TABLE IV

EQUILIBRIUM CONSTANT K_X OF EQUATION (19) AT 25°, IONIC STRENGTH APPROXIMATELY ONE

Components added R_1SSR_1 M	Na_2SO_3 M	Buffer	pH	ΔH of thioglycolic acid μa.	Σ Thioglycolic acid M	K_X × 10⁴
2.08 × 10⁻³	0.05	M NH_3, M NH_4Cl	9.32	3.01	8.41 × 10⁻⁴	5.0
1.04 × 10⁻³	.10	0.4 M NH_3, 0.4 M NH_4Cl, 0.3 M KCl	9.37	2.30	6.43 × 10⁻⁴	5.0
1.04 × 10⁻³	.05	0.5 M NH_3, 0.5 M NH_4Cl, 0.3 M KCl	9.30	1.93	5.40 × 10⁻⁴	4.8
2.08 × 10⁻³	.05	0.1 M NH_3, M NH_4Cl	8.30	4.99	1.35 × 10⁻³	2.4
1.04 × 10⁻³	.0125	0.1 M NH_3, M NH_4Cl	8.31	1.95	5.29 × 10⁻⁴	2.2
2.08 × 10⁻³	.0125	0.1 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.65 M KCl	7.36	6.19	1.70 × 10⁻³	5.8
1.04 × 10⁻³	.1025	0.08 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.71 M KCl	7.34	3.36	9.23 × 10⁻⁴	5.0
1.04 × 10⁻³	.025	0.05 M Na_2HPO_4, 0.05 M NaH_2PO_4, 0.73 M KCl	6.69	3.72	1.01 × 10⁻³	6.9
1.04 × 10⁻³	.0063	0.05 M Na_2HPO_4, 0.05 M NaH_2PO_4, 0.78 M KCl	6.38	3.29	9.17 × 10⁻⁴	5.0
					Average	4.7

$SO_3^=$ and partly as HSO_3^-. It is possible to calculate the equilibrium constants for the R_1SSR_1–sulfite system.

$$R_1SSR_1 + SO_3^= \overset{a_1 \quad d}{\rightleftharpoons} \overset{b \quad b_1}{R_1S^- + R_1SSO_3^-}, \quad K_X = \frac{bb_1}{a_1d} \tag{19}$$

$$R_1SSR_1 + HSO_3^- \overset{a_1 \quad c}{\rightleftharpoons} \overset{a \quad b_1}{R_1SH + R_1SSO_3^-}, \quad K_Y = \frac{b_1a}{a_1c} \tag{20}$$

Denoting the dissociation constant of the sulfhydryl group of thioglycolic acid as $K_2 = b[H^+]/a$, and the second constant of sulfite as $K_4 = d[H^+]/c$ the following relation between K_X and K_Y is obtained

$$K_X = \frac{K_2}{K_4} K_Y \tag{21}$$

The experimental results and the values for the equilibrium constant K_X at 25° in mixtures of ionic strength of 1 and of various pH and concentrations of reactants are listed in Table IV.

The average value of K_X at 25° is 4.7 × 10⁻⁴ and the change in free energy ΔF_X is −4,500 cal. K_X as calculated from equation (21) is 1.40 and ΔF_Y is +200 cal. at 25°. It is of interest to note that the constant of the reaction with sulfite and cystine containing free amino groups (K_I in equation 12) is 21 times as large as that of dithiodiglycolate. The difference becomes considerably greater when the reaction between cystine, containing two NH_3^+ groups is considered (equation 15).

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[CONTRIBUTION FROM THE NAVAL RESEARCH LABORATORY]

The Size of Soap Micelles in Benzene from Osmotic Pressure and from the Depolarization of Fluorescence[1]

BY C. R. SINGLETERRY AND LORRAINE ARKIN WEINBERGER

The size of oil-soluble soap micelles in non-polar solvents may be determined from the depolarization of the fluorescence emitted from a dye adsorbed by the micelle. The depolarization results from Brownian rotation of the dye-containing micelle during the interval between light absorption and fluorescence emission, the average excited life being of the order of 5 × 10⁻⁹ second. Micelle sizes calculated from fluorescence depolarization have been compared with those obtained by osmotic pressure measurements in similar solutions and found to be slightly smaller but closely proportional to the latter. For a 0.6% solution of calcium xenylstearate in benzene, fluorescence gives a gram micellar weight of 22,500, while osmotic pressure indicates a weight of 23,700. The fluorescence technique provides a convenient and rapid tool for the investigation of very small colloidal particles or of macromolecules in any system in which a suitable fluorescent material can be quantitatively associated with the colloidal phase. It is applicable at much higher dilutions than viscometry, osmometry or light scattering. The precision of the method decreases as the product of the gram micellar volume by solvent viscosity increases. With present techniques results become mainly qualitative if ηV exceeds 1500 (poises × cm.³).

