[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Diffusion Measurements, at 25°, of Aqueous Solutions of Amino Acids, Peptides and Sugars

BY L. G. LONGSWORTH

RECEIVED JULY 31, 1953

Using the method of free diffusion from an initially sharp boundary between solution and solvent, the spreading of the boundary with time being followed with the aid of Rayleigh interference fringes, the diffusion coefficients of 20 amino acids, 9 peptides and 3 sugars have been determined in dilute aqueous solution at 25°. The materials selected permit a study of the effect, on the diffusion coefficient, of such factors as polymerization, chain length, branching and polarity, and the results are discussed with the aid of the Stokes–Einstein relation and the apparent molal volumes. A procedure is also suggested for the evaluation of the change in the diffusion coefficient across the boundary when the fringe spacing is not Gaussian.

In a recent paper[1] the results of diffusion measurements on dilute aqueous solutions of some amino acids, peptides and sugars at 1° were presented. With the aid of essentially the same experimental procedure as described in that paper these materials have now been examined at 25°, thus affording an indication of the effect of temperature upon their diffusion. Moreover, in obtaining the results at the higher temperature a larger variety of materials has been investigated in order to learn something about the effects, on the diffusion coefficient, of such factors as chain length, branching and polarity. It is the purpose of this paper to present these results and to discuss them with the aid of the Stokes–Einstein relation and the apparent volumes of the substances concerned.

Experimental

As in the work at 1° a Tiselius electrophoresis cell, modified to facilitate the formation of Rayleigh fringes, has been used as a diffusion cell. The boundary was shifted to the center of the channel and sharpened at this level with the aid of the capillary siphoning procedure.[2] It has been observed that if the tip of the capillary is ground to an angle of 45° the boundary becomes sharp, during siphoning, near the mean level of this tip instead of below it when a flat tip is used. In each experiment the diffusion was from a dilute solution into water and the concentrations were such as to give approximately fifty fringes. Four photographs were taken during the sharpening for use in the determination of a zero-time correction, Δt, and the fractional part of the total number, J, of fringes, followed by ten exposures at increasing intervals as the diffusion proceeded. The positions of every other fringe in each exposure were determined with the aid of a comparator and a diffusion coefficient computed from the separation of the 2nd and 26th, the 4th and 28th, etc. The times at which the photographs were taken were selected so that these separations varied from about 1 mm. in the first exposure to 5 mm. in the last one. Thus with the more rapidly diffusing materials the first exposure was made five minutes after the interruption of the sharpening flow at zero time and the final one about two hours later. To facilitate the alignment of the photographic plate in the comparator the movement of the adjustable cross lines on the screen of that instrument has been graduated. This permits the use of an average value for the fractional part of J, as obtained from the four exposures made during sharpening, in the alignment.

Except for the amino benzoic acids, which were recrystallized from water, all of the materials listed in Table I have been used without further purification and the sources are indicated in column 2 of that table. Where more than one source is given at least one experiment was done on a sample from each source. In no case did either the specific refraction or the diffusion coefficient for samples of a given material from different sources differ by more than the uncertainty in the measurements, *i.e.*, 0.1%.

The solutions were prepared by direct weighing of both solute and solvent, correction to vacuum being made with the aid of the assumption that the density of an amino acid or peptide for which this datum is not available is the same as that of alanine. Except for asparagine and raffinose, which were weighed as the mono- and pentahydrate, respectively, all materials were dried in vacuum over anhydrous calcium sulfate at room temperature. Since the diffusion coefficient is not very sensitive to concentration no study was made of the extent to which this drying procedure removed traces of moisture. Such traces would, however, be a source of error in the specific refractions, a by-product of the diffusion measurements, and this should be considered in using the values in column 4 of Table I.

Since the apparent volumes of sarcosine and threonine could not be found in the literature[3] they were determined from density measurements on 1, 2, 3 and 4% solutions, using a procedure described previously.[4] Since no significant variation of the volume within this range of concentration was noted the values in column 8 of Table I are averages. Also, Dr. Schachmann of the University of California has called the author's attention to the fact that the volume for raffinose used previously[1] is inconsistent with the values for the mono- and disaccharide. This value was computed from a single density measurement in the literature.[5] On re-examining this source, which includes both density and viscosity measurements on a series of raffinose solutions, it was found that neither the specific viscosities nor the specific volumes that may be computed from these data are smooth functions of the concentration. Consequently the densities of some raffinose solutions were redetermined to obtain the volume given in Table I.

