Thermodynamic Dependence of Interfacial Transfer Kinetics of Nonionized Barbituric Acid Derivatives in Two-Phase Transfer Cell

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Abstract □ A theory was developed to describe interfacial transport kinetics of a series of drug homologs in a two-phase transfer cell. When tested, the theory held true for 5,5-disubstituted barbituric acid derivatives in a preequilibrated octan-1-ol = (pH 5) aqueous buffer system maintained at 37° and stirred symmetrically at 50 and 100 rpm. Theoretical prediction of transfer kinetics was not possible in such a cell if the phases were stirred asymmetrically. For symmetric stirring, successful prediction of the transfer kinetics of any homolog in the series was possible from a knowledge of the partition coefficient and transfer kinetics of the parent compound, the partition coefficient of the homolog, and some easily determined system variables. The viscosity and density of the two phases and the phase-volume ratio were needed to define a system constant dependent on the solute diffusion coefficient, interfacial area, donor phase volume, and the boundary layer thickness for diffusion in the donor phase. A method is described to enable estimation of this constant from a knowledge of the transfer kinetics of the parent compound. The rank order of compounds in terms of their observed first-order transfer rate constants is shown to be dependent on the characteristics of the solvent system and stirring conditions employed, as well as on the physical chemistry of the solutes. The results are discussed in light of previously documented investigations.

Keyphrases □ Transfer kinetics—thermodynamics, nonionized barbituric acid derivatives in the two-phase transfer cell □ Barbituric acids—derivatives, thermodynamic dependence of interfacial transfer kinetics in the two-phase transfer cell □ Two-phase transfer cell—thermodynamic dependence of interfacial transfer kinetics of nonionized barbituric acid derivatives □ Thermodynamics—interfacial transfer kinetics of nonionized barbituric acid derivatives in the two-phase transfer cell

To exert a biological effect, a drug moiety must reach its receptor site by passing through a series of hydrophilic and hydrophobic regions. The ease with which this transfer is accomplished affects the duration and intensity of the resultant biological response.

BACKGROUND

Two model systems used to investigate the transfer of solute from hydrophilic to hydrophobic regions are the two- and three-phase transfer cells (1, 2). Gordon and Sherwood (1) used a two-phase system of water and butanol to study the transfer of various solutes across the interface. Their studies provided data that supported the two-film theory of Lewis and Whitman (3). An aqueous *n*-octanol (pH 4.3) two-phase system was employed by Schumacher and Nagwekar (4) to study the transfer of sulfonamides. They (5) also varied the composition of the organic phase in the cell to determine the effect on the transport of the same drugs. The effect of varying the aqueous phase pH on salicylic acid transfer was also investigated (6). Since the transfer of ions through lipid membranes is fundamental to cell function, Ting *et al.* (7) used the two-phase transfer cell to investigate this transport for monovalent cations *in vitro*.

The three-phase transfer cell used (2) to study water diffusion and the transport of various salts through interfaces was modified by Perrin (8). It was used to study the transfer of solutes from an aqueous compartment of pH 2 through a lipid membrane to a second aqueous compartment at pH 7.4 to simulate gastric absorption of salicylic acid and aminopyrine. Doluisio and Swintosky (9) mimicked *in vivo* conditions using a three-phase rocking Y-tube apparatus in which the interfacial area available for drug transfer varied with respect to time. Correlations between *in vivo* drug absorption data and *in vitro* rate constants for three-phase transfer (where the pH of the aqueous compartments was varied) were also attempted for a series of sulfonamides (10).

Neither the solvent system nor the stirring arrangement associated with the respective phases of two- or three-phase transfer cells is standardized in the literature. This situation presents a problem when attempting to compare results among studies. Indeed, it was observed (11) that both the magnitude and rank order of the rate constants for drug transfer are significantly dependent on the design of the cell and the agitation conditions used.

This study demonstrates how the transport kinetics of a series of homologs may be successfully predicted from a knowledge of the transfer kinetics of the lead compound, its partition coefficient, the partition coefficients of the remaining homologs, and some simply determined system-dependent parameters in a symmetrically stirred two-phase transfer cell of known dimensions.

THEORY

Byron *et al.* (12) used a two-phase transfer cell, containing equal volumes of a preequilibrated aqueous phase and light liquid petrolatum (Fig. 1), to study the kinetics of interfacial transport of a model solute (cyclohept-2-enone) in the presence and absence of competing degradation in the aqueous phase. Their results supported the validity of the two-film theory (3) with assumptions of no significant interfacial resistance, no thermal, electrical, and osmotic gradients, and steady-state diffusion in the two-phase transfer cell. Values for the ratio of the forward and reverse first-order rate constants for the partitioning process (k_{12}/k_{21}, Scheme I) from kinetic analysis agreed with equilibrium studies to determine the partition coefficient K_D. Thus, the cell (Fig. 1) was shown to conform kinetically to Scheme I when the stirring speed remained constant, provided that the partition coefficient of the compound was concentration independent over the concentration range studied and the compound was stable for the duration of the experiment.