Introduction

The usefulness of fluorescent dyes as indicators of the presence of micelles of oil-soluble soaps in non-aqueous systems,[2] and the existence of a relationship between the degree of polarization of the emitted fluorescence and the volume of the micelle have been indicated in preliminary publications.[3] The phenomenon provides a convenient and powerful tool for the determination of particle size in colloidal systems. The present communication presents more detailed information concerning the spectral phenomena observed, the experimental procedures, and the factors affecting inferences of particle size from fluorescence depolarization.

When a small amount of an oil-soluble, micelle-forming soap is added to a dilute benzene solution

(1) The opinions or assertions contained in this communication are the authors' and are not to be construed as official or reflecting the views of the Navy Department. Article not copyrighted.

(2) The enhancement of the fluorescence of several dyes in aqueous systems containing soap micelles was reported by M. L. Corrin and W. D. Harkins, THIS JOURNAL, **69**, 679 (1947).

(3) Lorraine Arkin and C. R. Singleterry, *ibid.*, **70**, 3965 (1948); Lorraine Arkin and C. R. Singleterry, *J. Colloid Sci.*, **4**, 537 (1949).

of Rhodamine B, there is a many-fold increase in light absorption and a notable enhancement of the fluorescence efficiency of the dye. The first effect is specific to dyes for which an equilibrium between colored and colorless forms is possible.[4] The second phenomenon is a rather general result of the association of a fluorescent dye with a colloidal phase in which it is protected from collisions with molecules which might produce external quenching or increase the probability of internal conversion of the absorbed energy.[5-11]

The absorption and fluorescence of Rhodamine B in benzene are also enhanced by the presence of molecularly soluble materials such as alcohols and organic acids, although it can be seen from Fig. 1 that the effect of such a solute is several orders of magnitude less than that of a similar amount of micelle-forming soap in dilute systems. Consequently, increased fluorescence of Rhodamine B in benzene cannot be taken as evidence of micelle formation unless the fluorescent emission is shown to be polarized to an extent consistent with the size of the micelles postulated.

The estimation of micellar volume from observations of the degree of polarization of the emitted light is based upon an equation derived by F. Perrin[12] for the degree of polarization of the fluorescence emission of a dye in true molecular solution

$$\frac{1}{p} = \frac{1}{p_0} + (1/p_0 - 1/3)\frac{\tau RT}{V\eta} \qquad (1)$$

where

τ = average life of excited molecule before emission of fluorescent energy, seconds
V = molal volume of dye or of dye and associated solvent molecules, cc.; the equation is based on the assumption that the unit undergoing rotation is spherical
η = viscosity of the solution, poises
p_0 = degree of polarization that would be observed for completely immobilized dye molecules excited with plane-polarized light.

The value of p_0 may be obtained by extrapolation from the values for p observed with sufficiently dilute solutions in a series of solvents of increasing viscosity. For a solution with random orientation of fluorescent molecules p_0 cannot exceed 0.50; for dyes of the type of Rhodamine B p_0 usually lies between 0.40 and 0.45.

The degree of polarization, p, is given by the relation

$$p = \frac{I_z - I_x}{I_z + I_x} \qquad (2)$$

The quantities I_x and I_z are defined by the condition that, if the plane-polarized light entering the solution in a direction parallel to the x-axis has its electric vector oriented parallel to the z-axis, then I_x and I_z are the intensities of fluorescence observed in a direction normal to the plane of the x- and z-axes when the analyzer is set to pass light as indicated by the subscript.

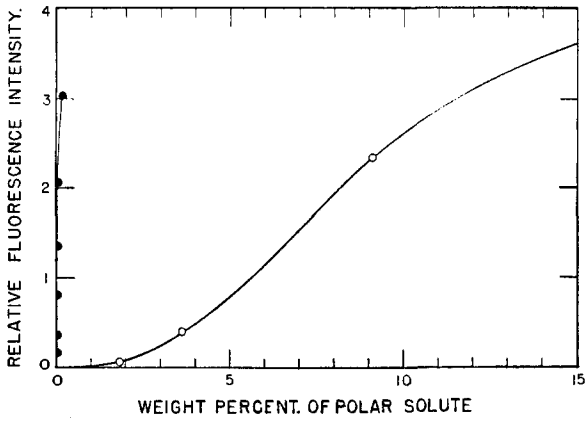

Fig. 1.—Fluorescent intensities of Rhodamine B as a function of the concentration of polar solute in benzene: O, methanol in 7×10^{-7} M dye; ●, calcium xenylstearate in 3.6×10^{-7} M dye.