The thermostat temperature was determined with a Parr thermometer, graduated at intervals of 0.02°, whose Bureau of Standards calibration was checked against a platinum resistance thermometer in this Laboratory. During an experiment the temperature remained constant, within 0.01°, as measured on a Beckmann thermometer, but deviations from 25° of as much as 0.08° occurred during the course of the work. The data of Table I have been corrected to 25.00° with the aid of the Stokes relation.

Results

The experimental results assembled in Table I are largely self-explanatory. Optically active forms of a few of the amino acids in column 1 have been studied but in no case did the result differ significantly from that obtained with the *dl*-modification. The concentrations, column 3, are in grams of solute per 100 g. of solution and the quotient, column 4, of this into the total number, J, of fringes is a measure of the specific refraction of the solute. Here $J = a\Delta n/\lambda$, where the channel depth, a, is 2.505 cm., Δn is the difference of refractive index between solution and solvent and λ, referred to air as unity, is 5461×10^{-8} cm. With the apparent molal volumes, column 8 of Table I, molecular re-

(1) L. G. Longsworth, THIS JOURNAL, **74**, 4155 (1952).

(2) D. S. Kahn and A. Polson, *J. Phys. Colloid Chem.*, **51**, 816 (1947).

(3) E. J. Cohn and J. J. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 159.

(4) L. G. Longsworth, THIS JOURNAL, **59**, 1483 (1937).

(5) E. W. Washburn and G. Y. Williams, *ibid.*, **35**, 750 (1913).

TABLE I

DIFFUSION COEFFICIENTS OF AMINO ACIDS, PEPTIDES AND SUGARS AT 25°

B, Bureau Stds.; D, Dougherty; G, General Biochemicals; H, Hoffman-LaRoche; M, Merck; Ma, Matheson; Mn, Mann; N, Nutritional Biochemicals; P, Pfanstiehl; S, Synthetical Labs.

1	2	3	4	5	6	7	8	9	10
	Source	Concn., wt. %	Sp. ref., J/concn.	Δt, sec. Obsd.	Calcd.	$D \times 10^5$	V, cc./mole	$DV^{1/3} \times 10^5$	D_{25}/D_1
Glycine	S	0.599	82.88	18	17	10.554	43.5	37.12	2.049
Diglycine	N	.577	87.50	11	9	7.909	77.2	33.68	2.087
Triglycine	H, N	.578	86.80	9	10	6.652	113.5	32.21	2.095
Glucose	P	.780	65.20	9	9	6.728	111.9	32.42	2.145
Sucrose	B, P	.769	65.62	9	8	5.209	209.9	30.96	2.158
Raffinose	N, P	.759	67.30	8	9	4.339	306.6	29.26	2.159
α-Aminopropionic acid (alanine)	M, Ma, N	.638	78.96	10	8	9.097	60.6	35.73	2.107
β-Aminopropionic acid (β-alanine)	M, N	.614	83.34	10	8	9.327	58.9	36.29	2.073
N-Methylglycine (sarcosine)	G	.636	74.05	8	9	9.674	62.7	38.43	...
β-Hydroxy-α-aminopropionic acid (serine)	M	.630	79.55	9	9	8.802	60.8	34.61	2.098
α-Aminobutyric acid	N	.626	79.72	17	9	8.288	76.5	35.18	2.130
α-Aminoisobutyric acid	N	.640	76.47	10	9	8.130	78.1	34.75	...
β-Hydroxy-α-aminobutyric acid (threonine)	G, M	.640	78.60	10	9	7.984	76.9	33.95	...
α-Aminovaleric acid (norvaline)	N	.635	80.15	10	9	7.682	92.7	34.77	...
α-Aminoisovaleric acid (valine)	M	.616	80.50	12	8	7.725	91.3	34.79	2.166
α-Aminocaproic acid (norleucine)	M	.642	79.48	9	12	7.249	108.4	34.56	2.178
α-Aminoisocaproic acid (leucine)	M, N	.652	79.85	8	9	7.255	107.5	34.50	2.177
Asparagine	N	.578	84.18	9	10	8.300	78.0	35.47	2.077
Glutamine	D	.676	83.71	8	9	7.623	...		...
Proline	H, N	.646	78.00	10	10	8.789	81.0	38.03	2.099
Hydroxyproline	M	.636	77.19	11	10	8.255	84.4	36.21	2.100
Histidine	N	.560	93.37	11	9	7.328	99.3	33.94	2.123
Phenylalanine	M, N	.500	103.15	12	10	7.047	121.3	34.88	2.172
Tryptophan	H, M	.451	116.41	11	10	6.592	144.1	34.56	2.167
Glycylalanine	Mn	.604	86.32	7	8	7.221	93.9	32.82	...
Alanylglycine	H	.601	84.13	9	8	7.207	94.5	32.83	...
Glycylleucine	H	.579	85.67	9	9	6.231	139.8	32.34	2.172
Leucylglycine	H	.616	82.99	7	9	6.129	143.2	32.07	2.165
Leucylglycylglycine	H	.606	83.59	10	10	5.507	178.5	31.01	...
o-Aminobenzoic acid	Ma	.488	106.80	10	10	8.40	96.7	38.56	...
m-Aminobenzoic acid	Ma	.472	109.67	10	13	7.741	90.3	34.73	...
p-Aminobenzoic acid	Ma	.454	117.84	11	9	8.425	97.3	38.75	...

