ments: Run 1 at 30°, *p*H 4.33 and Run 2 at 30°, *p*H 4.27.

TABLE II

Run No.	Temp., °C.	$k_2' \times 10^5$ min.$^{-1}$	Run No.	Temp., °C.	$k_2' \times 10^5$ min.$^{-1}$
1	30	1.38	8	45	9.76
2	30	1.38	9	50	14.1
3	35	2.50	10	50	12.6
4	35	2.46	11	55	19.5
5	40	4.75	12	55	19.9
6	40	4.25	13	60	43.1
7	45	8.84	14	60	41.0

The values of k_2' can be readily calculated from the slopes of the straight lines obtained. Table II is a complete summary of calculated values of k_2' determined at various temperatures in this investigation.

The Arrhenius equation represents the rate behavior of chemical reactions as a function of temperature

$$k_2' = se^{-\Delta H_a/RT} \qquad (12)$$

Figure 3 plots log k_2' *vs.* $1/T$ for calculation of the heat of activation, ΔH_a, for the reaction of (HCN) and (AcH). The value of ΔH_a from Fig. 3 is found to be +22.9 kcal./mole. The frequency factor, s, in the Arrhenius equation is found to be 4.36×10^{11} min.$^{-1}$ by substitution of known values of k_2', T, and the calculated value of ΔH_a.

TEXAS CITY, TEXAS

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

Diffusion Measurements, at 1°, of Aqueous Solutions of Amino Acids, Peptides and Sugars

BY LEWIS G. LONGSWORTH

RECEIVED MARCH 31, 1952

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, D, of 13 amino acids, 4 peptides and 5 sugars have been determined in dilute aqueous solution at 1°. Since the molecular weight, M, and volume, V, of these materials are known it has been possible to obtain empirical relations between D and M or V. These relations should be of value in providing preliminary estimates of molecular weight from diffusion measurements on structurally similar materials, such as polypeptides, where conventional methods are not applicable.

As is well known Stoke's law, $D = kT/6\pi\eta r$, relating the diffusion coefficient, D, to the radius, r, of the solute particle and the viscosity, η, of the solvent, is valid only for large molecules in dilute solutions and even for these the radius computed from the diffusion coefficient generally exceeds, as a result of hydration and asymmetry, that obtained from the molal volume, V, *i.e.*, $r = (3V/4\pi N)^{1/3}$. Here N is Avogadro's number whereas the k and T in Stoke's relation are the Boltzmann constant and the absolute temperature, respectively. Except in the case of extreme asymmetry the product $DV^{1/3}$ is, however, roughly constant for large molecules, whereas for small ones Polson[1] has noted a decrease in the product $DM^{1/3}$ with increasing molecular weight, M. Consequently he has proposed[2] the empirical equation

$$D = \frac{A'}{M^{1/3}} + \frac{B'}{M^{2/3}} + \frac{C'}{M} \qquad (1)$$

with three adjustable constants. Actually the simpler relation

$$D = A/(M^{1/3} - B) \qquad (2)$$

fits his data about as well as (1) and has the advantage that a plot of $DM^{1/3}$ *vs.* D is linear. In terms of molal volumes the expression is

$$D = a/(V^{1/3} - b) \qquad (3)$$

As a further test of the validity of these empirical relations the diffusion coefficients of a variety of substances of known molecular weight and volume have been determined and are reported later in this paper. Since these relations are potentially useful in providing preliminary estimates of molecular weight from diffusion measurements in the case of such materials as the polypeptides,[3] the present study has been restricted to structurally similar substances. Moreover, since the method that has been employed has proved to be a practical one for precise diffusion measurements, and permits the use of much existing electrophoresis equipment with only minor changes, the experimental procedures are also described in some detail.

Experimental

Method.—The method used in the present research is that 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.[4,5] The modified Tiselius cell shown diagrammatically in Fig. 1 has been used as a diffusion cell in conjunction with the capillary siphoning procedure of Polson[6] for sharpening the boundary initially. The modification of the cell consists in the addition of duplicates of the channel windows at W. Together with the thermostat water these windows provide a comparison path of essentially the same optical properties as the boundary channel.

