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# Determination of the thickness of the unstirred layers in the moving-drop method for measuring aqueous-nonaqueous interfacial transport rates

D.M. Miller

*Agricultural Canada Research Centre, London, Ontario (Canada)*

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**A technique for measuring the thickness of the unstirred layer in the aqueous phase surrounding a rising octanol drop is described. Measurements of the rate of movement of both acetic and valeric acids from the drop into buffered solutions were made using an apparatus previously described (Miller, D.M. (1986) Biochim. Biophys. Acta 856, 27-35). The rate of movement of valeric acid was shown to be dependent on the pH of the buffer while that of acetic acid was not. The thickness of the unstirred layer in the buffered solution (w.u.l.) was found to be 7.0 $\mu$m, and the thickness of the unstirred layer in the drop (o.u.l.) was estimated to have an upper limit of 4.6 $\mu$m. The rate constant for the movement of valeric acid from the octanol phase to the buffer, $k_{ow}$, has a value restricted to the range 21 to 46 $\mu$m s$^{-1}$ while that for movement in the opposite direction, $k_{wo}$, ranges from 600 to 1300 $\mu$m s$^{-1}$. The permeability of the w.u.l. is 120 $\mu$m s$^{-1}$, while that of the o.u.l. has a lower limit of 37 $\mu$m s$^{-1}$. Thus diffusion through the w.u.l. was not rate-limiting during movement into the drop, but was during movement outward. The rate of uptake of acetic acid was found to be 39 $\mu$m s$^{-1}$ and was independent of pH, from which it was concluded that the resistance of the w.u.l. was negligible for this compound.**

## Introduction

The passive movement of all but the smallest solute molecules through a cell membrane is usually considered to take place in a similar manner to that across a lipid bilayer. This, in turn, occurs in three steps. The solute must (i) cross the interface separating the bilayer from the solution on one side, (ii) diffuse through the bilayer and (iii) again cross an interface into the solution on the opposite side. Discussions on the kinetics of passive membrane transport usually contain the implicit assumption that the slow step is diffusion within the membrane and that interfacial transport is virtually instantaneous. Stein [1], for example, says almost nothing about the latter but concentrates instead on movement within the membrane. Perhaps the reason for this neglect is the lack of reliable measurements of interfacial transport rates, which prompted the author to initiate such a study [2] and resulted in the publication of values for the rates of movement of $^3$HHO, and four straight-chain alcohols across the water/octanol interface by the moving-drop technique. This employs an apparatus in which a 30-$\mu$l octanol drop is suspended in an aqueous solution of the compound being investigated. The drop is injected into the solution, removed after a measured time interval and the amount of compound it contains determined. By repeating this procedure on a number of drops for differing time intervals, $k_{ow}$, the rate constant for transfer of the compound from the solution to the drop is calculated. For time intervals of sufficient length, the concentration in the drop becomes constant and $K_{pc}$, the partition coefficient, is calculated as this concentration divided by that in the aqueous solution. Finally, the rate constant for movement in the opposite direction (from the aqueous solution to octanol) is determined as $k_{wo} = K_{pc}k_{ow}$. The latter constant can also be measured directly in a second apparatus in which a water drop is suspended in an octanol solution.

One of the major problems in this type of measurement is the assessment of the effect of unstirred layers, thought to be present on both sides of the interface,

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Correspondence: D.M. Miller, Agricultural Canada Research Centre, 1400 Western Road, London, Ontario, Canada, N6G 2V4.