fractivities[6] may thus be computed but the ratio of column 4 is adequate for the purposes of this paper.

The zero-time corrections, Δt—columns 5 and 6, are included to indicate the sharpness of the initial boundary. Moreover, as a result of minor improvements in manipulation the agreement between the observed and computed values of this correction is somewhat better than at the lower temperature.[1]

Although the diffusion coefficients, column 7, are given to four figures the uncertainty in this measurement is of the order of 0.1%. For reasons not yet understood the diffusion of *o*-aminobenzoic acid was non-ideal in both space and time and the value given in the table is correspondingly uncertain.

Discusson

In a comparison of the diffusion of a substance with its other physical properties complications arising from solute–solute interaction can be avoided by the use of values extrapolated to zero concentration. Although the coefficients of Table I are differential ones at a mean solute concentration of about 0.3%, the lowest concentration compatible with the desired precision in the measurements, no attempt has been made to extrapolate these values to infinite dilution. The available evidence indicates, however, that such an extrapolation would not change the order in which the materials of Table I diffuse. Thus a qualitative comparison of the values of that table is justified and the data are adequate for a consideration of such factors as polymerization, chain length, branching and polarity.

The Stokes–Einstein relation for a large spherical particle of radius r diffusing in a continuous medium of viscosity η affords a starting point. The relation is

$$D = kT/6\pi\eta r \qquad (1)$$

where k is the Boltzmann constant and T the absolute temperature. As a first approximation r is taken as proportional to the cube root of the molecular volume V, and in water at 25° equation 1 becomes

$$D \times 10^5 = 33.06/V^{1/3} \qquad (2)$$

from this it may be anticipated that an increase in

(6) N. Bauer and K. Fajans, Chapter 20 in "Physical Methods of Organic Chemistry," second edition, Interscience Publishers, Inc., New York, N. Y., 1949, p. 1160.

the volume will have a progressively diminishing effect in reducing the diffusion coefficient. This is illustrated by the glycine series of Table I, and by the mono-, di- and trisaccharide, where the difference in the coefficient is greater for the monomer and dimer than for the dimer and trimer. It may be noted, however, that the product, $DV^{1/3}$, is not constant, as it would be if Stokes' relation were valid, but decreases with increasing size. Except for the aromatic and heterocyclic amino acids the data of Table I may be represented, with an average deviation of 2%, by the empirical modification of the Stokes relation

$$D \times 10^6 = 24.182/(V^{1/3} - 1.280) \qquad (3)$$

In Fig. 1 the values of $DV^{1/3}$ from Table I are plotted as ordinate against the diffusion coefficient as abscissa. Here the sloping line represents equation 3 whereas the Stokes constant is indicated by the horizontal one.

The data of Table I also illustrate the diminishing effect of the progressive addition of the $-CH_2-$ group to the molecule. Thus the successive decrements in $D \times 10^6$ for the α-amino derivatives of the normal acids from acetic through caproic are 1.457, 0.809, 0.606 and 0.433.

