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Theoretical and experimental studies on the origin of pH–absorption shifts

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Summary

The in vitro pH–absorption profile of aniline has been examined using a rotating diffusion cell assembly. With 2,2,4-trimethylpentane as the lipoidal phase, it has been found that a reduction in the thickness of the diffusion layers leads to a higher apparent rate of absorption. For the physical model studied a theoretical relationship has been derived between the apparent rate of absorption and the speed of rotation of the diffusion cell used. Studies at different values of pH have been used to test a new theoretical approach for evaluating the influence of an aqueous diffusion layer on the pH–absorption rate profile. It is found that although the aqueous diffusion layer is responsible for a pH–absorption shift, the extent of any shift depends entirely upon the experimental conditions both in front and behind the membrane.

Introduction

The therapeutic activity of a drug is partly dependent upon not only the *fraction* of the given dose which enters the general circulation, but also the *rate* of its absorption. A passive diffusion of drug is the most significant route of absorption for the majority of drugs, with the physicochemical properties of both the drug and the (absorbing) membrane being the major determinants of the rate of absorption

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(Martin, 1980; Seydel and Schaper, 1982; Higuchi et al., 1981). It is apparent that further examination of the process of absorption can lead both to an improvement in the description of drug quantitative structure–activity relationships and, in drug formulation, to possible manipulation of the absorption step.

The important features of the membrane with respect to absorption are, inter alia, its lipophilic character (Higuchi et al., 1981), the presence of water pores (Higuchi et al., 1981), the 'virtual pH' at the surface of the membrane (Higuchi et al., 1981), the area of membrane available for absorption (Levine, 1970), and the presence of 'stagnant' diffusion layers (Hayton, 1980). The characteristics of a drug which can influence its rate of absorption are size, form and lipophilicity, and, for drugs which are weak acids or bases, particularly the extent of their ionization.

According to the classical pH–absorption theory (Shore et al., 1957; Schanker et al., 1958; Hogben et al., 1959) the rate of gastrointestinal drug absorption is dependent upon the lipophilicity of the drug and is directly proportional to the fraction of the unionized form present. Two types of deviation from the classical pH–absorption curves may be described. First, an upwards shift of the lower part of the curve due to a substantial permeation of the ionic form of the drug through the membrane (Crouthamel et al., 1971; Kakemi et al., 965; Nogami and Matsuzawa, 1961, 1962). Second, there can be a parallel shift of the pH–absorption curve from the pH–dissociation curve (to the right for acids and to the left for bases). The extent of the consequential movement in the position of the inflection point of the pH–absorption curve may be regarded as a measure of the pH-shift. In vivo these pH-shifts are regularly observed for series of drugs with similar pK_a and increasing lipophilicities (Beckett and Moffat, 1968, 1969; Kakemi et al., 1969a; Koizumi et al., 1964a and b; Schaper, 1982). Biological phenomena which may be responsible for these shifts include the presence of a 'microclimate' pH at the surface of the membrane (Hogben et al., 1959; Blair and Matty, 1974; Blair et al., 1975), and binding of drug to the surface of the (intestinal) mucosa (Kakemi et al., 1969; Kakemi et al., 1967, 1969b; Bridges et al., 1976).

Various theoretical models have been developed to explain these pH-shifts. The general non-linear absorption model of Higuchi and Ho (Suzuki et al., 1970a and b; Ho et al., 1972) demonstrates that the presence of significant 'unstirred layers' at the surface of the membrane can lead to observed pH-shifts. Although this model can account for a number of experimental findings (including the gastric, intestinal and rectal absorption of sulphonamides, the gastric absorption of barbiturates, and the buccal absorption of *n*-alkanoic acids — Suzuki et al., 1970b; Ho and Higuchi, 1971; Vora et al., 1972), it is difficult to use due to the necessary estimation of many physicochemical parameters (Plá-Delfina and Moreno, 1981).

The extraction theory of Wagner and Sedman (1973) assumes that the distribution of a drug between a bulk-phase solution and a barrier environment is responsible for observed pH-shifts. In their model the diffusion rate constant out of the membrane is the limiting factor in the rate of absorption, with the 'unstirred diffusion layer' assumed as not being a resistance to the penetration of a dissolved compound.

Whilst Winne (1977) has shown that neither model can unequivocably explain all

available in vivo absorption- distribution data, there are a number of observations which lead to a preference for the diffusion-model over the extraction model. In particular, it has been found that the in vivo rates of absorption of a number of drugs are clearly not directly related to the flow-rate of the (intestinal) blood stream, which is contrary to the extraction-model (Ochsenfahrt and Winne, 1969; Winne and Ochsenfahrt, 1967). Further, it has been shown in vitro that a reduction in the thickness of the diffusion layers brings about an increase in the rate of absorption (Lovering and Black, 1974; Tsuji et al., 1978), (although in these studies the origin of a pH-shift as a consequence of a diffusion layer effect was not fully proved).

In order to demonstrate a pH-shift an apparent dissociation constant is calculated first from the absorption data and is then compared with the actual dissociation constant. The apparent dissociation constant is calculated in such a way that for an acid or a base Eqs. 1 or 2, respectively, best fit the experimental data, i.e.

$$k_{app} = k_u \left[\frac{a_{H^+}}{a_{H^+} + K'_a} \right] \tag{1}$$

$$k_{app} = k_u \left[\frac{K'_a}{a_{H^+} + K'_a} \right] \tag{2}$$

where k_{app} and k_u are the apparent first-order rate constant for absorption and the absorption rate constant for the undissociated form of the drug (s^{-1}), respectively; a_{H^+} is the hydrogen ion concentration in the bulk solution, and K'_a is the apparent dissociation constant of the drug. Such a procedure is extremely dependent upon the accuracy of the absorption data. However, such data at just around the pK_a point are scarce, and interpretation of in vivo data is complicated further by the possibility that pH-shifts may arise because of absorption of the ionic form of the drug (Winne, 1977).