The concentration C_1 of the solute in the donor aqueous phase was described by:

$$(C_1 - C_1^\infty) = (C_1^0 - C_1^\infty)\, e^{-(k_{12}+k_{21})t} \qquad \text{(Eq. 1)}$$

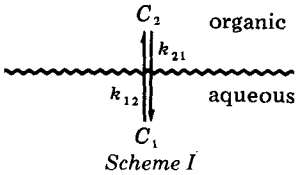
where $C_1 = C_1^0$ and $C_2 = 0$ at time $t = 0$, C_1^∞ is the equilibrium concentration of the aqueous phase, and k_{12} and k_{21} are the first-order forward and reverse rate constants for the transfer process. Thus, a plot of $\ln(C_1 - C_1^\infty)$ *versus* time is linear with a negative slope S, defined by the sum of the forward and reverse rate constants ($k_{12} + k_{21}$) in Scheme I.

The partition coefficient (K_D) may be determined either by kinetic analysis (12) or equilibrium studies from:

$$K_D = k_{12}/k_{21} = C_2^\infty/C_1^\infty \qquad \text{(Eq. 2)}$$

where C_2^∞ and C_1^∞ are the equilibrium concentrations in the organic and aqueous phases, respectively. When a transfer experiment conforms to Scheme I, Eq. 1 may be rewritten in terms of K_D, C_1^0, and the observed first-order rate constant, S ($= k_{12} + k_{21}$), by substituting for $C_2^\infty = (C_1^0 - C_1^\infty)$ in Eq. 2 and C_1^∞ in Eq. 1 and rearranging to give:

$$C_1 = [C_1^0/(K_D + 1)][K_D e^{-St} + 1) \qquad \text{(Eq. 3)}$$



Scheme I

0022-3549/81/1100-1265$01.00/0

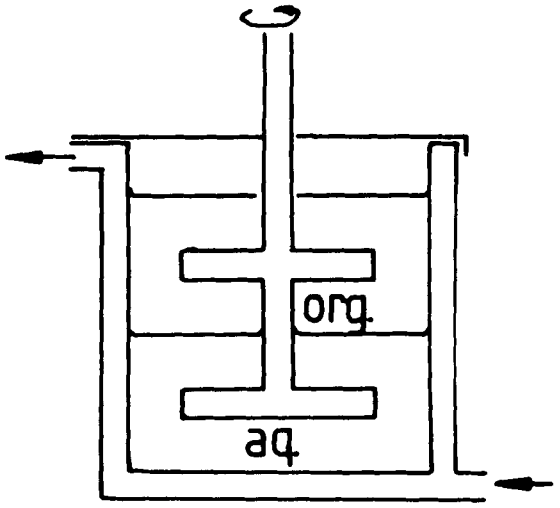


Figure 1—*Two-phase transfer cell with symmetric stirring.*

Equation 3 defines the C_1 *versus* time profile in the two-phase transfer cell given the aqueous donor phase concentration at $t = 0$, the partition coefficient, and the sum of the forward and reverse rate constants for the partitioning process.

Assuming the validity of the two-film theory (3) and Fick's first law of diffusion, Byron *et al.* (12) showed that the sum of the forward and reverse first-order rate constants in the two-phase transfer cell should be described by:

$$(k_{12} + k_{21}) = S = \frac{D_1 D_2 A(K_D V_2 + V_1)}{V_1 V_2 (D_2 K_D h_1 + D_1 h_2)} \quad \text{(Eq. 4)}$$

where subscripts 1 and 2 refer to the aqueous and organic phases, respectively, and S is dependent on the solute diffusion coefficients (D), the phase volumes (V), the diffusion boundary layer thicknesses at the interface (h), and the solute partition coefficient (K_D). It follows from Eq. 4 that, if this theory and its assumptions hold true for the two-phase transfer cell given D_1, D_2, V_1, V_2, h_1, h_2, A, and K_D, the C_1 *versus* time profile could be predicted theoretically from Eq. 3 if an initial solute concentration C_1^0 were assumed.

The partition coefficients (K_D) of solutes in specified solvent systems at fixed temperature may be determined empirically. Alternatively, K_D can be predicted for unsynthesized analogs (13, 14) from a knowledge of the partition coefficient of a lead compound and its substituents. In a transfer cell, the interfacial area and phase volumes may be kept constant throughout the experiment. The dependence of S on K_D (Eq. 4) for a given solvent system in the transfer cell at fixed stirring speed, however, still necessitates accurate theoretical prediction of D_1, D_2, h_1, and h_2. Theoretical predictions of absolute values for these parameters is problematic. Moreover, they will determine the nonlinear dependence of S on K_D and, thus, the changes in C_1 *versus* t profiles (Eq. 3) that may be expected when an homologous series is ascended.

