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

Experimental Tests of an Interference Method for the Study of Diffusion

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Introduction

When a diffusing boundary is placed in the path of a lens, *e. g.*, at C in Fig. 1, the light through the boundary is deflected, generally downward, by the gradients of refractive index therein and the image of an illuminated horizontal slit, S, is spread over a rectangular area in the focal plane, P. The upper edge of this rectangle is formed by light that passes, without deflection, through the homogeneous layers of solvent and solution above and below the boundary, respectively. The lower edge of the rectangle is formed by light that suffers a maximum deflection in the boundary. In Fig. 1 the gradients in the boundary are indicated by the curve abcde with a maximum at the level c.

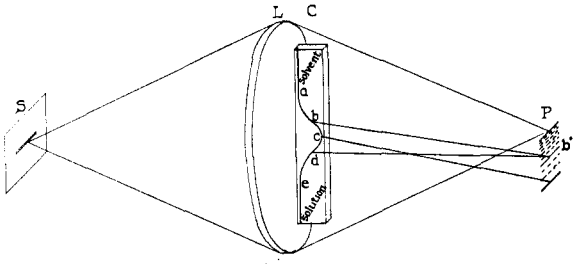

Fig. 1.

As diffusion proceeds and the boundary spreads the rectangle of light in the image plane P contracts by the upward movement of its lower edge. At any instant the light in the rectangle does not, however, vary monotonely with the height but consists of a pattern of interference fringes. The qualitative explanation of this interference is due to Gouy[1] and may be given with the aid of Fig. 1. If b and d are two levels in the boundary having the same value of the refractive index gradient the pencils of light through these levels are deflected to the same position, b′, in the image plane, P. Having originated in a common source and arriving at a common focus, but having followed two paths of different optical length, these pencils may, depending upon this path difference, reinforce or cancel each other. Each fringe in the focal plane, P, thus corresponds to conjugate levels on either side of the maximum at c for which the path length difference is such as to give constructive interference.

In a recent review dealing with the subject of diffusion[2] the author published a photograph that he had taken of these interference fringes and suggested that a quantitative theory of their spacing might aid in the study of this process. In a paper accompanying the present one,[3] Kegeles and Gosting have developed the necessary theory. Although it is one of my purposes to describe experiments that confirm this theory, it will also be shown that with its aid the interference fringes may be used for precise measurements of diffusion. In fact the sensitivity of the new method is such that it has afforded a useful critique of some of the experimental procedures in current use and these results of the investigation will also be presented.

Experimental

Since it is an advantage to be able to observe directly, with the aid of a schlieren camera, the gradients in the boundary the optical equipment employed in this research is that used in electrophoresis[4] except that a combination plate holder and focal plane shutter for the photography of the fringes has been made interchangeable with the schlieren diaphragm. This addition to the schlieren apparatus is shown in perspective in Fig. 2. The holder takes 3 × 6 cm. plates which are cut from the standard 9 × 12 cm. size. A plate is inserted through an opening, provided with a dark slide, in the back of the holder with the emulsion facing the sector-shaped shutter leaf, L. In making an exposure the 1 r. p. m. synchronous clock motor, M, rotates the shutter until one of the openings between the leaves L and L′ has passed in front of the emulsion. Although adjustment of this opening provides one method of controlling the exposure additional controls are necessary as will be described below.

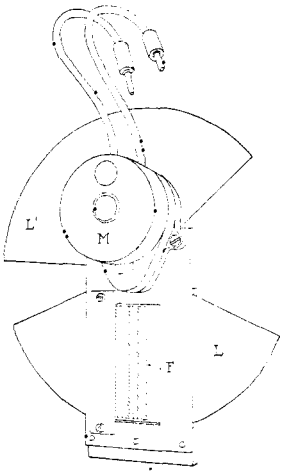

Fig. 2.