whose resistance to solute movement can be so high as to mask other processes at the interface. In the earlier publication, however, it was argued that if the measured octanol/water partition coefficients, $K_{pc}$, equaled the ratio $k_{wo}/k_{ow}$, where both rate constants were determined directly, the effect of unstirred layers was negligible. This was found to be true for all the compounds except butanol and it was concluded that for compounds having a low $K_{pc}$, the resistance of the unstirred layers was insignificant. Hladky [3] has rejected this conclusion, pointing out that such an equality is inevitable for all compounds since all processes must balance at equilibrium. He also showed that $J$, the overall transport rate from octanol to water, may be described by the expression $J = k'_{ow}C_o - k'_{wo}C_w$, where $C_o$ and $C_w$ are the concentrations of the compound in octanol and water and $k'_{ow}$ and $k'_{wo}$ are *apparent* rate constants. These, in turn are related to the *true* rate constants, $k_{ow}$ and $k_{wo}$, by the expressions $k'_{ow} = Fk_{ow}$ and $k'_{wo} = Fk_{wo}$, where

$$F = \frac{P_o P_w}{P_o P_w + k_{wo} P_o + k_{ow} P_w}$$

and $P_o$ and $P_w$ are the permeabilities of the octanol and water unstirred layers. If the thicknesses of these layers are $\delta_o$ and $\delta_w$, and the diffusion coefficients of the compound in the two media are $D_o$ and $D_w$, then $P_o = D_o/\delta_o$ and $P_w = D_w/\delta_w$. Next Hladky makes the assumption that the rate constants are large relative to the unstirred layer permeabilities allowing him to obtain an estimated maximum value for $\delta_o$ and $\delta_w$ using the equations

$$\frac{D_w}{k'_{wo}} = \frac{D_w k_{ow}}{D_o k_{wo}}\delta_o + \delta_w = \frac{D_w}{D_o K_{pc}}\delta_o + \delta_w$$

$$\frac{D_o}{k'_{ow}} = \frac{D_o k_{wo}}{D_w k_{ow}}\delta_w + \delta_o = \frac{D_o}{D_w}K_{pc}\delta_w + \delta_o$$

These expressions predict that if the original data are plotted as $D_w/k'_{wo}$ vs. $D_w/D_oK_{pc}$ and $D_o/k'_{ow}$ vs. $D_oK_{pc}/D_w$, straight lines should result from which an estimate of the unstirred layer thicknesses may be obtained. From the result of this analysis, Hladky obtained the values $\delta_o = 6$ µm and $\delta_w = 10$ µm. Finally he concluded that: "the variation in the rates of transfer observed for water and the short-chain alcohols in the octanol/water moving-drop experiments can be explained quantitatively on the basis of rate-limiting convection or diffusion".

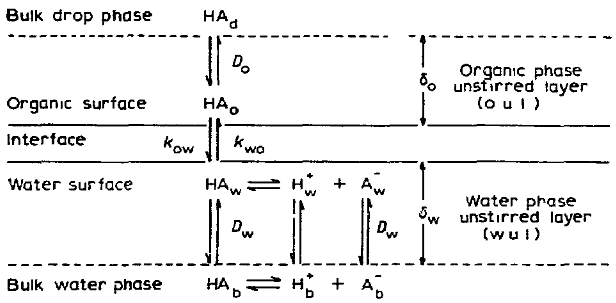
Such a conclusion is not warranted, however, since if uptake into the octanol phase were limited by diffusion, $k_{wo}$ for the series of compounds would have values in direct proportion to their diffusion coefficients, whereas in fact, the opposite is true. For example, water, which has a diffusion coefficient 2,4-times that of butanol, has a $k_{wo}$ which is 30-times less than $k_{wo}$ for butanol.

Nevertheless, a more reliable assessment of the effects of unstirred layers is warranted and so, to obtain another measure of the thickness of these layers in the water phase (w.u.l.), and to test Hladky's conclusion, a new procedure was devised. In this procedure, which is based on an earlier report by Gutnecht and Tostenson [4], the loss of a weak acid from an octanol drop into buffered solutions of differing pH is measured. Under these conditions, if the w.u.l. has a significant effect on the movement of the acid away from the surface of the drop, there will be a difference in the rate of loss into solutions of different pH. This results from the fact that when the pH is high, each acid molecule leaving the drop, will, upon entering the aqueous phase, immediately dissociate to an ionized form which cannot return to the organic phase. By contrast, a molecule of acid leaving the drop surface at low pH will not dissociate and may therefore be reabsorbed if its movement is restricted by the w.u.l., causing a decrease in the overall rate of loss compared to those at high pH. On the other hand, if the rate of diffusion through the w.u.l. is much higher than that of reabsorption by the drop, i.e. if $P_w \gg k_{ow}$, the rate of loss will be independent of pH.