Before the boundary is first formed in the left-hand side of the channel at the junction of the bottom and center sections of the cell, a thin-walled glass capillary is inserted, as shown in Fig. 1, with its tip near the center of the channel. As soon as the boundary is formed it is caused to rise in the channel by withdrawal of liquid through the capillary.

(1) A. Polson, *J. Phys. Colloid Chem.*, **54**, 649 (1950).

(2) A. Polson and D. van der Reyden, *Biochim. Biophys. Acta*, **5**, 358 (1950).

(3) K. O. Pedersen and R. L. M. Synge, *Acta Chem. Scand.*, **2**, 408 (1948).

(4) J. St. L. Philpot and G. H. Cook, *Research*, **1**, 234 (1948).

(5) Harry Svensson, *Acta Chem. Scand.*, **5**, 72 (1951).

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

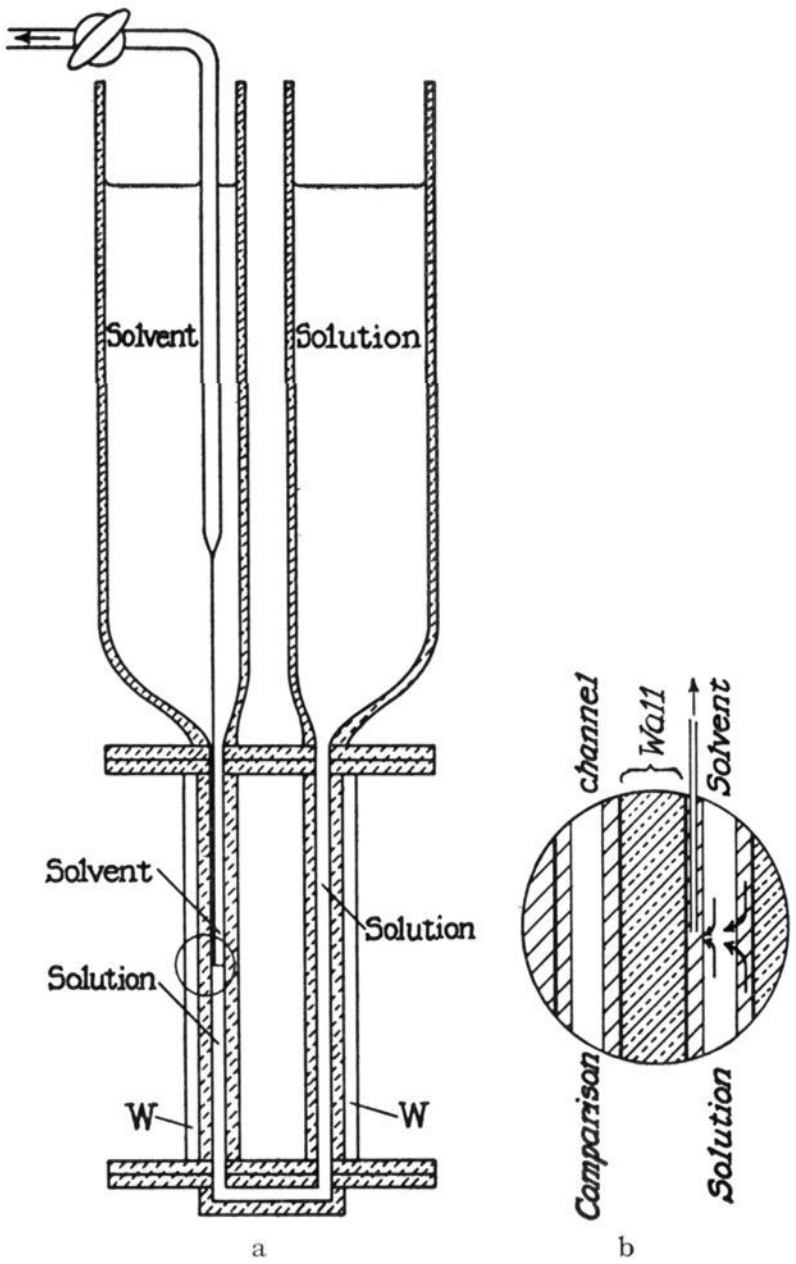

Fig. 1.—The modified Tiselius cell.