The most interesting aspect of Table I, however, is the effect, on the diffusion, of branching and polarity in isomeric compounds. Included in the table are three pairs of the α-amino derivatives of the normal and iso acids. In all three cases the change in volume on branching is accompanied, in accord with Stokes' relation by a change of opposite sign in the diffusion coefficient. With the valeric and caproic acids branching leads to a more compact diffusing entity whereas the converse is true for the butyric acids. In the case of the four-carbon compounds a similar behavior has been noted in preliminary studies of the diffusion of the isomeric butyl alcohols. Here the diffusion coefficient decreases in the order: normal, iso, secondary and tertiary, with ΔD for the secondary and tertiary much greater than for the first three members of the series. A shift of methyl groups to the carbon to which the hydroxyl group is attached appears to enhance the effective volume of the diffusing unit.

As the work of Lyons and Thomas[7] on glycine, and of Dunlop and Gosting[8] on its uncharged isomer, glycolamide, has shown, polarity also plays an important role in determining the effective volume of the diffusing entity. Another example is afforded by the aminobenzoic acids, Table I. Here the ortho and para acids have essentially the same volume and diffusion coefficient whereas the values for the meta compound are quite different. Owing to the lack of resonance in the benzene ring in the case of the meta compound this exists in aqueous solution largely as the dipolar ion, whereas the ortho and para acids are present in the uncharged state.[9] The observed results are the ones to be expected, then, if the assumption is made that the charge decreases the apparent volume by condensing, through electrostriction, the water in its immediate neighborhood and that some, at least, of this condensed solvent becomes part of the diffusing entity. Consequently what Scheraga and Mandelkern[10] call the effective hydrodynamic volume is larger for the meta acid than for the ortho and para compounds.

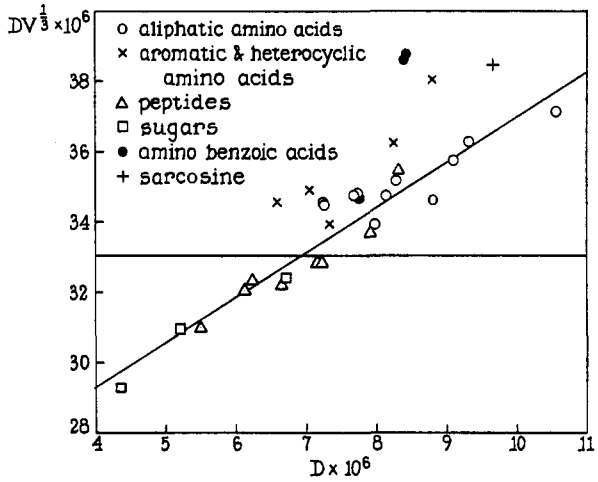

Fig. 1.—The diffusion coefficient as a function of the apparent molal volume.

The foregoing picture of the possible role of polarity is also consistent with the results for the hydroxy derivatives of Table I. The volumes of both serine and threonine are about the same as those of the unsubstituted amino acids. From this it would appear that the increment in volume due to the hydroxyl oxygen is balanced by a decrement due to electrostriction by this polar group. The increased electrostriction results, however, in an increase in the hydrodynamic volume and the diffusion coefficient is depressed accordingly. In the case of the prolines the volume of the hydroxy compound is somewhat greater than that of proline itself but the depression of the diffusion coefficient is out of proportion to this change. Although the sugars are not present in solution as dipolar ions the similarity of their diffusion with that of the aliphatic amino acids and peptides may be a reflection of the polar character of their many hydroxyl groups.

The results for alanine and its isomers, *i.e.*, sarcosine and β-alanine, are difficult to interpret. The greater volume and more rapid diffusion of sarcosine would indicate less electrostriction, but the pK values and dielectric increment of this material[11] do not suggest that its electrical properties are significantly different from those of alanine. In the case of the two alanines the smaller volume of the β-isomer is ascribed[3] to increased electrostriction resulting from the greater charge separation but this interpretation is incompatible with its more rapid diffusion. Possibly the methyl group on the α-carbon of alanine depresses the diffusion rate of this material as in the case of the aminobutyric acids and the butyl alcohols.