In this report, an expression is derived which describes this effect quantitatively and allows the thickness of the w.u.l. to be measured. This was applied to the data obtained from the measurement of the rate of loss of both valeric and acetic acids from an octanol drop and it was found that while valeric acid, which has a high octanol/water partition coefficient (and presumably a high $k_{wo}$) showed considerable differences over the pH range employed, acetic acid showed none. From this it is concluded that passage through the w.u.l. is the slow step in the exit of valeric acid from an octanol drop, but not in the exit of acetic acid. Thus Hladky's conclusion applies to the movement of valeric acid, but not to that of acetic acid, in the rising drop measurements.

## Theoretical

Consider a weak acid HA which dissociates into $H^+$ and $A^-$. If $K_a$ is its dissociation constant, then $\alpha$, the fraction of the total acid concentration which is in the undissociated form (HA), is $[H^+]/([H^+] + K_a)$, or if $C$ is the total concentration of the acid, i.e. $C = [A^-] + [HA]$, then $[HA] = \alpha C$ and $[A^-] = (1 - \alpha)C$. Only the undissociated form, HA, enters the octanol, so that $C_d$, the total concentration in the drop, $= [HA_d]$. Both forms are present in the water, however, and the mechanism of movement into and out of the drop may be represented by Scheme I, where $k_{ow}$ and $k_{wo}$ are the rate constants (in units of $\mu m\ s^{-1}$) for movement of HA across the interface in the directions octanol to water



Scheme I.

and water to octanol, respectively, $D_o$ is the diffusion coefficient of the acid in octanol and $D_w$ the diffusion coefficient of both HA and $A^-$ in water, and $\delta_o$ and $\delta_w$ are the thicknesses of the o.u.l. and w.u.l., respectively. (Subscript 'd' refers to the interior of the drop, 'o' and 'w' to the octanol and water sides of the interface and 'b' to the bulk water phase).

The net rate of movement of acid across the interface, i.e. the number of moles, $n$, entering the drop per unit time, is

$$\frac{dn}{dt} = A_d(k_{wo}[HA_w] - k_{ow}[HA_o]) = A_d(\alpha k_{wo}C_w - k_{ow}C_o)$$

where $A_d$ is the area of the drop, $C_w = [HA_w] + [A^-_w]$ and $C_o = [HA_o]$. The permeability constant of the w.u.l. is $P_w = D_w/\delta_w$, so that the number of moles of acid per second passing through this layer toward the drop is $A_dP_w([HA_b] - [HA_w] + [A^-_b] - [A^-_w])$. Letting $C_b = [HA_b] + [A^-_b]$ (the total concentration of acid in the bulk water phase) the rate may be written as $A_dP_w(C_b - C_w)$. Finally, the rate of movement of acid inward through the o.u.l. will be $A_dP_o([HA_o] - [HA_d]) = A_dP_o(C_o - C_d)$, where $P_o = D_o/\delta_o$ is the permeability constant of the o.u.l. If $V_d$ is the volume of the drop, then the concentration in the drop will be $C_d = n/V_d$ and $dn/dt = V_d dC_d/dt$.

Assuming that concentrations in the unstirred layers rapidly become time independent, i.e. under pseudo-steady-state conditions, all these rates will be equal, and the rate equation becomes:

$$\frac{V_d}{A_d} \cdot \frac{dC_d}{dt} = P_w(C_b - C_w) = \alpha k_{wo}C_w - k_{ow}C_o = P_o(C_o - C_d)$$

Since the concentrations at the interface, $C_o$ and $C_w$, cannot be measured, these two quantities are eliminated from this equation to give the rate equation in terms of the measureable concentrations ($C_b$ and $C_d$):

$$\frac{dC_d}{dt} = \frac{3}{r} \cdot \frac{P_oP_w(\alpha k_{wo}C_b - k_{ow}C_d)}{\alpha k_{wo}P_o + k_{ow}P_w + P_oP_w}$$

where $r$ is the radius of the drop.