In this present study we have used a rotating diffusion cell assembly (Albery et al., 1976) to examine the influence of the thickness of the effective diffusion layer on the pH–rate of absorption profile of aniline between water and 2,2,4-trimethylpentane. This model system has been chosen so as to preclude possible absorption of the ionized form of the drug (Kinkel et al., 1981). The results obtained have been used in the development of a new theoretical approach, based on the diffusion-model, which can be used to analyze found pH-shifts in a simple manner.

Theory

The physical model under study is described in Fig. 1, which gives the diffusion of dissolved aniline from an aqueous buffer phase to an organic acceptor phase (2,2,4-trimethylpentane) via a hydrophobic membrane saturated with the organic liquid. The hydrodynamic conditions attendent upon the use of a rotating diffusion cell permit the liquid stream next to the interface(s) to be described mathematically

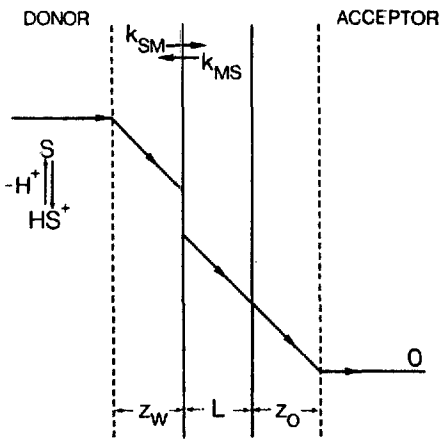

Fig. 1. Physical model, showing concentration gradients upon diffusion of unionized aniline, (S), from a donor aqueous phase through an aqueous diffusion layer of thickness z_w, a 2,2,4-trimethylpentane membrane of thickness L, and a 2,2,4-trimethylpentane diffusion layer of thickness z_o to sink acceptor phase of 2,2,4-trimethylpentane. k_{SM} and k_{MS} are the rate constants for interfacial transport between the aqueous stagnant layer and the membrane.

(Levich, 1962). With mixing, the concentrations in both bulk donor and acceptor phases are homogeneous, with rapidly changing concentration gradient(s) being found only in the area(s) directly next to the membrane surface where the movement of the liquids of nil. These area(s) are regarded as the effective boundary layers, δ. The thickness of such a layer may be defined as (Levich, 1962):

$$z = 0.643\, \nu^{1/6} D^{1/3} w^{-1/2} \tag{3}$$

where z, ν, D and w are the effective thickness of the diffusion layer (cm), the kinematic viscosity ($cm^2 \cdot s^{-1}$), the diffusion coefficient of the solute ($cm^2 \cdot s^{-1}$) and the speed of rotation of the cell (Hz), respectively. From Fick's first law of diffusion, it follows that under sink conditions in the acceptor phase that the steady-state flux of a solute may be given by (Winne, 1977):

$$J = P_{eff} C_w \tag{4}$$

where J, P_{eff} and C_w are the net flux per unit of surface ($mol \cdot s^{-1} \cdot cm^{-2}$), the effective permeability coefficient ($cm \cdot s^{-1}$) and the concentration of solute in the bulk donor phase ($mol \cdot cm^{-3}$), respectively. For the model described in Fig. 1 it now follows that

$$\frac{1}{P_{eff}} = \frac{1}{P_w} + \frac{1}{P_m} + \frac{1}{P_o} \tag{5}$$

where subscripts w, m and o refer to the water, membrane and organic phases,

respectively. From Eqn. 5 it is possible to show (Albery et al., 1976; Zwolinski et al., 1949) that:

$$\frac{1}{P_{eff}} = \frac{z_w}{D_w} + \frac{1}{\alpha\lambda k_{SM}} + \frac{L}{\alpha D_o K_d} + \frac{z_o}{D_o K_d} \tag{6}$$

where λ is the distance of one molecular step at the interface from the water phase to the membrane phase (cm); k_{SM} is the rate constant for the transfer from the solution to the membrane (s^{-1}); K_d is the distribution coefficient (molar concentration scale) being equal to k_{SM}/k_{MS}; and L and α are the thickness and porosity of the membrane, respectively. From Eqns. 3 and 6 it follows that:

$$\frac{1}{P_{eff}} = \left[\left(0.643\nu_w^{1/6}D_w^{-2/3}\right) + \frac{\left(0.643\nu_o^{1/6}D_o^{-2/3}\right)}{K_d}\right]w^{-1/2} + \frac{1}{\alpha\lambda k_{SM}} + \frac{L}{\alpha D_o K_d} \tag{7}$$

Thus a plot of $1/P_{eff}$ versus $w^{-1/2}$ gives a straight line with a slope, B, equal to $[0.643\nu_w^{1/6}D_w^{-2/3} + (0.643\nu^{1/6}D_o^{-2/3})/K_d]$, and an intercept C equal to $[(1/\alpha\lambda k_{SM}) + (L/\alpha D_o K_d)]$. From Eqn. 7 it is seen that at high values of K_d the effective permeability constant is determined principally by the diffusion through the unstirred water layer.