The estimate of V is dependent not only on the measured quantities p, η and T, but upon the value selected for p_0, and upon the average life, τ, of the excited molecule. The value of p_0 can be obtained by a very short extrapolation from convenient measurements. Direct determination of the average life of the excited dye molecule, however, involves exacting and highly specialized techniques.[13,14] In the case of Rhodamine B it is fortunately possible to estimate the value of τ applicable to a given measurement from Szymanowski's data for this dye in methyl alcohol and in glycerol by means of the relation between the quantum efficiency, Q, of a fluorescent process and the average excited life,[8] τ

$$\frac{\tau'}{Q'} = \frac{\tau}{Q} \qquad (3)$$

If the spectral character of the absorption and of the fluorescent emission are identical for two systems the efficiency of one may be established from the known efficiency of the other by a comparison of the ratios of absorption coefficient to fluorescent intensity when these quantities are measured under comparable conditions.

Experimental Procedure

Soaps.—Xenylstearic acid prepared and purified by the Oils and Fats Division of the Eastern Regional Laboratory of the Department of Agriculture[15,16] was used for the preparation of the soap studied. The neutralization equivalent of this acid was 440 (theory, 436.7). The acid is a mixture of isomers in which the 9- and 10-substituted forms are believed to predominate. The arylstearic acid was dissolved in isopropyl alcohol containing 17.5 volume per cent. of carbon dioxide-free water and neutralized to the phenolphthalein end-point with aqueous sodium hydroxide solution.

Calcium xenylstearate was precipitated by adding the original alcoholic solution of the corresponding sodium salt to a 10% excess of 1.5% aqueous calcium chloride solu-

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(9) J. Grant, *Textile Colorist*, **62**, 9 (1940).
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(12) F. Perrin, *J. Phys. Radium*, [VI] **7**, 390 (1926).
(13) E. Gaviola, *Z. Physik*, **42**, 853 (1927).
(14) W. Szymanowski, *ibid.*, **95**, 440 (1935).
(15) A. J. Stirton and R. F. Peterson, *Ind. Eng. Chem.*, **31**, 856 (1939).
(16) A. J. Stirton, B. B. Schaeffer, Anna A. Stawitzke, J. K. Weil and W. C. Ault, *J. Am. Oil Chemists Soc.*, **25**, 365 (1948).

tion adjusted to a *p*H of 10.5. The gummy calcium soap so obtained was transferred to benzene and centrifuged to remove water droplets. The benzene solution was refluxed under nitrogen to remove solubilized water and the clear dry solution, containing about 10% of soap, was dried under vacuum from the frozen state and sealed in ampoules under nitrogen. Analysis showed free acid, less than 0.1%; calcium, 4.47% (theory, 4.40%). Careful control of the *p*H of aqueous solutions in contact with the soaps or their solutions in non-polar solvents is required to prevent extensive hydrolysis at the water interface as a result of the selective partition of the products of hydrolysis between the two phases.

Dye.—Rhodamine B Extra hydrochloride (du Pont) was dried and extracted with benzene to remove small amounts of an oil-soluble material. The dye was then recrystallized from absolute ethanol to which a few drops of concentrated HCl were added to ensure precipitation of the hydrochloride. This preparation was used to evaluate the actual dye content of the commercial material, which was found to have 91% of the color activity of the purified dye. No significant spectral differences between the original and the purified dye were noted and the commercial form was used for most of the measurements.

Solvents.—Reagent grade benzene meeting A.C.S. specifications was percolated through silica gel before use. Bis-1-(2-methylpropyl)-4-ethyloctyl sebacate was percolated through Florosil. The absolute methanol was of reagent quality and was used without further treatment. U.S.P. glycerol (94.7%) was treated with activated charcoal and active alumina and filtered. This material is believed to correspond closely to the "normal commercial" glycerol used by Szymanowski for his measurements of the excited life of Rhodamine B in this solvent.[14]

Fluorescence Measurements.—All measurements of fluorescence were made with a Brice–Speiser light-scattering photometer[17], using 30 × 30 mm. square cells. The instrument was modified to incorporate a holder for secondary filters and a collimating grid that restricted to three degrees in the vertical plane and six degrees in the horizontal plane the maximum angle from which emitted light could reach the photomultiplier window. When observations were made with polarized light, readings were corrected for a small polarization remaining in the reference beam after passage through an opal glass diffusor, and for a slight asymmetry of response of the photomultiplier tube to polarized light. Fluorescence intensities were expressed in arbitrary units. Repeated measurements of the relative intensity for a given solution showed an average deviation of 0.4%. Fluorescence intensities employed in the estimate of fluorescent efficiency were corrected for the attenuation of the reference beam during its passage through the solution, and for reabsorption of the fluorescent emission within the cell.