As the boundary rises mixing occurs but when it reaches the tip of the capillary both solution and solvent begin to enter and it again becomes sharp. At 1° a siphoning rate of 2 to 3 ml./min. is used until 90 to 100 ml. have been withdrawn. During the later stages of this sharpening process four photographs are taken for use in determining the fractional part, F, of the total number, J, of fringes and a zero-time correction as will be described later in this report.

The experiment is started at zero time by closing the siphon cock, thereby stopping the sharpening flow. Immediately thereafter the bottom section is shifted, to prevent any movement of liquid from one side of the cell to the other, and the capillary removed.

Recording the Fringes.—The Rayleigh fringes are photographed with the aid of a modified Philpot–Svensson camera.[7] The modifications consist (a) in the rotation of the source slit from the horizontal to the vertical, (b) the use of a mask at the cell with a pair of vertical slits for the boundary and comparison channels, respectively, and (c) removal of the schlieren diaphragm. As Svensson has emphasized,[5] this optical system, although more complex than the Philpot–Cook arrangement used previously by the author,[8] makes less exacting demands on the cylindrical lens. Moreover, an achromatic cylinder, supplied by the Perkin–Elmer Co., has been used in the present work.

Ten photographs are taken at increasing intervals during a diffusion period that varies from two hours for small molecules to several days for proteins. These, together with the four taken during the sharpening, are all recorded on a single 9 × 12 cm. plate. In the author's equipment an image of the arc of the G. E. AH 4 lamp is projected, through a 77 A green filter, onto a 0.02 × 20 mm. opening in the Gaertner bilateral source slit with a 28 mm. lens of 32 mm. focal length. Since the slits in the cell mask are 1.6 mm. wide a 4 to 6 second exposure then overexposes somewhat a CTC panchromatic plate. The overexposure is desirable in order to improve the definition of the interference minima since it is these, rather than the maxima, whose positions are determined with the aid of a comparator.

Enlargements of that part of the fringe photographs conjugate to the boundary are shown in Fig. 2. That at a was recorded just prior to interruption of the sharpening flow whereas b and c were obtained after diffusion for 900 and 1800 seconds, respectively. Since the vertical coördinates, H, of a pattern are proportional to the height, h, in the channel, the points at which successive diagonal fringes intersect a vertical represent the levels in the boundary between which the optical path differs by one wave length of light. The set of vertical fringes on the right in each pattern can be ignored. They are due to a second pair of slits in the cell mask and represent an invariant system that will eventually be used as an aid in the alignment of the photograph in the comparator. At present, however, the glass plate used in separating these fringes from those conjugate to the boundary is not sufficiently flat to warrant this refinement in the procedure.