Prediction of Diffusion Coefficients—The Stokes–Einstein relationship (15) predicts the diffusion coefficient (D_{AB}) of a diffusing solute (A) in a specified solvent (B) as:

$$D_{AB} = \frac{RT}{6\pi\eta a N_A} \quad \text{(Eq. 5)}$$

where the coefficient is a function of the gas constant (R), the absolute temperature (T), Avogadro's number (N_A), the viscosity of B, η, and the radius (a) of the diffusing solute molecule. Whereas Eq. 5 has been shown to break down for solutes with molecular weights significantly less than 1000 (16), Wilke and Chang (17) developed Eq. 6 to predict D_{AB} for solutes in dilute solution. The Wilke equation:

$$D_{AB} = (7.4 \times 10^{-10})\,(\psi_B M_B)^{1/2} T/(\eta \bar{V}_A^{0.6}) \quad \text{(Eq. 6)}$$

is empirical, although it is based on Eq. 5. Experimental determinations of D_{AB} for numerous low molecular weight solutes (A) in various solvents (B) fall within 10% of the values predicted by Eq. 6 (17, 18). Equation 6 is written in the centimeter-gram-second system and takes into account the associating tendencies of the solvent molecules as an association parameter (ψ_B), their molecular weight (M_B), and replaces the molecular radius (Eq. 5) with $\bar{V}_A$, the molar volume of the solute (A) in cm^3/mole as a liquid at its normal boiling point. Typical solvent association parameters (ψ_B) were listed by Wilke and Chang (17).

Prediction of Boundary Layer Thicknesses for Diffusion at Interface—The boundary layer theory first introduced by Prandtl (19) and extended by Schlichting (20) assumes a condition of "no slip" at a solid–liquid or immiscible liquid–liquid interface due to molecular adhesion. This approach introduced the concept of a stagnant layer adjacent to an interface, where fluids are observed to behave in a nonideal fashion. If the velocity profile in one phase of the two-phase transfer cell is considered, then, provided a horizontal plane (Fig. 1) in the fluid adjacent to the stirring paddle approximates a rotating disk, Schlichting's boundary layer theory (21) predicts that the angular velocity of the fluid (ω) is directly proportional to the kinematic viscosity of the fluid, $\nu(=\eta/\rho$, where ρ is density), and inversely proportional to the square of the distance from the paddle (x^2) in the vertical direction (Fig. 1). Thus

$$\omega \propto \nu/x^2 \quad \text{(Eq. 8)}$$

such that ω will asymptotically approach zero as $x^2 \to \infty$. Moreover, it follows from Eq. 8 that in the case of symmetric stirring in a two-phase transfer cell:

$$\omega_1/\omega_2 = \nu_1/\nu_2 \quad \text{(Eq. 9)}$$

where subscripts 1 and 2 refer to the aqueous and organic phases, respectively. Equation 9 can be derived by writing Eq. 8 for each phase of the symmetrically stirred transfer cell and holding the distance x from the stirring paddle constant, such that $0 < x_1 = x_2 < x_i$, where x_i is the distance between the stirring paddle and the interface. It follows from Eq. 9 that for two immiscible solvents of different kinematic viscosities (Fig. 1), one solvent must move relative to the other under conditions of symmetric stirring where the angular velocities of both paddles are equal and constant.

Thus, when the condition of no slip is assumed at the interface, the bulk of the aqueous phase may be conceptualized as a disk with angular velocity (ω) rotating relative to a stationary organic phase or as an organic phase with angular velocity ($-\omega$) rotating relative to a stationary aqueous phase. Schlichting's original work was based on the estimation of the boundary layer thickness as the distance from an interface required to enable the velocity of the fluid under consideration to approach that of the bulk. Marked differences occur in the types of equations used, dependent on whether velocity, thermal, or diffusive boundary layer thicknesses are to be calculated. Levich (22) introduced an equation, based on Schlichting's work, permitting the prediction of the boundary layer thickness for diffusion adjacent to a rotating-disk electrode in a stationary fluid. Levich's equation was shown to hold true for systems of the type depicted in Fig. 1 (23) and can be written for h_1 and h_2 (Eq. 4) such that:

$$h_1 = 1.62(D_1/\nu_1)^{1/3}(\nu_1/\omega)^{1/2} \quad \text{(Eq. 10)}$$

and

$$h_2 = 1.62(D_2/\nu_2)^{1/3}(\nu_2/\omega)^{1/2} \quad \text{(Eq. 11)}$$

where ω is the angular velocity of one phase relative to the other.

