A Sensitive Method for Measuring Diffusion Coefficients in Agarose Gels of Electrolyte Solutions[1]

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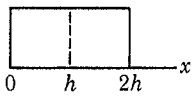
A transient-state method for measuring diffusion coefficients in agarose gels is described. Assuming agarose affects results only by increasing the length of the diffusion path and calibrating the diffusion apparatus against known diffusion coefficients, the method yields free-solution values. This assumption holds as long as the system under study does not interact appreciably with agarose. The applicability and precision of the method were demonstrated by comparing measured diffusion coefficients of NaCl, Na^+ in NaCl, and $BaCl_2$ with literature values. The lowest concentrations studied at which the method yielded free-solution values of diffusion coefficients were $C = 0.05\ M$ for D_{NaCl}, $C = 0.01\ M$ for D_{Na}, and $C = 0.5\ M$ for D_{BaCl_2}. At lower concentrations, agarose apparently interacted sufficiently with diffusing species to decrease their mobility. Where applicable, the method is very convenient and fast; 25 measurements can be made in a normal workday. The standard error of the mean of 50 determinations was generally <0.2% of the mean.

In connection with studies on diffusion of electrolytes in multicomponent aqueous solutions, it was necessary to develop a sensitive method of measuring diffusion coefficients in cells having boundary conditions as represented by cell A. The cell is closed with walls at



Cell A

$x = 0$ and $x = 2h$, where x is the direction in which diffusion occurs. The plane $x = h$ is the boundary between half-cells, each of which is initially homogeneous. These boundary conditions have been used by others to study diffusion in aqueous solution,[3] in clays,[4,5] and in beds of glass beads.[6]

In this work, the half-cell solutions are stabilized by preparing them as 2% agarose gels. Measuring diffusion coefficients in gels has experimental advantages over measuring them in free solutions; the gel suppresses convection due to mechanical and thermal disturbances and allows the boundary between half-cells to be sharply formed. If the gel is inert and diffusing species are small enough that the gel does not exert a sieving effect, then the gel should influence results only by creating a tortuous diffusion path, hence making the half-cell length, h, appear greater than it is. This is exactly what Slade, Cremers, and Thomas[7] concluded from their study of self-diffusion coefficients of Na^+ and Cs^+ (present as chlorides) in agar gels. If the only effect of the gel is to increase path length, then one should be able to use a salt of known diffusion coefficient to determine a factor which will convert diffusion coefficients measured in 2% agarose to free-solution diffusion coefficients.

It is known, of course, that agarose is not completely inert. The Bio-Rad agarose powder used in this study is specified by the manufacturer to have a sulfur content of <0.1% and hence should have a cation-exchange capacity (CEC) of <0.0625 mequiv/g; the Na CEC of our supply was determined, by isotopic dilution, to be 0.032 mequiv/g. In 0.05 N electrolyte solutions with 2% agarose, about 1.3% of the cations present should be associated with agarose. Thus, one might expect that the gel may be considered inert at electrolyte concentrations >0.05 N but not at lower concentrations. Slade, *et al.*,[7] believed that agar behaved inertly in their studies even though its CEC usually accounted for most of the cations present. The question of agarose inertness is, to our cell, analogous to the problem of inertness of the porous diaphragm for the diaphragm cell.[8,9]

In the following, we shall describe the mathematical analysis by which diffusion coefficients are computed and the diffusion apparatus and shall demonstrate the validity, precision, and accuracy of the method when used to measure salt- and self-diffusion coefficients in binary systems.

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(3) E. Cohen and H. R. Bruins, *Z. Phys. Chem.*, **103**, 337, 349, 404 (1923); **113**, 157 (1924).

(4) A. H. Bloksma, *J. Colloid Sci.*, **12**, 40 (1957).

(5) R. G. Gast, *ibid.*, **17**, 492 (1962).

(6) A. Klute and J. Letey, *Soil Sci. Soc. Amer. Proc.*, **22**, 213 (1958).

(7) A. L. Slade, A. E. Cremers, and H. C. Thomas, *J. Phys. Chem.*, **70**, 2840 (1966).