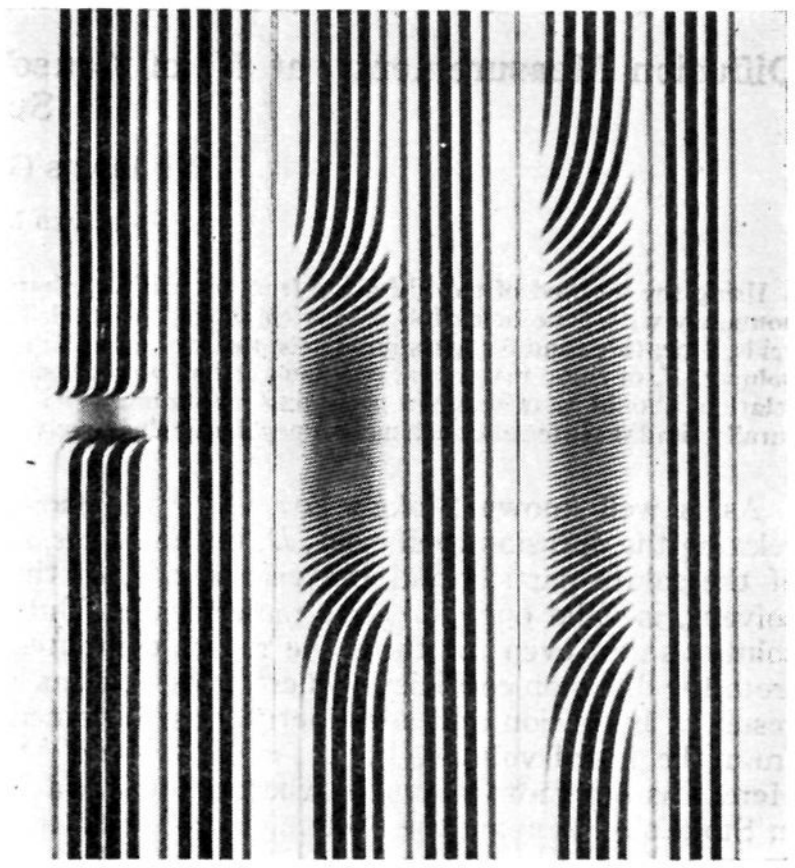

Fig. 2.—Rayleigh interference fringe photographs of the boundary between H_2O and 0.75% levulose during sharpening (a) and after diffusion for 900 (b) and 1800 sec. (c).

The Camera Constant.—It is an advantage of the Rayleigh system that the vertical magnification, $\Delta H/\Delta h$, can be more readily measured than its counterpart, the "b" distance, in the Gouy method. A glass scale is simply substituted for the diffusion cell, with the scale lines in the median plane and in the path of the light from one slit only, and photographed. The vertical fringes are then intersected by sharply defined "images" of the scale lines and a comparison of the intervals in the fringe photograph with those of the real scale affords not only a value of $\Delta H/\Delta h$ but also a check on the constancy of this ratio over the height in the channel. Since the glass plate on which the scale is ruled has approximately the same thickness as the channel window it duplicates the effect of this window if placed between the rulings and the camera.

Measurement of the Fringe Patterns.—The "comparation" of the patterns and ensuing computations are the time-consuming elements in the work and it is a pleasure to acknowledge the competent assistance of Miss Anne Churchill in these operations. In order to eliminate eye strain in the use of the comparator a 4 mm. achromat of 8 mm. focal length, together with a first surface mirror, is employed to

(7) L. G. Longsworth, *Ind. Eng. Chem., Anal. Ed.*, **18**, 219 (1946).
(8) L. G. Longsworth, *Rev. Sci. Instruments*, **21**, 524 (1950).

project a 40× enlarged image of the fringes onto a 9 × 10 cm. vertical ground glass screen. In addition to fixed cross hairs this screen is also provided with an adjustable set for use as described later in this section.

Since it is a measure of the total concentration change across the boundary the total number, J, of fringes is an important parameter in the computations. If a is the cell thickness, Δn the difference of refractive index between solution and solvent and λ the wave length of the light, then $J = a\Delta n/\lambda$. In general J is a compound number, the integral portion of which is the number of diagonal fringes intersecting a vertical, whereas the fractional part, F, is determined as follows. In Fig. 2a, for example, it can be seen, by holding a straight edge on one of the vertical fringes conjugate to the homogeneous solution below the boundary, that these are not in line with those above that are conjugate to the solvent. The ratio of this lateral displacement to the separation of adjacent vertical fringes is F.

In the case of a pattern taken during the sharpening process the vertical fringes are aligned parallel to the ungraduated cross-axis movement of the comparator so that the graduated axis can be used to determine, to the nearest 1 or 2 microns, the displacement in passing from solution to solvent. Since the lateral separation of the vertical fringes in the author's apparatus is 186 μ, this permits the determination of F to 0.01 fringe. After the patterns recorded during sharpening are thus measured the plate is turned through 90° so that the vertical fringes are now parallel to the graduated axis.

