and 2.94 g. of absolute ethanol. Ethyl γ-(*o*-methoxyphenyl)-butyrate (14 g., 0.063 mole) was then added dropwise with stirring and the mixture refluxed for twelve hours. The reaction mixture was decomposed with water and extracted with ether. A red viscous oil remained after distillation of ether.

5-Methoxy-3,4-dihydro-1,2-naphthalic Anhydride (VIII).—The above oxalyl ester was added slowly to 120 cc. of concentrated sulfuric acid, the temperature being kept between 20–25°, and the mixture allowed to stand for two hours at room temperature. The solution was poured onto ice, the yellow solid filtered and recrystallized from benzene–ligroin, yield 5.2 g. (36% based on starting ester VIb), m. p. 169–170°.

Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 68.17; H 4.62.

5-Methoxy-1,2-naphthalic Anhydride.—The dihydro-acid (4.0 g., 0.017 mole) and 0.55 g. (0.017 mole) of sulfur was heated at 230–235° for thirty minutes. After cooling the solid mass was recrystallized twice from benzene and then sublimed. The yellow solid melts at 228–229°; yield 3.2 g. (80%).

Anal. Calcd. for $C_{13}H_8O_4$: C, 68.42; H, 3.53. Found: C, 68.35; H, 3.57.

5-Hydroxy-1,2-naphthalic Anhydride (IV).—The above methoxynaphthalic anhydride (1.2 g., 0.005 mole) was refluxed for seven hours with 25 cc. of glacial acetic acid and 25 cc. of 48% hydrobromic acid. Upon cooling the solution to room temperature, an amorphous tan solid formed. Further concentration of the mother liquor yielded additional material. The combined solids were sublimed and then recrystallized from water, m. p. 271–272°, yield 0.5 g. (47%).

Anal. Calcd. for $C_{12}H_6O_4$: C, 67.29; H, 2.82. Found: C, 67.11; H, 2.87.

5-Methoxy-1,2,3,4-tetrahydro-1,2-naphthalic Anhydride.—The dihydro-anhydride (VIII) was hydrogenated over platinum oxide in glacial acetic acid. The solvent was removed under reduced pressure and the residue sublimed in vacuum. The yellow solid was recrystallized from benzene–ligroin, m. p. 144–146°.

Anal. Calcd. for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.35; H, 5.01.

Summary

5-Hydroxy-1,2-naphthalic anhydride, a possible metabolite of 1,2,5,6-dibenzanthracene, and several of its derivatives have been prepared by means of the Bougault reaction.

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[CONTRIBUTION FROM PULP MILLS RESEARCH, DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON]

A Study of Diffusion in Agar Gels by a Light Absorption Method

BY VINCENT F. FELICETTA, AARON E. MARKHAM, QUINTIN P. PENISTON AND JOSEPH L. MCCARTHY

Introduction

While precision methods are now available for determination of diffusion coefficients in solution systems,[1,2,3,4] these in general require elaborate apparatus and painstaking techniques. There is still, therefore, need for a relatively simple, rapid and generally applicable procedure for evaluation of diffusion coefficients with moderate precision.

The advantages to be gained by use of gels to immobilize one or both of the initial phases are considerable. Difficulties in establishment of an initial sharp boundary are reduced and convection effects are almost eliminated. Moreover, quantitative determination of dispersion by diffusion is greatly simplified since the cell can be subjected to mechanical and thermal stress during analysis without appreciable disturbance of the established gradients. For light-absorbing substances, the analysis may be conducted by direct scanning of the cell with a slit spectrophotometer. With instruments now available concentrations may be measured for narrow bands along the axis of diffusion with a limit of error of a few tenths of one per cent. This compares favorably with refractive index gradient measurements and obviates objections which have been raised to older light absorption methods.[2]

Since uncertainty exists regarding the effects of gels on the diffusion process[5,6] we have undertaken a study of these factors in conjunction with techniques for conducting the determination of diffusion coefficients. Our study of agar in lieu of other gel forming substances was prompted from consideration of its moderately good optical transparency in the ultraviolet and the lack of chemical interaction between it and acidic or neutral substances. While agar gels are considerably more opaque than gelatin gels, this optical density due to light scattering is quite uniform and reproducible. It has been studied in considerable detail by Donnan and Krishnamurti[7] and by Hatschek.[8]

Studies by Sabin and Sobotka[9] indicate that the interstitial fluid in an agar gel contains appreciable amounts of dissolved substances which can cause a "barophoresis" effect in diffusion measurements.