After an infinite time, equilibrium will be reached between the drop and the water phase and the concentration in the drop will reach a constant value which we may term $C_\infty$. At this point, according to the previous equation, since $dC_d/dt = 0$, $\alpha K_{pc}C_b$ must equal $C_\infty$. Thus the rate equation assumes the form

$$\frac{dC_d}{dt} = \frac{3}{r}k_\alpha(C_\infty - C_d)$$

where

$$k_\alpha = \frac{P_oP_wk_{ow}}{\alpha k_{wo}P_o + k_{ow}P_w + P_oP_w}$$

This equation may now be integrated from the initial value of $C_d = C_0$ at $t = 0$, to $C_d = C$ at time $t$, to give the expression

$$\ln(C_\infty - C) = \ln(C_\infty - C_0) - 3k_\alpha t/r$$

$k_\alpha$ is, of course, a rate constant for the movement of the acid into water from an octanol drop ($k_{ow}$) at a specified value of $\alpha$, (determined by the pH) and can therefore be measured by the technique previously described [2]. This consists of plotting $\ln(C_\infty - C)$ as a function of time to give a straight line of slope $-3k_\alpha/r$. Thus, by multiplying the measured slope by $-642.5$ μm, $k_\alpha$ is obtained, since a drop of volume = 0.03 cm$^3$ was used to make the measurements. Furthermore, using this equation, and the expression (obtained by inverting the above equation for $k_\alpha$),

$$\frac{1}{k_\alpha} = \frac{K_{pc}}{P_w}\alpha + \frac{1}{P_o} + \frac{1}{k_{ow}} = \frac{\delta_wK_{pc}}{D_w}\alpha + \frac{\delta_o}{D_o} + \frac{1}{k_{ow}} \qquad (1)$$

It is possible to measure the value of $\delta_w$ by the following procedure.

The acid is dissolved in a series of solutions, each of which is buffered at a different pH, and, at each pH, used to measure the rate constant for the movement of the acid between octanol and water as previously described [2]. If our mechanism is correct, plotting the inverse of this rate constant, $k_\alpha$, against $\alpha$ should give a straight line with slope $K_{pc}/P_w$ and an intercept of $1/P_o + 1/k_{ow}$. Thus, since $P_w = D_w/\delta_w$, $\delta_w$ will be the slope of the line multiplied by $D_w/K_{pc}$.

This treatment assumes that ionic equilibria are instantaneous, that the volume of the aqueous phase is large relative to that of the drop and that the solutions are so well buffered that the aqueous concentration of protons is uniform right up to the drop surface.

## Experimental

The apparatus and procedures for the measurement of the rate constant $k_{ow}$ were the same as in the previous publication [2], in which 30 μl octanol drops in apparatus A were employed, but with the exception that the aqueous phase contained 100 mM citric acid buffer at various pH values. This buffer was chosen since it is effective over a wide range of pH and since only ionized and therefore octanol-insoluble forms are present at the pH values used here. Acetic and valeric acids were dissolved in the buffers at a concentration of 10 mM, which was 1/10 the concentration of the buffer. A small amount of [$^{14}$C]acetate was added to the acetate solutions allowing the concentration of acetic acid in the octanol phase to be determined by radioactive assay in a similar manner to that used in measuring $^3$HHO movement in the previous work. Valeric acid in the octanol phase was determined by the gas chromatographic methods used previously [2].

The partition coefficients were found from the measurements of uptake into octanol drops at infinite time as detailed in the previous paper [2].