In this present study the relationship between the degree of dissociation of the solute and the slope term, B, has been examined. For a pseudo-steady-state situation (i.e. where the reduction in the concentration in the donor phase is small compared to the concentration reduction over the membrane), and with a sink condition in the acceptor phase, it follows that:

$$J = -\frac{dQ/dt}{A} = -\frac{V_w}{A}\cdot(dC_w/dt) = P_{eff}\cdot C_w \tag{8}$$

where Q is the amount of solute (moles), A is the area of membrane available for absorption (cm^2), and V_w is the volume of the water phase (cm^3). From Eqn. 8 we may write that

$$\ln\left[\frac{C_{w(t)}}{C_{w(0)}}\right] = -\left[\frac{A}{V_w}\right]\cdot P_{eff}\cdot t = -k_{app}\cdot t \tag{9}$$

where (0) and (t) refer to the initial state and the state at time t, respectively. Combining Eqns. 7 and 9 gives

$$\frac{1}{k_{app}} = \frac{V_w}{A}\cdot(Bw^{-1/2} + C) = B'w^{-1/2} + C' \tag{10}$$

Since, in the present study, V_w and A are constant, k_{app}^{-1} can be directly correlated with $w^{-1/2}$ to obtain the B′ and C′ terms. In order to obtain the rate constant, the

decrease in concentration of the unionized form of aniline has been followed using ultraviolet spectroscopy.

Defining $C_u = f \cdot C_T$, (where C_u is the concentration of unionized solute and f is the fraction of total solute concentration in the donor phase, C_T, unionized), then, since at the low concentrations used f remains constant during any one experiment, we may write that

$$k_{app} = \frac{-\ln\left[\frac{C_{T(t)}}{C_{T(0)}}\right]}{t} = \frac{-\ln\left[\frac{C_{u(t)}}{C_{u(0)}}\right]}{t} \tag{11}$$

where, for aniline, $f = 1/(1 + 10^{pK_a - pH})$. This form of the model enables either C_T or C_u to be measured for the determination of k_{app}.

Experimental

Materials

Aniline was from Fluka (Hicol, Rotterdam, The Netherlands), and was vacuum-distilled. 2,2,4-Trimethylpentane (analytical grade) and all buffer components were from Merck (Amsterdam, The Netherlands), and were used as received. Water was freshly distilled using an all-glass still after deionization over a mixed-bed ion exchanger. The buffers used were phosphate buffers having an ionic strength of 0.2 $mol \cdot dm^{-3}$ (Christian and Purdy, 1962). Both aqueous and organic phases were pre-saturated with one another at the temperature of the experiment. Depending upon the pH studied the concentration of aniline in the aqueous phase varied between 1×10^{-3} $mol \cdot dm^{-3}$ and 1×10^{-4} $mol \cdot dm^{-3}$.

Methods

A rotating diffusion cell similar to that described in the literature (Albery et al., 1976), and modified to have an adjustable membrane holder, has been used to measure the rate of transport of aniline from aqueous buffer to 2,2,4-trimethylpentane. The aqueous donor phase (44 cm^3) and organic acceptor phase (300 cm^3) were separated by a porous polytetrafluoroethylene membrane (Millipore type FHUP04700; 0.5 μm pore size; 60 μm thick; porosity 0.85) saturated with 2,2,4-trimethylpentane. Four millilitres of 0.2 N HCl were added to the acceptor phase to maintain sink conditions; under no circumstance did the acid phase come in the proximity of the membrane. A scheme of the assembly is given by Fig. 2. The diffusion cell was driven via a set of pulleys, with the rotation speed being monitored constantly using a digital counter (Venner digital counter, Type TSA 6634a). The rotation speed varied by not more than 1% during an experiment. The donor phase was pumped through a spectrophotometer flow cell using a high pressure pump (Orlita, type DMP, 1515). All tubing was made from narrow-bore stainless steel, and was isolated to avoid fluctuations in the temperature of the pumped phase. Temperature was maintained at 25°C (±0.1°C) using a water bath and circulator.

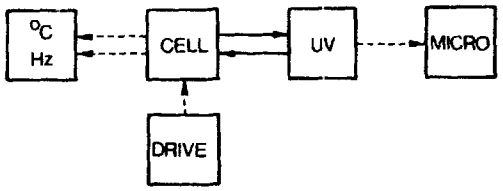

Fig. 2. Scheme of rotating diffusion cell assembly. Dashed lines indicate an information or a command link.

The rate of transport of aniline from the donor phase to the acceptor phase was followed constantly at the wavelength of maximum ultraviolet absorbance of the unionized form (230 nm) using a Pye-Unicam SP8-100 UV/VIS spectrophotometer. Over the concentration range studied aniline obeyed Beer-Lamberts law, enabling the rate constant to be calculated directly from the ln(absorbance) versus time profile. The rate of transport at every pH and rotation speed studied was calculated using linear regression by an APPLE II PLUS computer interfaced with the spectrophotometer.