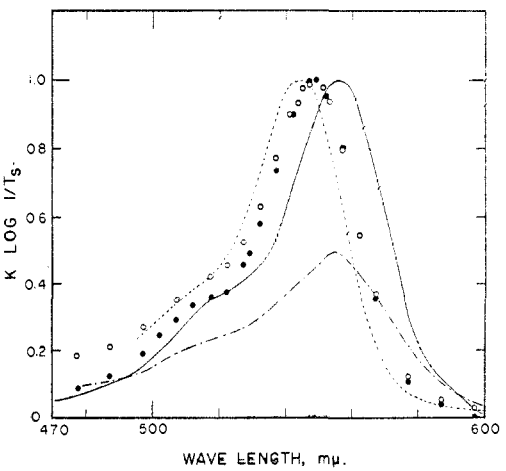

Fig. 2.—Absorption spectra for Rhodamine B in various solvent systems: ——, glycerol, 1.5 × 10^{-7} *M*; – – – – –, absolute methanol, 1 × 10^{-7} *M*; O, calcium xenylstearate in ditetradecyl sebacate, dye = 3 × 10^{-7} *M*; ●, calcium xenylstearate in benzene, dye = 4 × 10^{-7} *M*; —·—·—, dye in benzene, 3.6 × 10^{-5} *M*.

(17) R. Speiser and B. A. Brice, *J. Optical Soc. Am.*, **38**, 364 (1948).

The fluorescence of Rhodamine B was excited with the 546 mμ radiation from an AH3 mercury vapor lamp, isolated with Corning filters. It is essential for the method described here that excitation be by light absorbed in the band with which the fluorescence is directly associated. A Wratten 22 was used as a secondary filter in all cases. It reduced the amount of scattered primary light reaching the photomultiplier unit to a value too small for measurement under the conditions employed for the fluorescence observations.

Absorption spectra were measured with a Beckman spectrophotometer. Measurements of fluorescence and of absorption were made at 25°.

Osmotic Pressure Measurements.—The measurements of osmotic pressure were made in a modification of the osmometer described by Wagner.[18] The capillary tube was fused directly to the bell carrying the cellophane membrane, and the cells were filled or adjusted for level with a hypodermic syringe fitted with a special 22 gage needle. The liquid level in the osmometer capillary was compared with that in a similar (open) capillary clipped beside the former. The membranes employed were undried cellophane of 0.005 inch wet thickness, conditioned for use by transfer through acetone to benzene, and calibrated for the rate of fall of the benzene level in the capillary as a function of the effective liquid head.

The measurements of osmotic pressure were carried out in quadruplicate for each solution of calcium xenylstearate at 25° in a bath controlled to ±0.003°. The initial levels in the capillaries were adjusted to the approximate value expected. After thermal equilibrium had been established, the changes in level over a three-hour period were noted and the liquid heights adjusted to those estimated for the final equilibrium from the observed changes in head. Such estimates could be only approximations because the rate for a given membrane in benzene decreased slowly with time, and because convection effects were different if solvent was entering than if it was leaving the cell. When the difference between the observed and the equilibrium heads is only 1 or 2 mm., however, the uncertainty of the estimate is of the same order of magnitude as the experimental error in reading the height of liquid in the capillary.

Results

Light Absorption and Fluorescence of Rhodamine B.—The absorption of light by Rhodamine B varies both in intensity and in spectral range with the solvent system involved.[19] Absorption data for this dye in glycerol, in methanol and in benzene, as well as when solubilized by calcium xenylstearate in benzene and in bis-1-(2-methylpropyl)-4-ethyloctyl sebacate are presented in Fig. 2. Because of the extreme dilution of the solutions studied (10^{-6}- to 10^{-7} molar) and their consequent liability to appreciable loss of dye by fading or by adsorption on container walls, comparisons between molecular extinctions observed in the different solvents have only qualitative significance. We have instead plotted $k \log 1/T_s$ (where T_s is the transmittancy of the solution), with k so chosen as to make the maximum in each absorption curve unity. To avoid confusion in the figure the data for the very weak absorption of the dye alone in benzene has been plotted to one-half the scale of the other curves. The absorption curve for Rhodamine B in water is not plotted; it would fall very close to that for glycerol.

The molecular extinction coefficient of recrystallized Rhodamine B hydrochloride in water was 11.56 × 10^4 at the wave length of maximum absorption, 554 mμ. The absorption peak is shifted a few mμ toward longer wave length in alcohol or water solutions acidified with hydrochloric acid. The data reported were taken in neutral or weakly alkaline systems. In a saturated benzene solution 98% of the dye is present in colorless form. Aqueous solutions of Rhodamine B undergo hydrolysis at an oil–water interface to give a solution of the colorless lactoid form in the oil phase which is strongly adsorbed at the oil–water interface,[4] where it exists in the colored form. The color transition in the presence of soap micelles appears to be a similar phenomenon.