Diffusion coefficients were measured by the methods to be outlined in the following paper [5]. In this procedure, capillary tubes were filled with solutions of the acid in the solvent (either octanol or the buffer) and immersed in baths of the same solvent for an appropriate time interval. The total amount of acid remaining in the tubes was then determined and the diffusion coefficient calculated from the results. Octanol was used as a solvent for both acetic and valeric acids giving $D_o$ for both these compounds. Buffers at pH 2.5 and 7.0 were used as a solvent for valeric acid to provide diffusion coefficients for the free acid and the anion, respectively.

The viscosity of water-saturated octanol was measured using an Ostwald viscometer calibrated with pure water.

All measurements reported here were made at 25.0°C.

## Results

### *Acetic acid*

The acid dissociation constant, $K_a$, for this compound is quoted in the literature as $1.76 \cdot 10^{-5}$ mol $l^{-1}$ (p$K_a$ = 4.75). Preliminary measurements of the octanol to water transport rate indicated no change in the value of $k_\alpha$ over a wide range of $\alpha$, so several replicates were made at pH 2.1 and 6.8 only, giving the extreme values for $\alpha$ of 1.00 and 0.009, respectively. At both pH values, both measurements of uptake of acetic acid by, and exit from the octanol drop were made. (As pointed out previously [2], rate measurements using octanol drops result in a value for $k_{ow}$, regardless of which direction the solute is moving). Averaging the results for all measurements at each pH provided the values for $k_\alpha$ of 38.9 ± 1.4 $\mu$m s$^{-1}$ at pH 2.1 (six replicates ± S.D.) and 39.1 ± 0.7 $\mu$m s$^{-1}$ at pH 6.8 (four replicates).

The partition coefficient of acetic acid between an unbuffered aqueous phase and water saturated octanol was found to be 0.491 ± 0.004 (five replicates). The diffusion coefficient of acetic acid in octanol is 180 $\mu$m$^2$ s$^{-1}$ and in water is 1150 $\mu$m$^2$ s$^{-1}$ [5].

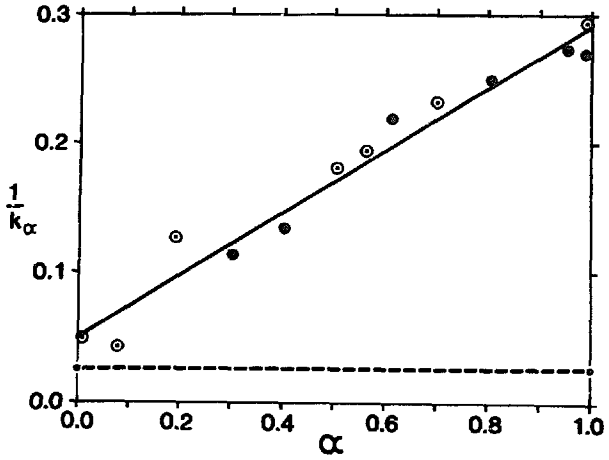


Fig. 1. Plot of the inverse transfer rate constant ($1/k_\alpha$) for valeric and acetic acids between an octanol drop and an aqueous citrate buffer, vs. $\alpha$, the fraction of the acid in the buffer in the undissociated form. Temperature 25°C. Open circles: loss of valeric acid by the drop. Solid circles: uptake of valeric acid by the drop. Solid line: least-squares fit to the valeric acid points. The slope of this line is 0.244 ± 0.014 s $\mu$m$^{-1}$, the intercept with the $\alpha = 0$ line is 0.049 ± 0.009 s $\mu$m$^{-1}$ and the correlation coefficient is 0.982. Broken line: line connecting the average $1/k_\alpha$ values for several measurements made using acetic acid at both $\alpha \approx 0$ and $\alpha = 1$. The slope of this line is not significantly different from zero, while the intercept is 0.0256 ± 0.0008 s $\mu$m$^{-1}$.