(8) R. H. Stokes, "Electrolyte Solutions," Academic Press, Inc., New York, N. Y., 1955, p 265.

(9) J. M. Nielsen, A. W. Adamson, and J. W. Cobble, *J. Amer. Chem. Soc.*, **74**, 446 (1952).

Mathematical Models

Consider a particular example of cell A in which a salt, i, is initially present at molar concentration C_i^0 on the left and $\hat{C}_i^0$ on the right. In the use of the cell, half-cells are brought together at time $t = 0$; diffusion is allowed to proceed for a given period; then half-cells are separated and the quantity of i is determined in each. We define the "fraction transferred," F_i, as the ratio of the quantity of i which has crossed the boundary $x = h$ in time t (Q_{it}), to that which would have crossed $x = h$ at infinite time ($Q_{i\infty}$); *i.e.*

$$F_i = \frac{Q_{it}}{Q_{i\infty}} = 2\frac{\hat{Q}_i - \hat{Q}_i^0}{Q_i^0 - \hat{Q}_i^0} \quad (1)$$

where $\hat{Q}_i$ is the quantity in the right half-cell at time t and Q_i^0 and $\hat{Q}_i^0$ are the quantities in the left and right half-cells at $t = 0$.

If Fick's second law

$$\frac{\partial C_i}{\partial t} = D_i \frac{\partial^2 C_i}{\partial x^2} \quad (2)$$

applies, then D_i may be computed from[10]

$$F_i = 1 - \frac{8}{\pi^2}S \quad (3)$$

where

$$S = \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp[-(2n+1)^2(\pi/2h)^2 D_i t] \quad (4)$$

In general, D_i for a salt will vary with its concentration hence the D_i computed from eq 3 will be the integral value over the concentration range C_i^0 to $\hat{C}_i^0$

$$D_i = \frac{1}{C_i^0 - \hat{C}_i^0}\int_{\hat{C}_i^0}^{C_i^0} D_i \, dC_i \quad (5)$$

For experiments in which $(C_i^0 - \hat{C}_i^0)$ is small, the computed D_i will essentially equal the differential value at the average concentration of salt in the cell, $\bar{C}_i = {}^1/_2(C_i^0 + \hat{C}_i^0)$. Except where noted, all diffusion coefficients referred to below are integral values.

Experimental Section

Diffusion coefficients of NaCl over the range $\bar{C} = 0.05$ to $\bar{C} = 1\ M$ were determined and used for calibrating diffusion cells. Initial half-cell solutions were $2\bar{C}$ M ^{22}NaCl on the left and water on the right (cell A). On the basis of the NaCl calibration, self-diffusion coefficients of Na^+ in 0.01–2 M NaCl were determined, using ^{22}Na tracer; in each case the cell consisted of $\bar{C}$ M ^{22}NaCl on the left and $\bar{C}$ M NaCl on the right. Diffusion coefficients of $^{133}BaCl_2$ were measured at $\bar{C} = 0.1$ and 0.5 M (initial half-cell solutions were $2\bar{C}$ M on left and water on right) in order to determine whether agarose influences the diffusion of a salt containing a divalent cation differently from how it does that of NaCl.

Two per cent agarose gels were prepared by adding the appropriate solution to agarose (100 ml of solution/2.209 g of agarose of 9.45% water content) in a conical flask. The flask was loosely stoppered and held in boiling water (about 10 min) to bring the agarose into solution. Gels were stored at room temperature in capped 15-ml vials until used (they were never stored more than 4 days).

Cylindrical half-cells of depth (h) 0.302 ± 0.001 cm and inside diameter 2.540 ± 0.001 cm were machined from stainless steel. Half-cells could be threaded into a stainless steel block such that when drawn tight their surfaces were flush with that of the block. Two blocks, each holding five half-cells, were used. Temperature was controlled at 25.0 ± 0.1° by circulating water from a bath through two 0.64-cm holes bored lengthwise through each block.