(18) R. H. Wagner, *Ind. Eng. Chem., Anal. Ed.*, **16**, 520 (1944).
(19) G. Braun, *J. Chim. Phys.*, **33**, 559 (1936).

Aged soap–dye solutions in benzene develop a new substance absorbing strongly at 525 mμ and fluorescing at a correspondingly shorter wave length. 546 mμ light is only weakly absorbed by the altered form. All measurements reported were made on freshly prepared solutions.

Because of the possibility of preparing micellar dispersions of soap in fluids of high viscosity, the limiting polarization p_0 of the fluorescent light emitted by Rhodamine B adsorbed on soap micelles can be measured almost directly. The liquid employed for this purpose was bis-1-(2-methylpropyl)-4-ethyloctyl sebacate. Dye and soap were added to the ester in a concentrated benzene solution. The measured viscosity of the completed solution was 61.0 centipoises.

From Perrin's equation it follows that the difference between the limiting polarization and that observed experimentally in a system showing some Brownian rotation of the dye molecules is

$$p_0 - p = pp_0(1/p_0 - 1/3)\frac{\tau RT}{\eta V} \qquad (4)$$

If V has a magnitude of 20,000 cc. per gram-micelle, τ equals 5.0×10^{-9} second, and η is 0.61 poise, then $p_0 - p$ will have a value of 0.004 when p_0 lies near 0.45.[20] Applying this correction, the resulting p_0 for the bis-1-(2-methylpropyl)-4-ethyloctyl sebacate system was 0.480. This value is slightly larger than those previously reported for Rhodamine B or for other dyes, possibly because depolarization by resonance transfer of energy or by normal absorption and re-emission is so slight in the extremely dilute dye solution studied.

Estimates of the average excited life, τ, of Rhodamine B adsorbed on soap micelles in nonpolar solvents can be made by comparing the ratio of total fluorescent emission to absorbed exciting light with the similar ratio for a Rhodamine B solution of known τ. There are, however, experimental complications that require consideration in the case of the soap–dye systems.

If the emitted fluorescence is partially plane polarized the intensity distribution will not be spherically symmetrical, and the measured intensity will be strictly proportional to the total emission only if the observations are made at a specific angle to the exciting beam. When the exciting light is unpolarized, and observation is made without an analyzer, the observed emission will be equal to the average of the emission in all directions at angles of 54.73° and 125.27° to the exciting light. Since available cells for measurements of fluorescence are not suitable for use at the required angle; it has been more convenient to make all observations at an angle of 90° to the exciting beam and to apply a correction computed from the measured degree of polarization of the fluorescent emission. For unpolarized exciting light this correction[21] is given by the expression

$$I_{av} = I_{90°}\left(\frac{6 - 2p}{6 - 3p}\right) \qquad (5)$$

where I_{av} is the intensity that would have been observed with complete depolarization of the emitted light, $I_{90°}$ is the observed intensity, and p is the degree of polarization of the light that would be emitted in the y direction under illumination in the x direction with *z-polarized* light. (The actual degree of polarization of the 90° emission excited by unpolarized light is, of course, less than p as defined.[21]) If however, the exciting light is vertically polarized and the analyzer is set to pass the vertical component of the emission the equation for the correction is

$$I_{z\,av} = I_{z90°}(3 - p)/(3 + 3p) \qquad (6)$$

where I_z is the observed 90 intensity and $I_{z\,av}$ is the average intensity that would be observed if depolarization were complete.

Another complication arises as a result of the difference in the maxima for absorption and for fluorescence of Rhodamine B in different systems. The sensitivity of the photomultiplier tube is greater if the wave length of the emitted light is less, and in the spectral range involved this effect is only partly compensated by the fact that the Wratten 22 secondary filter passes a smaller proportion of the fluorescent band lying at shorter wave lengths. If the wave length differences between fluorescence maxima are not too large, the true relative efficiency may be approximated by comparing the unknown system with two solutions of known efficiency whose fluorescence bands lie one on either side that of the unknown and making a suitable interpolation. Glycerol and methanol solutions of Rhodamine B have emissions that bracket that of the soap–dye dispersions in benzene in the required manner (Fig. 2).

The data reported for the fluorescence efficiency of Rhodamine B in different solvents and the average excited life of

TABLE I

FLUORESCENCE EFFICIENCY AND EXCITED LIFE OF RHODAMINE B

Solvent	τ, sec. ($\times 10^9$)	Source, ref.	Quantum efficiency	Source, ref.	τ/Q ($\times 10^9$)
Water	2.0 ± 0.4	13	0.25	22	8.0
			.28	22	7.1
Methanol	2.4	14			
Ethanol	1.6	8	.42	8	3.8
Glycerol	4.73	14	.70	8	6.75
	4.2	13			6.0
				Average ratio =	6.33

(20) P. Pringsheim, ref. 8, p. 373.