To obtain further information regarding the nature of the gel fluid a series of extraction experiments on agar gels prepared from "Difco Bacto-agar" have been conducted. These indicate that one-quarter to one-third of the agar can be extracted by cold water. The amount of

(1) J. W. McBain and T. H. Liu, THIS JOURNAL, **53**, 59 (1931).

(2) H. Neurath, *Chemical Revs.*, **30**, 357 (1942).

(3) H. S. Harned and D. M. French, *Ann. N. Y. Acad. Sci.*, **46**, 267 (1945).

(4) O. Lamm and A. Polson, *Biochem. J.*, **30**, 528 (1936).

(5) (a) L. Friedman and E. O. Kraemer, THIS JOURNAL, **52**, 1295 (1930); (b) L. Friedman, *ibid.*, **52**, 1305, 1311 (1930); (c) K. Klemm and L. Friedman, *ibid.*, **54**, 2632 (1932).

(6) V. Moravek, *Kolloid-Z.*, **49**, 39 (1929).

(7) F. G. Donnan and K. Krishnamurti, *Colloid Symposium Annual*, **7**, 1–16 (1930).

(8) E. Hatschek, *Kolloid Z.*, **48**, 246–248 (1929).

(9) A. B. Sabin and H. Sobotka, THIS JOURNAL, **50**, 1561–1572 (1928).

soluble agar decreases rapidly in successive treatments of the gel. On melting and regelation of the extracted gel only a small amount of additional extractable material is obtained. Extract solutions show high specific conductance and high viscosity (up to 2.0 centipoises at 25°). It is therefore believed that the extractable substances are low molecular weight agar molecules and that they occur dissolved in the gel fluid in equilibrium with the gel structure. Considerable increase in the viscosity of the gel fluid probably arises from this source.

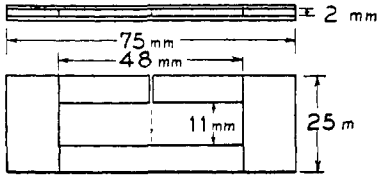
The use of a slit photometer for direct scanning of a diffusion cell has been applied to the determination of the diffusion coefficient of methylene blue in 5% gelatin by Eversole and Doughty.[10] The procedure used was that of free diffusion from a solution of fixed concentration into a gel of "infinite" length which had an initial zero concentration of the diffusing substance. Their calculation of the diffusion coefficient was based however on an erroneous expression for the concentration as a function of the distance along the axis of diffusion and the time.

In a later communication,[11] Eversole, Peterson and Kindsvater submitted a revised calculation of the earlier data based on integration of Fick's law for variation of the diffusion coefficient with concentration.

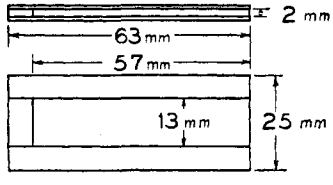
Experimental

Determination of Diffusion Coefficients

Two general techniques for determination of diffusion coefficients have been used. In the first of these, an initially sharp boundary is formed between two gel phases, one containing supporting electrolyte and the substance to be diffused and the other only the supporting electrolyte. The second technique is similar to that used by Eversole and Doughty, *i. e.*, diffusion from an aqueous solution at constant concentration across an initially sharp boundary into the gel phase. The procedure for scanning the diffusion cell at the end of the determination is identical for both methods and similar methods for calculation may be used.



Gel-to-gel diffusion.



Solution-to-gel diffusion.

Fig. 1.—Cells for diffusivity determination.

1. **The Diffusion Cells.**—The microdiffusion cells (Fig. 1) for both methods are constructed from two quartz microscope slides[12] of usual dimensions with sides and ends of ordinary glass. The cell components are joined using Cenco de Khotinsky cement. For gel-to-gel diffusion an opening is provided in the side for insertion of a thin stainless steel gate used in forming the boundary.

2. **Preparation of the Cell for a Determination.**—For most experiments "Difco" powdered agar has been used. This material has been found to contain water soluble substances which absorb ultraviolet light. Although no correction is necessary in the case of gel-to-gel diffusion for light absorption by these substances, they do result in low values of the diffusion coefficient due to increase in the viscosity of the gel fluid. Hence, the use of agar which has been exhaustively extracted with distilled water is recommended.

