

following values were obtained for dodecylamine hydrochloride at 20, 25, 30, 40, 50 and 60°: calcd., 74.20, 83.67, 95.02, 116.7, 139.6 and 164.3; found, 74, 83, 94, 115, 137 and 160. The values found are approximately those calculated, which is in agreement with the work of others upon colloidal electrolytes.² The values for dodecylamine acetate at 0, 20, 40 and 60° are as follows: calcd., 36.61, 67.10, 107.0 and 150.6; found, 45, 84, 130 and 180. Octadecylamine hydrochloride shows a calculated value of 157.9 and an actual value of 156 at 60°. The calculated values for octadecylamine acetate differ materially from the actual values. At 50° the calculated value is 118.7 and the actual value 530, while at 60° the calculated value is 140.8 and the actual value 825.

The slope of the second and third ranges is undoubtedly due to the formation of micelles. Micelle formation begins at approximately 0.013 molar for the dodecylamine salts and at approximately 0.0003 molar for the octadecylamine salts, and in both cases micelle formation begins at a slightly lower concentration as the temperature is increased. Both of the hydrochlorides show a slight increase in equivalent conductance with concentration over a portion of the third range.

Dodecylamine acetate-water systems show a gel area beyond 0.90 molar. It will be noted that there is no noticeable change in the slope of the

curve (Fig. 2) as the system goes from a solution to a gel.

Calculations of l_c/Λ_0 give the following values of n_c for dodecylamine hydrochloride at infinite dilutions: 0.273 at 20°, 0.275 at 25°, 0.322 at 30°, 0.325 at 40°, 0.309 at 50° and 0.312 at 60°. Values of n_c for dodecylamine acetate are 0.410 at 20°, 0.459 at 40° and 0.434 at 60°. It will be noted that in both cases n_c reaches a maximum at approximately 40°. Determinations of the transference numbers over the entire range of concentration for the salts reported in this paper are now in progress.

Summary

1. Conductivities and densities of aqueous solutions of dodecyl- and octadecylamine acetates and the corresponding hydrochlorides have been determined.

2. The conductivity curves show three ranges characteristic of colloidal electrolytes.

3. These salts appear as simple strong electrolytes in dilute solution as shown by the fact that in several cases the actual slopes are found to be almost exactly the values calculated from the Onsager equation.

4. Values for Λ_0 , l_c and n_c at infinite dilution have been calculated from the data obtained.

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A Nephelometric Method for Determination of Solubilities of Extremely Low Order

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In an investigation¹ of the reactions of carcinogenic and related polycyclic hydrocarbons, the need arose, for reasons set forth in detail in the following paper, to determine the solubilities of these substances in water, in aqueous media containing various solubilizing agents, and in natural biological fluids. The development of a method for this purpose was governed by two principal considerations. First, the method must be extremely sensitive, capable of measuring solubilities down to less than 1 microgram of solute per liter of solvent (*i. e.*, 0.001 part per million). Secondly, it must be sufficiently general in prin-

ciple to be applicable to a variety of complex solvents.

On the basis of preliminary experiments with various potential methods for measuring the amount of a given hydrocarbon held in solution, ultraviolet absorption spectrometry was rejected because of insufficient sensitivity and the possibility of interference by components of the solvent media, such as proteins. The fluorometric method, though applicable in a few cases, was found to be unsuitable as a general method because of insufficient sensitivity, and because of the possibility of errors due to quenching of hydrocarbon fluorescence by the solvent. The possibility of estimating the amount of hydrocarbon

(1) W. W. Davis, M. E. Krahl and G. H. A. Clowes, *THIS JOURNAL*, **62**, 3080 (1940).

colorimetrically after reaction with an added reagent capable of attacking only dissolved hydrocarbon was rejected because of the probability of interference from the solvent, and because of difficulty in finding reactions which would distinguish dissolved from suspended hydrocarbon.

The method finally adopted consisted, briefly, in preparing serial dilutions of a suspension of the hydrocarbon in water and determining nephelometrically the amount of hydrocarbon per unit volume beyond which further dilution caused no reduction in light scattering, which remained equal to that of the pure solvent.

Although developed primarily for estimation of the solubility of polycyclic hydrocarbons, the method appears applicable to other slightly soluble substances and to solvents other than water. This paper presents an account of the technique, the precautions required in using it, and its limitations. The following paper contains results obtained from application of the method to the specific case of solubility of polycyclic hydrocarbons in pure water.