In the early stages of diffusion the boundary is sharp and the fringe pattern is formed by the light through a comparatively small portion of the cell while that through the remainder of the cell is concentrated in the normal, *i. e.*, undeviated, slit image. Moreover, owing to the large gradients of refractive index in the boundary at this stage the pattern covers a relatively large area at the photographic plate. Consquently if the plate is exposed to give an adequate density in the fringe pattern the normal slit image is overexposed. Two methods are used simultaneously in overcoming this difficulty One is to mask the cell above and below the boundary so that the exposed homogeneous

(1) Gouy, *Compt. rend. acad. sci.*, **90**, 307 (1880).
(2) Longsworth, *Ann. N. Y. Acad. Sci.*, **46**, 211 (1945).
(3) Kegeles and Gosting, THIS JOURNAL, **69**, 2516 (1947).
(4) Longsworth, *Ind. Eng. Chem., Anal. Ed.*, **18**, 219 (1946).

layers of solution are comparable in thickness with the boundary layer. In order to avoid troublesome diffraction effects the edges of the mask should not be horizontal. Moreover, the homogeneous layers of solution exposed by the mask must not be too thin since the edges of the boundary, together with the edges of the mask, appear to have the properties of a Young double slit[5] and introduce fine structure into the normal slit image. As the boundary spreads and the fringe system is compressed the fringes nearest the normal slit image may be confused with this fine structure. By viewing the boundary with the aid of the schlieren camera, the mask may be adjusted for each photograph so that the exposed portion of the cell is about twice the boundary thickness. As the gradients approach the ends of the cell, however, this becomes impossible and thereby limits the duration of the experiment.

Another method of controlling the exposure is to place directly in front of the plate, several pieces of an appropriate Wratten gelatin filter, *e. g.*, number 58 if green light is used, with each successive piece displaced horizontally a small distance. Three such pieces are shown at F in Fig. 2. Thus one end of the image of a fringe is formed by light that has undergone no absorption while that forming each successive portion of the image has penetrated an increasing number of thicknesses of the absorbing filter. A photograph obtained in this manner is shown in Fig. 3. With a sufficient number of steps in the filter one can always find a fringe segment that is properly exposed. In locating maxima a segment is used where the step filter has absorbed most of the energy and the fringe is thus barely visible on the plate. In the case of minima, segments are used where the fringes are overexposed and the minima appear as narrow, clear lines. In the present research preference has been given to the minima since in the location of these the cross-hairs in the eyepiece of the comparator microscope are not obscured. It will be clear, however, that the comparator must be arranged so that the plate can be moved parallel to the fringes as well as normal to them. This has been done by adapting a smooth, but ungraduated, cross-axis movement to a Gaertner comparator reading directly to microns.

The use of a step filter at the plate has also made it possible to estimate the variation, with the height, of the light intensity in a single fringe without recourse to a microphotometer and the accompanying problems of plate calibration. Since a contrast plate is used the optical density at the edge of a fringe changes quite abruptly. Thus the level at which the change occurs can be determined for different segments of the fringe image and plotted against the anti-logarithm of the number of thicknesses of absorbing filter in the path of the given segment. The use of a contrast plate is also

(5) Monk, "Light—Principles and Experiments," McGraw-Hill Book Co., New York, N. Y., 1937, p. 125.

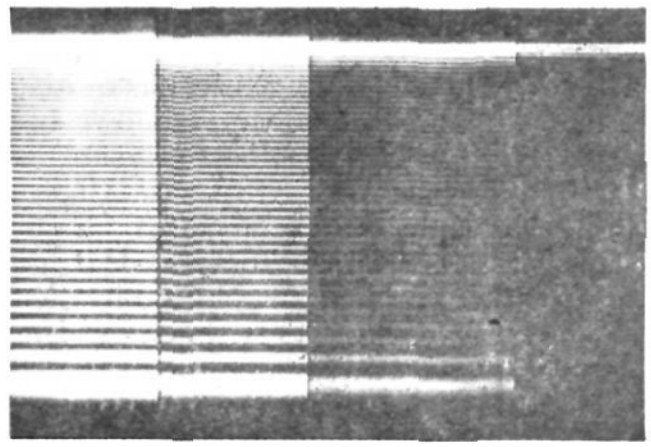

Fig. 3.

desirable in order to obtain adequate resolving power.

In order to obtain well defined fringes the light should be essentially monochromatic. The G. E. H4 mercury vapor lamp, together with the Wratten filter #77A for isolation of the mercury green line at $\lambda = 5461$ Å., has proved to be a satisfactory source.

If, as in the author's equipment, the schlieren lens is used at unit magnification another requirement for well-defined fringes is that the slit width be one fourth, or less,[6] of the minimum separation between fringes. Since patterns containing as many as 25 fringes per millimeter have been recorded it is clear that slit widths as small as 0.01 mm. must be used occasionally and that the parallelism of the slit jaws must be considerably better than this. A Gaertner bilateral spectrometer slit reading directly to the nearest 0.01 mm. has proved satisfactory.