Also listed in Table I are the results for two additional pairs of isomers of the same charge

(7) M. S. Lyons and J. V. Thomas, THIS JOURNAL, **72**, 4506 (1950).
(8) P. J. Dunlop and L. J. Gosting, *ibid.*, **75**, 5073 (1953).
(9) Page 124 of reference 3.
(10) H. A. Scheraga and L. Mandelkern, THIS JOURNAL, **75**, 179 (1953).
(11) Page 146 of reference 3.

type but with different charge separations, *i.e.*, asparagine and glycylglycine on the one hand and glutamine and glycylalanine, or alanylglycine, on the other. Although the volumes of the isomers appear to be about the same the amides diffuse more rapidly than their isomeric peptides. Since ΔD for asparagine and glycylglycine is about the same as for glutamine and its isomeric peptides, these results throw no light on the suggestion made by Steward and Thompson[12] that asparagine has a ring structure in aqueous solution. It may also be noted here that in the case of the two isomeric peptides of glycine and alanine, and also those of glycine and leucine, the diffusion coefficient parallels the inverse volume.

Except for histidine the aromatic and heterocyclic amino acids of Table II diffuse more rapidly than aliphatic amino acids of similar volume. The results are too meager, however, to say whether this is due to the greater compactness of the ring structure or to decreased electrostriction resulting from a smaller proportion in which these substances may be present as dipolar ions. In this connection it may be noted that butanol,[13] glycolamide[8] and urea,[14] for which $DV^{1/3} \times 10^6$ is 37.87, 43.75 and 48.86, respectively, also diffuse more rapidly than equation 3 would predict, *i.e.*, these points lie above the sloping line of Fig. 1.

In the last column of Table I is given the ratio of the diffusion coefficient at 25° to that at 1°. The tendency of the temperature coefficient of diffusion to increase somewhat with the particle size was noted by Öholm.[15] The fact that most of the deviations of the ratios of column 10 from the Stokes value of 2.105 ($= 298.1\eta_1/274.1\eta_{25}$) are positive could be interpreted as due to decreasing hydration with increasing temperature but such a quantitative application of Stokes' relation to particles as small as those of Table I is not justified. For the same reason the fact that the experimental values of $DV^{1/3}$, column 9 of Table I, do not deviate excessively from a Stokes factor of 33.06 $\times 10^{-6}$ should not be taken as evidence for the identity of the hydrodynamic volume with that obtained from solution densities. It is of interest that equation 3 may be modified so that the numerator is the Stokes constant, *i.e.*

$$D \times 10^6 = 33.06/(1.367V^{1/3} - 1.750) \quad (3')$$

The coefficient of the thermodynamic "radius," $V^{1/3}$, can then be interpreted as a factor for its conversion to a hydrodynamic value, thus making a crude average allowance for asymmetry and hydration, whereas the constant in the denominator is a reflection of the failure of the Stokes law as the size of the diffusing particle approaches that of the solvent. It cannot be too strongly emphasized, however, that empirical relations of this type should be used only if the data necessary for the more complete methods of Oncley[16] and of Scheraga and Mandelkern[10] are not available.

(12) F. C. Steward and J. F. Thompson, *Nature*, **169**, 739 (1952).

(13) P. A. Lyons and C. L. Sandquist, THIS JOURNAL, **75**, 3896 (1953).

(14) L. G. Gosting and D. F. Akeley, *ibid.*, **74**, 2058 (1952).

(15) L. W. Öholm, *Medd. K. Vetenskapsakad Nobelinstitut*, **2**, 23 (1913).

(16) J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941).

Acknowledgment.—I am indebted to Dr. L. J. Gosting of the University of Wisconsin and to Dr. Lyons of Yale University for a preview of their recently published papers. It is also a pleasure to thank Dr. D. A. MacInnes of these Laboratories for his criticism of this manuscript and Miss Anne Churchill for skillful assistance.

Appendix

Skew Boundaries.—Although most of the boundaries studied in this research have been essentially Gaussian, deviations have been noted that are of interest. A marked effect was observed in the diffusion of butanol, which was studied, with the aid of the Rayleigh fringes, for comparison with the results of Lyons and Sandquist[13] using the Gouy method. In work with the optical methods a strict proportionality between the refractive index and concentration is assumed and skewness can thus arise from a variation, with the concentration, of either the diffusion coefficient, D, or the specific refraction, dn/dc, or both. In the case of most of the materials that have been studied with modern precision both dD/dc and d^2n/dc^2 are negative in dilute solution whereas for butanol these coefficients are of opposite sign. The possibility exists, then, that the two sources of skewness may partially compensate each other in the one case and combine to enhance the effect in the other. To test this suggestion the computations summarized in Table II have been made. These are based on interpolations from graphs of distorted Gauss curves and the results may be anticipated as follows. Although extreme variations of the specific refraction introduce skewness, the graphical procedures described below are inadequate for predicting whether or not the effect will be detectable in the less extreme cases usually encountered in practice. The skewing effect of a given variation in D is, on the other hand, more marked and here the graphical treatment affords a simple method for the evaluation of this change. It is hoped that the procedure suggested here will point the way for the development of more precise analytical methods.