Using the final pattern that is recorded during sharpening, a minimum conjugate to the solvent is focused on the fixed cross hairs while the adjustable set is fixed on one conjugate to the solution. All patterns obtained during the diffusion period are then aligned so that the corresponding solvent and solution fringes focus on the fixed and adjusted cross hairs, respectively. In this work no attempt has been made to correct for the slight curvature of the fringes that exist with solvent throughout the channel. Although amounting to about 0.1 fringe over the entire 86 mm. height, this distortion of the "base line" is concentrated near the ends of the channel and does not introduce an appreciable error if the boundary is not allowed to spread too far.

Treatment of the Data.—Since the computations are based on the assumption that the concentration distribution in the boundary is Gaussian, the Federal "Tables of Probability Functions"[9] can be used. In fact, these tables are practically indispensable since their completeness virtually eliminates the need for interpolation.

In most instances the concentration has been such that $J \simeq 50$. The position of every other diagonal fringe is then tabulated and, as is shown in Table I for a typical case, a value of $(4Dt)^{1/2}\Delta H/\Delta h$ is computed from the separation,

TABLE I

TREATMENT OF FRINGE DATA-PATTERN OF 0.75% LEVULOSE AFTER DIFFUSION FOR 13003 SEC.

1	2	3	4	5
jk	jl	$\Delta H = H_l - H_k$, cm.	$\Delta h/(4Dt)^{1/2}$ $J = 50.52$	$(4Dt)^{1/2}\Delta H/\Delta h$
2	26	0.5296	1.2673	0.4179
4	28	.4566	1.0939	.4174
6	30	.4182	1.0031	.4169
8	32	.3962	0.9490	.4175
10	34	.3828	.9172	.4174
12	36	.3770	.9017	.4181
14	38	.3755	.9000	.4172
16	40	.3797	.9118	.4164
18	42	.3913	.9390	.4167
20	44	.4117	.9864	.4174
22	46	.4441	1.0654	.4168
24	48	.5037	1.2081	.4169
			Av.	.41722

$4(\Delta H/\Delta h)^2 = 4.140$, $Dt = 0.042047$, D (uncor.) $= 3.234 \times 10^{-6}$

(9) Federal Works Agency, Works Project Administration, Supt. of Documents, Washington, D. C., 1941.

column 3, of the pair whose numbers, jk and jl, are given in columns 1 and 2. As shown in column 5 twelve values are obtained in this manner and their constancy validates the assumption of Gaussian distribution. Their mean is used, as shown at the bottom of Table I, to compute a value of D.

After evaluating and applying the zero-time correction[10] to the value of D from each of the ten patterns, these are then averaged to give the result for the experiment. This procedure is superior to that previously used[8] since approximately equal weight is assigned to all comparator readings and the necessity for finding the center of the boundary is avoided. The normalizing factor, column 4 of Table I, is obtained as follows.

In the Federal tables the normalized "concentration"

$$\frac{2}{\sqrt{\pi}}\int_0^x e^{-\alpha^2}\mathrm{d}\alpha$$

is tabulated in the right-hand column of each page as a function of normalized "distance," $x = -h/(4Dt)^{1/2}$, in the first column, and varies from 0 to 1 as x varies from 0 to infinity. Since the fringe number, j, is a measure of the concentration in the boundary and is equal to $\frac{1}{2}J$ at the center where $h = 0$, these numbers may be converted to the same scale as that of the Federal tables by means of the proportion $(j - \frac{1}{2}J)/\frac{1}{2}J$. Consequently values of $(2j - J)/J$ are computed for $j = 2, 4, 6$, etc.; these are found in the right-hand column and the corresponding values in the first column transcribed opposite the appropriate j. Since the Federal tables cover only the positive half of the symmetrical probability function, the sign of $(2i - J)/J$ is ignored, provided $j_k < \frac{1}{2}J < j_l$, and the values of $h_j/(4Dt)^{1/2}$ for $j_k = 2$ and $j_l = 26$, for example, are added to give the first entry in column 4 of Table I.

Solutions.—As is indicated in Table II the materials included in this study were obtained from the best available sources. They were used without further purification, the solutions being prepared by direct weighing of both solute and solvent. Although the probable presence of moisture in some of the samples does not affect the validity of the diffusion measurements it would be a source of error in the relative specific refractions that can be computed from the data of Table I.