Nephelometer.—An early model 100-mm. Bausch and Lomb Dubosq colorimeter, fitted with the latest type of optical system, and used with the Bausch and Lomb

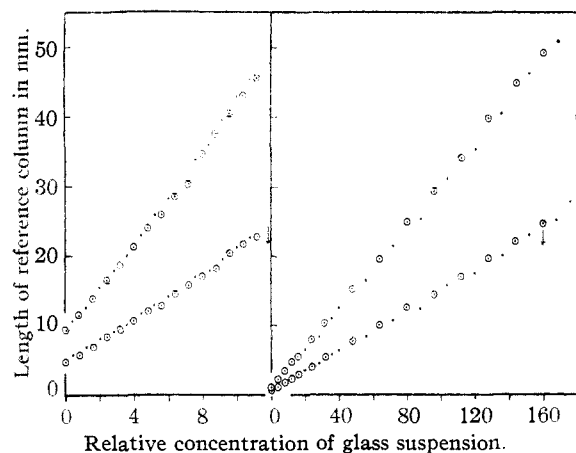


Fig. 1.—Linear relation of reference column length to concentration of suspended particles (Pyrex glass particles) at two concentrations of reference suspension and two fixed lengths of sample column. In Figs. 1a and 1b the reference suspensions of glass particles had concentrations which gave light scattering equivalent to those of suspensions containing 12 micrograms per liter and 160 micrograms per liter of 10-ethyl-1,2-benzanthracene, respectively. These reference points are indicated by arrows. In each case the lower curve was obtained with a 25 mm. length of sample column, the upper curve with a 50 mm. length of sample column. Experimental points marked ● were all obtained in one experiment; points marked ○ were all obtained in a second experiment.

nephelometer illuminator, served as the basic instrument. The following modifications were made to reduce instrumental light. (a) The flat-bottom nephelometric tubes were replaced by Pyrex tubes having sloping bottoms and painted on the outside with dull black paint up to a point approximately 2.5 cm. above the bottom tip. This reduced the light scattered by the end of the tube directly into the visual field. (b) A black metal shield, with a slit 7 mm. wide in front of each tube, was fitted on the nephelometer between the illuminator and the nephelometric tubes; this reduced to a minimum the light scattering from irregularities and dust particles on the sides of the tubes. (c) A black metal apron was fitted around each plunger support, extending about 5 mm. below the end of the plungers. These aprons shielded the ends of the plungers from direct illumination and reduced the light scattering from dust particles, lint, or air bubbles on the ends of the plungers.

To reduce heating of the solutions by the intense beam of the illuminator, a water cell, 2-cm. thick, covering the whole beam of the illuminator, was mounted between the light source and the nephelometric tubes.

In accordance with the usual criteria for a satisfactory nephelometer² the instrument gave a linear relationship between length of column and concentration of suspension (Fig. 1a and 1b). Suspensions of Pyrex glass, which has a solubility too low to be detected by the present method, were used to standardize the instrument; suspensions of hydrocarbons could not, of course, be used for this purpose because of the solution of a fraction of the hydrocarbon. For any nephelometer operating under constant conditions there is a characteristic amount of instrumental light. The degree to which this fixed amount of instrumental light displaced the origin of the nephelometric curves along the reference column axis increased as more dilute reference suspensions were used. Compare the curves of Figs. 1a and 1b with each other and those of Fig. 2.

Solvent Water.—To attain the high sensitivity required for satisfactory use of the method, it was essential that the solvent water or solution be as free as possible from dust and other light scattering elements. The water was received directly into a carefully cleaned 4 gallon Pyrex bottle from a copper still in an air conditioned, virtually dust-free room. This bottle was tightly closed with a carefully cleaned rubber stopper carrying a glass tube siphon and an air inlet tube. As the water was withdrawn through the siphon for use, the space was filled by air which had been previously passed through a fritted glass filter from which it bubbled through water. Joints in the siphon line were made with pure gum rubber, and the delivery was controlled by a pinch clamp; a stopcock could not be used because it contaminated the water with minute particles of glass and stopcock grease.

Hydrocarbon Suspensions.—The hydrocarbons employed were the same as those previously used in surface film studies.¹ The hydrocarbon under examination was dissolved in Merck Reagent acetone to a concentration of 1 mg. per cc.; 1 cc. of this solution was then delivered into about 110 cc. of water. The water had previously been

(2) J. H. Yoe, "Photometric Chemical Analysis," John Wiley and Sons, Inc., New York, N. Y., 1929, Vol. II, "Nephelometry," p. 65.