The materials selected for study were potassium chloride and the protein, ovalbumin. A reagent grade potassium chloride was recrystallized once with centrifugal drainage and fused in air in platinum. The solutions of this salt were prepared by direct weighing of both solute and solvent, the density data of the "I.C.T." being used to convert the concentrations to a volume basis. The author is indebted to Dr. Gertrude Perlmann for the thrice recrystallized sample of ovalbumin. As will be shown below, these two materials represent a thirty-fold change in the diffusion coefficient and by their use defects in the experimental procedures have come to light that would have been missed if only one had been studied.

Both the Tiselius and the Lamm cells have been used in this research. In the Tiselius cell[7] an initially sharp boundary between solution and solvent is formed by a shearing mechanism after which it is slowly shifted into view from behind the opaque horizontal plates used in its formation. This cell, which is the one used in electrophoresis, is a cemented cell and the optical quality of the windows is such that the resulting distortion of the normal slit image interferes somewhat with

(6) Jenkins and White, "Fundamentals of Physical Opties," McGraw-Hill Book Co., New York, N. Y., 1937, p. 140.

(7) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

its location. In the Lamm cell[8] the boundary is formed by the removal of a partition between solution and solvent. Optically this cell is quite good. When using it the thermostat windows become the factor that limits the quality of the normal slit image and work is in progress to remove this limitation. Mechanically the Lamm cell is open to the objection that it is difficult to avoid leakage and also to avoid the trapping of solution in the crevices around the movable partition. Although a Neurath cell[9] was not available for test it should be free from this defect. For the study of diffusion from an initially sharp boundary the cell suggested by Claesson[10] appears to overcome most of the difficulties encountered in the present research and is now under construction.

Test and Application of the Theory

The most important test that the theory of Kegeles and Gosting must meet is whether or not it predicts accurately the spacing of the fringes for normal diffusion. Since results to be presented below indicate that at moderate concentrations the diffusion coefficient of potassium chloride changes less than a per cent. for a 0.1 N increment of concentration the diffusion of this salt over appropriate concentration intervals should give gradient curves that are essentially Gaussian. The results given in Table I for the fringe pattern obtained after the diffusion of 0.6 N potassium chloride into 0.4 N potassium chloride for 7500 seconds at 0.5° are typical. In the first column of the table the values of j indicate a minimum, zero being assigned to the lowest one in the pattern. In order to reduce the size of the table every fifth minimum only, after the first two, is listed. As will be shown later in this paper the lowest fringes deviate from the theory *when diffraction is neglected.* The figures in the second column of Table I are the observed distances of the minima from the normal slit image whereas the next column contains the factor, given by the theory as will be described below, by which the fringe displacement is divided in obtaining the value of C_t, column 4. If the theory were valid the entries in the fourth column would be a constant[3] and this is seen to be the case.

As is indicated in Table I, not all minima are measured but only those at a regular interval such that 8 to 10 values are represented by the final average. Since the somewhat greater precision with which the higher minima can be located partially compensates for their smaller displacement no weighting of the constants in taking the average has been attempted. However, the minima nearest the normal slit image are not used since accidental errors in their location are unduly magnified and since these correspond to the weak gradients in the boundary most likely to be disturbed by temperature fluctuations, etc. In the present example the pattern contained 60 minima but nothing above j_{40} was used in the computations. The diffusion coefficient to be computed from the constant, C_t, of Table I is thus an integral value over the concentration interval from about 0.43 to 0.57 N.

TABLE I

AN EXPERIMENTAL TEST OF THE THEORY

Interference fringes photographed after diffusion, in the Lamm cell (a = 1.600 cm.), of 0.6 N potassium chloride into 0.4 N potassium chloride for 7500 seconds at 0.5°.

1	2	3	4	5	6	7
j	Y, cm.	e^{-z^2} (j_m = 60.3)	Y/e^{-z^2} (= C_t) (j_m = 60.3)	C_t (j_m = 60.2)	C_t (j_m = 60.4)	C_t[b] (j_m = 60.1)
0	0.5989	0.9355	0.6402[a]			0.6300[a]
1	.5675	.8874	.6395	.6397	.6394	.6320[a]
5	.4822	.7548	.6388	.6390	.6386	.6335
10	.4040	.6329	.6383	.6387	.6397	.6337
15	.3402	.5328	.6385	.6390	.6379	.6340
20	.2848	.4457	.6390	.6397	.6381	.6347
25	.2354	.3683	.6392	.6402	.6379	.6348
30	.1904	.2983	.6383	.6398	.6368	.6342
35	.1497	.2344	.6387	.6408	.6368	.6343
40	.1127	.1762	.6396	.6422	.6367	.6346
45	.0785	.1226	.640[a]			.635[a]
50	.0476	.0745	.639[a]			.632[a]
55	.0205	.0320	.641[a]			.629[a]
Mean			.6389	.6399	.6378	.6342
Average deviation			.0004	.0008	.0007	

[a] Not included in mean. [b] Computed with a path length difference of $j + 1/2$ wave lengths as the condition for destructive interference.