Results

Table 1 gives the mean of 4 measured apparent rate constants determined at six pHs (pH 4–pH 8) and at 4 rotation speeds. Each rate constant has been calculated from the corresponding ln(absorbance) versus time relationship determined over 1800 s. With n = 18 these relationships all have correlation coefficients greater than 0.999. The relative standard deviations of the rate constants compare favourably

TABLE 1

MEAN APPARENT RATE CONSTANTS, $\bar{k}_{app}$ [a], AND THEIR RELATIVE STANDARD DEVIATIONS, FOR THE TRANSFER OF ANILINE AT DIFFERING VALUES OF pH AND WITH DIFFERING SPEEDS OF ROTATION

Rotation speed (Hz)	pH											
	4.00		4.66		5.00		6.00		7.00		8.00	
	$\bar{k}_{app}$	rel.S.D. (%)	$\bar{k}_{app}$	rel.S.D. (%)	$\bar{k}_{app}$	rel.S.D. (%)	$\bar{k}_{app}$	rel.S.D. (%)	$\bar{k}_{app}$	rel.S.D. (%)	$\bar{k}_{app}$	rel.S.D. (%)
1.368	1.027	9.34	2.300	5.17	3.253	2.26	4.097	2.32	4.390	5.78	4.415	4.85
2.551	1.214	7.21	2.936	5.64	3.900	4.31	5.142	2.67	5.512	1.97	5.517	4.15
3.378	1.280	6.19	3.259	5.06	4.289	4.24	5.701	3.29	5.874	1.86	6.085	2.05
5.682	1.471	6.74	3.789	3.80	4.810	3.64	6.667	4.65	6.815	3.13	7.038	3.94

[a] All values have been multiplied by 10^5, have units of s^{-1}, and are the means of 4 separate determinations.

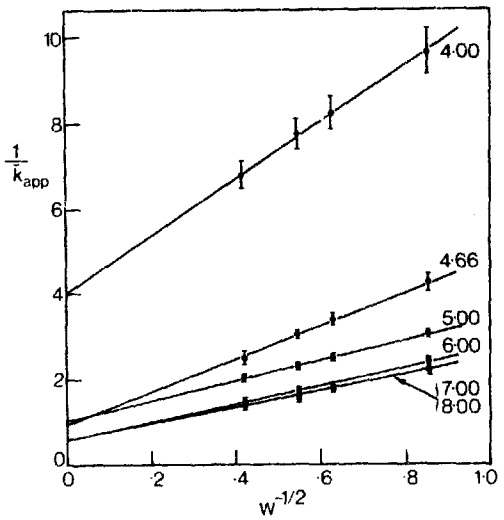

Fig. 3. Relationship between reciprocal mean apparent rate constants for absorption $(\bar{k}_{app})^{-1}$, ($10^4 \times$, s), and reciprocal square-root of the speed of rotation of the diffusion cell, $w^{-1/2}$ ($Hz^{-1/2}$), at different pH values of the aqueous phase. (Relative standard deviations are given with each data point, and values of pH are given next to each datum line. Lines drawn are the regression lines as given in Table 2).

with those obtained by other workers using similar rotating diffusion cells. The increase in the relative standard deviation at pH 4 reflects both the sensitive temperature dependency of the equilibrium between ionized and unionized forms, and the extremely small decrease in absorbance found. At 25°C aniline has a pK_a of 4.6 (Perrin, 1972) and at pH 7 a $\ln(K_d)$ (2,2,4-trimethylpentane/water) of -0.479 (Kinkel et al., 1981). Fig. 3 shows that as aniline becomes more dissociated the value of k_{app} falls and the value of the slope of the $1/k_{app}$ versus $w^{-1/2}$ increases. Table 2 gives the regression equations and statistics describing these various $1/k_{app}$ versus $w^{-1/2}$ relationships, and Fig. 4 shows the relationships found between the apparent rate constant and pH at differing speeds of rotation of the cell.

TABLE 2

REGRESSION EQUATIONS ACCORDING TO EQN. 10 FOR THE DATA GIVEN IN TABLE 1

pH	Regression equation [a]	r [b]	$F_{(1,2)}$ [c]
4.00	$k_{app}^{-1} = 66{,}400(4.63\%)w^{-1/2} + 40{,}880(4.74\%)$	0.9978	467
4.66	$k_{app}^{-1} = 39{,}570(2.92\%)w^{-1/2} + 9470(7.69\%)$	0.9992	1177
5.00	$k_{app}^{-1} = 23{,}080(3.13\%)w^{-1/2} + 11{,}010(4.15\%)$	0.9990	1019
6.00	$k_{app}^{-1} = 21{,}700(1.25\%)w^{-1/2} + 5830(2.95\%)$	0.9998	6354
7.00	$k_{app}^{-1} = 18{,}540(4.03\%)w^{-1/2} + 6830(6.91\%)$	0.9984	616
8.00	$k_{app}^{-1} = 19{,}500(1.74\%)w^{-1/2} + 5940(3.60\%)$	0.9997	3320

[a] Values after the regression coefficients are their relative standard deviations.

[b] Correlation coefficient.

[c] Variance ratio value.

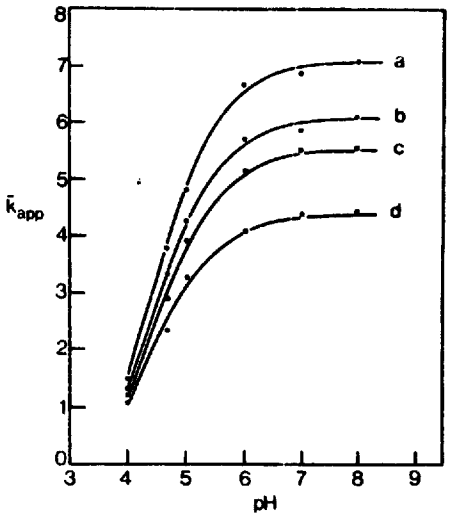

Fig. 4. Effect of diffusion cell rotation speed on the relationship between the mean apparent rate of absorption, $\bar{k}_{app}$, ($10^{-5} \times$, s^{-1}) of aniline at different values of pH. (a–d indicate speeds of rotation of 5.682 Hz, 3.378 Hz, 2.551 Hz and 1.368 Hz, respectively).