Prediction of absolute values for D_1 and D_2 and h_1 and h_2 remains problematic. The dimensionless ratios of the diffusion coefficients (D_1/D_2) and the diffusive boundary layer thicknesses (h_1/h_2) in the aqueous and organic phases, however, may be determined by writing Eq. 6 for D_1 and D_2 and solving for the quotient D_1/D_2, assuming T is constant, such that:

$$D_1/D_2 = (\eta_2/\eta_1)[(\psi_1 M_1)/(\psi_2 M_2)]^{1/2} \quad \text{(Eq. 12)}$$

and dividing Eq. 10 by Eq. 11 to give

$$h_1/h_2 = (D_1/D_2)^{1/3}(\nu_1/\nu_2)^{1/6} \quad \text{(Eq. 13)}$$

Solving Eqs. 12 and 13 for D_2 and h_2 and substituting in Eq. 4 give:

$$S = [(D_1 A)/(V_1 h_1)]\{R_1(K_D + r)/[R_1 K_D + (R_1)^{1/3}(R_2)^{1/6}(\psi_1 M_1)^{1/3}(\psi_2 M_2)^{-1/3}]\} \quad \text{(Eq. 14)}$$

where $R_1 = \eta_1/\eta_2$, $R_2 = \nu_2/\nu_1$, and $r = V_1/V_2$. Equation 14 is now in a form that enables the prediction of the sum of the forward and reverse first-order rate constants ($k_{12} + k_{21}$) in Scheme I for any compound in an homologous series, provided that S and K_D are known for the parent compound. K_D can be predicted for each compound under consideration, while the solvent system-dependent constants R_1, R_2, r, ψ_1, ψ_2, M_1, and M_2 may be determined easily.

The term $(D_1 A)/(V_1 h_1)$ must first be estimated experimentally for the system under consideration from a knowledge of the transfer kinetics

of the parent compound. This estimation can be made by rearranging Eq. 14 to give:

$$(D_1A)/(V_1h_1) = \{S[R_1K_D + (R_1)^{1/3}(R_2)^{1/6}(\psi_1M_1)^{1/3} (\psi_2M_2)^{-1/3}]\}/[R_1(K_D + r)] \quad \text{(Eq. 15)}$$

Viscometry and densitometry measurements on the mutually saturated binary solvent system under investigation (constant temperature) yields R_1 and R_2. The phase ratio (r) and molecular weights of the solvents (M_1 and M_2) are known. Wilke (17) quoted values for the association parameters ψ_1 and ψ_2, recommending values of 2.6 for water, 1.9 for methanol, 1.5 for ethanol, and 1.0 for benzene, ether, heptane, and other unassociated solvents.

The partition coefficient (K_D) may be determined experimentally from C_2^∞/C_1^∞ for the parent compound. The initiation of a transfer experiment by introduction of a bolus of the parent solute into the aqueous donor phase of the transfer cell and the study of its transfer kinetics at fixed temperature and stirring speed enable estimation of $S(=k_{12} + k_{21})$ according to Eq. 1. The term $(D_1A)/(V_1h_1)$ may then be determined from Eq. 15.

With fixed stirring conditions, constant temperature, and constant V_1, $A/(V_1h_1)$ should be independent of the solute under consideration. Moreover, it was shown (15) that the diffusion coefficients of nonassociated solutes change insignificantly within a homologous series because:

$$D \propto 1/\sqrt[3]{M_s} \quad \text{(Eq. 16)}$$

where M_s is the molecular weight of the solute. The term $(D_1A)/(V_1h_1)$ can thus be considered constant within a homologous series once estimated under defined conditions for the parent compound in the two-phase transfer cell. Therefore, Eq. 14 can be used to calculate S as a function of K_D within a homologous series.

Given the theoretical estimation of K_D for unsynthesized compounds the prediction of partitioning kinetics should be possible without exhaustive experiments. This theory was evaluated experimentally using a series of barbituric acid derivatives.

EXPERIMENTAL

Determination of S for a Series of 5,5-Disubstituted Barbituric Acid Derivatives—Transfer kinetics were studied for a series of 5,5-disubstituted barbituric acid derivatives (Table I) in the two-phase transfer cell shown in Fig. 1. The drugs in concentrations of $\sim 1.3 \times 10^{-4}$ M were introduced as solutes into the donor aqueous phase. The cell used contained preequilibrated octan-1-ol[1] and 0.06 M Sorensen's buffer (pH 5.0) (24). Preequilibration was effected by stirring for >6 hr prior to solute introduction. Phase volumes were kept constant such that $V_1 = V_2$, 90 ml ($r = 1$). During the transfer experiments, the phases were stirred[2] symmetrically (Fig. 1) with a 4-cm diameter stirring paddle positioned 1 cm from the interface in each phase, rotating at 50 or 100 (±0.5) rpm. The circular glass cell was 6.75 cm i.d.

The aqueous phase was pumped[3] through a flowcell in a spectrophotometer[4]. The concentration in the donor phase (C_1) was continuously monitored by automatically recording[5] the absorbance as a function of time at 222.5 nm (the isosbestic point for all the compounds) until equilibrium was attained. Control experiments monitoring 222.5 nm absorbance in the absence of the organic phase showed that Compounds I–VI were stable for the time course of a kinetic determination. Equilibrium partition coefficients determined at 37° were concentration independent in the range employed during the kinetic experiments. Plots based on Eq. 1 of $\ln(C_1 - C_1^\infty)$ *versus* t were linear for >95% of the process. The terminal slope (S) was determined in each case by linear regression analysis.

