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# The effect of cell size and shape on the resistance of unstirred layers to solute diffusion

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**The conventional flat plate model used to relate diffusion resistance to the thickness of the unstirred layer was shown to be inappropriate for microorganisms. Alternate models were developed, and they predict that diffusion resistance decreases as cell size decreases and that it depends on the shape of the cell.**

The rate of uptake of substances by cells may be determined by the rate of diffusion through an unstirred layer of water adjacent to the surface. The effect of the unstirred layer is usually quantified with a permeability coefficient, $P$, a resistance parameter, $R$, or a thickness parameter, $\Delta x$ [1–6]. Dainty [2] defined $P$ as diffusivity/thickness of the unstirred layer, i.e. $D/\Delta x$. By definition diffusion resistance, $R$, is the reciprocal of permeability, hence $R = \Delta x/D$ in Dainty's model. The parameter $\Delta x$ was operationally defined as the width of a uniform concentration gradient equal to the gradient at the cell surface. Although this hypothetical uniform gradient was a simplification of the real gradient across the unstirred layer, it was not unreasonable for diffusion to a flat surface. In the ideal situation of a truly discrete outer bound to the unstirred layer, the gradient would, in fact, be constant across the layer. Consequently, estimates of $\Delta x$ are often treated as rough estimates of the physical width of the unstirred layer.

For surfaces with a high degree of curvature with respect to the physical width of the unstirred layer the gradient in the ideal situation would not be constant, and the operationally defined thickness is no longer even a crude approximation of the physical thickness. Consequently, the formula for diffusion resistance derived from Dainty's definition of $\Delta x$ can yield very misleading results for small cells if $\Delta x$ is treated as if it were the physical width of the unstirred layer. Suitable formulae can be derived for various shapes, however, from a general definition for diffusion resistance:

$$R = \frac{C_o - C_s}{J} \quad (1)$$

where $J$ is the surface specific flux and $C_o$ and $C_s$ are the concentrations in the bulk phase and at the surface of the cell, respectively. Formulae for $J$ are obtained by solution of Fick's second law under steady-state conditions. Equations for spheres and cylinders of infinite length were obtained from Crank [7] and for prolate and oblate spheroids from Pasciak and Gavis [8]. These equations contain $C_o$ and $C_s$ as boundary conditions but after substitution into Eqn. 1, these variables can be eliminated. The resulting formulae (Table I) give diffusion resistance in terms of cell size and a parameter for the thickness of a 'shell' of water around the cell through which a substance must diffuse. Like the flat plate model it is assumed that the outer 'edge' is a uniform, discrete distance from the cell. The limitations that this assumption impose are discussed below.

Unstirred layers of five to several hundred microns thick have been reported [1,9–14], and the

behavior of the functions in Table I have been studied over this range. Some examples are presented in Fig. 1, and it is quite evident that the relationship between $\Delta x$ and $R$ differs markedly between the various geometric shapes. Unlike the flat plate model the diffusion resistance of spheroids does not increase infinitely as the thickness of the unstirred layer increases. For these shapes a limiting resistance is reached when $\Delta x$ is approx. 10-times the cell radius. This limiting resistance can be calculated from the formulae in the last column of Table I.

In conventional usage and in the present derivations it was assumed that the thickness of the unstirred layer was a uniform, discrete distance. In reality the outer 'edge' of the unstirred layer is a diffuse region in which mixing with the bulk phase decreases as the distance to the surface of the cell decreases. Consequently, the formulae that relate the thickness of the unstirred layer to diffusion resistance should be treated primarily as mathematical constructs which describe the behavior of ideal systems rather than as predictive formulae. However, the formulae for limiting resistances may yield, in certain instances, reasonably accurate predictions for real systems. These formulae do not contain a term for the thickness of unstirred layer. For these equations, the assumption of a uniform discrete boundary is irrelevant, and the formulae should be applicable to real systems when the unmixed zone is sufficiently large that the limiting resistance has been reached.

Unfortunately, for cell suspensions the relationship between $\Delta x$, cell size and mixing conditions is unknown. Most work on diffusion resistance has been performed with flat membranes [9,10,13,14]. In these systems the unstirred layer has a minimum thickness of about 30 $\mu$m under vigorously stirred conditions [9], but it is substantially thicker ($\sim$ 100 $\mu$m) in gently stirred solutions [1,9,10, 12–14]. Assuming that these estimates are applicable to cell suspensions one would predict that the diffusion resistance of any cell with a radius less than about 3 $\mu$m (1/10 of the minimum thickness) would be independent of mixing conditions and could be calculated with the formulae for the limiting resistance. For larger cells the formulae would be applicable in gently stirred solutions, but vigorous mixing would cause the real diffusion resistance to differ from the predicted value. The major uncertainty here concerns the applicability of the estimates of $\Delta x$ obtained with flat mem-

