombi and Szöllösy[9] who corrected the observed efficiencies for the large amount of fluorescence reabsorption. The observed efficiencies also were corrected using equation 4. It was found that the corrected points fell very close to those of Budó, et al. (see Fig. 1). This observation seemed to justify the use of the much simpler equation (equation 4).

Discussion

A decrease in the fluorescence efficiency of a solute as the concentration is increased is due to the quenching of excited solute molecules by unexcited ones (self-quenching). If the quenching requires the close encounter of the two solute molecules, the change in the fluorescence efficiency with concentration should obey the Stern–Volmer law

$$Q_0/Q = 1 + k_s C \qquad (7)$$

Q_0 = efficiency at infinite dilution
Q = efficiency at the concn. c (mole/l.)
k_s = self-quenching constant

It has been considered by many authors notably Förster[3] (p. 176 and 218) that overlap between absorption and fluorescence spectra of a molecule indicates a close coupling between excited and unexcited molecules which can cause quenching even when the molecules are separated over several molecular diameters. Self-quenching by this mechanism obeys the Stern–Volmer law only over a limited concentration range. In the work de-

scribed here, deviations from the Stern–Volmer law were traced to reabsorption effects (see Figs. 2 and 3).

Bowen and Sahu[20] found the efficiency of 9,10-diphenylanthracene to be 1.0 whereas Bowen and Cook[21] gave the figure 0.80 at low concentrations. As in our work (Table II) these figures may differ as a result of the reabsorption of fluorescence.

The efficiencies of 9-cyanoanthracene and perylene in petrol ether and ethanol at infinite dilution were calculated from equation 7 using these estimates for k_s

9-cyanoanthracene, petroleum ether k_s	= 300
9-cyanoanthracene, ethanol	= 100
perylene, petroleum ether	= 200
perylene, ethanol	= 80

Since the solutions are so dilute, large errors in these estimates would not greatly affect Q_0.

The rate constants for self- and oxygen-quenching (Table IV) have been estimated using methods similar to those of Bowen.[6] The fluorescence lifetimes of the substances were calculated from the equation of Förster[3] (p. 158) using the absorption spectra determined in ethanol solution. k_1 is the rate constant for self-quenching, k_2 that for oxygen-quenching. It appears from Table IV that self-quenching is less efficient than oxygen quenching, that is, may not be diffusion controlled, but this question can only be answered satisfactorily by investigating the effect of viscosity on self-quenching.

Appendix I

Absolute Quantum Fluorescence Efficiency of the Quinine Bisulfate Standard.—The absolute quantum efficiency of fluorescence of a 5×10^{-3} M solution of quinine bisulfate in 1 N H_2SO_4 at 25° was measured by the method described previously.[5] A more stable a.c. apparatus described above replaced the d.c. method used in ref. 5. Five measurements gave the following results (figures are relative quanta sec.$^{-1}$ cm.$^{-2}$.): (1) exciting radiation scattered from MgO (1 mm.) = 9800 ± 30; (2) radiation scattered into the photomultiplier from MgO with a non-fluorescent red dye in the quantum counter = 40 ± 3; (3) fluorescence from the quinine bisulfate solution = 685 ± 5; (4) radiation scattered into the photomultiplier from the quinine bisulfate solution with a non-fluorescent red dye in the quantum counter = 4 ± 1; (5) radiation scattered from the exciting beam into the quantum counter by the cuvette window = 13 ± 1.

The radiation scattered from a very thick block of MgO which is used for the determination of absolute reflectivities, is about 1.5% higher than from a 1 mm. block; thus (1) must be increased by 1.5%. The previous equation used for calculating absolute quantum efficiencies of fluorescence (ref. 5) is not quite correct; the absolute reflectivity of MgO at 366 mμ is 0.93[22] and not 0.96 which was

(20) E. J. Bowen and J. Sahu, J. Phys. Chem., **63**, 4 (1959).