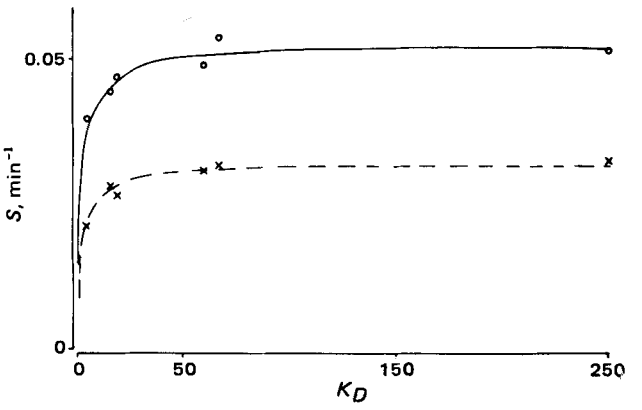
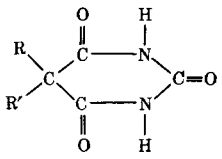


Figure 2—*Dependence of* S ($=k_{12} + k_{21}$) *(Eq. 1; Scheme I), on* K_D *for I–VI (Table I) at 50 (X) and 100 (O) rpm at 37°. The dashed (50 rpm) and solid (100 rpm) profiles are theoretical curves based on Eq. 14 after calculation of* $(D_1A)/(V_1h_1)$ *from Eq. 15.*

Table I—Structures, Molecular Weight, pKa Values and Source of Barbituric Acid Derivatives Used in the Study



Compound	R	R′	Molecular Weight	pKa[a] (25°)	Source
I	CH_3CH_2–	$CH_3—CH_2$–	184.20	7.91	[b]
II	CH_2=$CHCH_2$–	CH_3CH(–CH_3)–	210.23	7.91	[c]
III	CH_2=$CHCH_2$–	CH_3CHCH_2(–CH_3)–	224.25	7.68	[c]
IV	CH_2=$CHCH_2$–	CH_3CH_2CH(–CH_3)–	224.25		[d]
V	CH_2=$CHCH_2$–	$CH_3(CH_2)_2CH$(–CH_3)–	238.27	8.08	[c]
VI	CH_2=$CHCH_2$–	CH_2=$CHCH_2$–	208.21	7.79	[c]

[a] Reference 25. [b] Hopkins and Williams, Essex, England. [c] Ganes Chemical Works, Carlstadt, N.J. [d] Sterling-Winthrop Research Institute, Rensselaer, N.Y.

Partition Coefficients—Values of K_D were estimated in triplicate by equilibrating I–VI at 37° in the solvent system previously defined. The phase ratio (r) was adjusted such that concentrations in the aqueous phase gave $0.1 < (\text{Abs})_{222.5\text{ nm}} < 0.9$, and K_D was calculated from:

$$K_D = [(C_1^0 - C_1^\infty)r]/C_1^\infty \quad \text{(Eq. 17)}$$

where C_1^0 and C_1^∞ are the initial and final concentrations in the aqueous phase.

Viscometry and Densitometry—The viscosities and densities of mutually saturated octan-1-ol and 0.06 M Sorenson's buffer (pH 5.0) were determined in replicate relative to water at 37° using an Ostwald U-tube viscometer and a 10-ml specific gravity bottle. Mean values for η and ρ enabled estimation of R_1 and R_2 (Eq. 14).

Dependence of Transfer Kinetics on K_D—Values for the ratios R_1 and R_2 were determined as previously described. Molecular weights M_2 and M_1 for preequilibrated octan-1-ol and 0.06 M Sorenson's buffer (pH 5.0) were assumed to be 130.23 and 18.02, respectively, as if they were pure octanol and water. The association parameters ψ_1 and ψ_2 were assigned values of 2.6 and 1.0 (17). Values for S were determined from experimental data for C_1 *versus* t for each compound at both stirring speeds. Plots of $\ln(C_1 - C_1^\infty)$ *versus* t were subjected to linear regression analysis to determine the value of the linear negative slope $S(=k_{12} + k_{21})$ according to the logarithmic form of Eq. 1. Values for the coefficient $(D_1A)/(V_1h_1)$ were calculated for I–VI based on $r = 1$ and their experimentally determined values for S at 50 and 100 rpm using Eq. 15. Thus, two mean values for the coefficient were calculated at the different stirring speeds. The theoretical dependence of $S(=k_{12} + k_{21})$ on K_D was determined for the octan-1-ol buffer system at 37° using Eq. 14. Calculated values for S were compared to those determined experimentally for I–VI.

[1] Specgrade, Fisons Ltd., Loughborough, England.
[2] Model KQT59 stirrer, Citenco Ltd., Herts, England.
[3] Model RPD lab pump, Fluid Metering Inc., Oyster Bay, NY.
[4] Model SP500, Pye-Unicam, Cambridge, England.
[5] Model 26000 recorder, Bryans Southern Instruments, Mitcham, Surrey, England.