With half-cells positioned in a block, a vial of melted agar (3 min in boiling water) was used to overfill each of the five half-cells; the excess agarose was excluded by passing a 2 × 3 in. glass slide over each half-cell.[11] Five minutes was allowed for the agarose to gel; then glass slides were removed and a tightly fitting aluminum cover was placed over the block to prevent evaporation. At least 15 min was allowed for temperature equilibration before initiating diffusion.

Labeled half-cells (*i.e.*, those containing radioisotope) were turned up flush with their block. Unlabeled half-cells were removed from their block and placed, gel side down, on the block containing labeled half-cells, taking care to exclude air between the unlabeled gels and the surface of the block. Diffusion was initiated by sliding each unlabeled half-cell into a position directly over the corresponding labeled half-cell at a known time; block and cells were covered again.

After given times, diffusion across $x = h$ was stopped by removing the initially unlabeled half-cells. Quantities of radioisotope were determined by counting half-cells enclosed in stainless steel covers in the well of a NaI,Tl scintillation crystal. Fractions transferred were calculated from

$$F = 2\frac{\hat{Q}}{Q^0} = \frac{2\hat{R}}{R + \hat{R}} \quad (6)$$

(10) Methods of integrating such equations are given in several text and reference books, including H. S. Carslaw and J. C. Jaeger, "Conduction of Heat in Solids," Oxford University Press, London, 1959.

(11) Considerable preliminary experimentation was required to determine the best procedures to use in molding agarose in the steel half-cells and forming the boundary between opposite half-cells. Agarose must be poured when warm, and it contracts on cooling. To compensate for contraction, it was necessary to position half-cells in the blocks such that their surfaces were slightly recessed from the block surface. Reference marks were scratched on each half-cell and on the edge of each block receptacle so as to be able to position half-cells reproducibly. A circular scale, marked at 5° intervals, was used to set a half-cell to any desired position. In order to offset variations between half-cells, each was individually positioned to eliminate differences in the quantity of ^{22}NaCl–agarose contained. Using this criterion, half-cells were made identical within ±0.3%. The "average" half-cell was recessed below the block an estimated 0.009 cm before pouring agarose; this was the least recession that ensured that gels did not contract below the surface of half-cells and hence ensured gel continuity at $x = h$.

Table I: Calibration of Diffusion Cells by Comparing Observed and Free-Solution Diffusion Coefficients of NaCl, 25.0°

$\bar{C}$, M	t, min	N[a]	D_{obsd}[b]	D_{soln}[b,c]	Cell factor	Corrected D[b]	Corrected σ	Corrected SE
0.05	35.00	60	1.3980	1.5112	1.0810	1.512	0.021	0.003
0.10	35.00	50	1.3770	1.4938	1.0848	1.489	0.018	0.003
0.20	35.00	40	1.3693	1.4830	1.0830	1.481	0.015	0.002
0.50	10.00	24	1.3685	1.4797	1.0813	1.480	0.019	0.004
0.50	15.00	25	1.3674	1.4797	1.0821	1.479	0.013	0.003
0.50	25.00	25	1.3694	1.4797	1.0805	1.481	0.013	0.003
0.50	50.00	24	1.3689	1.4797	1.0809	1.481	0.008	0.002
1.00	35.00	46	1.3797	1.4901	1.0800	1.492	0.014	0.002

[a] N is the number of measurements included in the means listed in columns 4 and 7. [b] In units of 10^{-5} cm²/sec. [c] Reference 14.

where $\hat{R}$ is the net counting rate of the initially unlabeled half-cell and R is that of the initially labeled half-cell. Counting statistics were such that the standard deviation of F values attributable to counting error was usually <0.0015.

D values were computed from eq 3 and 4 using the nominal value[12] h = 0.302 cm. Computation was performed by a Hewlett-Packard Model 9100A desk computer using an iterative procedure; t and F were the only input required.

All of the data (D values previously corrected for cell variation[13]) were subjected to analysis of variance to determine whether there was variability between runs other than that expected on the basis of variation within runs—none could be detected (ratios of mean-square-between to mean-square-within averaged 0.8).