(21) E. J. Bowen and R. J. Cook, J. Chem. Soc., 3059 (1953).

(22) F. Benford, S. Schwarz and G. P. Lloyd, J. Opt. Soc. Am., **38**, 964 (1948).

Table III

Absolute Quantum Fluorescence Efficiencies, Self- and Oxygen-quenching Constants at 25°

Substance	Solvent	Concn., M	L	Q	k_a	Q_0	$k(O_4)^a$
Quinine bisulfate	1 N H_2SO_4	5 × 10^{-3}	0.510	0.508	14.5	0.546	<5
Anthracene	Benzene	2 × 10^{-3}	.228	.241	27.5	.256	128
Anthracene	Pet. ether[b]	3 × 10^{-3}	.220	.241	103	.316	240
Anthracene	Ethanol	3 × 10^{-3}	.228	.249	28.5	.270	158
Anthracene	H. par.[c]	3 × 10^{-3}	.295	.323	4 ± 2	.328	..
9-Bromoanthracene	Benzene	1.5 × 10^{-3}	.048	.050	18 ± 2	.052	25
9-Bromoanthracene	Pet. ether	1.5 × 10^{-3}	.022	.024	[e]	(.025)	13
9-Bromoanthracene	Ethanol	1.5 × 10^{-3}	.018	.019	10 ± 5	.020	12
9,10-Dibromoanthracene	Benzene	1.5 × 10^{-3}	.202	.213	20.5	.220	45
9,10-Dibromoanthracene	Pet. ether	10^{-3}	.081	.088	[e]	(.10)	38 ± 5
9,10-Dibromoanthracene	Ethanol	9 × 10^{-4}	.096	.103	[e]	(.11)	41
9-Cyanoanthracene	Benzene	1.5 × 10^{-3}	.885	.796	79	.878	190
9-Cyanoanthracene	Pet. ether	9.2 × 10^{-4}	.755	.660	[e]	(.85)	140
9-Cyanoanthracene	Ethanol	1.5 × 10^{-3}	.845	.740	[e]	(.85)	240
9,10-Diphenylanthracene	Benzene	1.5 × 10^{-3}	1.000	.840	<2	.84	210
9,10-Diphenylanthracene	Pet. ether	10^{-3}	1.002	.83	<2	.83	295
9,10-Diphenylanthracene	Ethanol	10^{-3}	0.975	.81	<2	.81	280
9,10-Di-(1-naphthyl)-anthracene	Benzene	1.5 × 10^{-3}	.745	.63	[e]	(.61)	140
Perylene	Benzene	2 × 10^{-3}	.945	.800	52.5	.890	210
Perylene	Pet. ether	5.5 × 10^{-4f}	.913	.790	[e]	(.88)	228
Perylene	Ethanol	3.5 × 10^{-4f}	.990	.840	[e]	(.87)	195
Anthranilic acid	Benzene	8 × 10^{-3}	.548	.536	10.5	.582	220
Anthranilic acid	Ethanol	1.3 × 10^{-2}	.575	.558	5.3	.588	290
Methyl anthranilate	Benzene	2.7 × 10^{-2}	.565	.549	1.0	.565	210
Methyl anthranilate	Pet. ether	2.7 × 10^{-2}	.380	.376	2.5	.402	240
Methyl anthranilate	Ethanol	2.7 × 10^{-2}	.681	.656	1.6	.685	250
2-Naphthylamine	Benzene	2.7 × 10^{-2}	.497	.486	1 ± 0.3	.50	480
Acridone	Ethanol	1.5 × 10^{-3}	.845	.780	43	.825	170
Diphenylhexatriene	Benzene	10^{-3}	.796	.750	..	...	225
Diphenylhexatriene	Pet. ether	6 × 10^{-4}	.640	.610	..	...	490

[a] $k(O_2)$ = oxygen-quenching constant. [b] Unpurified petrol ether gave k_a = 99 and Q_0 = 0.285. [c] Heavy paraffin, 150 centipoise. [d] Petrol ether, 0.43 centipoise. [e] Not sufficiently soluble to allow the measurement of the self-quenching constant. [f] Exciting radiation not completely absorbed; the fluorescence was corrected by equation 6.

