THEORY OF DIFFUSION IN GELS

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ABSTRACT It has been shown that when concentration of solute is expressed as amount per unit volume of gel—solvent—solute system, Fick's laws for diffusion in a gel take the same form as for diffusion in solvent alone, except that the usual coefficient must be replaced by a new coefficient, D', equal to $D(1 - \alpha\varphi)/(1 - \varphi)$, where φ is the effective volume fraction of the gel substance and α is a coefficient of obstruction equal to 5/3 if the gel substance can be considered to be made up of randomly oriented rods. An equation was derived for the total amount of solute entering the gel, which is analogous to but not identical with the equation for the total amount of solute crossing the initial boundary in free diffusion. The effect of slice thickness was investigated by a mathematical procedure involving the solutions of approximate differential equations. It was shown that even for slices so thick that 95 per cent of the solute in the gel is contained in the first two, a correction factor equal to the square of the slice thickness divided by $48D't$ permits one to obtain accurate measurements of D' from the mean concentration and the position of the midplane of the slice.

INTRODUCTION

It is sometimes necessary to determine diffusion coefficients of biologically important solutes in extremely dilute solutions. The usual method of free diffusion at an interface is ill adapted to such measurements because of the essentially uncontrollable hazard of convection. One way to circumvent this difficulty is to allow the solute to diffuse into or out of a gel.

Many investigators have used diffusion in gels as a means of determining diffusion coefficients. In general, the results obtained are within 5 or 10 per cent of those obtained by the method of free diffusion. The purpose of the present communication is to examine the relevant aspects of the theory of diffusion in gels for the purpose of ascertaining whether greater precision is possible in principle.

Two convenient methods of determining diffusion coefficients both involve bathing the exposed surface of a gel in a cylindrical vessel with a constant concentration of solute. The coefficient of diffusion can be determined either from the total amount of solute which penetrates the gel in a given time or by slicing the gel at the end of the experiment and measuring the concentration of solute as a function of distance from the interface. For both methods, two questions must be answered. How does one take into account the effective volume occupied by the gel substance? In what way does the gel substance obstruct the diffusion of solute? These two questions are somewhat related. The first must be answered both to permit one to specify what is meant by solute concentration in the gel and in order to answer the second. A third question arises in connection with the technique of determining diffusion coefficients by analyzing successive slices of the gel. What error is introduced as a result of finite slice thickness?

The Obstruction Effect. There are three ways a gel might interfere with the diffusion of solute. The first is strong binding of the solute by the gel substance. In the subsequent treatment, this kind of obstruction is neglected; the results, therefore, are valid only for systems in which binding does not occur. A second type of obstruction would be observed if the gel network were so fine that the effective pores were smaller than the solute particles, in which case the gel would be a filter. For agar agar gels, at least, this type of obstruction is probably not important. Preliminary observations carried out by Mr. Allen T. Ansevin in the author's laboratory indicate that *E. coli* bacteria can penetrate a 1.5 per cent agar agar gel under the influence of an electric field. Such gels, therefore, should not act as filters for smaller particles such as viruses, proteins, etc. A third type of obstruction is to be expected. The solute particles must detour around the gel substance particles or threads, thereby causing an effective increase in path length for the diffusing solute or an effective decrease in the coefficient of diffusion.