The factors of column 3 of Table I were interpolated from a plot of e^{-z^2} *vs.* $f(z)$ for values of $f(z)$ equal to $(j + 3/4)/j_m$ where z is the reduced height in the cell and $f(z)$ is defined by equation (12) of the accompanying paper.[3] The path difference parameter, j_m, is given by the relation

$$j_m = a(n_s - n_0)/\lambda \qquad (1)$$

between the cell dimension, a, parallel to the light path, the refractive indices, n_s and n_0, of the solutions forming the boundary and the wave length, λ, of the light. Although j_m does not need to be an integer, it should not exceed by more than one the integral number of fringes in the pattern. Although it is an essential feature of the method that the number of fringes in a pattern remain invariant as diffusion proceeds, it is clear that counting this number will not give a sufficiently precise value for j_m since only the next smallest integer to the true value can be obtained in this manner. The following procedure for evaluating j_m has, therefore, been used.

Values of C_t for different assumed values of j_m in the neighborhood of the number of fringes actually counted are tabulated and the one yielding the best constant for different values of j is thus found. In Table I the values of C_t in column 4 were computed for j_m = 60.3 while those in columns 5 and 6 are for j_m = 60.2 and 60.4, respectively. Here the average deviation from the mean is least for j_m = 60.3. Repetition of this procedure

(8) Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, Ser. IV. **10**. No. 6 (1937).

(9) Neurath, *Science*, **93**, 431 (1941).

(10) Claesson, *Nature*, **158**, 834 (1946).

for each of the nine plates obtained in this experiment leads to the results shown in column 2 of Table II. The tendency of j_m to decrease in the later photographs is typical and is probably due to an error in the location of the normal slit image resulting from the smaller proportion of light concentrated in this image as the gradients approach the ends of the cell. Although it is hoped that this effect can be eliminated in the new, tall cells becoming available, correction for it in the present research has been made as follows. Taking j_m as constant for all times and equal to 60.3, the average value from the early photographs, the position of the normal slit image is then adjusted until the average deviation of C_t from the mean value for each pattern is again a minimum. The necessary adjustments are seldom more than a few microns.

TABLE II

OBSERVED VARIATION, WITH THE TIME, OF THE NUMBER, j_m, OF FRINGES AND OF THE DIFFUSION COEFFICIENT, D', IN THE DIFFUSION OF 0.6 INTO 0.4 N POTASSIUM CHLORIDE AT 0.5°

1 t, seconds	2 j_m	3 $D' \times 10^6$ (j_m = 60.3)	4 $D \times 10^6$ (Δt = 31)
1200	60.4	9.509	9.270
2280	60.5	9.424	9.297
3360	60.2	9.398	9.312
5520	60.1	9.334	9.282
7500	60.3	9.337	9.298
8760	60.4	9.304	9.280
11820	60.4	9.327	9.302
14280	59.8	9.273	9.253
17520	60.0	9.306	9.290
		Mean	9.287
		Av. dev.	0.15%

A diffusion coefficient, D', is then computed for each exposure with the aid of the relation

$$D' = j_m^2\lambda^2 b^2/4\pi C_t^2 t \qquad (2)$$

in which b, the optical distance from the center of the diffusion cell to the photographic plate, is 182.0 cm. in the author's apparatus.[4] The values obtained in the present example are given in the third column of Table II and tend, at least in the early stages of diffusion, to decrease with increasing time. This is undoubtedly due to initial mixing for which a correction is made as follows.