TABLE II

RATIOS OF THE FRINGE SEPARATIONS IN SKEW BOUNDARIES TO THOSE IN AN IDEAL BOUNDARY

1	2	3	4	5
D_1/D_2	1.0	0.7228	0.7228	0.7228
$\frac{(dn/dc)_1}{(dn/dc)_2}$	0.5	1.0	0.5	2.0
$\frac{N_2}{N_1}$				
0.96–0.48	1.015	0.944	0.965	0.893
.92– .44	1.003	.954	.973	.903
.88– .40	0.995	.967	.976	.915
.84– .36	.988	.976	.979	.926
.80– .32	.980	.987	.982	.939
.76– .28	.974	.999	.988	.951
.72– .24	.971	1.010	.993	.965
.68– .20	.966	1.020	.998	.981
.64– .16	.962	1.030	1.003	.998
.60– .12	.962	1.041	1.009	1.019
.56– .08	.959	1.050	1.015	1.039
.52– .04	.956	1.061	1.022	1.061
Average	0.978	1.003	0.992	0.966
Av. dev.	0.016	0.032	0.015	0.045

In preparing Table II the variation, with the concentration, of the diffusion coefficient has been taken as linear, thus permitting the use of Stokes'[17] second table and reference to his paper is essential to an understanding of what follows. A linear variation of the specific refraction, dn/dc, also has been assumed so that if his relative concentration $(c - c_2)/(c_1 - c_2)$ is designated C, and the relative refractive index $(n - n_2)/(n_1 - n_2)$ as N, then for $(dn/dc)_1/(dn/dc)_2 = 0.5$ for example, $N = 1.3333C - 0.3333C^2$, etc. Here the subscript 1 refers to the homogeneous solution on one side of the diffusing boundary and 2 to the solvent, or more dilute solution, on the other side. For each value of C in a column of Stokes' table a value of N was computed and

(17) R. H. Stokes, *Trans. Faraday Soc.*, **49**, 887 (1952).

plotted as ordinate against the reduced height $z = x/2\sqrt{Dt}$ as abscissa. On such a plot the values of z for a constant ordinate increment represent the positions of the Rayleigh fringes. The ratio of the separation of the pair indicated in column 1 of Table II to that of the same pair from a normal plot (*i.e.*, one of which $D_1/D_2 = (dn/dc)_1/(dn/dc)_2 = 1$) is tablulated in the subsequent columns of that table. As is indicated in the first column an increment of 0.04 in N was selected, thus duplicating the conditions used in this research. In order that the uncertainty in the graphical interpolations would not obscure trends in the computed fringe spacings, the assumed variations in D and dn/dc, given at the head of each column, are greater than those usually encountered in practice.

With dn/dc constant and $dD/dc < 0$, column 3, the ratio increases with dilution, but the mean value does not differ significantly from unity. Since the diffusion coefficient is proportional to the square of this ratio it appears that the method of computation used by the author yields a mean value when D varies linearly with C and the specific refraction is constant. On the other hand if D is constant and dn/dc varies sufficiently, column 2, an incorrect value for the coefficient is obtained although in the absence of independent data this fact would not be recognized experimentally. Comparison of the figures in column 4 with those in columns 3 and 5 indicates that the ratio is more nearly constant if the concentration dependence of D and dn/dc has the same sign, a result suggested above in connection with the diffusion of butanol.