Results

The experimental results are assembled in Table II, which is largely self explanatory. The values of Δt, column 6, are the times, in seconds, that must be added to the recorded times in order to obtain diffusion coefficients independent of that variable in each experiment. These will be referred to as the observed zero-time corrections, whereas those in column 7 are obtained independently as follows.

Although the boundary is too sharp during the siphoning for resolution of most of the diagonal fringes it is always possible to locate one or two at each edge. In Fig. 2a, for example, the separation of the 1st and 49th fringe is 0.323 mm., indicating that some 95% of the total concentration change at the boundary occurs within this distance. By reversing the procedure outlined above one may compute that this is the separation these two fringes would have after the diffusion of an infinitely sharp boundary for 10 seconds. The probable significance of the differences between the observed and computed values of Δt will be considered later in this report.

In columns 8 and 9 of Table II are listed, respectively, the mean value of D and the average deviation, from this mean, of the individual results for each of the ten exposures. Since systematic errors such as vibration and solute impurities would not contribute to these deviations they are probably not an accurate index of the precision of the measurements. It is encouraging, however, that the reproducibility in duplicate experiments is of the order of 0.1% and that in the two cases where a comparison is possible the results are in agreement with those obtained with the aid of the Gouy method. Thus the value of 5.151×10^{-6} for glycine may be compared with 5.153×10^{-6} computed from the function of Lyons and Thomas[11] at the mean concentration, $\bar{c}$, of 0.3. In the case of sucrose the value 2.408×10^{-6} at $\bar{c} = 0.375$ given by the relation of Gosting and

(10) L. G. Longsworth, THIS JOURNAL, **69**, 2510 (1947).

(11) Margaret S. Lyons and Jean V. Thomas, *ibid.*, **72**, 4506 (1950).

TABLE II

DIFFUSION COEFFICIENTS OF AMINO ACIDS, PEPTIDES AND SUGARS AT 1°

SL, Synthetical Laboratories; NB, Nutritional Biochemicals; M, Merck; E, Eastman; HL, Hoffman-LaRoche; B. Baker; P. Pfanstiehl; BS, Bureau Stds.

1 Material	2 Source	3 V,[a] cc./mole	4 Concn.,[b] wt. %	5 J	6 Δt, sec. Obsd.	7 Δt, sec. Calcd.	8 $D \times 10^5$	9 Av. dev., %
Glycine	SL	43.5	0.578	50.63	21	11	5.151	0.06
dl-β-Alanine	NB	58.9	.618	53.51	13	13	4.500	.03
dl-α-Alanine	M	60.6	.624	51.24	16	11	4.317	.08
dl-Serine	M	60.8	.602	50.11	12	11	4.195	.04
dl-α-Amino-*n*-butyric acid	E	76.5	.600	49.32	9	11	3.891	.05
dl-Valine	NB	91.3	.600	49.65	14	14	3.566	.07
dl-Leucine	NB	107.5	.590	48.72	12	33	3.333	.08
dl-Norleucine	M	108.4	.618	50.91	16	23	3.328	.05
l-Proline	NB	81.0	.559	45.12	10	18	4.187	.02
Hydroxy-*l*-proline	M	84.4	.559	44.72	12	14	3.930	.06
l-Histidine	NB	99.3	.601	58.44	7	11	3.452	.04
dl-Phenylalanine	NB	121.3	.601	64.02	10	14	3.244	.06
l-Tryptophan	HL	144.1	.559	66.96	20	15	3.042	.04
Diglycine	NB	77.2	.594	54.12	10	12	3.790	.06
Triglycine	NB	113.5	.553	49.79	13	15	3.175	.07
Glycyl-*dl*-leucine	HL	139.8	.554	49.30	15	14	2.869	.05
dl-Leucylglycine	HL	143.2	.546	46.62	16	11	2.831	.05
Glucose	B	111.9	.747	50.42	19	13	3.137	.07
Galactose	P		.746	51.22	6	10	3.131	.07
Levulose	P		.748	50.52	12	10	3.230	.04
Sucrose	BS	209.9	.747	50.28	19	12	2.414	.04
Raffinose·5H_2O	P	300.8	.895	52.46	17	16	2.010	.05

[a] V = apparent molal volume at 25°—see ref. 13. [b] Brass weights in air of density 0.0012.