Table II—Theoretical and Experimental Estimates for S ($=k_{12} + k_{21}$) at 50 and 100 rpm in Transfer Cell and Mean Octanol–pH 5.0 Buffer Partition Coefficients at 37° for I–VI

		50 rpm			100 rpm		
Compound[a]	K_D^b	S_{th}^c	S_{obs}^d	Percent Error[e]	S_{th}^c	S_{obs}^d	Percent Error[e]
I	4.5	2.18	2.10	3.8	3.57	3.80	−6.1
II	15.7	2.78	2.83	−1.8	4.55	4.41	3.2
III	19.3	2.84	2.64	7.6	4.66	4.68	−0.4
IV	60.0	3.08	3.06	0.7	5.04	4.89	3.1
V	67.0	3.09	3.17	−2.5	5.07	5.36	−5.4
VI	250.0	3.18	3.23	−1.5	5.21	5.14	1.4

[a] Table I. [b] Observed, mean of three determinations. [c] Equation 14; expressed in $min^{-1} \times 10^2$. [d] ($k_{12} + k_{21}$) based on kinetic analysis (Eq. 1) ($min^{-1} \times 10^2$). [e] 100 ($S_{th} - S_{obs}$)/S_{obs}.

RESULTS

Determination of S for a Series of 5,5-Disubstituted Barbituric Acid Derivatives—Plots of $\ln(C_1 - C_1^\infty)$ *versus* t based on Eq. 1 were linear for >95% of the partitioning process for each compound studied at either 50 or 100 rpm. Observed terminal slopes, S_{obs} ($=k_{12} + k_{21}$), are documented in Table II after determination for each of the derivatives of I–VI by linear regression (correlation coefficient $r > 0.99$, $n > 10$).

Partition Coefficients—Partition coefficients as mean values from triplicate determinations for I–VI at 37° in the solvent system previously described are shown in Table I, these values are based on Eq. 17, with an experimental deviation from the mean of <8%.

Viscometry and Densitometry—Mean viscosity and density values of the aqueous and organic phases used in the study were determined as 0.7005×10^{-2} and 4.806×10^{-2} poise and 1.0017 and 0.8148 g/ml, respectively, at 37°.

Dependence of Transfer Kinetics on Partition Coefficient—Mean values for the coefficient $(D_1A)/(V_1h_1)$ were determined for I–VI as 3.21×10^{-2} ($\pm 0.13 \times 10^{-2}$) and 5.26×10^{-2} ($\pm 0.21 \times 10^{-2}$) min^{-1} at 50 and 100 rpm, respectively (Eq. 15). The dashed and solid curves of S *versus* K_D in Fig. 2 were generated using Eq. 14 for the octanol buffer system with $(D_1A)/(V_1h_1)$ held constant at these mean values for 50 and 100 rpm. They show the theoretical dependence of the sum of the forward and reverse rate constants k_{12} and k_{21} (Scheme I) on the equilibrium partition coefficient K_D. Experimental determinations of S ($=k_{12} + k_{21}$), determined by kinetic analysis of C_1 *versus* t profiles for I–VI at the two stirring speeds (from data anlysis using Eq. 1), are shown. The percentage error involved when determining S from Eq. 14 (S_{th}) as opposed to experimentally in the two-phase cell (S_{obs}) at either stirring speed is shown in Table II to be ≤8%. Table II summarizes these errors and the values of S_{th}, S_{obs}, and K_D at 50 and 100 rpm for I–VI.