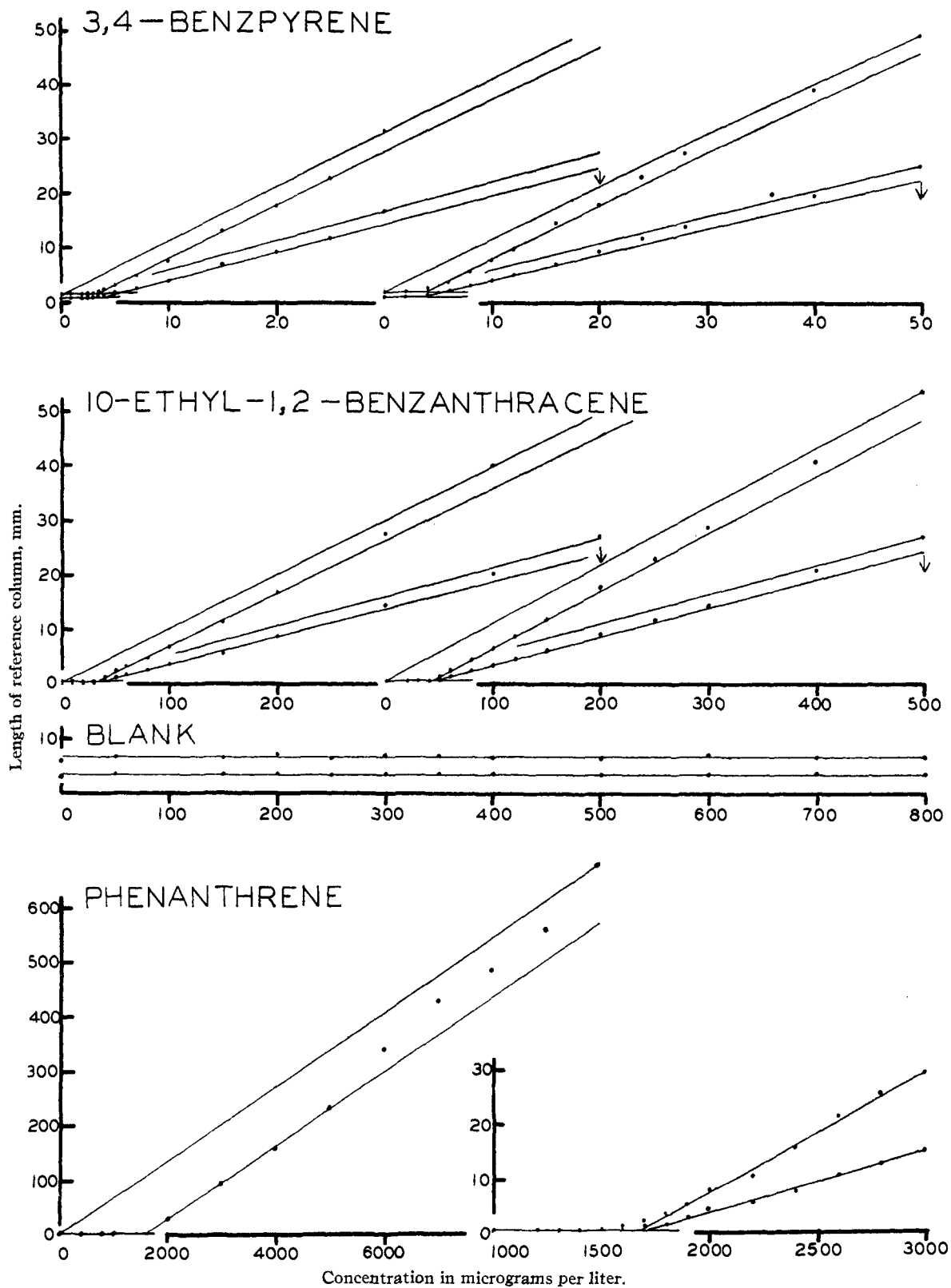


Fig. 2.—Nephelometric curves for three hydrocarbons and a blank (see text). For 3,4-benzpyrene and 10-ethyl-1,2-benzanthracene four pairs of curves are given for each hydrocarbon. In each case the two pairs of curves at the left correspond to one solubility determination with column lengths of 25 mm. (lower pair of curves) and 50 mm.