For gel-to-gel diffusion an aqueous solution containing the supporting electrolyte and agar, both at one and one-half times the desired concentration, is prepared by heating in a water-bath at 100° for thirty minutes using occasional stirring. The sol is filtered through a pad of glass wool and is held at 60°. One volume of a solution of the sample, at three times the desired concentration, is added to two volumes of the agar sol. The solvent sol is prepared by adding one volume of water to two volumes of the agar sol. Both sample and solvent sols are then at the same agar and supporting electrolyte concentrations (usually 0.585% and 0.02 molar, respectively) and contain agar prepared under identical conditions. The cell is now filled to the gate opening with the sample sol, the gate is inserted and the sol is allowed to gel and cool to 25°. The upper half of the cell is now carefully cleaned with lens paper, the gate is removed and the cell is filled with the solvent sol precooled to just above the setting temperature. The cell is now cooled rapidly to 25°, the removable end is replaced, and all openings are sealed with paraffin wax. It is then placed in a thermostat at 25 ± 0.1° and 100% relative humidity for the diffusion period.

For solution-to-gel diffusion the solvent agar gel is cast in the diffusion cell. The sample solution, containing no agar but supporting electrolyte in the same concentration as the solvent agar gel, is placed in a wide-mouth bottle. It should contain 100 or more times the amount of sample actually diffused into the gel. The diffusion cell may be suspended from the stopper so that the open end dips just below the solution surface (upward diffusion), or it may be fixed open end upward to the bottom of the bottle with the solution just covering the open end (downward diffusion). The bottles and cells for a series of determinations are mounted in a shaking rack of slow period in a thermostat at 25 ± 0.1° for the diffusion process.

3. **Ultraviolet Absorption Analysis.**—At the end of the desired diffusion period the cell is mounted in a carriage designed to fit the sample chamber of a Beckman Quartz Spectrophotometer. The carriage is equipped with a screening slot to provide a uniform width of cell for light transmission with index lines to locate the boundary position, and also with a vernier micrometer rack for moving the cell across the light path of the instrument. The spectrophotometer is set for the wave length corresponding to maximum absorption for the sample and is adjusted to zero optical density at the end of the cell containing the pure solvent gel. Values of optical density are then read at one millimeter intervals throughout the length of the cell (Fig. 2). The times of starting and ending the ob-

(10) W. G. Eversole and E. W. Doughty, *J. Phys. Chem.*, **39**, 289 (1935); *ibid.*, **41**, 663 (1937).

(11) W. C. Eversole, J. D. Peterson and H. M. Kindsvater, *J. Phys. Chem.*, **45**, 1398 (1941).

(12) Obtainable from the Thermal Syndicate, Ltd., 12 E. 64th St., N. Y., N. Y.

servations are noted and the mean time is taken as that of the end of the diffusion period.

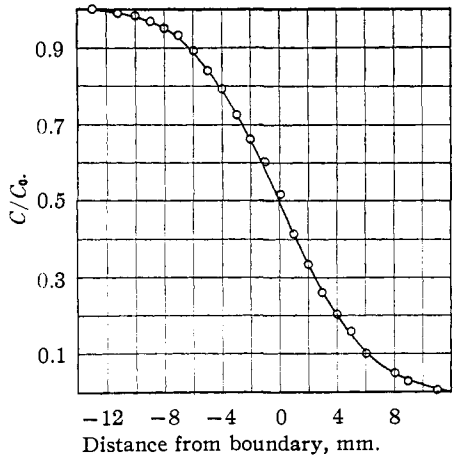


Fig. 2.—Observed diffusion data: gel-to-gel diffusion of orange *II*; detn. no. 46, D = 40.2 mm.²/day, time = 0.285 day.

4. Calculation of the Diffusion Coefficient

Case I, Gel-to-Gel Diffusion.—Assuming the diffusion coefficient to be independent of concentration, Fick's law for this case may be integrated to the expression

$$\frac{C}{C_0} = 0.5 - \frac{1}{\sqrt{\pi}}\int_0^{\frac{x}{\sqrt{4D\cdot t}}} e^{-\beta^2}\,d\beta$$

where C_0 represents the boundary concentration; C, the concentration at distance x and time t, and D is the diffusion coefficient. X is taken as the distance from the initial boundary, positive in the direction of diffusion. The observed optical density readings are converted to fractions of the original concentration by assuming the validity of the Lambert–Beer law and that the total change in optical density throughout the cell represents the original concentration. These C/C_0 values