The Zero-Time Correction.—Since initial mixing causes the concentration distribution at any instant to appear as though the boundary had been formed before this actually occurred, a small, but constant, increment, Δt, must be added to the observed time, t, of equation 2 in order to obtain a corrected value, D, for the diffusion coefficient. The relation is

$$D' = D\left(1 + \frac{\Delta t}{t}\right)$$

and a plot of D' *vs.* $1/t$ should yield a straight line whose intercept is D and whose slope is $D \cdot \Delta t$. Such a plot of the data of Table II is shown in Fig. 4 and gives $D = 9.287 \times 10^{-6}$ and $\Delta t = 31$ seconds. Application of this zero-time correction to the observed values of Table II leads to the results in the last column where the average deviation from the mean is 0.15%.

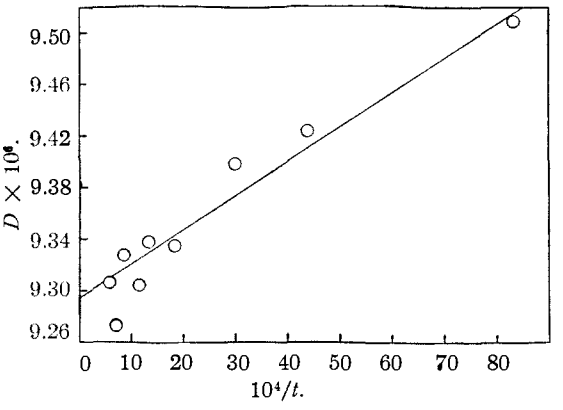

Fig. 4.

The Diffusion of Potassium Chloride in the Lamm Cell.—The results, computed as outlined above, for the solutions of potassium chloride listed in the first two columns of Table III are given in the third column of that table.

TABLE III

SUMMARY OF RESULTS ON THE DIFFUSION OF POTASSIUM CHLORIDE IN THE LAMM CELL AT 0°

1 Solution, N KCl	2 Solvent, N KCl	3 $D \times 10^6$ cm.²/sec.	4 No. of plates	5 Av. dev., %	6 Δt, sec.	7 j_m, obsd.	8 j_m, comptd.
0.2	Water	9.016	8	0.05	16	63.6	64.13
.4	0.2	9.056	7	.13	56	61.6	62.00
.6	.4	9.111	9	.15	31	60.3	60.56
.8	.6	9.259	8	.08	10	59.3	59.42
1.0	.8	9.410	8	.19	6	58.3	58.51

For the comparison with the theory shown in Fig. 5, and discussed below, these values have been corrected from 0.5 to 0° by means of the viscosity–temperature factor, 273.1 $\eta_{0.5}$/273.6 η_0 = 0.9810. In the next two columns are listed the number of plates analyzed in each experiment and the average deviation from the mean. The rather erratic

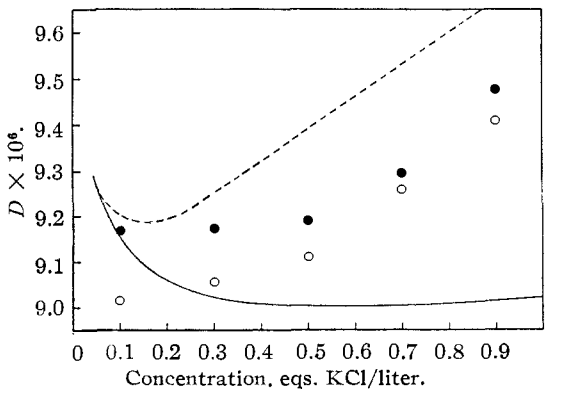

Fig. 5.

fluctuations in the zero-time correction, column 6, are probably due to the fact that several different filling procedures were tested in this series of experiments. Of particular interest are the values of the path difference parameter, j_m, column 7, obtained from the fringe patterns as described above and as computed with the aid of equation 1 from the refractive indices of the solutions that have been measured independently in a hollow prism cell,[4] column 8. In each case the observed value for j_m is less than the computed value, a discrepancy[10a] that may, possibly, arise from a slight distortion of the boundary due to variation of both the diffusion coefficient and the equivalent refraction with the concentration. In this case the neglect of the fringes near the normal slit image in minimizing, by adjustment of j_m, their fluctuations of C_t would influence the value of the path difference parameter.

Although the Onsager–Fuoss theory[11] can be expected to yield only approximate values at the concentrations of Table II it is of interest, nevertheless, to compare the experimental results with that theory. This is done in Fig. 5 where their relation is plotted as the full curve and, after correction for the viscosity as suggested by Gordon,[12] as the dashed curve. In making the computations the limiting cation transference number of potassium chloride at 0° was taken as 0.4960,[13] the limiting equivalent conductance as 81.50,[14] and the mean ion radius as 3.78 Å.[15] The experimental values are indicated by the circles in Fig. 5 and are plotted at the mean concentration for each interval. If the values of j_m that are obtained independently from refractive index measurements are used instead of those from the fringe photographs, the diffusion coefficients that result are indicated by the dots of Fig. 5. At present it is not possible to say whether the circles, or the dots, represent the best values. In either case the experimental points tend to lie between the two curves of Fig. 5, indicating, possibly, that a viscosity exponent of unity in the Gordon relation is too large.