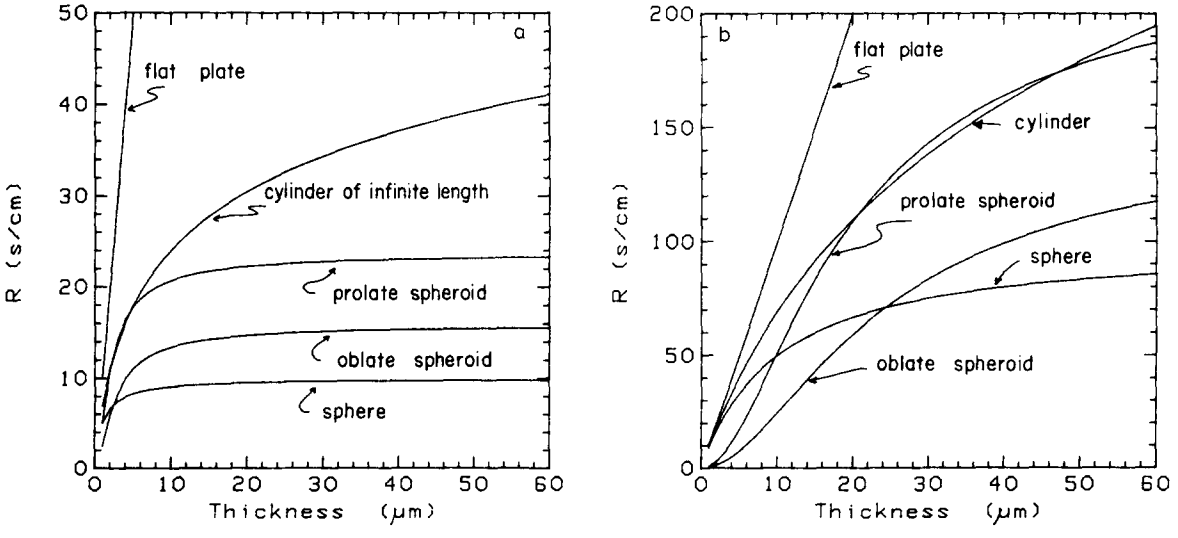


Fig. 1. Diffusion resistance calculated from the models presented in Table I. Diffusion resistance to curved surfaces is much less than to a flat surface, and in the case of spheroids the resistance does not increase infinitely as in the flat plate model. (a) For the cylinder and sphere a radius of 1 $\mu$m was assumed; for the prolate and oblate spheroids the major and minor radii were 2 and 1 $\mu$m, respectively. (b) Same as (a) except that dimensions were increased 10-fold. Note the vertical scale difference between (a) and (b). $D = 10^{-5}$ cm/s.

TABLE I

FORMULAE FOR ESTIMATING THE RESISTANCE OF UNSTIRRED LAYERS TO SOLUTE DIFFUSION TO VARIOUSLY SHAPED SURFACES

See text for explanation.

| Model | Approximate formula for $R$ assuming $\Delta x$ is a discrete distance | Eqn. | Formula for $R$ assuming $\Delta x \gg x_s$, $x_1$ or $x_2$ | Eqn. |
|---|---|---|---|---|
| Flat plate | $R = \frac{\Delta x}{D}$ | (2) | – | |
| Cylinder of infinite length | $R = \frac{x_s}{D} \ln\left(\frac{x_s + \Delta x}{x_s}\right)$ | (3) | – | |
| Sphere | $R = \frac{x_s \Delta x}{(x_s + \Delta x) D}$ | (4) | $R = \frac{x_s}{D}$ | (4a) |
| Prolate spheroid | $R = \frac{A}{8\pi D x_1 e}\left[\ln\left(\frac{1+e}{1-e}\right) - \ln\left(\frac{\sqrt{\Delta x^2 + x_1^2} + x_1 e}{\sqrt{\Delta x^2 + x_1^2} - x_1 e}\right)\right]$ | (5) | $R = \frac{A}{8\pi D x_1 e} \ln\left(\frac{1+e}{1-e}\right)$ | (5a) |
| Oblate spheroid | $R = \frac{A}{4\pi D x_1 e}\left[\tan^{-1}\left(\frac{x_1 e}{x_2}\right) - \tan^{-1}\left(\frac{x_1 e}{\sqrt{\Delta x^2 + x_2^2}}\right)\right]$ | (6) | $R = \frac{A}{4\pi D x_1 e} \tan^{-1}\left(\frac{x_1 e}{x_2}\right)$ | (6a) |

$D$, is the diffusion coefficient.
$\Delta x$, is the thickness of the unstirred layer.
$x_s$, is the radius of the cell (Eqns. 3 and 4).
$x_1$, and $x_2$ are the major and minor axes (Eqns. 5 and 6).
$e$, is eccentricity, $e = \sqrt{1 - (x_2^2/x_1^2)}$.
$A$, is the area of the spheroid
for Eqn. 5: $A = 2\pi x_1^2 + 2\pi \frac{x_1 x_2}{e^2} \sin^{-1} e^2$
for Eqn. 6: $A = 2\pi x_1^2 + 2\pi \frac{x_2^2}{e^2} \ln \sqrt{\frac{1+e^2}{1-e^2}}$.

branes to cell suspensions. Sha'afi et al. [11] reported an unstirred layer of 5 $\mu$m for red blood cells which suggests that the unstirred layer of cell suspensions is much less than for flat membranes. However, this method was based on a mathematical model for diffusion to a flat surface. As shown above, the relationship between diffusion resistance and $\Delta x$ for spheroids is markedly different than for a flat surface. Also, Miller [4] pointed out that the cell density imposes an upper limit to the thickness of the unstirred layer. To obtain unstirred layers greater than 100 $\mu$m fairly dilute cell suspensions must be used.