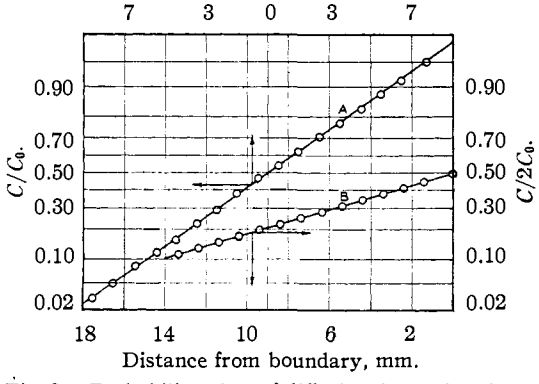


Fig. 3.—Probability plots of diffusion data: A, gel-to-gel diffusion of Orange II; detn. no. 121, D = 42.5 mm.²/day, time = 0.247 day; B, solution-to-gel diffusion of l-tryptophan; detn. no. 263, D = 60.4 mm.²/day, time = 1.037 days (corrected for agar diffusion).

are plotted *versus* x ($C/C_0 = 0.5$ when $x = 0$) on "probability paper" (Keuffel and Esser, No. 359–23) (Fig. 3). The best fitting straight line is located visually and its intercepts at one or more values of x are noted. The value of the probability integral is then calculated

$$\frac{2}{\sqrt{\pi}}\int_0^{\frac{x}{\sqrt{4D\cdot t}}} e^{-\beta^2}\,d\beta = 1 - \frac{2C}{C_0}$$

and the corresponding value of $x/4D\cdot t$ is obtained from tabulated values of this integral.[13] (For convenience a graph of $D\cdot t$ *vs.* C/C_0 may be prepared for certain x values (see Fig. 4)).

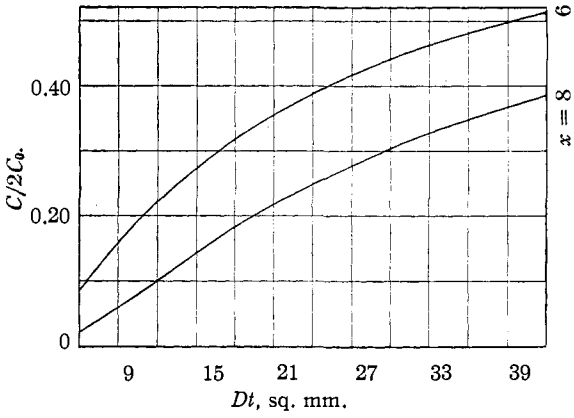


Fig. 4.—Calculation curves from probability integral.

We have found the above method of calculation satisfactory for most cases in that very good linear plots are usually obtained from the data and values are quite reproducible. Diffusion coefficients of Orange II have been calculated both by the above probability integral procedure and also by a modification of the statistical method of Pearson and Lamm as described by Neurath[2] involving calculation of the second moment of the derivative of the diffusion curve (Table I). This latter method of calculation makes no assumptions regarding the shape of the diffusion curve so that the close agreement shown between the two methods of calculation is a surprising confirmation of the validity of the probability integral solution to the differential diffusion equation. While the statistical method yields a lower standard deviation for the set of determinations than the probability integral method, there is probably a slight systematic error in determining the derivatives for high values of x so that low values of the diffusion coefficient are obtained.

Case II, Solution-to-Gel Diffusion.—Calculation of the diffusion coefficient for this case is somewhat more complicated than the above treatment for two reasons: Firstly, a correction must be applied to the observed optical density data to compensate for the diffusion of light absorbing substances from the agar to the solu-

(13) "Tables of Probability Functions," Vol. I, Federal Works Agency, Works Progress Administration, New York, N. Y. (1941).

TABLE I

GEL TO GEL DIFFUSION OF ORANGE II

Temperature 25.0 ± 0.2°; 2500 Å.