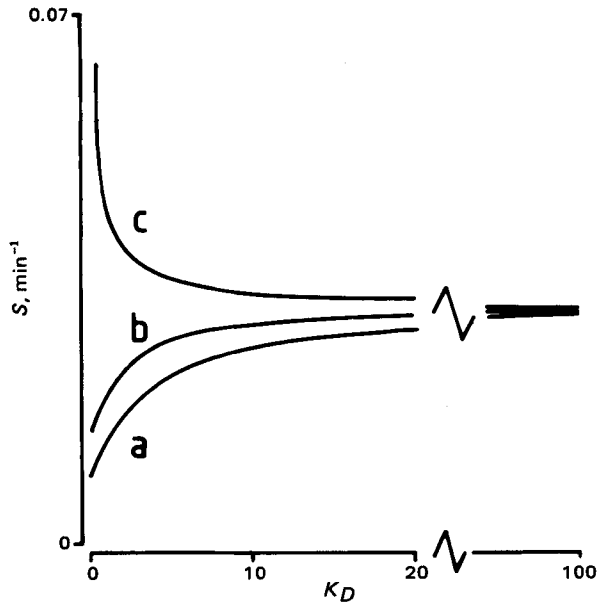


Figure 3—*Theoretical dependence of* S *on* K_D *(Eq. 14) in the two-phase transfer cell at 50 rpm for (a) the octanol–aqueous system used in this study, (b) a butanol–aqueous system at 35°, and (c) a chloroform–aqueous system at 35°. The values of the coefficient* $(D_1A)/(V_1h_1)$ *and* r *were held constant at* 3.21×10^{-2} min^{-1} *and 1, respectively. Association parameters* (ψ) *were assigned values of 2.6, 1.0, 1.0, and 1.0 for water* (M = *18.02), octanol* (M = *130.23), butanol* (M = *74.12), and chloroform* (M = *119.38), respectively (17). The profile a was generated with* $R_1 = 0.149$ *and* $R_2 = 8.435$, *their experimentally determined values. For curves b and c, literature values (28, 29) were utilized for the pure solvents such that* $R_1 = 0.340$ *and* 1.430 *and* $R_2 = 3.522$ *and* 0.480 *for butanol–water and chloroform–water systems, respectively.*

DISCUSSION

The theory describing the dependence of the sum of the forward and reverse first-order rate constants (S) in Scheme I on K_D was derived in this study for solutes introduced into a donor phase of a stirred transfer cell (Eq. 14). For this theory to hold true, the solute must conform to Scheme I and neither dissociate nor associate in the organic or aqueous phases at experimental concentrations. An aqueous buffer (pH 5.0) was chosen to ensure that the barbituric acid derivatives were insignificantly ionized (<1%) in the aqueous phase of the cell, provided the pKa values quoted in Table I at 25° were ≥7 at 37°. These studies showed that the partition coefficients of I–VI varied insignificantly with concentration in the experimental ranges. This finding agrees with previous observations (26). Therefore, it was unlikely that these compounds existed significantly as other than unionized monomers in either phase.

The validity of the two-film theory in the two-phase transfer cell was established previously (1, 12). Experimental conditions were chosen such that both phases were mutually saturated, and the transfer cell was maintained at 37° to eliminate temperature gradients. Evaporation of water *via* diffusion through the upper organic layer was minimized by covering the cell and was negligible during 24 hr at 37°. The adequacy of Eqs. 1 and 3 in describing the experimental data, together with the absence of a visible lag time, implies rapid achievement of steady-state diffusion conditions in the transfer cell. The assumption of steady-state diffusion is involved in the derivation of Eq. 4. Similarly, the agreement between the continuous data for C_1 *versus* t and Eqs. 1 and 3 supports the assumption of an effectively constant interfacial area, A, at both 50 and 100 rpm.

The remaining assumptions necessary to develop this theory and Eq. 14 require that solvent flow in each phase is essentially laminar (low Reynold's number) and that the bulk of the organic phase rotates in relation to the bulk of the aqueous phase. This approach enabled prediction of the dimensionless term h_1/h_2 by assuming the validity of the Levich equation (Eqs. 10 and 11) and the existence of symmetric stirring. Similarly, the validity of Wilke's equation (Eq. 6) and his solvent association parameters, ψ_B, were necessary for determination of D_1/D_2. Wilke's equation for low molecular weight nonassociating solute molecules in dilute solution enabled calculation of $(\psi_1 M_1)^{1/3}(\psi_2 M_2)^{-1/3}$ for use in Eqs. 14 and 15. The absence of significant interfacial resistance, osmotic gradients, effective constancy of diffusion coefficients within a series of drug derivatives, and the validity of these latter assumptions can only be verified by testing the dependence of S on K_D and by comparing the theory to empirical observations.

Figure 2 and Table II show the theoretical and experimental dependence of S ($=k_{12} + k_{21}$) (Scheme I) on K_D for I–VI in an octanol–aqueous system at 37°. The excellent agreement between experiment and theory (theoretical prediction of S varies <8% from empirical determinations, Table II) demonstrated the validity of the assumptions for the two-phase transfer cell. Moreover, Fig. 2 and Eq. 14 demonstrate clearly how the transport kinetics of a series of drug analogs may be predicted from a knowledge of the transport kinetics of the parent compound and its partition coefficient. Given either experimental determination of the partition coefficient of subsequent analogs or its theoretical prediction for even unsynthesized compounds (13, 14), a single estimation

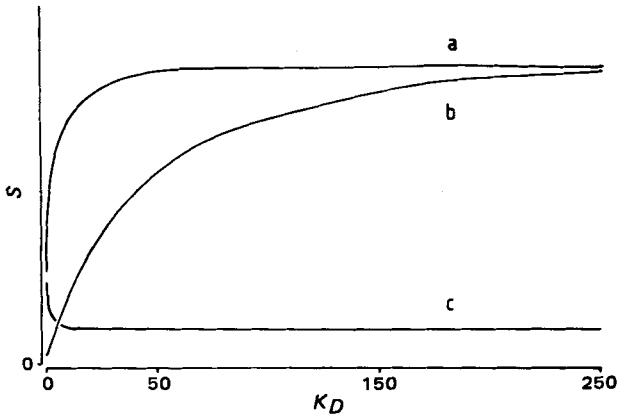


Figure 4—*Theoretical dependence of* S *on* K_D *(Eq. 4) for conditions of symmetric (a) and asymmetric (b and c) stirring in an octanol–aqueous system at 37° in the two-phase transfer cell. The ratio of boundary layer thicknesses for diffusion,* h_1/h_2*, were varied such that* $h_1/h_2 = 1.12$ *(curve a; the present study with symmetric stirring), 0.1 (curve b), and 10 (curve c). Other variables were held constant (see text).*