Morris[12] is slightly less than the 2.414×10^{-5} of Table II. In dilute solutions at 1°, however, their relation is weighted in favor of the low values that they obtained with large concentration differences. In the present research no such dependence on the concentration difference has been noted. Although not listed in Table II the author has also studied sucrose at $\bar{c} = 0.75$, with $\Delta c = 1.5$, to give $D = 2.400 \times 10^{-5}$ and at $\bar{c} = 1.12$, with $\Delta c = 0.75$, to give $D = 2.385 \times 10^{-5}$. Actually the mean values given by the Rayleigh method are in good agreement with the Gouy results for $\Delta c = 0.5$.

Moreover, three samples of alanine from different sources gave values of 4.314, 4.317 and 4.322×10^{-5}, respectively, whereas the results for two samples of valine were 3.566 and 3.567×10^{-5}.

Discussion

Limitations of the Tiselius Cell.—Although not a factor in the present research, in which only dilute solutions have been studied, it is a limitation of the cell shown in Fig. 1 that the liquid in the comparison path is the thermostat fluid. Due, presumably, to the pressure broadening of the spectral lines in the AH4 lamp, the Rayleigh fringes become increasingly fuzzy as the path difference between the boundary and comparison channels increases. Although the fringes are still usable with path differences of 500 waves, in a study of differential diffusion in concentrated solutions it becomes necessary to insert additional strips of glass in the comparison path. Duplicates of the channel windows have been used for this purpose, each strip increasing the path length by some 800 waves, but these introduce slight additional distortion.

Another limitation is the relatively large volume of solution required. This results from the necessity for rinsing the bottom half of the boundary channel free of solvent during the sharpening process. It is in this connection that a comparison of the observed and computed zero-time correction is of interest. In the present research the relatively small differences between the two corrections are probably due to departures from a Gaussian distribution in the sharpened boundary and the screening of the channel walls by the masking slit. The screening effect is illustrated in Fig. 1b, which is an enlarged diagram of the channel in the neighborhood of the boundary. Here the unshaded portions represent the two slits in the mask. Since the walls of the channel are indicated by the heavy vertical lines it will be noted that these are screened by the mask. Hence the camera does not see the boundary at the walls where it is doubtless more diffuse, during sharpening, than in the center. This diffuseness at the walls contributes to the observed, but not to the computed, zero-time correction.

In some preliminary work on the diffusion of proteins, however, the observed correction was many times the computed value, the excessive difference being due to incomplete rinsing. With inadequate sharpening flow, the solution in the center of the channel just below the boundary may have the same composition as in the reservoir but solvent may still be adhering to the walls. If the flow is stopped and the experiment started at this stage lateral diffusion of solute toward the wall leads to an observed zero time correction much greater than the computed value. In the study of proteins it is possible to conserve some material by shifting and sharpening the boundary for a brief time only. The flow is then stopped for an hour or more, during which the protein diffuses into the stagnant film of solvent in

[12] L. G. Gosting and Margaret S. Morris, THIS JOURNAL, 71, 1998 (1949).

contact with the wall, after which the flow is resumed for a sufficient period to replace this diluted protein with fresh, full-strength solution from the reservoir.

A large zero-time correction is a source of error since its validity depends upon the assumption that the concentration distribution in the sharpened boundary is Gaussian and an examination of Fig. 2a indicates some asymmetry. The alternative is to keep the correction as small as possible and to check it with the two independent methods outlined here.

Diffusion and Molecular Size.—The data of Table II may be represented by the relation $D = 11.66 \times 10^{-6}/(M^{1/3} - 1.893)$ with an average deviation of 4.7%. As is shown in Fig. 3 the scatter is random and does not differentiate the three classes of compounds represented, *i.e.*, aliphatic amino acids, aromatic and heterocyclic amino acids and carbohydrates. Empirical relations such as this are proving of value in providing preliminary estimates of molecular weight from diffusion measurements. Since a substituent analysis on a polypeptide, for example, leaves open the question of whether the molecular weight is the minimum value or some simple integral multiple thereof, a diffusion measurement may resolve the problem.