Detn.	Time, days	Sample concn., mg./l.	Agar concn., %	KCl concn., *M*	Diffusion coefficient: Probability integral method, sq. mm./day	Diffusion coefficient: Statistical method, sq. mm./day
49	0.280	100	0.585	0.02	43.0	41.9
52	.292	100	.585	.02	42.1	40.4
72	.247	100	.585	.02	39.4	38.4
82	.235	100	.585	.02	40.6	40.6
99	.297	100	.585	.02	38.2	38.3
81	.234	100	.836	.02	41.2	39.6
80	.239	100	1.25	.02	40.0	38.6
79	.242	100	1.67	.02	39.9	40.0
71	.255	100	0.585	None	44.5	42.5
73	.239	100	.585	0.05	39.7	40.2
74	.228	100	.585	.20	39.9	39.1
97	.302	25	.585	.02	44.6	40.3
98	.300	50	.585	.02	41.7	39.9
100	.295	200	.585	.02	39.7	37.7
120	.241	100	.7	.02	42.5	42.2
121	.247	100	.7	.02	42.5	41.8
		Mean value			41.2	40.1
		Average deviation			1.54	1.14
		Standard deviation			1.76	1.41

tion. The extent of this correction will depend on the pretreatment of the agar, the agar concentration and the time of diffusion. We have found it convenient to prepare tables for the amount of correction at various distances from the boundary and various agar concentrations for several diffusion times. Suitable interpolation to fit the conditions of a diffusion experiment is then made to obtain the corrections to the observed data.

Secondly, since scanning of the diffusion cell cannot be extended to the actual gel-solution boundary due to non-uniformity of the light path

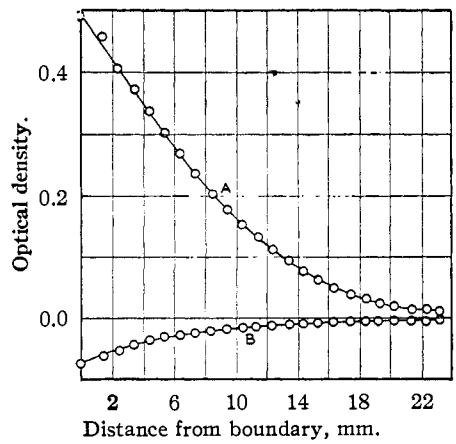


Fig. 5.—Observed diffusion curves: solution-to-gel diffusion of *l*-tryptophan; detn. no. 275, D = 57.9 sq. mm./day, time = 0.884 day: A = observed data, B = agar correction data.

in this region, an extrapolation is necessary to obtain the initial or boundary concentration. While this is theoretically objectionable, one is guided by the fact that the derivative of the observed diffusion curve approximates one-half of a normal probability distribution. The observed curve will thus be nearly linear over the range of the extrapolation with a maximum slope at the boundary. Figure 5 shows optical density data obtained in a diffusion determination on tryptophan with application of the agar correction and the extrapolation.

The corrected optical density values are now converted to values of $C/2C_0$ and are plotted *versus* x on probability paper (Fig. 3). The best fitting straight line is drawn as for Case I and the intercept at a particular value of x is observed. The value of $D \cdot t$ is obtained as before from tables of the probability integral or a graph as in Fig. 4.

Results and Discussion

For four series of determinations on tryptophan at two different agar concentrations using both upward and downward diffusion (Table II), pronounced dependence of diffusion rate on agar

TABLE II

SOLUTION OF GEL DIFFUSION[a] OF TRYPTOPHAN

Detn.	Agar concn., %	Time, days	*D*, sq. mm./day
	Upward Diffusion		
262	0.627	1.041	59.0
263	.627	1.037	58.9
264	.627	1.029	58.4
253	.627	0.831	57.5
254	.627	.836	58.6
255	.627	.843	58.6
	Mean value		58.50
	Standard deviation		0.49
	Probable error of mean		0.15
259	1.254	0.990	54.5
260	1.254	1.003	55.0
261	1.254	1.012	54.7
256	1.254	0.850	54.1
257	1.254	.859	54.4
258	1.254	.865	55.4
	Mean value		54.68
	Standard deviation		0.42
	Probable error of mean		0.13
	Downward Diffusion		
271	0.627	0.850	55.3
272	.627	.862	55.9
273	.627	.868	56.4
	Mean value		55.87
268	1.254	0.827	52.1
269	1.254	.837	54.4
270	1.254	.846	55.1
	Mean value		53.87

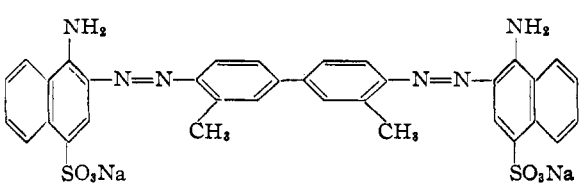
[a] One hundred mg. of tryptophan per liter; 25 ± 0.1°; 0.02 *M* potassium chloride; 2800 Å.

concentration is indicated. Downward diffusion yields slightly lower values than upward diffusion, as would be expected from the observations of Sobotka and Sabin,[9] since the density of the fluid in the gel is probably greater than that of the sample solution and convection would therefore be greater with upward diffusion.