### *Valeric acid*

The value of the dissociation constant for this acid is $1.51 \cdot 10^{-5}$ mol $l^{-1}$ (p$K_a$ = 4.82). Unlike acetic acid, the valeric acid transfer rate was not constant as the pH was changed, and so $k_\alpha$ was measured for both exit and uptake by an octanol drop rising through buffer at a variety of pH values. The results are shown in Fig. 1 where the inverse of the rate constant ($1/k_\alpha$) is plotted against $\alpha$.

$K_{pc}$ for this compound was found to be 29.3 ± 2.1 (six replicates). The results of the diffusion coefficient measurements were 170 $\mu$m$^2$ s$^{-1}$ in octanol, 853 ± 21 $\mu$m$^2$ s$^{-1}$ for the pH = 2.5 buffer and 820 ± 21 $\mu$m$^2$ s$^{-1}$ in the pH = 7.0 buffer solution.

The viscosity of water-saturated octanol was measured as 7.18 ± 0.03 cP, or 8.08-times that of pure water at 25.0°C.

## Discussion

Fig. 1 shows that for valeric acid, a plot of the reciprocal of the rate constant vs. $\alpha$ closely approximates a straight line. According to the above mechanism then, $\delta_w$ equals the slope of this line times $D_w/K_{pc}$. Letting $D_w$ equal 840 $\mu$m$^2$ s$^{-1}$, the average for its value at the two extremes of pH, $\delta_w$ is found to be 7.0 $\mu$m. This is close to Hladky's value of 10 $\mu$m, but is more reliable since it does not require the assumption that the rate constant is large relative to the unstirred layer permeability. One might conclude from the similarities of these two values that Hladky's conclusion is correct, in the case of the w.u.l. at least, and that therefore the assumption is justified – and indeed it is for valeric acid which has a high partition coefficient and, as we shall see below, probably a high $k_{wo}$. For compounds with low partition coefficients, however, the assumption that diffusion in the w.u.l. is limiting is not valid, as shown dramatically by the acetic acid transport measurements. Here, since a plot of $1/k_\alpha$ vs. $\alpha$ has a slope $(K_{pc}/P_w)$ which is virtually zero, $P_w$ must be very large relative to $P_o$ and (or) $k_{ow}$.

An estimate for $\delta_o$ can be made using the relationship that $(\delta_o/D_o) + (1/k_{ow})$ equals the intercept of the straight line plot in Fig. 1. For valeric acid this reduces to the expression: $\delta_o = 8.3-(170/k_{ow})$ from which we can conclude that $\delta_o$, the thickness of the unstirred layer in the octanol drop, cannot be greater than 8.3 $\mu$m, its value for an infinitely large $k_{ow}$. A similar calculation on the acetic acid data gives an upper limit for $\delta_o$ of 4.6 $\mu$m.

Since conditions were constant, the unstirred layers must have had the same thickness during all the measurements. Thus the lower of these two 'upper' limits must apply, and using this value, we can estimate the valeric acid rate constants by rearranging the above expression to $k_{ow} = 170/(8.3 - \delta_o)$. This leads to a value for $k_{ow}$ which is restricted to the range 21 $\mu$m s$^{-1}$ when $\delta_o$ equals its lowest value, zero, to 46 $\mu$m s$^{-1}$ when $\delta_o = 4.6$ $\mu$m. its highest possible value. Furthermore, due to this restriction on the magnitude of $\delta_o$, $P_o$ (which equals $D_o/\delta_o$) must have a lower limit of 37 $\mu$m s$^{-1}$, and even at this value it approximates the highest possible value for $k_{ow}$ and hence passage through the o.u.l. can never be rate-limiting for valeric acid.

Having established the limits for $k_{ow}$ for valeric acid, we may do the same for $k_{wo}$ since it is equal to $k_{ow} \cdot K_{pc}$. This leads to a range for $k_{wo}$ of 600–1300 $\mu$m s$^{-1}$. Such values greatly exceed that of $P_w$, at 120 $\mu$m s$^{-1}$, and as a consequence, for this compound, as for all compounds with a high partition coefficient [5], passage through the w.u.l. is rate-limiting for movement in the direction water to octanol.