of $(D_1A)/(V_1h_1)$ (Eq. 15), after determination of R_1, R_2, r, and $(\psi_1M_1)^{1/3}(\psi_2M_2)^{-1/3}$, is possible from kinetic analysis for S $(=k_{12}+k_{21})$ (Eq. 1) for the parent compound in the transfer cell. The agreement between theory (Eq. 14) and experiment (Fig. 2) demonstrates that the $(D_1A)/(V_1h_1)$ estimate (Eq. 15) applies to the entire series at a fixed stirring rate. Therefore, once the system is defined, the dependence of S on K_D can be evaluated. Prediction of C_1 *versus* t profiles for the remaining compounds in the series thus is possible for a given donor phase concentration at $t = 0$, C_1^0, using Eq. 3.

Variation of Solvent Systems and Stirring Conditions—Neither the solvent systems nor the stirring conditions associated with the two-phase transfer cells documented in the literature were standardized. Gordon and Sherwood (1) employed an aqueous–butanol system while Zecchi *et al.* (6) used benzene as the organic phase. Symmetric (27) and asymmetric (1) stirrers also were described. The dependence of transfer kinetics on K_D in systems containing either various organic phases or asymmetric stirring methods will be different from that shown in Fig. 2 for the symmetrically stirred aqueous–octanol system.

Values for the solvent system dependent variables R_1, R_2, ψ_1, ψ_2, M_1, and M_2 were obtained for butanol–water and chloroform–water systems at 35° (17, 28, 29) and are documented (Fig. 3). The coefficient $(D_1A)/(V_1h_1)$ was held constant at 3.21×10^{-2} min^{-1}. The theoretical dependence of S on K_D for these two systems with symmetric stirring and $r = 1$ is shown in Fig. 3 according to Eq. 14. The theoretical curve for the octanol–aqueous system used in the present study is shown for comparison. Although K_D is itself a function of the solvent system employed, at a given K_D value in Fig. 3 the gradient dS/dK_D may be positive, negative, or zero, depending on the solvent system employed. This behavior disproves the often stated assumption that S values are proportional to K_D values.

To determine the effect of asymmetric stirring on the dependence of S on K_D for a given solvent system, it is necessary to solve Eq. 12 for D_2 and to substitute in Eq. 4. The value for the ratio D_1/D_2 was maintained at 4.032, its calculated value from Eq. 12 for the aqueous–octanol system at 37°. The phase volumes V_1 and V_2 were held constant and equal. Equation 4 was used to determine the dependence of S on K_D for $h_1/h_2 = 1.12$ (the ratio for symmetric stirring in the aqueous–octanol system at 37°), 0.1, and 10.0. Figure 4 illustrates the likely effect of asymmetric stirring where h_1/h_2 would be expected to change on the resultant dependence of S on K_D for a specified solvent system at fixed temperature. The gradient dS/dK_D at a fixed K_D value may be positive, negative, or zero, depending on the stirring conditions employed.

The results from extrapolation of the present theory are shown in Figs. 3 and 4. Studies are in progress to test these extrapolations experimentally in appropriate systems. These implications are fundamental to transport kinetic studies employing two- or three-phase transfer cells. Augustine and Swarbrick (10) used a three-phase transfer cell to rank a series of compounds dependent on a parameter proportional to k_{12} (Scheme I). Other investigators (9) attempted to discriminate between compounds on the basis of their partitioning kinetics. However, it is clear from Figs. 3 and 4 that such a procedure in an unstandardized transfer cell is nothing more than a study of solvent systems and stirring paddles, provided that the present theory is correct. The observation (Figs. 3 and 4) that dS/dK_D may be positive, negative, or zero at fixed K_D means that S $(=k_{12}+k_{21})$ and, therefore, C_1 *versus* t profiles for a series of compounds may be ranked merely by the experimental solvent and stirring conditions.

The choice of solvent is a subject for debate in that the solvent system itself defines the partition coefficient of the compounds studied. The use of asymmetric stirring in two- and three-phase cells however, necessitates the absolute estimation of individual values for h_1 and h_2 (Eq. 4). Absolute boundary layer thicknesses can only be approximated (20). The dependence of S on K_D must be established empirically under asymmetric stirring conditions. The theoretical approach described in this paper, which is only applicable to symmetric stirring, utilizes the dimensionless ratio h_1/h_2 in the derivation of Eq. 14, which predicts the kinetics of transfer as a function of K_D.

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