Linear extrapolation of the mean values in Table II to zero agar concentration assuming an uncertainty equal to the standard deviation yields diffusion coefficients for tryptophan (aqueous 0.02 *M* potassium chloride) of 62.2 ± *ca.* 2.0 sq. mm./day for upward diffusion and 58.0 sq. mm./day (± *ca.* 2.0) for downward diffusion with the downward diffusion results probably more reliable.

Data on tryptophan at 20° have been obtained by Polson[14] using the refractive index method.[4] Converting his data to present units, and adjusting to 25° by the viscosity relation he suggests, a value of 60.4 sq. mm./day (± *ca.* 1.0) is obtained. That this value is in close agreement with results of the present study indicates that the gel diffusion method can be used to obtain results which may be only slightly less precise than more elaborate methods.

While the diffusion coefficient of tryptophan shows a pronounced dependence on agar concentration (Table II), such an effect has not been noted for the dyes Orange II and Azogrenadin S (Table III). Values of E. Valko[15] for these substances at 25° (0.02 *M* sodium chloride) using the porous disk method of McBain and Liu[1] are 44.4 sq. mm. per day for Orange II and 40.4 sq. mm. per day for Azogrenadin S with which present values are in satisfactory agreement. The lack of any dependence on agar concentrations for these substances in spite of their increased molecular size compared to tryptophan suggests that there is no substantial mechanical retardation of diffusion by the agar molecules for neutral or acidic substances of molecular size at least up to that of Azogrenadin (molecular weight is 464 for the dyestuff ion). For basic substances however there is possibility of salt formation with carboxylic acid groups or sulfate groups which form a part of the agar structure.[16] This would result in a reduction of the rate of diffusion as has been observed for tryptophan. In this regard our observations on the basic dye Benzopurpurin 4B show it to be almost completely immobilized in an agar gel although Valko[14] has reported a



(14) A. Polson, *Biochem. J.*, **31**, 1903 (1937).
(15) E. Valko, *Trans. Faraday Soc.*, **31**, 230–245 (1945).
(16) W. G. M. Jones and S. Peat, *J. Chem. Soc.*, 225–231 (1942).

diffusion coefficient in water of 18.8 sq. mm./day at the same sample and electrolyte concentrations (50 mg./l. and 0.02 *M* sodium chloride).

Significantly lower values for the diffusion coefficient of Orange II are found by the gel-to-gel method (Tables I and III). Similar results have been obtained on all substances for which both methods of study have been used, *i.e.*, the gel-to-gel method yields results which are 10–15% lower than those obtained by the solution-to-gel method. It is believed that this effect is due to the viscosity of the fluid in the gel. This has been shown by Sobotka and Sabin[9] to contain appreciable amounts of dissolved material which our own studies indicate is of rather marked influence in raising the viscosity of the gel fluid over that of water. We have been unable to devise an experiment other than diffusional processes which we feel will give a true measure of the viscosity of this fluid as it actually occurs in equilibrium with the gel structure. Extraction experiments, however, suggest that it may well be high enough to account for differences observed in diffusion coefficients when determined by the gel-to-gel and solution-to-gel procedures. In the former method the fluid in the gel may be considered to be uniform throughout the cell so that diffusion of the sample is uniformly reduced by the dissolved agar. The lack of any effect due to gel concentration probably results because the fluid in the gel is "saturated" with agar molecules and thus the concentration of dissolved agar is not dependent on the over-all agar concentration in the gel. In the case of solution-to-gel diffusion, there is simultaneous diffusion of dissolved agar from gel to solution. Gradients for both processes are at a maximum at the boundary where the concentration of dissolved agar in the fluid of the gel will be zero. Impedance to diffusion of the sample is therefore reduced in the region where the greatest transport of sample occurs so that higher diffusion coefficients are obtained.