Discussion

The Higuchi-Ho non-linear absorption model regards the absorption of a drug as a passive diffusion through a significant diffusion layer before the membrane, followed by a passive transport through the membrane itself. This two-compartment diffusion model assumes further that only the unionized form of the solute can permeate through the lipid membrane, and that (in vivo) the acceptor compartment is a perfect sink (due to blood flow) (Suzuki et al., 1970a and b; Ho et al., 1972). Under these circumstances Eqn. 5 reduces to:

$$\frac{1}{P_{eff}} = \frac{1}{P_w} + \frac{1}{P_m} \tag{12}$$

This model assumes also that both unionized and ionized solute forms may diffuse through the stagnant water layers (Suzuki et al., 1970a and b; Ho et al., 1972; Winne, 1977; Tsuji et al., 1978; Lovering et al., 1974). P_w may be thus written as:

$$P_w = (1 - f) \cdot P_{w,i} + fP_{w,u} \tag{13}$$

where $P_{w,i}$ and $P_{w,u}$ are the permeability coefficients for the ionized and unionized forms in the water layer, respectively. Assuming that $P_{w,i}$ is practically the same as $P_{w,u}$, then we may write

$$P_w = P_{w,u} = P_{w,i} \tag{14}$$

Similarly, for the permeability coefficient through the membrane, P_m, we may write

$$P_m = P_{m,i}(1 - f) + P_{m,u} \cdot f \tag{15}$$

Since $P_{m,i}$ will be null, from Eqns. 12, 14 and 15 it follows that

$$\frac{1}{P_{eff}} = \frac{1}{P_{w,u}} + \frac{1}{f \cdot P_{m,u}} \tag{16}$$

which may be recast as

$$k_{app} = \left[\frac{A}{V_w}\right] \cdot \left[\frac{P_w}{1 + (P_w/f \cdot P_{m,u})}\right] \tag{17}$$

In order to identify a pH–absorption shift an absorption function term, AF, must be introduced. This term is defined as the ratio of the apparent rate constant to the maximal possible rate constant, (i.e. the apparent rate constant of the unionized solute) (Winne, 1977), viz:

$$(AF) = \frac{\text{absorption rate}}{\text{maximum absorption rate}} = \frac{k_{app}}{k_{app}^{max}} = \frac{P_{eff}}{P_{eff}^{max}} \tag{18}$$

Combining Eqns. 16 and 18 gives the absorption in permeability coefficient terms, viz:

$$(AF) = \frac{\left[\frac{P_{m,u}}{P_w} + 1\right]}{\left[\frac{P_{m,u}}{P_w} + \frac{1}{f}\right]} \tag{19}$$

which gives that the absorption function is dependent upon both f and the ratio $P_{m,u}/P_w$. (For multiple-barrier systems, equations similar to Eqn. 19 may be also written — Winne, 1977).

Since P_w is dependent upon the thickness of the aqueous diffusion layer, i.e.

$$P_w = \frac{D_w}{z_w} \tag{20}$$

then a significant diffusion layer thickness will cause the AF term to deviate from f. This model for explaining a pH–absorption shift is based entirely upon the assumption that Eqn. 14 is valid.

In the case of a rotating diffusion cell, P_w is, according to Eqn. 3, dependent upon $w^{1/2}$; thus, in an experimental assembly similar to the two-compartment diffusion cell model, Eqn. 16 may be then rewritten as:

$$\frac{1}{P_{eff}} = Bw^{-1/2} + \frac{C}{f} \tag{21}$$

It follows from Eqn. 21 that for the relationship between either $1/P_{eff}$ (or $1/k_{app}$) versus $w^{-1/2}$ only the intercept (and not the slope term B) is dependent upon f — the fraction of solute undissociated.

In the present study using a water–oil—oil arrangement (Experimental), it has been observed (Fig. 5) that the slope term, B, is dependent upon f. This indicates one of the two situations. First, (and in accordance with the Higuchi-Ho model), that:

$$\frac{1}{P_{eff}} = \frac{1}{P_w} + \frac{1}{f \cdot P_o} + \frac{1}{f \cdot P_{m,u}} \tag{22}$$

Since P_o is dependent upon $w^{1/2}$, the slope term, B (characterized in Eqn. 22 by P_w and P_o), is here influenced by the value of f. For the second case, our observations can also mean that total flux is determined only by the flux of the unionized form through every individual barrier. This second situation would give rise to

$$\frac{1}{P_{eff}} = \frac{1}{f \cdot P_w} + \frac{1}{f \cdot P_o} + \frac{1}{f \cdot P_{m,u}} \tag{23}$$

such that the absorption function, (AF), then becomes

$$(AF) = \frac{\dfrac{1}{P_w} + \dfrac{1}{P_o} + \dfrac{1}{P_{m,u}}}{\dfrac{1}{f}\left(\dfrac{1}{P_w} + \dfrac{1}{P_o} + \dfrac{1}{P_{m,u}}\right)} = f = 1/1 + 10^{(pK_a - pH)} \tag{24}$$

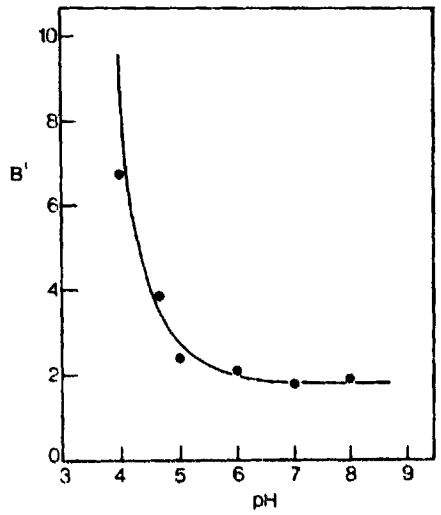

Fig. 5. Relationship between pH and the slope term B' (10^4, $s^{1/2}$) of Eqn. 10. Datum points correspond to experimentally determined values, whilst the drawn line is the theoretical curve computed using Eqn. 24.