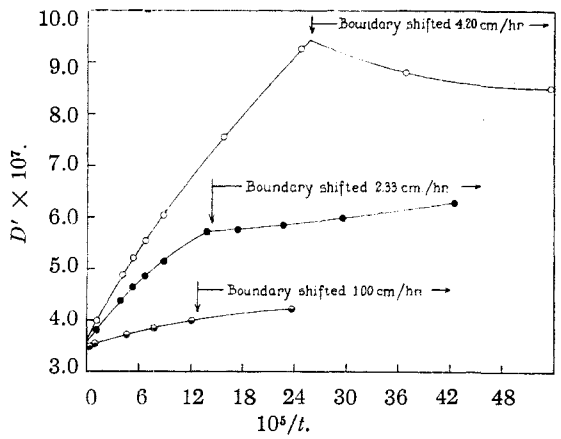

Fig. 6.

The Tiselius Cell as a Diffusion Cell.—Although the Tiselius cell has been used by the author,[16] and others, for diffusion measurements on proteins the results of this research indicate that it is not suitable for precise work. Due, probably, to slight mixing during shifting of the boundary the number of fringes in the pattern for a given initial concentration difference is consistently less than in a Lamm cell of the same thickness. Moreover, the boundary is disturbed not only by its formation at zero time but also over the period in which it is being shifted into view. Consequently no simple zero-time correction can be applied. This is illustrated in Fig. 6 where the observed values, D', in three experiments on an ovalbumin solution at different rates of shifting the boundary are plotted against the reciprocal of the time. During the period that the boundary is being shifted, and for several hours thereafter, the fringe spacing is not in accord with the theory. The values of D' for this period that are plotted in Fig. 6 were computed using the value of j_m from later photographs and the displacement of the lowest minimum. After the boundary has been shifted to the proper level in the cell, D' decreases with increasing time but the variation is not linear in $1/t$ and extrapolation to $1/t = 0$ is uncertain. Similar results have been obtained for the diffusion of bovine serum albumin in the Tiselius cell. In Fig. 6 the variation in the extrapolated value is from 3.5 to 3.6 $\times 10^{-7}$. This is lower than the value, 3.8 $\times 10^{-7}$, obtained earlier[16] for ovalbumin in the same solvent. Although the boundaries were shifted at the low rate in the earlier work and no schlieren scanning photographs were taken for the first fifteen hours, *i. e.*, $10^5/t \leq 2$, the graphical analysis of the plates used at that time was not sufficiently sensitive to indicate a variation of D' with t and no zero-time correction was applied. The results shown in Fig. 6 suggest that, owing to the neglect of this correction, the earlier values are high by a few per cent.

Owing, apparently, to its much more rapid diffusion, when potassium chloride is studied in the Tiselius cell the disturbing effects encountered with proteins are scarcely detectable. Although the boundary is being shifted during an appreciable part of the total period of observation all of the fringe patterns are normal and a zero-time correction can be made. The disturbing influences become evident only on comparison with results in

(10a) This discrepancy has been eliminated in experiments with the Claesson cell that became available during the preparation of this paper. In this cell, which is 82 mm. tall and for which a = 2.4845 cm., a potassium chloride concentration difference of 0.1 N is used and the initial mixing is so slight that a coefficient obtained after diffusion for as little as ten minutes differs from the final value by a very few tenths of a per cent.

(11) Onsager and Fuoss, *J. Phys. Chem.*, **36**, 2689 (1932).

(12) A. R. Gordon, *J. Chem. Phys.*, **5**, 522 (1937).

(13) Allgood, LeRoy and Gordon, *ibid.*, **8**, 418 (1940).

(14) Gunning and Gordon, *ibid.*, **10**, 126 (1942).

(15) Scatchard and Prentiss, THIS JOURNAL, **55**, 4355 (1933).