In early work on diffusion in gels, this third effect was assumed to be negligible, but the questionableness of this assumption was pointed out by Williams and Cady (1). This obstruction effect is an old problem of nineteenth century physics and is involved in the Poisson theory for induced magnetism, the Lorentz-Lorenz theory for the index of refraction, and the Clausius-Mosotti theory for the dielectric constant of dispersed systems. The application to diffusion of solute in a constant solute gradient around an obstacle is the exact physical analog of the conduction of an electric current in a vessel containing non-conducting obstacles. Important contributions to this theory were made by Lord Rayleigh (2) and more recently by Fricke (3). Specific application to diffusion was considered by Wang (4) without, however, placing the problem within its historical perspective. The decrease in conductivity or in diffusion coefficient, as the case may be, is, in dilute systems, proportional to the volume fraction occupied by the gel substance. This can be expressed by the relationship, $D_g = D(1 - \alpha\phi)$, where D_g is the coefficient of diffusion in the gel, D is the coefficient of diffusion in the solvent involved in the gel, ϕ is the ratio of the volume occupied by the gel substance, that is, the obstructing material, to the total volume of the gel, and α is a constant which depends upon the shape and the orientation of the obstructing particles. For spheres, α has a value of 3/2; for very long thin rods perpendicular to the gradient, α has a value of 2; for such rods, or for ellipsoids of revolution with an axial ratio of infinity, randomly oriented, α has a value of 5/3. Values of α for ellipsoids with different axial ratios have been presented by Wang and can easily be inferred from the treatment by Fricke. The simple linear correction is inadequate for concentrated obstructing material, but values of $(1 - \alpha\phi)$ are accurate to 0.4 per cent for randomly oriented rods when ϕ is 0.05. At least partial experimental confirmation of the theory is provided by the results of Oker-Blom (5) on the conductivity of suspensions of sand in gelatin gels. Similar and, in some ways, better confirmation is afforded by studies of Fricke and Morse (6) on the electrical conductivity of cream.

Fick's First and Second Laws. Fick's first law with appropriate modification for the obstruction effect takes the form of Equation 1. The symbol,

$$dS = -D(1 - \alpha\phi)Q(dc/dx)\,dt \tag{1}$$

dS, means the amount transported across an area, Q, in time, dt, when the concentration gradient is a constant equal to dc/dx and the coefficient of diffusion in the absence of gel is D.

The meaning of the symbol α has already been discussed in detail. It is necessary at the moment to consider the meanings of dc/dx and of ϕ. In dc/dx of Equation 1, c means amount of solute per unit volume of liquid, exclusive of gel substance. It is often convenient experimentally and it leads to simple relationships theoretically to express the concentration of solute in terms of amount per total volume of the system, solute, solvent, and gel substance. If concentration thus expressed is represented by c', the relationship $c' = c(1 - \phi)$ holds. Fick's first law for diffusion in the presence of an obstructing material like a gel can then be written in the form of Equation 2, in which D', the effective coefficient of diffusion in the gel, is equal to D, the coefficient of diffusion in the absence of gel, multiplied by $(1 - \alpha\phi)/(1 - \phi)$.

$$dS = -D\frac{(1 - \alpha\phi)}{(1 - \phi)}Q(dc'/dx)\,dt = -D'Q(dc'/dx)\,dt \tag{2}$$

The meaning of ϕ remains to be clarified. It is the fraction of the total volume of the system from which solute is excluded. In the simplest case, this is the fraction of the total volume of the system occupied by the obstructing material. However, when the interaction between the obstructing material, in our case the gel material, and the other components of the system is complex, ϕ will have a different meaning. The value of ϕ can be determined experimentally by some such means as freezing point depression when a solute is dissolved in the gel. In this manner Schantz and Lauffer (7) showed that the value of ϕ for a 1.5 per cent agar agar gel is approximately 0.05 when the solute is sodium chloride. Since freezing point depression measurements can be made only with very low molecular weight solutes, it is necessary to assume that the volume not free to dissolve the solute of interest is the same as that not free to dissolve the low molecular weight material. There are circumstances under which this assumption might lead to error.

In principle, $\alpha\phi$ can be determined by conductivity measurements; such determinations are simple only when the obstructing substance is a perfect insulator. Furthermore, it is necessary in this instance also to assume that the obstructing volume fraction, ϕ, is the same when the solute is the electrolyte used in the conductivity measurement and when it is the substance of interest in a diffusion experiment.