(21) The partly polarized emission from a small volume of a fluorescent dye solution excited by unpolarized light may be considered to consist of a completely unpolarized fraction having a spherically symmetrical intensity distribution and of a remainder for which the electric vector is restricted to the yz plane but has a completely random orientation with respect to the y- and z-axes. Consider for the moment only this polarized fraction. Its intensity is symmetrical about the x axis, but varies with the angle of observation, θ, as in the familiar case of Rayleigh scattering, *i.e.*

$$I\theta = I_{90°}(1 + \cos^2\theta) \qquad (a)$$

Integration shows the average of the intensity over all angles to be

$$I_{av} = I_{90°}(1 + 1/3) \qquad (b)$$

Consequently the angle of observation for which the observed intensity is equal to the average intensity over all angles will be arc cos 1/3, or 54.73°.

The intensity distribution for this polarized fraction would have been the same if the fraction had consisted entirely of two equal plane-polarized components with their electric vectors oriented parallel to the y- and z- axes, respectively. If now only the z component be supposed present, the intensity observed normal to the xz plane will be unchanged, but the average of the intensity over all angles will be reduced to 2/3 $I_{90°}$. If now the observation normal to the xy plane be made through a Nicol analyzer oriented to pass the z component of the emission, the intensity will still be equal to the original 90° intensity of equation (b). If, however, the z-component of the emission is completely depolarized as a result of Brownian rotation, $I_{z90°}$ will be only one-third as great as before depolarization.

Returning to a consideration of actual fluorescent emission, two cases are of interest:

(A) *The fluorescence is excited by natural radiation and is observed without an analyzer:* Let $I_{90°}$ be the observed intensity normal to the xz plane, having a polarization p_N.

Let I_{av} be the average of the intensity taken over all angles.

$$I_{av} = I_{90°}(1 - p_N + 4/3p_N) = I_{90°}(1 + p_N/3) \qquad (c)$$

If it is desired to express I_{av} for the above case as a function of p_p (the polarization observed for the 90° emission from the same system when the excitation is by z-polarized light), use is made of the relation, $p_N = p_p/(2 - p_p)$ (P. Pringsheim, ref. 8, p. 213). This gives (still for emission excited with natural light and observed without an analyzer)

$$I_{av} = I_{90°}(6 - 2p_p)/(6 - 3p_p) \qquad (d)$$

The quantity p_p is designated elsewhere in this paper simply as p.

(B) *The fluorescence is excited with plane-polarized light having its electric vector parallel to the z-axis:* Let $I_{90°}$ be the intensity observed normal to the xz plane without an analyzer, and $I_{z90°}$ the intensity of the z component of the same emission.

$I_{z\,av} = I_{av}/2$ will be the intensity of the z-component if complete depolarization takes place.

Then

$$I_{z\,av} = I_{90°}[(1 - p_p)/2 - p_p/3] = [(3 - p_p)/6]I_{90°} \qquad (e)$$

$$I_{z90°} = I_{90°}[(1 - p_p)/2 + p_p] = [(1 + p_p)/2]I_{90°} \qquad (f)$$

so

$$I_{z\,av} = I_{z90°}(3 - p_p)/(3 + 3p_p) \qquad (g)$$

(22) S. I. Vavilov, *Z. Physik*, **22**, 266 (1924); **42**, 311 (1927); H. Hellstroem, *Arkiv Kemi Mineral. Geol.*, **12A**, No. 17, 1 (1937).

the dye molecule in the same or analogous systems are presented in Table I.

The data are not altogether consistent, and Vavilov considered his original measurements of fluorescence efficiency to have an absolute uncertainty of 10 or 15%. The ratio τ/Q of Table I, except for the ethanol solution, is seen to be constant within such limits of uncertainty. Szymanowski's careful measurements of τ for Rhodamine B in methanol and in glycerol are used here to estimate τ for the same dye in soap–benzene systems. Since his observations were made on solutions, respectively, twenty thousand and one thousand times as concentrated as the corresponding solutions used in this study, his data for τ have been extrapolated to the appropriate lower concentrations. This extrapolation leads to $\tau = 2.6 \times 10^{-9}$ second in methanol and to $\tau = 5.0 \times 10^{-9}$ second in glycerol. The ratio (q') of the average fluorescence intensity ($I_{z\,av}$) to the absorbency for unit path ($1/L \log 1/T_0$) for a given solution is taken to be proportional to the quantum efficiency of Rhodamine B fluorescence, subject to the restrictions resulting from differences in average wave length for the emission from different solvent systems.