(upper pair of curves); the two pairs of curves at the right correspond to a second independent determination and are presented to illustrate the reproducibility of the observations. In each pair of curves the upper is an arbitrary curve drawn through the origin with the slope of the experimental curve, this being the curve which would be obtained if no hydrocarbon were dissolved in the water. The lower curve in each pair is drawn through the linear B segment (see text) of the curve determined by the experimental points, which are given by the black dots. The arrows indicate the concentrations of hydrocarbons used in the reference suspensions. The solubility of the hydrocarbon is the amount per unit volume at the point of intersection of the extrapolated A and B segments (see text) of the curve.

For phenanthrene, the pair of curves at the left corresponds to one determination with a Pyrex glass reference suspension equivalent to 375 micrograms per liter of 10-ethyl-1,2-benzanthracene. This determination was carried out with varying sample column lengths; the results were then recalculated to give the reference column lengths which would correspond to a constant sample column length of 50 mm., the calculated reference column lengths being given on the ordinate axis. The two curves at the right correspond to two determinations with a Pyrex glass reference suspension equivalent to 1250 micrograms per liter of 10-ethyl-1,2-benzanthracene and sample column lengths of 25 mm. (lower curve) and 50 mm. (upper curve).

placed in a tared round-bottom Pyrex flask fitted, through a glass stopper, to a distilling head carrying a stopcock in the outlet tube. The acetone solution was delivered under the water surface with vigorous rotation of the flask. A glass tube was placed in the flask to prevent bumping; the acetone was then removed under vacuum, along with about 10 cc. of water. The weight of the flask and contents was then determined. In this way the concentration of hydrocarbon in this "parent suspension" was adjusted to 1 mg. per 100 cc. The flask was kept evacuated until the dilutions were begun; this helped to protect the "parent suspension" from dust-laden air.

A series of dilutions was made from the "parent suspension" as follows. Pyrex test-tubes of 20-cc. capacity were cleaned in chromic acid, rinsed with distilled water and acetone, dried in an inverted position. Just before use they were turned upright in a test-tube rack and loosely capped with clean glass vials. About 10 cc. of solvent water, prepared as described above, was added to each test-tube directly from the siphon. Appropriate volumes of the "parent suspension," or a suitable dilution of it, were then pipetted into the test-tubes, and the volume brought to a mark at the 15-cc. level by further addition of water. The addition from the small jet terminating the siphon also served to mix the diluted suspension thoroughly. This provided a series in which the concentration of hydrocarbon varied from zero to five or ten times the expected solubility, fifteen or more tubes being used in each series. These dilutions were then allowed to stand at room temperature for any desired time. The conditions of time (aging), temperature, and exclusion of light had to be fixed according to the substance under examination.

All experiments here reported were conducted at room temperature, $27 \pm 3^\circ$. Owing to other inaccuracies in the method to be discussed below, and to substantial technical difficulties, closer control of temperature was not attempted for the present experimental series.

The present experiments with polycyclic hydrocarbons were conducted, and the nephelometric readings made, under conditions of light exposure which had been shown, by preliminary observations, to allow no significant photochemical decomposition of the hydrocarbons.

The effect of particle size on solubility is considered in a later section.

Technique of Measurement.—The reference suspension was placed in the left nephelometer tube. The most concentrated suspension of the substance being studied

was used as reference, if this suspension was sufficiently stable, otherwise, a stable suspension of some other hydrocarbon, of Pyrex glass, or of a mixture of hydrocarbon and glass was used, the mixture being chosen to match the color of the hydrocarbon suspension.

The diluted sample suspensions were placed successively in the right nephelometer tube, progressing from lower to higher concentrations.

The length of the illuminated column in the right tube (test suspension) was set at either 25 mm. or at 50 mm.; the length of column in the left (reference) tube necessary to match the nephelometer fields was then determined. Reference column length will be shown to be a linear measure of light scattered. The lengths of column required in the reference tube to match a series of test suspensions were plotted against the total concentrations of hydrocarbon in the series of test suspensions.