Results and Discussion

Calibration with NaCl. The results of D_{NaCl} measurements are summarized in Table I. Integral free-solution values of D_{NaCl} for each cell (column 5) were calculated from eq 5 using the following equations to represent the concentration dependence of the differential diffusion coefficients, D_{diff}

$$D_{diff} = 1.6111 - 0.9330\sqrt{\bar{C}} + 2.724C \qquad 0 \leq C \leq 0.01$$

$$D_{diff} = 1.5913 - 0.5113\sqrt{\bar{C}} + 0.5483C \qquad 0.01 \leq C \leq 0.20$$

$$D_{diff} = 1.4909 - 0.0683\sqrt{\bar{C}} + 0.0619C \qquad 0.20 \leq C \leq 2.00$$

These expressions were obtained through a least-squares multiple regression of D_{diff} on $\sqrt{\bar{C}}$ and C; the D_{diff} values were taken from Harned and Owen.[14] The average deviation of regression values from listed[14] values is 0.05%; the largest deviation is 0.20%. Cell factors, D_{soln}/D_{obsd}, are the factors by which D_{obsd} values must be multiplied to yield free-solution values. The mean cell factor is 1.0816 and the standard error of the mean (SE) is 0.0006; this SE is almost exactly that expected on the basis of the precision of the mean D_{obsd} values. Constancy of the cell factor is evidence that agarose acts only to lengthen the diffusion path. The last three columns of Table I list the D_{obsd}, σ_{obsd}, and $(SE)_{obsd}$ values corrected by the factor 1.0816. It is evident that corrected D_{obsd}'s are in excellent agreement with free-solutions D's.

In order to evaluate whether there was significant initial mixing at the plane $x = h$, D_{NaCl} was measured for 0.5 M NaCl at four different time periods. If initial mixing takes place, D should decrease with time. The variation between D's measured at different times is insignificant and in any case is in the wrong direction to be explained by mixing—a linear least-squares regression of D *vs.* $1/t$[15] resulted in a zero-time correction of −0.7 sec.

Self-Diffusion of Na in NaCl. Sodium self-diffusion results are summarized in Table II. These values are in good agreement with those obtained by Mills using a diaphragm cell;[16] Mill's values are approximately 0.5% lower than ours. The two sets of data are presented in Figure 1, where the curve was drawn for our data, and assuming the curve was linear above $\bar{C}$ = 0.5.

(12) The 0.302-cm depth must be considered nominal. Because of the technique used in filling half-cells (see ref 11), 0.302 cm represents the minimum half-cell depth; gels actually protruded slightly above the steel half-cells.

(13) Despite having adjusted the settings of half-cells in order to eliminate differences between them (see ref 11), it became evident after many diffusion runs that there remained small, but statistically highly significant, variability between cells. Since all results are based on the means of many determinations, averaged across runs and cells, the cell variability would not affect the final average if there were no missing data. However, our experience has been that about 3–4% of measurements must be thrown out due to bubbles in the gels, accident, or outliers (if an individual D value was $>3\sigma$ from the mean, it was declared an outlier). Therefore, on the basis of about 50 runs, involving 50 determinations in each of the five cells, correction factors were calculated to make *each* cell yield the mean of *all* cells; the factors (applied to measured D's) ranged from 0.995 to 1.007. Besides allowing one to use runs in which there were missing data, the elimination of cell variability in this manner yields a better estimate of random error associated with the method.

(14) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solutions," Reinhold Publishing Corp., New York, N. Y., 1963, p 255. D's at rounded concentrations are based on the work of V. Vitagliano and P. A. Lyons, *J. Amer. Chem. Soc.*, **78**, 1549 (1956).

(15) L. G. Longsworth, *ibid.*, **69**, 2510 (1947).

(16) R. Mills, *ibid.*, **77**, 6116 (1955).

Table II: Self-Diffusion Coefficients of Na in NaCl, Corrected to Free-Solution, 25.0°

$\bar{C}$, M	D_{obsd}[a]	σ	SE
0.01	1.323	0.016	0.002
0.05	1.315	0.020	0.003
0.10	1.310	0.011	0.002
0.30	1.299	0.014	0.002
0.50	1.285	0.015	0.002
0.70	1.263	0.015	0.001
1.00	1.239	0.014	0.002
2.00	1.140	0.012	0.002

[a] In units of 10^{-5} cm²/sec.