Recently, Amidon et al. (1982) have found that the slope term, B, is dependent upon only the diffusion of the unionized form of a solute. Whilst measuring the rate of transport of an organic acid across a membrane using a rotating diffusion cell, these workers observed that with both donor and acceptor phases as 0.01 N HCl the value of B was a factor of two larger than when the acceptor phase was 0.1 $mol \cdot dm^{-3}$ phosphate buffer of pH 7.2. Further, in both cases the value of the intercept (Eqn. 10) was constant.

Since the Higuchi-Ho linear absorption model predicts a slope term B′ which is independent of the fraction of unionized solute, the findings of Amidon et al. appear contradictory. This has lead us to examine the flux equations in more detail.

For solute flux in the aqueous diffusion layer in front of a membrane, we may write:

$$J_1 = P_{w,1} \cdot \left(C^b_{w,1} - C^s_{w,1}\right) \tag{25}$$

where superscripts b and s refer to the bulk phase and the surface of the membrane, respectively, and $P_{w,1}$ is similar to Eqn. 13 with f_1 the fraction of unionized solute in front of the membrane. Similarly, with an aqueous acceptor phase, flux through the aqueous diffusion layer behind the membrane may be described by:

$$J_2 = P_{w,2} \cdot \left(C^s_{w,2} - C^b_{w,2}\right) \tag{26}$$

where for $P_{w,2}$ (Eqn. 13), f_2 (the fraction of unionized solute behind the membrane), is dependent upon the pH of the acceptor compartment.

Thus, for a water/oil/water assembly, flux through the membrane is given by:

$$J_m = P_m \cdot \left(f_1 \cdot C^s_{w,1} - f_2 \cdot C^s_{w,2}\right) \tag{27}$$

where P_m is:

$$P_m = (K_d \cdot D_{m,u})/L \tag{28}$$

At steady-state, the flux of the solute through every individual barrier must be equal, so that Eqns. 25, 26 and 28 may be recast as:

$$J = \frac{P_{w,1} \cdot C^b_{w,1} - (f_2/f_1)\left(P_{w,1} \cdot C^b_{w,2}\right)}{1 + (P_{w,1}/P_m \cdot f) + (f_2 \cdot P_{w,1}/f_1 P_{w,2})} \tag{29}$$

Under sink conditions, Eqn. 29 reduces to a form similar to Eqn. 4, i.e.

$$\frac{1}{P_{eff}} = \frac{1}{P_{w,1}} + \frac{1}{f_1 \cdot P_{m,u}} + \frac{f_2}{f_1 \cdot P_{w,2}} \tag{3}$$

Because with the rotating diffusion cell the diffusion layer thickness in front of the membrane is equal to the thickness of the diffusion layer behind the membrane,

Eqn. 30 reduces to:

$$\frac{1}{P_{eff}} = \frac{(f_1 + f_2)}{P_{w,1} \cdot f_1} + \frac{1}{f_1 \cdot P_{m,u}} \tag{31}$$

As $P_{w,1}$ is dependent upon $w^{1/2}$, the slope term B′ in the relationship between k_{app}^{-1} and $w^{-1/2}$ (Eqn. 10) in the water/oil/water system, is a function of the fraction of the unionized form of the solute both *before* and *behind* the membrane. Further, the magnitude of a pH–absorption shift in a water/membrane/water system depends upon the value of f_2. Clearly, as f_2 reduces to zero then the absorption function will be as given by Eqn. 19. If, however, f_2 equals $(1 - f_1)$ then the absorption function simply reduces to:

$$AF = f_1 \tag{32}$$

The validity of Eqn. 31 has been examined for aniline under a number of different conditions. Table 3 gives the results obtained for the transfer of aniline from an aqueous donor compartment through a membrane containing 2,2,4-trimethylpentane to an aqueous acceptor compartment, where both compartments are at various pHs. It is seen that changing the pH behind the membrane clearly alters the slope term B′, whereas changing the pH before the membrane whilst maintaining the pH behind the membrane at pH = 1, has no influence on the slope term B′. This latter observation means that a pH–absorption shift can be caused by the thickness of the aqueous diffusion layer before the membrane. (Similar results have been obtained by us for *p*-chloroaniline and *p*-methylaniline.)

The results given in Table 3 for transfer in a water/oil/water system, combined with those given above for the water/oil/oil system, show that for the latter system Eqn. 22, and not Eqn. 23 is correct, proving that the absorption function (AF) is not given simply by f.

TABLE 3

SLOPE (B′) AND INTERCEPT (C′) REGRESSION COEFFICIENTS FOR THE RELATIONSHIP BETWEEN k_{app}^{-1} AND $w^{-1/2}$ FOR THE TRANSPORT OF ANILINE IN A WATER/OIL/WATER SYSTEM

Diffusion cell conditions		Regression coefficients		
Donor phase pH	Acceptor phase pH	B′ ($s^{1/2}$)	C′ (s)	r
7	1	10,659 [a]	7026	0.998
4.6	1	9803	16,601	0.991
7	7	22,469	8080	0.997
7	4.6	16,706	6695	0.999

[a] Assuming a diffusion coefficient for aniline in water of 1×10^{-5} $cm^2 \cdot s^{-1}$ (Guy and Hadgraft, 1981), then for the present system with A = 2.67 cm^2, V_w = 44 ml and $\nu = 0.902 \times 10^{-2}$ $cm^2 \cdot s^{-1}$, and where the donor and acceptor compartments are at pH 7 and 1, respectively, a value for the slope term B′ of 10,400 $s^{1/2}$ may be calculated using $B' = 0.643\ D^{-2/3} \cdot \nu^{1/6} \cdot V_w / A$.