The Form and Interpretation of the Nephelometric Curves.—The form of the curves obtained by plotting reference column length against hydrocarbon concentration is illustrated by the curves for three hydrocarbons in Fig. 2. Each curve has a segment A, running parallel to the concentration axis, along which the light scattered is the same as that for the pure solvent; a segment B, along which the light scattered increases approximately proportional to $C_{\text{total}} - C_s$ (where C_s is the concentration of hydrocarbon in solution and is a constant for the experiment); a segment C, along which this relationship fails, the light scattered increasing more rapidly than this relationship demands; and a segment D, observed in a few experiments in the range of high hydrocarbon concentrations (not shown in Fig. 2), along which the light scattered increases linearly with C_{total} . At the intersection of segments A and B there is usually a slight rounding off which decreases with aging of the suspensions (see Fig. 3).

The tentative interpretation of the form of the nephelometric curves is as follows. Along segment A all of the hydrocarbon is in solution; the

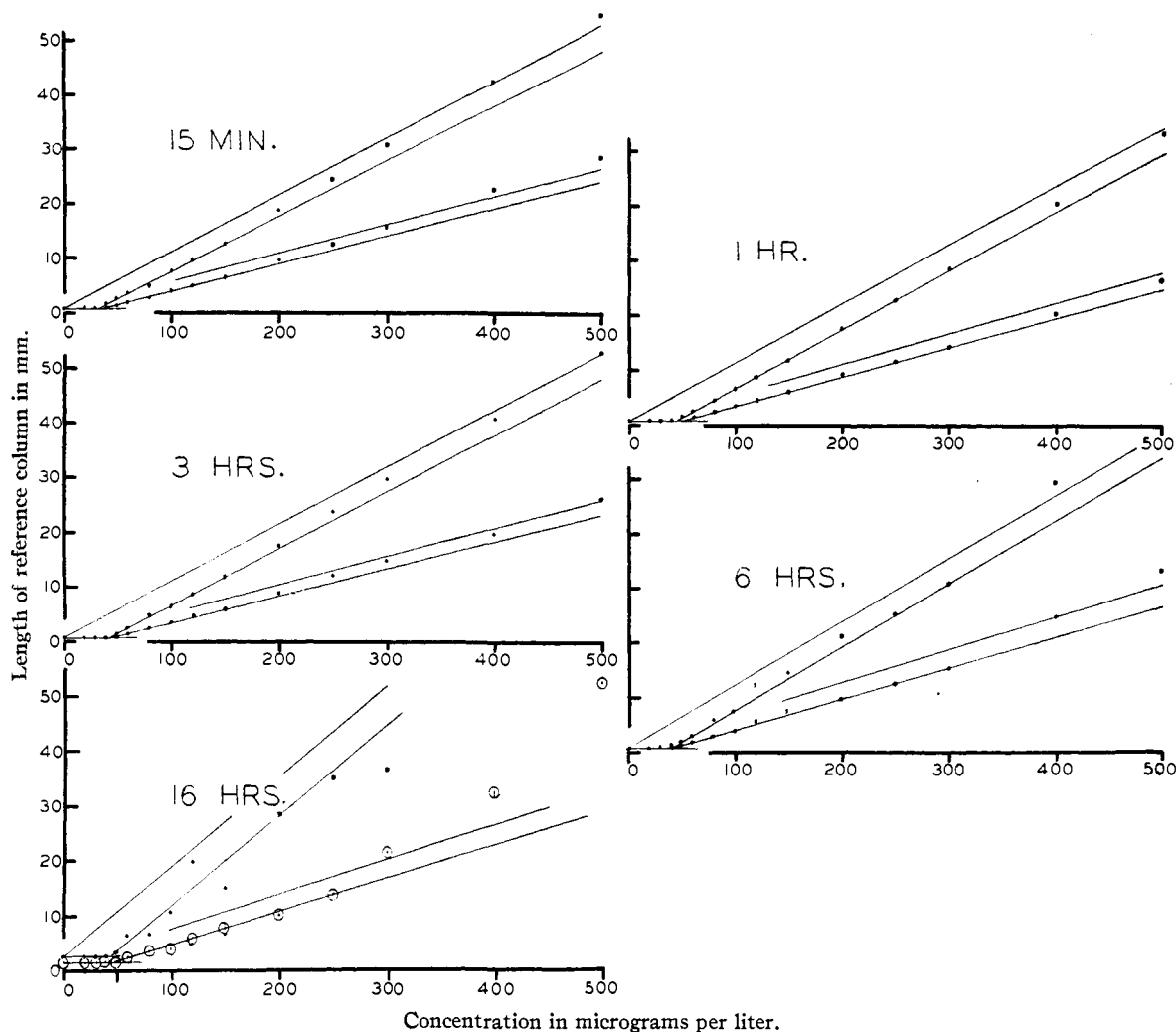


Fig. 3.—Nephelometric curves for suspensions of 10-ethyl-1,2-benzanthracene aged for the periods of time indicated. At each time, the upper and lower pairs of curves have the same significance as in Fig. 2, the reference suspension being in each case 500 micrograms of 10-ethyl-1,2-benzanthracene per liter aged for the same period as the experimental sample. In the sixteen-hour experiment, the points corresponding to the lower curve are circled to distinguish them from the points corresponding to the upper curve.