For acetic acid, $k_{ow}$ must exceed 40 $\mu$m s$^{-1}$, its value when $\delta_o$ is zero, and similarly $k_{wo}$ must exceed 20 $\mu$m s$^{-1}$. Estimating $D_w$ in the buffer as $\approx 1000$ $\mu^2$ s$^{-1}$, $P_w$ will be $\approx 140$ $\mu$m s$^{-1}$, while $P_o$ will have a lower limit of 40 $\mu$m s$^{-1}$.

All these values are summarized in Table I, where it can be seen that while $k_{wo}$ for valeric acid greatly exceeds the permeability of the unstirred layers, this is not the case for acetic acid. In fact, for acetic acid, neither $k_{ow}$ nor $k_{wo}$, is larger than the unstirred layer permeabilities, $P_o$ and $P_w$. Thus diffusion in the unstirred layers cannot be rate-limiting during acetic acid transfer across the octanol/water interface. These compounds, therefore, conform to the conclusion in the original publication [2] that, while diffusion in unstirred layers may be rate-limiting for compounds having a large $K_{pc}$ (valeric acid), they are not for compounds possessing low $K_{pc}$ (acetic acid).

TABLE I

*Summary of the rate constants and unstirred layer permeabilities*

Values (in $\mu$m s$^{-1}$) of the rate constants, and of the permeabilities of the unstirred layers, effective during the movement of acetic and valeric acids across an interface separating a rising 30 $\mu$l octanol drop from 100 mM citrate buffer. The thicknesses of the unstirred layers were $\delta_w = 7.0$ $\mu$m and $\delta_o \leqslant 4.6$ $\mu$m.

| | Valeric acid | Acetic acid |
|---|---|---|
| $k_{ow}$ | 21– 46 | $\geqslant 40$ |
| $k_{wo}$ | 600–1300 | $\geqslant 20$ |
| $P_o$ | $\geqslant 37$ | $\geqslant 40$ |
| $P_w$ | 120 | 140 |

The quantities derived above apply only to transfer between octanol and the buffers used, and will not be valid for measurements with water as the aqueous phase. This results from the fact that the presence of electrolytes increases the density of the aqueous phase, which, in turn, increases the rate of rise of the drop through the suspending medium. The result is an increase in stirring within the drop and a consequent decrease in the thickness of the unstirred layer (see below). In addition, there may well be an effect on the values of the rate constants themselves. Nevertheless, these effects are not likely to be drastic, so that the qualitative aspects of the above conclusion are still valid.

The low values for $\delta_o$ and $\delta_w$ may seem surprising at first sight, since to obtain such thin layers with living cells requires very vigorous and turbulent stirring unlike the relatively calm conditions used here. A study of the hydrodynamics of the moving drop, however, indicates otherwise. Such studies have shown that as the octanol drop rises through the water, friction between the two phases causes its surface to be dragged downward and the liquid within it set in motion. Hadamard [6] first derived equations describing the geometry of the streamlines which develop within a drop under these conditions. These streamlines, which have been confirmed by experiment [7], are plotted in Fig. 2. Now consider a small section of the interface of the drop such as the shaded band in Fig. 2. It can readily be seen that as this section moves from the leading (or top) edge of the drop to its equator, there is an increase in the area of the interface and therefore there must be a mass movement of both liquids into the interface. After passing the equator, the area decreases again resulting in a movement of material out of the interface. Thus, with the exception of a very small region at the equator,