Fick's second law, as applied to diffusion in a gel, can be derived from Fick's first law as expressed in Equation 2 by the usual method of calculating the amounts moving into and out of an infinitesimal section. When the definitions involved in Equation 2 are adhered to, Equation 3 is obtained, which is of exactly the same form as Fick's second law as applied to diffusion in the absence of gel. When this equation is integrated for the boundary conditions that the interface at $x = 0$ is bathed by a constant concentration of solute, c_o, that the gel is infinitely thick, and that the concentration at $t = 0$ is 0 for all values of x within the gel, Equation 4 is obtained. It must be remembered that c_o' in Equation 4 is equal to $c_o(1 - \phi)$. Essentially constant c_o' at $x = 0$ can be attained experimentally by stirring continuously a volume of external solution large enough to contain an amount of solute much greater than the total which diffuses into the gel. When the density of the solution is significantly different from that of the solvent, the experimental arrangement can be such that convection maintains the original concentration at the gel surface. For example, when the density of the solution is considerably greater than that of the solvent, convection will maintain the original concentration at the interface when it is below the solution.

$$\partial c'/\partial t = D' \, \partial^2 c'/\partial x^2 \tag{3}$$

$$dc'/dx = (-2c_o'/\sqrt{4\pi \, D't})e^{-x^2/4D't} \tag{4}$$

Total Solute Entering Gel. One of the methods of determining the coefficient of diffusion of a solute is to measure the total amount of solute, ΔS, which penetrates a gel in time, t, when a plane surface of area Q is bathed with solute of constant concentration, c_o. Such experiments can be carried out in a Petri dish. Care must be taken to maintain the bathing concentration constant either by replenishing the solute or by supplying originally much more than the total amount expected to penetrate and assuring constant mixing. It is desirable to cut a plug excluding the gel at the very edge of the Petri dish because of the meniscus. The thickness of the gel should exceed $(3/2)\sqrt{2D't}$. Under these circumstances almost no solute entering at the interface will diffuse back out.

The relationship between ΔS and D' can be derived by substituting the value of x at the interface between gel and solution, 0, into Equation 4 and by then substituting the resulting expression for dc'/dx into Equation 2 to yield Equation 5. Upon integration, one obtains Equation 6. Again it must be remembered that c_o' is equal to $c_o(1 - \phi)$. Polson has derived a similar expression, but without the factor 2, for diffusion without stirring across a plane (8).

$$dS = \frac{\sqrt{D'}\,Qc_o'\,dt}{\sqrt{\pi}\,\sqrt{t}} \tag{5}$$

$$\Delta S = \frac{2c_o'Q\,\sqrt{D't}}{\sqrt{\pi}} \tag{6}$$

Slicing Technique. Diffusion coefficients can also be determined by bathing an exposed, flat surface of a cylindrical block of gel with the solution and by then measuring the concentration of solute in the gel at various distances from the surface after time, *t*, of diffusion. A convenient experimental arrangement was recently developed by Schantz and Lauffer (7). After diffusion, the gel is extruded and cut into slices which are then analyzed by whatever method is appropriate to the solute under study.

If one defines z by Equation 7 and then substitutes into Equation 4, one obtains Equation 8, which can be integrated to give Equation 9. From the measured concentration within a slice, c', divided by the appropriately defined concentration in