Table IV

Calculated Rate Constants for Self- and Oxygen-quenching (25°)

Substance	Solvent	$10^9 \times \tau_e{}^a$ (sec.)	$10^9 \times \tau$[b]	$10^{-9} \times k_1$ (l. mole^{-1} sec.$^{-1}$)	$10^{10} \times k_2$
Anthracene	Ethanol	15.5	4.2	7.0	3.8
Anthracene	Pet. ether		4.9	20.9	4.9
Anthracene	Benzene		4.0	6.9	3.2
Quinine bisulfate	1 N H_2SO_4	20.0[c]	10.9	1.3	<0.05
9-Bromoanthracene	Ethanol	13.8	0.28	29	4.2
9-Bromoanthracene	Pet. ether		.35	...	3.7
9-Bromoanthracene	Benzene		.72	14	3.5
9,10-Dibromoanthracene	Ethanol	12.5	1.4	...	2.9
9,10-Dibromoanthracene	Pet. ether		1.3	...	2.9
9,10-Dibromoanthracene	Benzene		2.8	7.3	1.6
9,10-Diphenylanthracene	Ethanol	9.0[d]	7.3	<0.3	4.1
9,10-Diphenylanthracene	Pet. ether		7.5	<0.3	4.2
9,10-Diphenylanthracene	Benzene		7.6	<0.3	3.0
Perylene	Ethanol	5.7[d]	5.0	...	4.0
	Pet. ether		5.1	...	4.5
	Benzene		5.1	7.8	4.2
Acridone	Ethanol	15.2	12.5	3.1	1.3

[a] Solvent, ethanol. [b] $\tau = \tau l Q_0$ (Q_0 from Table III). [c] Calculated from the spectrum in 1 N H_2SO_4. [d] Bowen[4] gives 7.9 and 6.9 × 10^{-9} sec., respectively.

the result of a wide extrapolation from earlier data given by these authors. Theoretically determined values of $I_0/I_\mathrm{av.}$ given previously[5] are not correct since experimentally determined values were found to be closer to unity. The figures used

for calculating the absolute quantum fluorescence efficiency of the standard solution were:

$$(F/S) = (685 - 17)/(8900 - 40) = 0.075$$
$$n^2 = 1.81$$
$$I_0/I_\mathrm{av.} = 1.005$$

R_1 = 0.92 = relative reflectivity of MgO when illuminated at 45° compared with normal illumination

R_2 = 0.93 = absolute reflectivity at MgO at 366 mμ

R_e = 0.050 = exciting light lost by reflection off the cuvette face

R_f = 0.039 = fluorescence light lost by reflection off the cuvette face

R_0 and R_f were determined experimentally. The results agreed closely with those calculated from Fresnel's formula. (F/S) was decreased by 1% to allow for the lower transmission of the cuvette face at 366 mμ compared with longer wave lengths. Thus

$$Q = (F/S)4n^2(I_0/I_{av.})R_1R_2/(1 - R - R_f) = 0.510 \text{ at } 25°$$

The observed absolute efficiencies of other solutions were measured with reference to this standard as

$$Q_x = (x/s)\ 0.282n_x^2$$

where

x = relative quanta/sec. from the unknown

s = relative quanta/sec. from the standard

n_x = refract. index of the soln. containing the unknown x

Appendix II

Reabsorption of Fluorescence in a Disc-shaped Cuvette.—A disc-shaped cuvette of radius R and thickness l is illuminated with a narrow, parallel beam of radiation incident at an angle between 0 and 45° on the flat front face of the cuvette. The thickness of the window is considered negligible compared with l. The sides and back of the cuvette are painted with non-reflecting black paint to prevent internal reflection.