(16) Longsworth, *Ann. N. Y. Acad. Sci.*, **41**, 267 (1941).

the Lamm cell in that (a) the values from individual photographs show larger deviations from the mean, (b) the zero-time corrections are from 40 to 100 seconds, and (c) the differences between the observed and computed values of j_m are greater than in the Lamm cell.

Ovalbumin in the Lamm Cell.—On attempting to study ovalbumin in the Lamm cell the results could only be interpreted by assuming that a slow leak at the level of the partition developed during the experiment. Tests made by placing the filled cell on the pan of a balance and noting the loss in weight with time showed that the Lamm cell actually did leak, although in an unpredictable manner. Due, possibly, to the relatively short times involved no evidence has come to light that leakage interfered with the diffusion experiments on potassium chloride in this cell. These experiments will, however, be repeated in the new cells becoming available.

The Boundary Level.—In the development of the theory of the fringe spacing it is assumed[3] that the cell is illuminated with convergent light but that the maximum gradient in the boundary is at the level where the light enters normally. Although there is no difficulty in adjusting the cell support so that this condition is realized, to within a millimeter or so, it is of interest to determine if such adjustment is adequate. Consequently one of the experiments on potassium chloride in the Lamm cell was repeated with this cell 3 cm. below the proper level. Although the effect on the fringe spacing was not large it could be detected as a decrease, with time, of the observed values of j_m for which correction, by shifting the normal slit image position within permissible limits, could not be made. Placing the cell at the proper level, to within a millimeter say, appears, however, to be adequate and is practicable.

Effect of Diffraction.—In computing the intensity at a given level in the fringe pattern Kegeles and Gosting take into account the contribution of a considerable portion of the wave front on either side of the normals to the level in question. This leads, as a close approximation, to a path difference of $j + 1/4$ wave lengths as the condition for a maximum and $j + 3/4$ for a minimum. It is of interest to compare these conditions with the ones that are given by the usual ray-optical treatment, namely, j for a maximum and $j + 1/2$ for a minimum. It will be recalled that such a treatment identifies the ray with the normal and ignores the contributions of the secondary wavelets from neighboring points in the wave front. Except for the lowest maxima and minima the spacing is given about equally well by either set of conditions. In the case of the lowest fringes the more complete theory is, however, clearly superior. This is shown in Fig. 7 where the intensities, obtained as described earlier in this paper, in the two lowest maxima of the pattern represented by Table I are plotted as ordinates against the

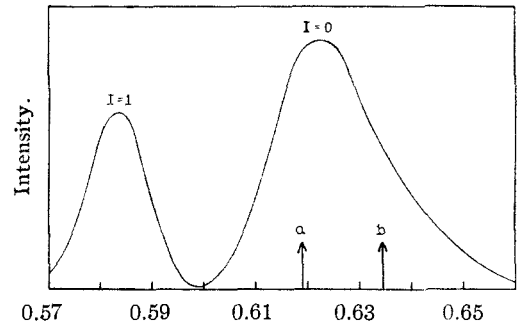

Fig. 7.

displacement from the normal slit image as abscissas. Here the lowest maximum occurs at $Y = 0.6228$ whereas the value computed from $C_t = 0.6389$, Table I, and $e^{-z^2} = 0.9696$, corresponding to $j = 0$ in $f(z) = (j + 1/4)/60.3$, is $Y = 0.6195$. The arrow, a, in the figure indicates this position. If, however, the condition for *minima* is taken to be a path difference of $j + 1/2$ wave lengths, the best value for C_t becomes 0.6342, column 7 of Table I, and the lowest *maximum* should be at this level, *i. e.*, arrow b. The difference, 0.0114, between this and the observed value is greater than the difference, -0.0033, when the more complete theory is used. Moreover, as Kegeles and Gosting point out, their condition for a maximum, $j + 1/4$ wave lengths, represents an approximation that is poorest for $j = 0$. Their detailed computation, with the aid of the Airy integrals, of the intensity distribution in the lowest fringe of a typical system indicates that the difference of -0.0033 cm. observed here is of the correct sign and magnitude. This, together with the fact that the observed skewness in the intensity distribution of the lowest fringe is also predicted, affords considerable confidence in the validity of their theory.

Although the quality of the approximation that the maxima and minima correspond to path differences of $j + 1/4$ and $j + 3/4$ wave lengths, respectively, improves rapidly as one moves up from the lowest maximum, it will be clear from Table I that the 0-th minimum is not used in the evaluation of C_t.