Data for the degree of polarization, p, relative fluorescence efficiencies, q', and the resulting estimates of average excited life are recorded for seven Rhodamine B solutions in Table II. The geometrical mean of the τ/q' values for methanol and for glycerol was used to calculate τ in the soap–dye systems.

TABLE II

CHARACTERISTICS OF THE FLUORESCENT EMISSION FROM RHODAMINE B HYDROCHLORIDE IN VARIOUS SYSTEMS UNDER EXCITATION BY PLANE-POLARIZED LIGHT (λ = 546 mμ)

System	Soap concn. fw/l. ($\times 10^4$)	Dye concn. moles/l. ($\times 10^7$)	Polarization of 90° fluorescence	$q' = I_{z\,av}/(1/L)\log 1/T_0$	τ/q' ($\times 10^8$)	τ, ($\times 10^9$) from mean τ/q'
Water		2.6	0.067	17.9		
Methanol		1.2	.027	28.0	0.9286	
Glycerol		1.5	.462	41.9	1.1933	
Ca xenylstearate in C_6H_6						
A′	73	3.0	0.300	35.6		3.75
B′	19.6	3.0	.283	34.7		3.65
C′	4.9	3.0	.283	36.0		3.79
Ca xenylstearate in bis-1-(2-ethylpropyl)-4-ethyloctyl sebacate	19.4	3.0	0.476	41.7		4.39

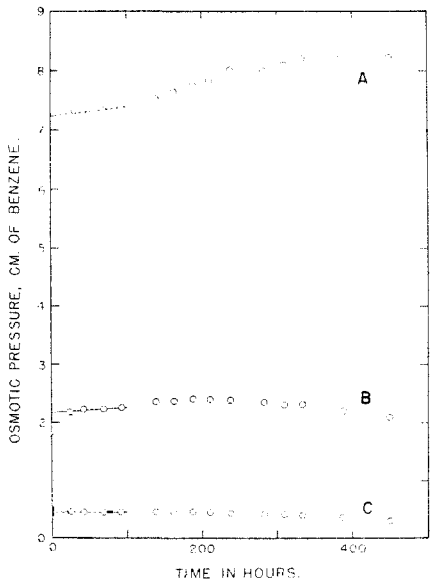

Fig. 3.—Osmotic pressure of solutions of calcium xenylstearate in benzene as a function of time: A, 0.592 g./100 ml.; B, 0.155 g./100 ml.; C, 0.0357 g./100 ml.

The values of τ from the final column of Table II were used to calculate the effective micellar volume in the three concentrations of calcium xenylstearate by means of equation (2). The gram micellar volumes so computed were, in the order of decreasing concentrations, 21,600, 18,100, and 18,800 cc., respectively. Since the apparent density of calcium xenylstearate in benzene is 1.04, the gram micellar weights will have slightly larger numerical values than the corresponding volumes (Table III). These solutions all contained approximately one mole of water per mole of soap.

Micelle Size from Osmotic Pressure.—The determination by osmotic pressure measurements of the size of calcium xenylstearate micelles in benzene solutions is complicated by the fact that this size is a sensitive function of the water content of the system.[3] Fluorescence observations show that the polarization of the emission from very dilute soap–dye systems reaches a constant minimum value at water concentrations corresponding to 10% or less of the amount required for saturation of the benzene. In more concentrated systems the viscosity of the solution and the polarization of the fluorescent emission change little after the addition of one mole of water per mole of soap. One mole of water per mole of soap was therefore added to the initial stock solution of calcium xenylstearate in benzene, and all of the benzene entering the solutions was dried and then 15% saturated with water at 25°. The benzene used outside the cells also contained this proportion of water.

After the initial adjustment of the osmometer levels to their approximate equilibrium values, the osmometers were read daily during a four-week period. The indicated equilibrium osmotic pressure was calculated for each interval from the previously determined rate constant and the change in level that had occurred during the interval. The initial osmotic pressure was obtained by extrapolating the plots of osmotic pressure during the first 100 hours to zero time.

The extrapolations obtained with different cells containing the same concentration of soap differed by as much as 5% for the two more dilute solutions and by a maximum of 1.5% for the most concentrated system. These differences exceed the uncertainties in the actual measurement of levels in the osmometers; they are believed to reflect uncontrolled variations in water content and membrane permeability from one cell to another.

The observations over a 500-hour period are summarized graphically in Fig. 3, where each plotted point represents the average for four cells. The two more concentrated solutions, in which the water content of the solvent was small as compared with the number of moles of soap present, showed a steady rise of osmotic pressure with time. This rise indicates that the soap systems were capable of taking up more water than was originally present, and that the average micelle size was reduced by the diffusion of water through the membrane from the water-bearing solvent outside. The change in slope that appears in the curves between 300 and 400 hours is probably a complex result of particle aggregation in the presence of excess water and of oxidative deterioration of the soaps.