From Eqn. 22 it follows that for a water/oil/oil arrangement, the slope term B is dependent upon P_w^{-1} and $(f \cdot P_0)^{-1}$. However, since P_0 depends upon the distribution coefficient of a solute between an aqueous and an organic phase, $(f \cdot P_0)^{-1}$ will be insignificant for a solute having a high K_d, whereas for solutes with a low K_d the $(f \cdot P_0)^{-1}$ term will be much more important compared to the P_w^{-1} term. Thus for a solute such as aniline, the slope term B (or B') is nearly completely dependent upon f, such that the absorption function can be *approximated* by f.

This is seen from Fig. 5, where the drawn line is the theoretical line of B' against pH as given by f, and is in close agreement with the experimental values, showing that, for aniline in a water/oil/oil arrangement, the aqueous diffusion layers cause no significant pH–absorption shift.

Conclusions

This study has examined the influence of diffusion layer thickness on the in vitro pH-absorption rate profile of aniline. It has been found that a reduction in the thickness of the diffusion layer leads to a higher apparent rate of absorption. For the physical models studied, derived relationships between k_{app}^{-1} and $w^{-1/2}$ are found to be linear, and, depending upon the fraction of the unionized form of the solute both before and behind the membrane, have slope terms which can be a measure of a pH-absorption shift.

Where the slope is invariant upon altering the pH of the donor compartment, then the pH–absorption shift is found to be maximal. Such a situation may be created by reducing the effective diffusion layer (for the unionized form of aniline) behind the membrane by reducing the pH to 1 (Table 3).

For cases where the slope term is nearly completely dependent upon the fraction of the unionized form present before the membrane — as is the case for solutes having a low distribution coefficient, (e.g. aniline) — in the water/oil/oil arrangement, then the absorption function may be approximated by f_1.

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References

Albery, W.J., Burke, J.F., Leffler, E.B. and Hadgraft, J., Interfacial transfer studied with a rotating diffusion cell. J. Chem. Soc. Faraday Trans., 1, 72 (1976) 1618–1626.

Amidon, G.E., Higuchi, W.I. and Ho, N.F.H., Theoretical and experimental studies of transport of micelle-solubilized solutes. J. Pharm Sci., 71 (1982) 77–84.

Beckett, A.H. and Moffat, A.C., The influence on alkylsubstitution in acids on their performance in the buccal absorption test. J. Pharm. Pharmacol., 20 (1968) 239S–247S.

Beckett, A.H. and Moffat, A.C., The influence of substitution in phenylacetic acids on their performance in the buccal absorption test. J. Pharm. Pharmacol., 21 (1969) 139S–143S.

Blair, J.A., Lucas, M.L. and Matty, A.J., Acidification in the rat proximal jejunum. J. Physiol. (Lond.). 245 (1975) 333–350.

Blair, J.A. and Matty, A.J., Acid microclimate in intestinal absorption. Clin. Gastroenterol., 5 (1974) 183–197.

Bridges, J.W., Houston, J.B., Humphrey, J., Lindup, W.E., Parke, D.V., Schillingford, J.S. and Upshall, D.G., Gastrointestinal absorption of carbenoxolone in the rat determined in vitro and in situ: deviations from pH-partition hypothesis. J. Pharm. Pharmacol., 28 (1976) 117–126.

Christian, G.D. and Purdy, W.C., The residual current in orthophosphate medium. J. Electroanal. Chem., 3 (1962) 363–367.

Crouthamel, W.G., Tan, G.H., Dittert, L.W. and Doluisio, J.T., Drug absorption IV. Influence of pH on absorption kinetics of weakly acid drugs. J. Pharm. Sci., 60 (1971) 1160–1163.

Guy, R. and Hadgraft, J., Interfacial transport of salicylic acid. J. Colloid Interface Sci., 81 (1981) 69–74.

Hayton, W.L., Rate-limiting barriers to intestinal drug absorption: a review. J. Pharmacokin. Biopharm., 8 (1980) 321–334.

Higuchi, W.I., Ho, N.F.H., Park, J.Y. and Komiya, I., Rate limiting steps and factors in drugs absorption. In L.F. Nimmo and W.S. Prescott (Eds.), Drug Absorption, MTPPress, Lancaster, U.K., 1981, pp. 35–60.

Ho, N.F.H., Higuchi, W.I. and Turi, J., Theoretical model studies of drug absorption and transport in the GI tract III. J. Pharm. Sci., 61 (1972) 192–197.

Ho, N.F.H. and Higuchi, W.I., Quantitative interpretation of in vivo buccal absorption of *n*-alkanoic acids by the physical approach. J. Pharm. Sci., 60 (1971) 537–541.

Hogben, C.A.M., Tocco, D.J., Brodie, B.B. and Schanker, L.S., On the mechanism of intestinal absorption of drugs. J. Pharmacol. Exp. Ther., 125 (1959) 275–282.

Kakemi, K., Arita, T., Hori, R. and Konishi, R., Absorption and excretion of drugs. XXXIII. Absorption of barbituric acid derivates from rat small intestine. Chem. Pharm. Bull., 15 (1967) 1883–1887.