$$z = x/\sqrt{2\,D't} \tag{7}$$

$$dc'/dz = \frac{-2c_o'}{\sqrt{2\pi}}\,e^{-z^2/2} = -Ke^{-z^2/2} \tag{8}$$

$$c'/c_o' = 2/\sqrt{2\pi}\int_z^\infty e^{-z^2/2}\,dz \tag{9}$$

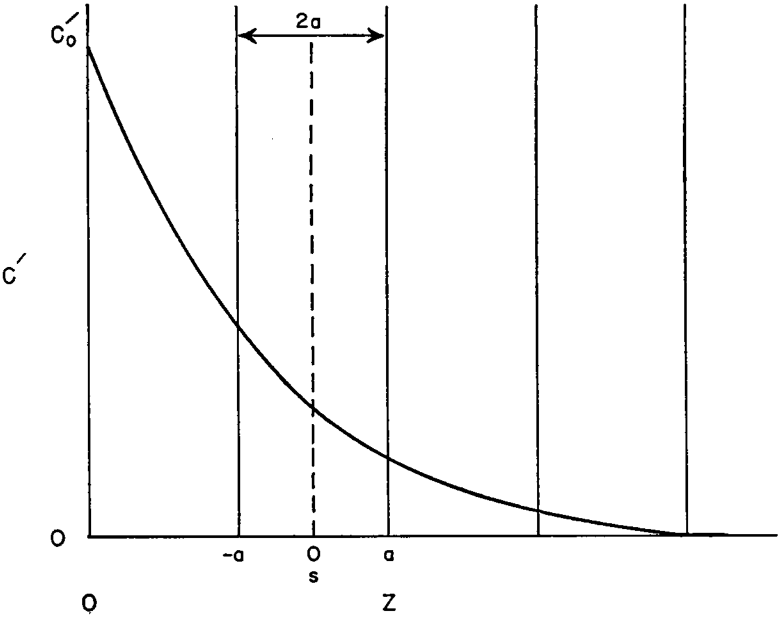

FIGURE 1 Relationship between c' and z in a gel cut into four slices, each of thickness $2a$.

the bathing solution, c_0', one can determine the value of z for a particular slice by using readily available tables of integrals of the normal curve. From the value of z thus determined, the distance from the interface to the characteristic position of the slice, x, and the time, t, one can determine D' by using Equation 7. An alternative procedure is to plot c'/c_0' against x on probability paper. This is in reality a plot of z against x for a particular time, t, from which D' can be evaluated.

The relationship between c' and z is curvilinear, somewhat as illustrated by the curve of Fig. 1. The mean concentration in a slice will approximate closely the concentration at the midplane only for extremely thin slices. Since it is customary to take the position of the midplane as the characteristic distance of the slice from the interface, an error is thereby introduced when thick slices are cut. Methods for determining diffusion coefficients from analyses on thick sections have been presented in tabular form (9-11). It is, however, possible to solve this problem analytically.

If one divides the gel into slices of equal thickness, as illustrated in Fig. 1, and considers the ith slice in a sequence in which 1 represents that adjacent to the gel-solution interface, as illustrated in Fig. 2, one can transform the abscissa with the

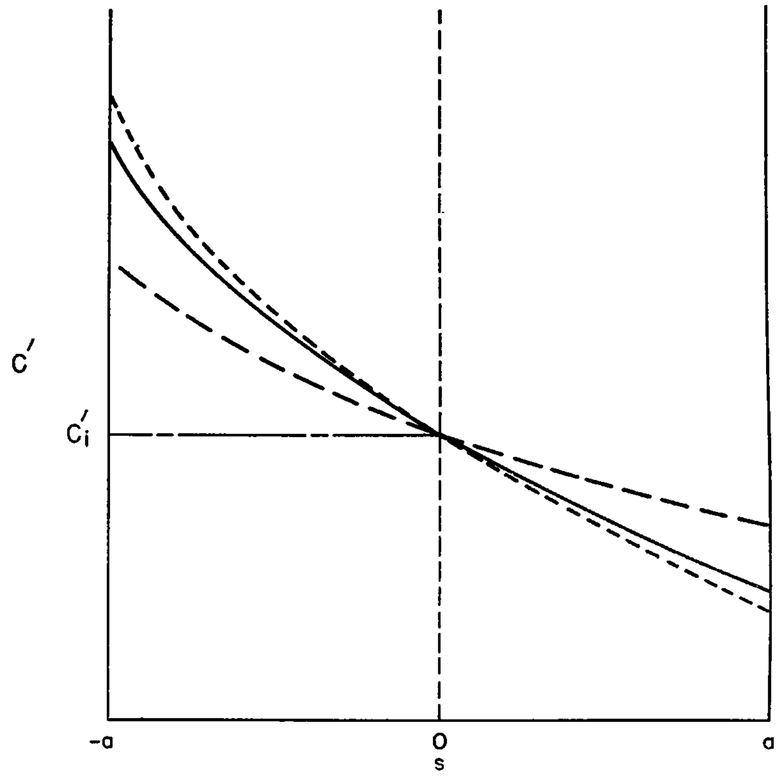

FIGURE 2 Relationship between c' and s in the ith slice of a gel. Solid line, integral of Equation 11a. Short-dashed line, Equation 12b. Long-dashed line, Equation 12c.