It might be expected that deviation from the theoretical diffusion equations would occur with the solution-to-gel method due to this change in viscosity with distance from the cell boundary. This should result in two effects: (a) a dependence of the observed diffusion coefficient on the time of the diffusion process with higher values for longer times, and (b) non-linearity of the concentration *versus* distance plots on probability paper with lower values of $D \cdot t$ indicated at greater distances from the boundary. Both of these effects are observed in our data for Orange II and for tryptophan although the time effect over the range studied (0.8 to 1.1 days) is only slightly greater than the probable error of the determination and the deviation from the theoretical probability curves is scarcely discernible. Also, in harmony with the above explanation for the difference in diffusion coefficients by the two methods

TABLE III

SOLUTION TO GEL DIFFUSION OF ORANGE II AND AZOGRENADIN S[a]

Detn.	Agar concn., %	Time, days	D, sq. mm./day
		Orange II	
285	0.627	0.805	44.7
286	.627	.811	46.7
287	.627	.818	44.7
	Mean value		45.3
288	1.254	0.840	45.9
289	1.254	.846	45.4
	Mean value		45.7
		Azogrenadin S	
294	0.627	0.875	38.1
295	.627	.851	36.8
296	.627	.865	38.5
	Mean value		37.8
291	1.254	0.844	38.3
292	1.254	.849	38.7
293	1.254	.860	36.8
	Mean value		37.8

[a] Fifty mg. of Orange II per liter; 100 mg. Azogrenadin S per liter; 25 ± 0.1°; 0.02 *M* potassium chloride; 2500 Å.

are the significantly higher results obtained by the gel-to-gel diffusion method when exhaustively extracted agar was used.

With regard to the relative merits of the two diffusion methods it has already been shown that the gel-to-gel method leads to a more straightforward calculation in that no extrapolation to obtain the boundary concentration and no corrections for light absorbing substances in the agar are required. In addition this method is more economical of sample since only enough material to fill half the cell is needed (about 1 ml. at 50–200 mg. per liter or 50–200 micrograms for strongly absorbing substances).

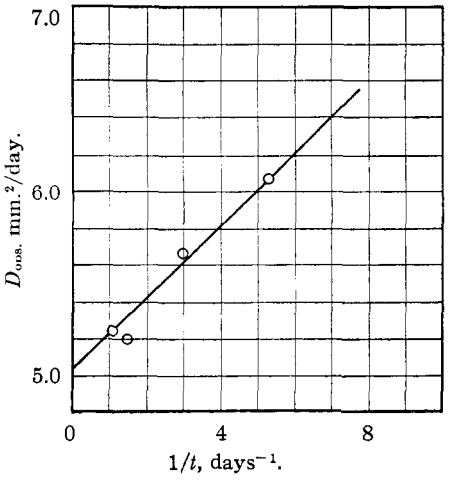


Fig. 6.—Effect of imperfect boundary on diffusion coefficient: high molecular weight lignin sulfonate fraction.

On the other hand, the technique for preparing the cells for the diffusion analysis is more difficult and a perfect boundary cannot be obtained. This leads to an apparent time dependence for the diffusion coefficient since the effect of an imperfect boundary approximates an extension of the diffusion time. For substances of low diffusivity or short diffusion periods this leads to serious error. The effect is illustrated in Fig. 6 which shows diffusion coefficients obtained for a sodium lignin sulfonate preparation of low diffusivity[17] using various times for the diffusion process. Assuming the error in diffusion coefficient is proportional to the error in time, then

$$\frac{D_{\text{correct}}}{D_{\text{observed}}} = \frac{t_{\text{obs}}}{t_{\text{obs}} + C}$$

where C represents the error in time equivalent to the imperfect boundary. Then

$$D_{\text{obs}} = D_{\text{cor}} + C.\ D_{\text{cor}}/t$$

In Fig. 6 D_{obs} is plotted *versus* the reciprocal of the time. The intercept thus represents the value of D_{cor} and the slope is equal to $D_{\text{cor}} \times C$. From the observed slope an equivalent time error equal to fifty-six minutes may be calculated. This of course applies only to the substance investigated for which $D_{\text{cor}} = 5.0$ sq. mm./day.

When the diffusion coefficient is dependent on the concentration of the diffusing substance the probability integral solution to Fick's law is no longer valid, and linear plots will not be obtained using probability paper. If, as is usually the case, the diffusion coefficient increases with decreasing concentrations, deviation from linearity will be toward higher values of C/C_0 (or $D{\cdot}t$) for greater distances from the boundary in the direction of diffusion. This is equivalent to the skewed probability curves obtained with the refractive index gradient method. We have not observed this effect with the low molecular weight substances hitherto investigated but do encounter it with some high molecular weight lignin sulfonic acid preparations. An average integral diffusion coefficient may still be calculated by the statistical method or values of D representative of particular concentration values may be obtained.[11] It should be noted that the effect is generally opposite to that caused by viscosity of the gel fluid so that some concentration dependence may be masked with the solution-to-gel diffusion method.