Kakemi, K., Arita, T., Hori, R., Konishi, R. and Nishimura, K., Absorption and excretion of drugs. XXXIII. The correlation between the absorption of barbituric acid derivatives from the rat small intestine and their binding to the mucosa. Chem. Pharm. Bull., 17 (1969) 248–254.

Kakemi, K., Arita, T., Hori, R., Konishi, R., Nishimura, K., Matsui, H. and Nishimura, T., Absorption and excretion of drugs. XXXIV. An aspect of the mechanism of drug absorption from the intestinal tract in rats. Chem. Pharm. Bull., 17 (1969) 255–261.

Kakemi, K., Arita, T. and Muranishi, S., Absorption and excretion of drugs. XXV. On the mechanism of rectal absorption of sulfonamides. Chem. Pharm. Bull., 13 (1965) 861–869.

Kinkel, J.F.M., Tomlinson, E. and Smit, P., Thermodynamics and extrathermodynamics of organic solute liquid–liquid distribution between water and 2,2,4-trimethylpentane. Int. J. Pharm., 9 (1981) 121–136.

Koizumi, T., Arita, T. and Kakemi, K., Absorption and excretion of drugs. XIX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (1) Absorption from rat stomach. Chem. Pharm. Bull., 12 (1964a) 413–420.

Koizumi, T., Arita, T. and Kakemi, K., Absorption and excretion of drugs. XX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides (2). Absorption from rat small intestine. Chem. Pharm. Bull., 12 (1964b) 421–427.

Levich, L.G., In Physicochemical Hydrodynamics, Prentice Hall, Englewood Cliffs, NJ, 1962, p. 69.

Levine, R.R., Factors affecting gastrointestinal absorption of drugs. Am. J. Digest. Dis., 15 (1970) 171–188.

Lovering, E.G. and Black, D.B., Diffusion layer effects on permeation of phenylbutazone through polydimethylsiloxane. J. Pharm. Sci., 63 (1974) 1399–1402.

Martin, Y.C., A practitioner's perspective of the role of quantitative structure–activity analysis in medicinal chemistry. J. Med. Chem., 24 (1981) 229–237.

Nogami, H. and Matsuzawa, T., Studies on absorption and excretion of drugs. I. Kinetics of penetration of acidic drugs, salicylic acid, through the intestinal barrier in vitro. Chem. Pharm. Bull., 9 (1961) 532–540.

Nogami, H. and Matsuzawa, T., Studies on absorption and excretion of drugs. II. Kinetics of penetration of basic drug, aminopyrine, through the intestinal barrier in vitro. Chem. Pharm. Bull., 10 (1962) 1055–1060.

Ochsenfahrt, H. and Winne, D., Der einfluss der Durchblutung auf die resorption von Arzneimitteln aus dem Jejunum der Ratte. Naunyn-Schmiedebergs Arch. Pharmakol., 264 (1969) 55–75.

Perrin, D.D., Dissociation Constants of Organic Bases in Aqueous Solution, Suppl., Butterworths, 1972, London.

Plá-Delfina, J.M. and Moreno, J., Intestinal absorption–partition relationships — a tentative functional nonlinear model. J. Pharmacokin. Biopharm., 9 (1981) 191–215.

Schanker, L.S., Tocco, D.J., Brodie, B.B. and Hogben, C.A.M., Absorption of drugs from the rat small intestine. J. Pharmacol. Exp. Ther., 123 (1958) 81–88.

Schaper, J., Absorption of ionizable drugs: nonlinear dependence on log P, pKa and pH — quantitative relationships. Quant. Struct.–Act. Relat., 1 (1982) 13–27.

Seydel, J.K. and Schaper, K.J., Quantitative structure–pharmacokinetic relationships and drug design. Pharmacol. Ther., 15 (1982) 131–182.

Shore, P.A., Brodie, B.B. and Hogben, C.A.M., The gastric secretions of drugs: pH-partition hypothesis. J. Pharm. Exp. Ther., 119 (1957) 361–369.

Suzuki, A., Higuchi, W.I. and Ho, N.F.H., Theoretical model studies of drug absorption and transport in the gastro intestinal tract I. J. Pharm. Sci., 59 (1970a) 644–651.

Suzuki, A., Higuchi, W.I. and Ho, N.F.H., Theoretical model studies of drug absorption and transport in the gastro intestinal tract II. J. Pharm. Sci., 59 (1970b) 651–659.

Tsuji, A., Miyamoto, E., Hashimoto, N. and Yamana, T., GI absorption of β-lactam antibiotics II. Deviation from pH-partition hypothesis in penicillin absorption through in situ and in vitro lipoidal barriers. J. Pharm. Sci., 67 (1978) 1705–1711.

Vora, K.R.M., Higuchi, W.I. and Ho, N.F.H., Analysis of human buccal absorption of drugs by physical model approach. J. Pharm. Sci., 61 (1972) 1785–1791.

Wagner, J.G. and Sedman, A.J., Quantitation of rate of gastrointestinal and buccal absorption of acidic and basic drugs on extraction theory. J. Pharmacokin. Biopharm., 1 (1973) 23–50.

Winne, D., Shift of pH-absorption curves. J. Pharmacokin. Biopharm., 5 (1977) 53–94.

Winne, D. and Ochsenfahrt, H., Die formale Kinetik der Resorption unter Berucksichtigung der Darmdurchblutung. J. Theor. Biol., 14 (1967) 293–315.

Zwolinski, B.J., Eyring, H. and Reese, C.E., Diffusion and membrane permeability, i. J. Phys. Colloid Chem., 53 (1949) 1426.