For solutions as dilute as these, the micelle weight calculated from the van't Hoff relation, $\pi V = nRT$, differs from that corresponding to the exact thermodynamic relation or to Morse's equation by an amount which is less than the experimental uncertainty of the measurements. The

TABLE III

THE SIZE OF HYDRATED CALCIUM XENYLSTEARATE MICELLES IN BENZENE SOLUTION, FROM MEASUREMENTS OF OSMOTIC PRESSURE AND OF FLUORESCENCE AT 25°

Solution	Concn. (g./100 ml.)	Osmotic pressure at $t = 0$, atm. $\times 10^{-2}$	Gram micellar wt. at $t = 0$ from osmotic pressure	Initial gram micellar weight from fluorescence
A	0.592	6.12	23,700	
B	.155	1.84	20,700	
C	.0357	0.38	23,000	
A′	.670			22,500
B′	.179			18,900
C′	.0445			19,600

van't Hoff equation was used to calculate the entries in column 4 of Table III.

The osmotic pressures and gram micellar weights in this table are those obtained by extrapolation of the data to zero time. The customary extrapolation to infinite dilution, which has been so valuable for the study of macromolecules in general, does not seem justifiable in this case, where the dispersed phase is an aggregate whose size is a function of the water content and possibly of the soap concentration.

The minimum micellar weights corresponding to the maxima in the osmotic pressure curves were 20,800 and 18,600 for the A and B solutions, respectively.

Discussion

The micelle weights determined osmotically and those deduced from the depolarization of Rhodamine B fluorescence differ by an average of 9.4% of the former. The difference is in a direction opposite to that which might have been expected if the micelles were solvated or if they were anisometric. Either of these conditions should decrease the rate of the Brownian rotation on which the depolarization depends, and lead to high estimates of the micelle volume or weight. If the dye molecule were not completely immobilized with respect to the soap micelle of which it was a part, but could engage in oscillation or rotation that altered its relative orientation, the observed depolarization would not reflect correctly the Brownian rotation of the micelle. The estimated micellar volume would then be too low. However, the inverted type of soap micelle that must be assumed in a non-polar solvent implies for a micelle containing fifty or less acid residues a compact polar core with which only the most polar element of the dye molecule would be directly associated. The latter is considered to coöperate with other amphiphatic units in micelle formation rather than to exist within the micelle in the manner of hydrocarbons solubilized by an aqueous soap system. Complete rotation of the dye molecule seems improbable in such a structure, but oscillations of a few degrees about an average position are possible. The close approach to the theoretical maximum of the polarization of the fluorescence from the dye soap in bis-1-(2-ethylpropyl)-4-ethyloctyl sebacate as well as the agreement between the micelle sizes obtained from osmotic pressures and from fluorescence seem to indicate that the dye has little freedom of rotation with respect to the micelle.

The osmotic and fluorescence methods place the micelle sizes for corresponding solutions in the same order, and it is concluded that the fluorescence method of determining micelle size provides good relative accuracy for the comparison of similar systems. The absolute accuracy at present depends upon measurements of the average excited life of the Rhodamine B molecule made under very different conditions; this accuracy may be considerably improved if direct determinations of the average excited life and fluorescence efficiency of Rhodamine B in soap–hydrocarbon systems become available.

In any case the ease and quickness with which the necessary measurements can be accomplished makes the fluorescence method a tool whose promise is not limited to the study of soap micelles, but extends to other colloidal units to which a suitable fluorescent dye can be adsorbed or chemically attached. The method has the further outstanding advantage of permitting studies of micelle or particle size in more dilute solutions than do such techniques as osmometry, viscometry or light scattering. Under suitable conditions useful measurement of the depolarization of the fluorescence can be made in solutions containing 0.001% by weight of a benzene-soluble soap.

The precision of the method decreases as p approaches p_0 in Equation (4). It is apparent from this equation that $p_0 - p$ is inversely proportional to the product, ηV, of the gram micellar volume by the solvent viscosity. With present techniques the results are only qualitatively significant if ηV exceeds 1500 poises $\times$ cm.^{3} If an otherwise suitable dye with a much longer average life can be employed, this limit may well be increased by a factor of 10 or more.

This work establishes the size of the micelles formed by a typical oil-soluble carboxylate soap in benzene in the presence of equimolal amounts of water. In addition, the general agreement between the sizes obtained from osmotic pressure and from fluorescence depolarization indicates that the life-time of an individual soap micelle must be one or more orders of magnitude greater than 10^{-8} second. The average residence time of a dye molecule in a given micelle must also be 10^{-7} second or longer. These limits are of interest because of their implications concerning the rate of exchange of molecules between the micelle and the solvent.

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