When dn/dc is constant the approximate linearity of the ratio with concentration, *e.g.*, column 3 of Table II, provides a simple method for the evaluation of D_1/D_2. If a ratio in this column is plotted against the corresponding mean concentration the slope, m, of the resulting line is characteristic for the assumed value of D_1/D_2. On preparing such graphs for $D_1/D_2 = 0.8806$, 0.7228, 0.5506, 0.3270 and 0.1407 the corresponding values found for $-m$ were 0.109, 0.264, 0.468, 0.836 and 1.250. A plot of these *versus* D_1/D_2 appears to be linear in the neighborhood of $D_1/D_2 = 1$ where $D_1/D_2 = 1 + 1.09m$. If, now, the reduced fringe separations of an actual photograph are normalized, through division by their average value, they may be plotted just as the ratios of Table II and D_1/D_2 evaluated from the slope of the resulting line. Figure 2 is such a plot for the butanol fringes. Here the small circles represent individual values from each of the last five photographs, whereas a large one is the average at a given mean concentration. The slope of the line through these points, as determined by least squares, is -0.0530 from which $D_1/D_2 = 0.942$. The results summarized in Table II suggest that

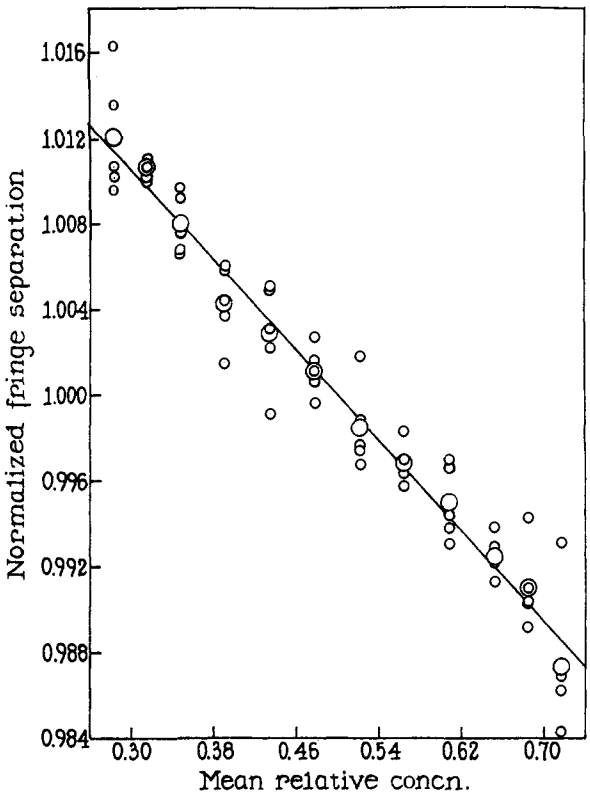

Fig. 2.—Effect on the fringe separation of a variation of the diffusion coefficient with the concentration in the boundary.

correction for the concentration dependence of the specific refraction might raise this somewhat closer to the value of 0.954 predicted by the results of Lyons and Sandquist. The graphical methods used here are inadequate, however, when $(dn/dc)_1/(dn/dc)_2$ is as near unity, namely, 1.03, as in this experiment. As mentioned above, the question must be left open as to whether or not the usual variations in the specific refraction are a source of detectable skewness.

NEW YORK, N. Y.

[CONTRIBUTION NO. 1676 FROM WESTINGHOUSE RESEARCH LABORATORIES]

Dissociation Constants of Some Phenols and Methylol Phenols

BY G. R. SPRENGLING AND C. W. LEWIS

RECEIVED MARCH 31, 1953

The acidities of phenol and of its methylol derivatives as well as of further methyl and methylol substituted phenols have been determined by the ultraviolet spectroscopic method. The relative values of pK thus obtained are estimated to be accurate within ± 0.03. A calculated correction to obtain the thermodynamic values is given. A discussion of the effect of acidity of substituted phenols on their relative reactivities is given.

The various phenols charged and methylol phenols engendered in the process of making phenolic resins differ quite largely in their speed of reaction with formaldehyde.[1] This reactivity can be expected to bear some relation to their acidity, since the anion of a phenol is more reactive toward electrophilic substitution than the undissociated molecule.[2]

The effect of substituents is not simple, particularly when the substituent varies widely in nature. A knowledge of the relative acidities may, however, give some insight into the relative rate of reaction of phenols with formaldehyde in buffered solutions when the directing substituents are comparatively simple and slightly active, such as the methyl or methylol groups.

For the measurement of these acidities, a method operating at very low concentration is preferred for two reasons: low solubility of many phenols does not permit higher concentrations; and calculation of the correction needed to obtain the thermo-

(1) M. M. Sprung, THIS JOURNAL, **63**, 334 (1941).

(2) L. Pauling, "The Nature of the Chemical Bond," 2nd Ed., Cornell University Press, Ithaca, N. Y., 1940, pp. 149–150, 204–205.