aid of Equation 10, where a is half the thickness of the slice measured in z units,

$$z = ba + s = ba(1 + s/ba) \tag{10}$$

b is $(2i - 1)$, and s can have values between $+a$ and $-a$. It is evident that ba is the z coordinate of the midplane of the slice. When this transformation is applied to Equation 8 one obtains Equation 11a. This equation cannot be integrated in terms of elementary functions, but two approximate differential equations, both capable

$$dc'/ds = -Ke^{-b^2a^2/2}\,e^{-bas}e^{-s^2/2} \tag{11a}$$

$$dc'/ds \geq -Ke^{-b^2a^2/2}\,e^{-bas} \tag{11b}$$

$$dc'/ds \leq -Ke^{-b^2a^2/2}\,e^{-a^2/2}e^{-bas} \tag{11c}$$

of such integration, can be written. Equation 11b was obtained by dropping the exponential involving s^2. This equation is accurate near the center of the slice where the values s are small. The other approximation, represented by Equation 11c was obtained by substituting a^2 for s^2. This equation yields the correct value for the gradient at the two outer edges of the slice. Equations 11b and 11c can be integrated

$$c' = c_i' - M(1 - e^{-bas}) \simeq c_i' - Mbas \tag{12b}$$

$$c' = c_i' - Me^{-a^2/2}(1 - e^{-bas}) \tag{12c}$$

to yield Equations 12b and 12c where c_i' is the concentration at the midplane and M is defined by the relationship, $M = (2c_0'/\sqrt{2\pi})\,(1/ba)e^{-b^2a^2/2}$. Equation 12$b$ is represented diagrammatically by the short-dashed curve of Fig. 2 and Equation 12c by the long-dashed curve. The simplified approximation of the right member of Equation 12b was obtained by expanding the exponential and dropping all terms above first order. The simplified form is accurate for small values of s, that is, near the center of the slice.

The mean value of c' can be calculated from Equations 12b and 12c by dividing the integral of $c'ds$ evaluated from $-a$ to $+a$ by the integral of ds evaluated from $-a$ to $+a$. In this way, Equations 13b and 13c were obtained. The mean concentrations with the subscripts b and c are the means calculated for the relationships

$$\bar{c}_a' \leq \bar{c}_b' = c_i' - M + (M/ba^2)\sinh(ba^2) \simeq c_i' + M(b^2a^4/6) \tag{13b}$$

$$\bar{c}_a' \geq \bar{c}_c' = c_i' - Me^{-a^2/2} + (M/ba^2)e^{-a^2/2}\sinh(ba^2) \simeq c_i' + (Me^{-a^2/2})(b^2a^4/6) \tag{13c}$$

described by Equations 12b and 12c, while the mean concentration with the subscript a is the actual mean concentration in the slice, of which the former are separate approximations, one too large and one too small. The simplified versions of the right members of Equations 13b and 13c were obtained by expanding the hyperbolic sine and dropping terms higher than third order. Since the hyperbolic sine expands into a rapidly converging series, these approximations are quite accurate. Equations 14*b* and 14*c* were obtained by substituting Equations 13*b* and 13*c*, respectively, into Equation 12*b*, in order to find the value of s representative of a concentration, c', equal to the mean concentration. Equation 12*b* was used instead of 12*c* for both operations because 12*b* is nearly exact for positions near the center of the slice. Since $e^{-a^2/2}$ rapidly approaches unity as a diminishes below values of 1/2, Equation 14 can be written instead of 14*b* and 14*c*. Equations 14, 14*b*, and

$$\frac{-s_{\bar{c}'}}{ba} \leq \frac{a^2}{6} \tag{14b}$$

$$\frac{-s_{\bar{c}}}{ba} \geq \frac{a^2}{6} e^{-a^2/2} \tag{14c}$$

$$\frac{a^2}{6} \simeq \frac{-s_{\bar{c}'}}{ba} \tag{14}$$

14*c* serve not only to specify the position in the slice characteristic of the mean concentration, but also to indicate that the uncertainty of this position is vanishingly small for values of a much below 1/2.