In the conduct of diffusion studies of polymer systems the effects of polydispersity must be considered. For a mixture of n components, assumed to diffuse according to Fick's law as individuals and without interaction, the concentrations of each of these can be expressed for the case of solution-to-gel diffusion

(17) A. E. Markham, Q. P. Peniston and J. L. McCarthy, THIS JOURNAL, **71**, in press (1949).

$$\frac{C_i}{C_{0,i}} = 1 - \frac{2}{\sqrt{\pi}}\int_0^{\frac{x}{\sqrt{4D_i t}}} e^{-\beta^2}\,d\beta$$

where C_i is the concentration of the "i-th" component; $C_{0,i}$ its boundary concentration and D_i its diffusion coefficient. If all of the components have the same extinction coefficient, optical density ratios will be equivalent to concentration ratios and summation can be performed directly

$$\frac{(O.D.)_x}{(O.D.)_0} = \frac{C_x}{C_0} = 1 - \sum_1^n \frac{2w_i}{\sqrt{\pi}}\int^{\frac{x}{\sqrt{4D_i t}}} e^{-\beta^2}\,d\beta$$

where W_i is equal to the weight fraction of the "i-th" component in the original mixture. However, if extinction coefficients for the different components vary, optical density ratios will not be equivalent to concentration ratios and the following expression is obtained

$$\frac{(O.D.)_x}{(O.D.)_0} = 1 - \sum_1^n \frac{2w_i e_i'}{\sqrt{\pi}}\int_0^{\frac{x}{\sqrt{4D_i t}}} e^{-\beta^2}\,d\beta$$

in which e_i is a relative extinction coefficient for the "i-th" component.

$$e_i' = e_i \Big/ \sum_1^n w_i e_i$$

Observed curves will deviate from the form of true probability curves since the summation term cannot be expressed as a single probability integral. Observed diffusion coefficients will depend on the character of the polydispersity distribution and the time of the diffusion process and could not be correctly represented by any simple additive formula, *e.g.*

$$D_{obs} \neq \sum_1^n D_i w_i$$

Actually, however, deviation from such an expression will not be large as may be seen from experiment.

When an equal weight mixture of two lignin sulfonic acid fractions[17] having diffusion coefficients of 13.9 ± 0.5 sq. mm./day and 5.7 ± 0.5 sq. mm./day was diffused for one day a diffusion coefficient of 9.3 ± 0.5 sq. mm./day was obtained in agreement with 9.8 ± 1.0 sq. mm./day, the arithmetic mean for values obtained on the two fractions.

Applying the equation developed above to this experiment and considering the two fractions to be individually monodisperse, the probability integral giving the closest fit to the summation curve would lead to a diffusion coefficient of 9.1 and the maximum deviation between the two curves would be 1.3% of the total boundary concentration. This should result in an observable deviation from linearity in the data when plotted on probability paper and is equivalent to the leptokurtic distribution observed for polydisperse systems with the refractive index gradient method.

Summary

1. Rapid and facile procedures have been developed for study of diffusion in gels by a direct scanning light absorption method, using a quartz spectrophotometer.

2. The course of diffusion in the gels studied is found to be in agreement with particular solutions of the differential diffusion equation which satisfy the extant boundary conditions.

3. Results obtained for the dyes Orange II and Azogrenadin S indicate that there is no *mechanical* hindrance to diffusion by the gel structure for the range of agar concentrations investigated. Dependence of the rate of diffusion on agar concentration occurs, however, for the amino acid tryptophan and the dye Benzopurpurin 4B. These two substances contain basic groupings and hindrance to diffusion may result from salt formation with acidic groupings in the agar structure or else from adsorption.

4. Extraction experiments on agar gels show that the agar used is not homogeneous with regard to solubility and that the viscosity of the fluid in the gel is made much higher than that of water by reason of the presence of dissolved agar molecules. Deviation of diffusion coefficients in gels from reported values in aqueous solution can be explained on this basis.

5. By appropriate experimentation in view of the above factors, absolute values for the diffusion coefficients of molecularly dispersed light absorbing substances can be obtained in good agreement with reported values in aqueous solution. Relative values can be obtained with a precision which compares favorably with the elaborate solution methods.

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