A small volume element, dz, part of the narrow exciting beam causing fluorescence in the solution, is situated a distance z behind the front face. Consider a cone of fluorescence radiation passing through the solution, the axis of the cone coinciding with the axis of the disc and the vertex being at z. The fraction of the radiation of a particular wave length λ' between the cones with vertical half angles θ and $\theta + d\theta$, absorbed by the solution is

$$1 - [\exp(-\alpha\lambda'\ l(\theta)]$$

where

$\alpha\lambda'$ = absorption coefficient of the solution at λ'

$l(\theta)$ = path length (varies with θ)

For the whole cuvette, the fraction absorbed is

$$M(\lambda',z,c) = \int_0^\pi \{1 - [\exp(-\alpha\lambda'l(\theta))]\} \sin\theta\ d\theta$$

$l(\theta)$ is not a simple function of θ but behaves as follows: In the hemisphere above the plane of the cuvette window (away from the solution)

$\theta = 0$ to θ_c l $(\theta) = z \sec\theta$ where $\theta_c = \text{Sin}^{-1}(1/n)$
   $= \theta_c$ to $\pi/2$ $= R \csc\theta$

In the hemisphere below the window (into the solution)

$\theta = 0$ to θ_1 $l(\theta) = (l - z) \sec\theta$ where $\theta_1 = \tan^{-1}/[R/(l - z)]$
   $= \theta_1$ to $\pi/2$ $= R \csc\theta$

The four integrals were evaluated graphically for different values of z and $\alpha\lambda'$ for a cuvette with $R = 15$ cm. and $b = 10$ mm. The results are plotted in Fig. 4.

The calculation of $M(\lambda'', z, c)$ (the fraction of secondary fluorescence reabsorbed) is in principle possible using the above method, but would be very laborious and therefore was not attempted here.

CONTINUOUS DISSOLUTION OF COPPER BY NITRIC ACID

By Edward A. Travnicek and James H. Weber

University of Nebraska, Lincoln, Nebraska

Received June 20, 1960

The kinetics of the reaction between copper and nitric acid were studied in a flow reactor in which a continuous dissolution process was performed. Experiments were carried out at 20, 30, 40 and 50° over a range of acid concentration of 3 to 5.5 N. The results are consistent with those of previous investigators who believe the nitrous acid produced in one step of the dissolution process acts as an autocatalyst for the reaction. At acid normalities of 4.3 and 5.5, the energy of activations— as defined by Arrhenius—were found to be 9.73 ± 0.30 kcal./g. atom of copper and 7.83 ± 0.14, respectively, over the temperature range of 30–50°.

The continuous dissolution of metals is a phenomenon which has been investigated only infrequently. While the practical applications of this type of process are limited, experimentation in this area can be of scientific interest. In contrast, a number of studies have been made of the batchwise dissolution of metals. This type of experiment often yields the information necessary to determine the rate-controlling step, be it chemical reaction or diffusion, of the process involved. Frequently, however, this is not the case.

Furthermore, the results obtained from experiments conducted batchwise by different groups of investigators are often difficult to compare. The reason is that quite different methods may have been employed. For example, one investigator may rotate a cylindrically shaped metallic sample in the dissolving medium, while another may immerse a thin metallic sheet in an agitated bath of the medium.

On the other hand, in a continuous process the flow rate and, in turn, the flow pattern of the dissolving medium over the surface of the metal can be controlled and reproduced. This, of course, presupposes a properly shaped sample of metal.

In this investigation, which follows the work of Johnson, Hobson and Weber[1] and Kissinger,[2] the

(1) R. L. Johnson, M. Hobson and J. H. Weber, *Ind. Eng. Chem.*, **50**, 1194 (1960).

(2) R. D. Kissinger, Master's Thesis, Univ. of Nebraska, 1958.