Table III: Diffusion Coefficients of $BaCl_2$, Corrected to Free-Solution, 25.0°

$\bar{C}$, M	D_{obsd}[a]	σ	SE	D_{soln}[a]
0.1	1.128	0.019	0.003	1.173
0.5	1.162	0.017	0.003	1.164

[a] In units of 10^{-5} cm²/sec.

Diffusion of $BaCl_2$. The results of D_{BaCl_2} measurements are given in Table III, as are the free-solution values from Harned and Owen.[14] The equations relating D_{diff} to C_{BaCl_2} for use in eq 5 were obtained as described above for NaCl, and are

$$D_{diff} = 1.3855 - 2.396\sqrt{\bar{C}} + 9.245C \qquad 0 \leq C \leq 0.01$$

$$D_{diff} = 1.3222 - 0.9941\sqrt{\bar{C}} + 1.522C \qquad 0.01 \leq C \leq 0.10$$

$$D_{diff} = 1.1811 - 0.1063\sqrt{\bar{C}} + 0.1029C \qquad 0.10 \leq C \leq 1.00$$

While the agreement at $\bar{C} = 0.5\ M$ is within our experimental error, our value at $\bar{C} = 0.1\ M$ is lower than the solution value by much more than can be attributed to random experimental error.

Limitations and Advantages of the Method. The assumption that agarose affects D_{obsd} only by introducing tortuosity seems to hold as long as solutions are sufficiently concentrated that interaction between the salt (or its ionic constituents) and agarose is negligible. Our results indicate that interaction is negligible for 1:1 electrolytes as long as $\bar{C} > 0.05\ M$; the lower concentration limit ($\bar{C} = 0.05\ M$) at which the method yields free-solution D's corresponds to that of the diaphragm cell. The $BaCl_2$ results indicate that the applicability of the method for other than 1:1 electrolytes is more limited. We are not aware of any diaphragm-cell results for salts of divalent cations, but Stokes has suggested that the diaphragm cell limit may be at higher concentrations for these.[8]

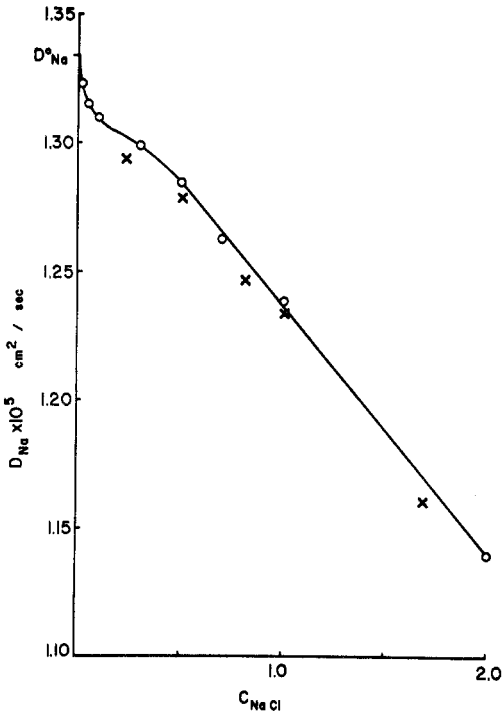

Figure 1. Self-diffusion coefficients of Na^+ at 25.0°: O, this work; ×, Mills.[16]

Diffusion coefficients measured are integral values. One could, of course, obtain differential values by keeping the quantity $(C_i^0 - \hat{C}_i^0)$ small; experimental precision will decrease as $(C_i^0 - \hat{C}_i^0)$ becomes smaller.

The method is very convenient and fast; 25 determinations (five runs) can be made in a normal workday. By obtaining large numbers of measurements (about 50), D can be estimated to a high degree of accuracy—estimated standard errors were generally <0.2% of the mean.