residual apparent turbidity is due principally to instrumental light. While some light scattering occasionally appears below the A-B intersection, this is not a general phenomenon and is not reproducible; it is attributed to the slow dissolution of the last few particles required to saturate the solution. Along segment B the water is saturated with hydrocarbon, and the light scattering, in excess of the instrumental light, is proportional to the hydrocarbon which remains undissolved, that is, to $C_{\text{total}} - C_s$.

The solubility of the hydrocarbon is the amount of hydrocarbon per unit volume at the point of intersection of the extrapolated A and B segments of the curve (see Fig. 2 for examples). The ex-

trapolation of the B segments may be performed by inspection or by determining the slope by the least square method. The latter method does not seem justified at present, other uncertainties or inaccuracies in the method being greater than that involved in extrapolation by inspection.

The interpretation of the C and D segments, which do not enter into consideration for the solubility determinations, is beyond the scope of the present paper.

Application of the Method to 3,4-Benzpyrene, 10-Ethyl-1,2-benzanthracene and Phenanthrene.—Nephelometric curves for suspensions of 3,4-benzpyrene, 10-ethyl-1,2-benzanthracene, and phenanthrene are given in Fig. 2.

For each hydrocarbon two curves were made with sample column lengths of 25 and 50 mm. The two solubility values so obtained were averaged. Disproportion between points on the two curves occurred if there were contaminating dust particles or bubbles in the test suspension.

The two pairs of curves shown for each hydrocarbon were obtained on separate days and illustrate the degree of reproducibility obtained. The duplicate solubility values obtained for the three substances in the order named were, in micrograms per liter, 3.9 and 4.0, 35 and 45, and 1600 and 1700.

Control Experiments.—A blank series of "suspensions" was prepared by putting pure acetone through the same procedure as used for the acetone solutions of hydrocarbon. A perfectly horizontal nephelometric base line resulted (Fig. 2, Blank); the light scattering was exactly equal to that from water alone in the instrument.

The principal source of uncertainty as to the validity of the present method arises from the fact that the solubility of a substance from small particles increases as the size of the particle is diminished. When only macroscopic particles are involved this factor is negligible, but when the particles are less than one micron in diameter this factor may become important.

The ratio of solubility from small particles of radius r to that from large particles is given by the equation

$$S_r/S = e^{2\sigma M/\rho r RT}$$

which involves the density of the particles ρ , the molecular weight of solute M , the absolute temperature T , the radius of the particles in cm. r , the interfacial free energy between solute particle and solvent σ , and the gas constant R .^{3,4,5}

The average particle sizes of perylene, 3,4-benzpyrene, 10-amyl-1,2-benzanthracene, and 10-ethyl-1,2-benzanthracene in suspensions prepared as described were determined by counting the particles ultramicroscopically. A quartz micro slide chamber was used in conjunction with a cardioid condenser. Series of dilutions of appropriate concentrations were made up and counted. From the dimensions of the field (diameter 2.58 μ , depth 6.35 μ), and the known dilution of the suspensions, the number of particles per liter of parent suspension and the average size were calcu-

lated. An example with the very slightly soluble perylene may be cited. The values used were: $M = 252$, $\rho = 1.25$, $R = 8.32 \times 10^7$ ergs, $T = 300^\circ \text{A.}$, $\sigma = 70$ ergs/sq. cm. No experimentally determined σ value for solid perylene-water is available. The value of σ is therefore chosen arbitrarily by comparison with interfacial systems of known σ , namely, for the liquid interface between benzene and water where $\sigma = 35$ ergs/sq. cm. and for the solid-liquid interface between lead iodide and water where⁴ $\sigma = 130$ ergs/sq. cm. The system solid hydrocarbon-water would seem to be more closely related to the first than the second. The value of σ used for calculation is therefore probably rather too high than too low. Determinations of average particle size for perylene, in repeated experiments, gave $r = 0.091 \times 10^{-4}$ cm. These figures make $S_r/S = 1.13$ for perylene. Analogous experiments yielded S_r/S values of 1.11 for 3,4-benzpyrene and 1.00 for 10-ethyl-1,2-benzanthracene. Thus it appears that, for hydrocarbons having solubilities below 10 micrograms per liter, the observed solubilities may be at most 10-15% too high owing to the extremely small particle size. For hydrocarbons having solubilities greater than 10 micrograms per liter, the particle size is, on the basis of these results, not a disturbing factor.