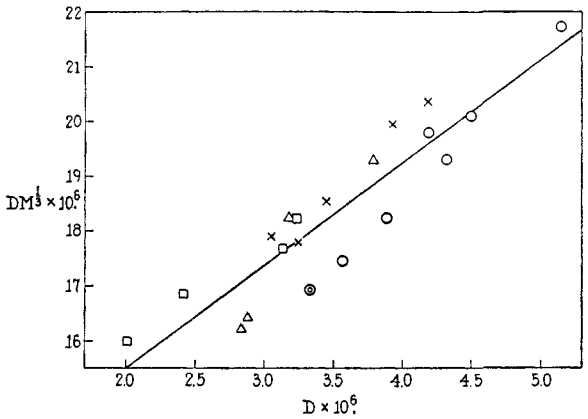

Fig. 3.—The diffusion coefficient, D, as a function of the molecular weight, M: O, aliphatic amino acids; X, aromatic and heterocyclic amino acids; Δ, peptides; □, sugars.

If the materials listed in Table I are assumed to have essentially the same molal volume at 1° as at 25°, the temperature for which the volumes have been reported,[13] it is also possible to examine the validity of equation 3. This is done in Fig. 4 where it will be noted that, except for the cyclic amino acids, the scatter is much less than in Fig. 3. If phenylalanine, tryptophan and the prolines are omitted, the remaining data may be represented by $D = 10.772 \times 10^{-6}/(V^{1/3} - 1.450)$ with an average deviation of 1.4%. It may also be noted that in all cases of isomerism, *i.e.*, α- and β-alanine, leucine and norleucine, glycylleucine and leucylglycine, where the volumes differ by a few cc. at most, the diffusion coefficient faithfully parallels the inverse cube root of the volume. Moreover, in a homologous series such as the α-amino acids, or mono-, di- and triglycine, the linearity of $DV^{1/3}$ with D is closer than the average deviation of 1.4% mentioned above.

(13) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Polypeptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 159.

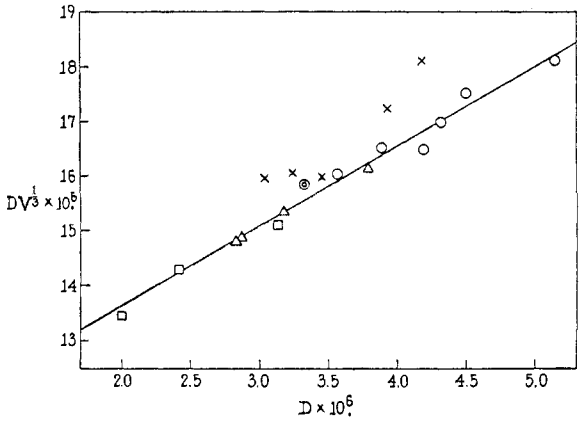

Fig. 4.—The diffusion coefficient, D, as a function of the apparent molal volume, V: O, aliphatic amino acids; X, aromatic and heterocyclic amino acids; Δ, peptides; □, sugars.

Except for histidine, however, the cyclic amino acids have appreciably higher diffusion coefficients than their volumes would lead one to expect. Of these cyclic acids only histidine contains more than one amino and one carboxyl group. If increasing asymmetry at constant volume reduces the diffusion coefficient of small molecules as it does in the case of large ones, then the cyclic acids are more symmetrical than the aliphatics.

In this research no study has been made of the effect of polarity, *e.g.*, by comparing the diffusion of an amino acid with its uncharged isomer, but this may be a factor in the case of histidine. Others have shown[13] that polarity reduces the volume, as a result of electrostriction of the solvent by the charged groups, but if some of the compressed solvent moves with the solute such a volume decrease would be accompanied by a reduction in the diffusion rate rather than the increase predicted by equation 3. Although the molar volume appears to be a more significant property for the interpretation of the diffusion results than the molecular weight, it is clear that other characteristics must also be considered in the final analysis of this intricate problem.

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