A comparison of diffusion resistance calculated with formulae in Table I to the measured resistance for *Anacystis nidulans* [15,16] suggests that the formulae do yield reasonably accurate predictions of diffusion resistances. The bacciliform shape of this organism is intermediate between a prolate spheroid and a cylinder. The limiting resistance was calculated for a prolate spheroid of the same surface area as the cell, an eccentricity determined by its long and short axes and with $D = 1.1 \cdot 10^{-5}$ cm$^2$/s. A value of 26.3 s/cm was obtained. For a cylinder of infinite length, $R$ depends on $\Delta x$, and I have used the results from flat membranes as a guide. Since the medium was gently stirred, $\Delta x$ values of 100 to 200 $\mu$m seemed reasonable, and these values yielded $R$ values of 30 to 34 s/cm. Bearing in mind that the results from these two models should represent the extremes, one would predict that diffusion resistance for *Anacystis* should lie between 26 and 34 s/cm. The mean ($\pm$S.E.) of four experiments was 29.9 ($\pm$0.7) s/cm [15,16]. Although the agreement between the theoretical prediction and the estimate for this one organism may be fortuitous, these results are certainly encouraging.

A variety of qualitative and quantitative predictions may be deduced from the formulae for limit-

ing resistance which could be used to further test the models. For a sphere the resistance should be proportional to the radius of the cell. Since spherical bacteria, cyanobacteria, and algae are common, it should be possible to find a series of spherical miroorganisms for which diffusion resistance could be measured using the technique described by Mierle [15]. If Equation (4a) is correct diffusion resistance should be a linear function of the cell radius, and the slope should equal the reciprocal of the diffusivity. The effect of cell shape may be more difficult to test quantitatively because cells that are exactly prolate or oblate spheroidal in shape are less readily available. However, it can be deduced from Equations (5a) and (6) that the diffusion resistance of a prolate spheroid should be greater than that of a sphere of equivalent volume, whereas the diffusion resistance of an oblate spheroid of eccentricity $> 0.92$ should be slightly less than or equal to a sphere. These predictions could be tested by comparing the measured diffusion resistance of baccillar and discoid shaped cells, respectively, to the limiting resistance predicted for a sphere of equivalent volume. A few complicating factors should always be kept in mind, however. Many microorganisms have gelatinous or membranous sheaths, fibrillar coatings, or cell walls which differ from organism to organism. The diffusivity in these coatings may differ from the diffusivity in pure water, and the predictions of the formula for limiting resistance do not take this factor into account.

While it may be premature to discuss at length the implications of the relationship between the predicted values of diffusion resistance and cell size and shape, a few points merit comment, if only to stimulate further work in the area. Firstly, Dainty [2] has emphasized that measurements of membrane permeability may be biased by the unstirred layer, and he has suggested that estimates of membrane permeability greater than a few times one tenth the permeability of the unstirred layer (i.e. the reciprocal of diffusion resistance) should be considered suspect. Using the flat plate model the permeability of the unstirred layer is on the order of $10^{-4}$ cm/s, and Dianty's criterion is a few times $10^{-5}$ cm/s. For small cells the permeability of the unstirred layer is much greater than for a flat plate. For example a cell with a 1 $\mu$m radius would have an unstirred layer with a permeability of about 0.1 cm/s and Dainty's criterion would be a few times 0.01 cm/s. Since the membrane permeability of small nonionic substances is in the range $10^{-2}$ to $10^{-5}$ cm/s, the bias that has been encountered with flat membrantes [13] could be avoided by using small cells or liposomes. Secondly, the unstirred layer thickness, $\Delta x$, has been used frequently to quantitate the effect of unstirred layer. For diffusion to flat surfaces this is reasonable because by definition $\Delta x$ is linearly related to diffusion resistance for this system. For other shapes, however, the relationship is not linear and differs from shape to shape. Consequently, $\Delta x$ is an unsuitable parameter for comparing systems of different shapes. Furthermore, transport functions which incorporate the effect of diffusion in terms of $\Delta x$ (e.g. Winne [5]) must be treated as shape specific transport functions. If the diffusion resistance parameter, $R$, (or its reciprocal, permeability) is used, generalized transport functions can be derived [15]. Thirdly, the effect of size on diffusion resistance predicted by Equations 4a–6a suggests that small cells could have a competitive advantage over large cells in nutrient limited systems. Primary productivity in oceans [17] and lakes [18] and phosphorus transport in lakes [19] is, in fact, dominated by very small ($\sim 3$ $\mu$m diameter) cyanobacteria. This suggests that the relationship between size and diffusion resistance may play a fundamental role in microbial ecology.

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