Another potential source of uncertainty in the present method lay in the necessity of allowing sufficient time for each hydrocarbon to saturate the solution, while at the same time not allowing sufficient time for the hydrocarbon dispersions to increase in particle size by crystal growth or clumping to a point which would disturb the nephelometric behavior of the series. Although each hydrocarbon had to be considered individually, numerous control experiments indicated that a period of one hour was sufficient to establish the A and B segments of the curves. Typical results for 10-ethyl-1,2-benzanthracene after fifteen minutes, one, three, six and sixteen hours, are shown in Fig. 3. It will be noted that the equilibrium was reached very slowly in the region where the final stage in the process involved nearly saturated solutions and a relatively small number of particles, *i. e.*, in the region near the intersection of the A and B segments. Extrapolation of the other points on the A and B segments gave solubilities (in micrograms per liter) of 35, 45, 42, 40 and 44 at 15 minutes, one, three, six and sixteen hours, respectively, thus making it pos-

(3) M. L. Dundon and Edward Mack, Jr., *THIS JOURNAL*, **45**, 2479 (1923).

(4) M. L. Dundon, *ibid.*, **45**, 2658 (1923).

(5) I. M. Kolthoff, *Z. anal. Chem.*, **86**, 34 (1937).

sible to complete the solubility determination well within the period of stability of the dispersion. Here, as in the case of other hydrocarbons studied, the aging of suspensions caused a change in turbidity without changing the solubility figure.

To rule out any solubilizing effect of acetone which might not be removed by evacuation of the "parent suspension," control experiments were made in which no acetone was removed. In a typical experiment, a "parent suspension" of 10-ethyl-1,2-benzanthracene contained 1% acetone. This was divided into two parts, one part being freed of acetone in the usual manner and the other not; both parts were subjected to the requisite dilution for the solubility determination. The solubility where the acetone was removed was 40 ± 5 micrograms per liter; the solubility where no acetone was removed and the dilution of the "parent suspension" was such as to provide an acetone concentration of 70 mg. per liter was exactly the same as after acetone removal, 40 ± 5 micrograms per liter.

As a further control, a mixture of 1 part absolute methyl alcohol and 3 parts methyl acetate was substituted for acetone as the original hydrocarbon solvent. This procedure gave the same solubility as previously observed, 42 micrograms per liter.

Probable Scope of the Method.—The principal conditions for the application of this solubility method are: (1) The substance under examination must form dispersions which are stable over a period relatively longer than that necessary for the substance to saturate the water. (2) The particle size must be determined and found to be

within a range wherein the solubility from the particle is close to that from the bulk. (3) The particles of the substance must differ from the water in refractive index to an extent sufficient to give light scattering high enough to yield nephelometric readings.

The method appears applicable to the determination of solubilities in pure or mixed solvents of large lipid soluble organic molecules of many types, possibly including the biologically important fatty acids, sterols, phosphatides, and other natural substances.

While the solubility of certain lipoidal substances in water may be so low as to yield no definite solubility figure, it is nevertheless possible to use the method to measure the ability of dissolved substances to effect solution of an insoluble lipoidal substance in an aqueous medium.

The authors are indebted to Dr. G. H. A. Clowes and Dr. M. E. Krahl for discussion of various aspects of this work.

Summary

1. A nephelometric method capable of measuring solubilities of polycyclic hydrocarbons down to approximately 1 microgram per liter is described.

2. To illustrate the application of the method, the solubilities of 3,4-benzpyrene, 10-ethyl-1,2-benzanthracene, and phenanthrene in pure water have been determined and found to be (in micrograms per liter), respectively: 4.0 ± 0.5 , 40 ± 5 , and 1650 ± 50 .

3. The extensions and limitations of the method are discussed.

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