The product ba in Equation 10 is the z coordinate of the midplane of the slice, which can be represented by the symbol z_m. If $z_{\bar{c}'}$ represents the position corresponding to the mean concentration, then, from Equations 10 and 14, one can obtain Equation 15.

$$z_{\bar{c}'} = z_m\left(1 - \frac{a^2}{6}\right) \tag{15}$$

Since Equation 7 holds for z_m and x_m, the z and x values of the midplane, it can be combined with Equation 15 to obtain Equation 16.

$$\sqrt{2\,D't} = \frac{x_m}{z_{\bar{c}'}}\left(1 - \frac{a^2}{6}\right) \tag{16}$$

In Equation 16, the symbol, a, means the half-thickness of the slice measured in z units. If we define p as the total slice thickness measured in x units and utilize Equation 7, we obtain Equation 17.

$$\frac{\sqrt{2\,D't}}{1 - p^2/(48\,D't)} = \frac{x_m}{z_{\bar{c}'}} \tag{17}$$

The remarkable thing about Equations 15, 16, and 17 is that they are essentially independent of the position of the slice, that is, of the value of i. The truth of this assertion can be ascertained by considering Equations 11 *a*, *b*, and *c*. When i and b are large, Equations 11*b* and 11*c* approach equality to each other and, therefore, to 11*a*. When i is small, for example, one, then the exponents in Equations 11*a*, *b*, and *c* differ considerably; however, it is one of the properties of the normal curve that the ordinate, represented by dc'/dz in this case, is quite insensitive to changes in the abscissa near the origin. Therefore, for small values of i the gradients described by Equations 11*a, b,* and *c* do not differ greatly, even though the exponents do.

A slice thickness of a equal to 1/2 in z units, the upper limit for the safe replacement of Equations 14*b* and 14*c* by 14 and, therefore, of the reasonable accuracy of Equations 16 and 17, means a slice thickness of one standard deviation unit in terms of the normal curve. With such a slice thickness, approximately 95 per cent of all of the diffused solute will be found in the first two slices. This means, then, that Equations 16 and 17 can be used with reasonable accuracy for the determination of D' even if the slices are cut so thick that almost all of the diffused solute is found in the first two.

Interpretation of a gel-slicing diffusion experiment is carried out in the following manner. The slice of total thickness, p, is analyzed and c', the amount of solute per unit volume of gel system, is obtained. The concentration per unit volume of solution bathing the gel is multiplied by $(1 - \varphi)$ to yield c_0'. From c'/c_0', the value of z is obtained with the aid of Equation 9 and either a table of integrals of the normal curve or probability paper. Next, either from the ratio of the distance from the interface of the midplane of the slice, x_m and the z value thus obtained, symbolized as $z_{\bar{c}'}$, or from the slope of the plot on probability paper, D' is evaluated by using Equation 17. The diffusion coefficient thus obtained is corrected for the effect of gel by dividing by $(1 - \alpha\varphi)/(1 - \varphi)$ to give, D, the coefficient of diffusion in the absence of a gel.

Diffusion of several test substances into 1.5 per cent agar agar gels was studied by Schantz and Lauffer (7). Values of D' were obtained in the manner just indicated. Values of D calculated from them by setting α equal to 5/3, the theoretical value for randomly oriented rods, and φ equal to 0.05, the value determined experimentally for 1.5 per cent agar agar gels by the freezing point method, were in very good agreement with those in the literature for free diffusion.

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