Conclusion

Owing to the ease and reproducibility of 1 or 2 microns with which the fringes can be located the method described here can be recommended for precise work. It has revealed hitherto unsuspected sources of experimental error that will be corrected in the future. Until the theory of the fringe spacing due to skew boundaries is developed the method is restricted to normal diffusion and thus can be made to yield differential values for a solute whose diffusion coefficient is concentration dependent only by reducing the concentration difference across the boundary. This suggests that the horizontal dimension of the cell

should be increased but, owing to the decrease in the magnitude of the density gradients that stabilize the boundary, more careful control of the temperature would be required. Since it appears essential to have homogeneous layers of solution of appreciable thickness above and below the boundary, the period of observation can only be extended by increasing the height of the cell. Although the fringes are compressed as diffusion proceeds the resolving power of the available photographic emulsions[17] is such that this is not a limiting factor.

It is a pleasure to acknowledge my indebtedness to D. A. MacInnes of these laboratories for his care in the review of this manuscript and to Gerson Kegeles of the University of Wisconsin for clarifying correspondence throughout the course of the investigation.

(17) "Photographic Plates for Use in Spectroscopy and Astronomy," Eastman Kodak Co., 5th edition, 1946, Rochester, N. Y.

Summary

In an accompanying paper Kegeles and Gosting have developed the theory of the spacing of the interference fringes that are formed in the focal plane of a lens when an illuminated horizontal slit serves as the light source and a diffusing boundary is placed in the path of the light. In the present paper this theory is confirmed experimentally and a method is suggested for the use of the fringes in the evaluation of diffusion coefficients. Moreover, results for the diffusion, at 0.5°, of aqueous potassium chloride solutions in the Lamm cell are presented and compared with the Onsager–Fuoss theory. The difficulties that were encountered in the use of this cell for the study of proteins, and in the use of the Tiselius electrophoresis cell as a diffusion cell for both salts and proteins, are also reported.

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The Theory of an Interference Method for the Study of Diffusion

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Introduction

In 1880 Gouy[1] discovered a new interference phenomenon produced from a single wave front which had been distorted on passage through a column of liquid containing a diffusion boundary. Gouy gave a qualitative explanation of his observation, but he published no photographs, and presented no mathematical theory. In a recent review dealing with the subject of diffusion, Longsworth[2] gave an account of this phenomenon and published a photograph that he had taken of the interference fringes. This review stimulated the development of the quantitative theory to be presented in this paper, relating the space and intensity in the interference fringe system to the diffusion coefficient. In the report accompanying this one, L. G. Longsworth[3] presents an experimental verification of this theory, as well as his experimental development of the interference phenomenon into a precision method for the study of diffusion.

A brief qualitative description of the original experiment[1] is repeated here, with the aid of Fig. 1, in order to introduce the mathematical treatment. To make his observations, Gouy collimated the light from an illuminated horizontal slit and, after passing it through a diffusing salt boundary, brought it to focus with a telescope. In the illuminated rectangle at the focal plane of the telescope objective, several decades of fringes could be counted, and the fringe system showed peculiarities foreign to those produced by multiple slits. The originally plane wave front on leaving the diffusion cell takes the form, in projection onto the plane of the paper (Fig. 1), of the refractive index function in the column. For ideal diffusion, this distorted wave front is symmetrical about the inflection point corresponding to the level of the maximum refractive index gradient. Because of this symmetry, two normals from any given level Y in the focal plane may be drawn to the wave front, at points denoted X and X'. This means, according to geometrical optics, that rays passing through two symmetrical levels in the diffusion column are brought to a focus together a distance Y below the undeviated slit image. Gouy pointed out that the disturbance at Y could be calculated as the superposition of the disturbances originating in the wave front at X and X', with a phase difference determined by the difference in path lengths from Y to the wave front at X and X', respectively. The theory of the phenomenon derived on this basis is not in complete accord with experiment,[3] however, and according to wave optics it is necessary to take account of the disturbances arising at all other points in the wave front which must also contribute something to the intensity at Y. In the calculations to be presented, the treatment of the phenomenon will be undertaken from these two different starting assumptions, and the results will be compared. As the assumption of geometrical focussing forms the basis of the refractometric optical methods which are applied to studies of the molecular kinetic properties of dissolved solutes. this comparison

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(1) Gouy, *Compt. rend.*, **90**, 307 (1880).

(2) Longsworth, *Ann. N. Y. Acad. Sci.*, **46**, 211 (1945).

(3) Longsworth, THIS JOURNAL, **69**, 2510 (1947).