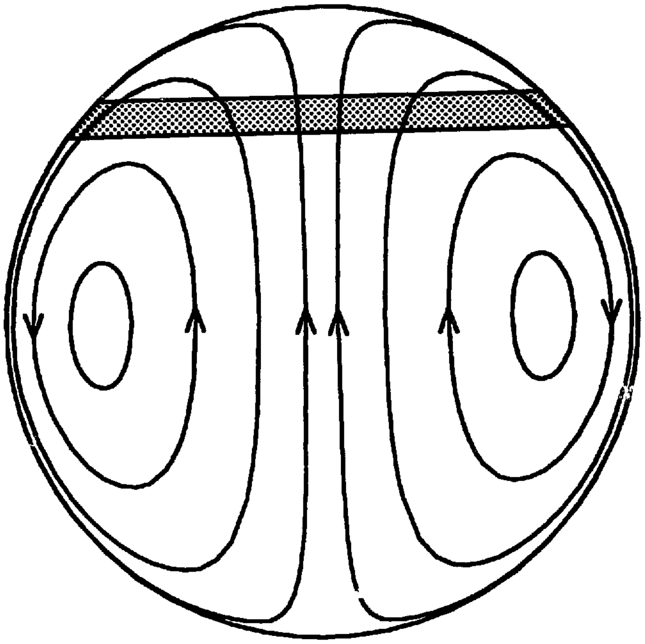


Fig. 2. Streamlines in an octanol drop rising through water. The arrows indicate the direction of the flow. The shaded band represents a portion of the drop interface which is being drawn from the top, or leading edge of the drop, to the bottom.

there is, in fact, no unstirred layer at the surface of the drop, since mass flow is occurring in a direction which is perpendicular to the surface. The amount of this mass flow will decrease as the surface approaches the equator, however, so that over part of the surface, the rate of transport of a compound by diffusion may approach or even exceed that by mass transport. Thus the 'thickness' of the unstirred layers will be an average or operational quantity only, and will not necessarily correspond to that found by rheological studies.

The analysis of the data presented in this and the following publication [5] is based on the conventional approach first enunciated by Dainty [8], according to which the total resistance to movement of the compounds between the two phases is considered to be the sum of two components, the first determined by the magnitude of diffusion within the two liquids and the second by interfacial transport. Since diffusional resistances will be inversely proportional to the value of the diffusion coefficients, the resistance on the two sides of the interface will be $\delta_o/D_o$ and $\delta_w/D_w$ where $\delta_o$ and $\delta_w$ are the thicknesses of two unifrom, discrete, unstirred layers. In reality, however, it is sufficient to state only that there exist two diffusion zones of an unspecified nature, and that therefore $\delta_o$ and $\delta_w$ are simply proportionality constants whose magnitude is determined by the physical nature of the measuring system. These two constants must be evaluated, however, to determine $P_o$ and $P_w$ and ultimately $k_{ow}$ and $k_{wo}$, which is the prime purpose of this investigation. Furthermore, the methods employed here are appropriate to this goal since they measure rates of transport, and hence permeabilities, directly. Thus although $\delta_o$ and $\delta_w$ may correspond to unstirred layer thicknesses, it is not necessary for the present purposes to assume that they do.

One more concern about circulation within the drop, is that the presence of surface active compounds may partially or completely inhibit it. This results from the fact that when these compounds are adsorbed at the interface, they are swept toward the trailing edge of the drop where they form a condensed film which prevents the transfer of momentum to the interior fluid, and when present in high enough concentration, will totally cover the surface and inhibit all circulation [9]. This has been demonstrated with the present apparatus through experiments in which lipids extracted from human erythrocytes were dissolved in octanol used as the drop phase in measuring interfacial transport rates of various compounds. The presence of the lipids in concentrations more than sufficient to form a monolayer on the drop surface was found to reduce the transport rates to as little as .. third of their value in the absence of lipids. Under these conditions the drops act as solid particles, which, as a consequence, are surrounded by much thicker unstirred layers than those around circulating drops. Thus the decrease in the rate constants arising from the presence of monolayer films is further proof that in the experiments described here and the following paper [5], circulation within the drop is indeed effective in reducing the diffusion resistance, and accounts for the small values of $\delta_o$ and $\delta_w$ actually measured.

## Acknowledgement

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