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Theoretical Model-Based Equations for the Linear Free Energy Relationships of the Biological Activity of Ionizable Substances. 1. Equilibrium-Controlled Potency

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Because of the ambiguities of how to treat ionization in empirical equations which relate biological activity to partition coefficient by use of a $(\log P)^2$ term, a theoretical approach to the problem is proposed. Based on a simplified view of assays of potency following in vitro or continuous infusion administration of drugs, equations have been derived from a combination of mass law, equilibrium, and extrathermodynamic assumptions. In general form the equations which relate potency to partition coefficient (P) and degree of ionization (α) are the following. If the neutral form reacts with the receptor, $\log (1/C) = -\log [1 + \Sigma^m(d_i P^{c_i}) + \Sigma^n[a_j/P^b(1 - \alpha_j)]] + X$. If the ionic form reacts with the receptor, $\log (1/C) = -\log [1 + (1 - \alpha_n)/(\alpha_n)[\Sigma^m(d_i P^{c_i}) + \Sigma^n[a_j/P^b(1 - \alpha_j)]]] + X$. In this generalized model there are m nonaqueous compartments and n aqueous compartments of different pH. The parameters a, b, c, and d can be interpreted in terms of the model. The shape of the $\log (1/C)$ vs. $\log P$ curve may be asymptotic, linear, or composed of two portions of unequal slope which meet at an optimum or a bend. With the use of these equations it is possible to examine whether the ion or the neutral form is the active species and whether there is hydrophobic bonding to the receptor and/or an inert compartment. The models may be further extended to include terms other than $\log P$ and α.

During the past 10 years a great deal of progress has been made in the study of quantitative structure–activity relationships. In particular, the extrathermodynamic or linear free energy approach has been used to analyze the relationships in many series of compounds.[1,2] However, very little attention has been paid to the nature of these relationships when the drugs involved are partially ionized at the pH of the biological system.[1,3,4] This report deals with the derivation of equations for the relationship between potency, partition coefficient, and degree of ionization for closed, i.e., equilibrium, systems. Such equations may be useful in the correlation of potency in certain in vitro and continuous infusion assays.

The main problem to be discussed is how to correctly account for the effect of ionization in the modeling of biological partitioning. It is known that the partition coefficient (P) of the ionic species of a compound is approximately 15 000× lower than that of the neutral form of the same compound.[5] Thus for all practical purposes, if the concentration of the neutral form is at least 0.001 that of the ionized form, then the ion does not contribute to the observed partition coefficient. This may be expressed in equation form

$$\log P \text{ (obsd, solvent X)} = \log P \text{ (neutral, solvent X)} + \log (1 - \alpha) \quad (1)$$

in which α is the fraction of drug ionized at the pH of measurement. Some workers have assumed that one should therefore use the partition coefficient between buffer of biological pH and an organic solvent to model the hydrophobic effect of the compound in the biological system.[6] Others have divided the observed potency ($1/C$) by the fraction un-ionized ($1 - \alpha$) in order to "correct" the potency to be that of the neutral form.[3,4] The following discussion will evaluate the merits of each approach as well as suggest the possible complications.

The problem with using the extrathermodynamic approach with ionizable substances in biological systems is that in general there is not a simple linear relationship between the $\log P$ of a substance and its biological potency. This nonlinear relationship has traditionally been fit by

a parabola, which was empirically chosen because it fit a large number of sets of data.[7]

Riggs[8] has clearly described the theoretical and practical distinction between empirical and theoretical equations. Empirical equations are often useful for summarizing observed relationships. Their primary deficiencies are that if such an equation does not fit the data there is no guide as to why this is so or what to do next. Theoretical or model-based equations are frequently more general in applicability. Since they are derived from theory, the fit or lack of fit to such equations may be used to refine or discard the model. The disadvantages of such equations are that in specifying a model one may lose sight of the fact that the model is only an imperfect representation of the biological system, that the equations may contain more adjustable parameters and thus be less attractive statistically than an empirical equation, and the mathematical nature of the equations may be such that the fitting of the data to the equation is difficult. In spite of these limitations, the question of how to treat ionization in quantitative structure–activity studies is answered only by the theoretical approach.

Other workers have derived model-based equations for structure–activity studies. Higuchi and Davis[9] proposed a model identical with the one proposed below. They did not consider the problem of ionization, make the extrathermodynamic assumptions, nor include the amount of drug at the receptor in the material balance. Flynn and Yalkowsky and various co-workers derived equations based on a kinetic model.[10-13] They also did not consider the influence of ionization on the relationships.

In terms of the models to be considered below, it is important to recognize what statistical fits to the model-based equations can distinguish and what they cannot distinguish. In particular, if drug is in equilibrium in a number of discrete nonaqueous biological *phases* which in the model are not the ones in which the biological activity occurs, and if the equilibrium constants for partitioning into these phases are related to log P by the same equation, to the analysis these phases will appear as one *compartment*. Similarily, all aqueous *phases* in equilibrium at the same pH will appear as one *compartment* in the analysis.[14]

Our approach to the examination of the influence of ionization on structure–activity relationships is thus to suggest explicit models, apply extrathermodynamic assumptions to each step in the model, and ultimately derive equations to be fit. These equations will thus contain terms which account for the effect of ionization on each of the log P dependent steps. In other words, although there is a linear relationship between physical properties and individual rate or equilibrium constants, the overall relationship between biological and physical properties may be of any mathematical form.

The step-by-step algebraic derivation of each equation will not be presented. A general derivation is given in the Appendix. The following procedure is used. (1) The equilibrium and mass law expressions which apply to the model are stated. (2) A general equation for the dependence of the observed biological activity on these constants is formulated. It is assumed that concentrations may be used in the equilibrium constants. (3) Next, it is assumed that each equilibrium constant is a function of the logarithm of some solvent–water partition coefficient, P. This is the usual extrathermodynamic assumption. (4) The extrathermodynamic relationships are substituted into the model-based equation to give the biological activity as a function of log P and log $(1 - \alpha)$.

Models in Which the Biological Response Is a Linear Function of the Amount of Drug at the Receptor, and the Partitioning of Drug to the Receptor Is a Function of Hydrophobicity Only. In the models to be considered in this section it is assumed that potency is a linear function of the concentration of drug at the receptor, that is, that the members of the series differ in affinity and not in intrinsic activity. It is the model from which was formulated the typical dose–response curve formalism and the notions of intrinsic activity and affinity.[15]

Log P has been defined as an additive constitutive parameter.[16] This implies that hydrophobicity is a fundamental characteristic of a molecule. Verification of the fundamental nature of hydrophobicity is the demonstration that for a series of compounds their log P between any one solvent and water is proportional to that between a second solvent and water.[17] The fundamental character of hydrophobicity also indicates that if the partition coefficients of one compound between different solvent systems are to be compared, then the comparison should be made between the partition coefficients of the species which is involved in the particular hydrophobic equilibrium; the partition coefficients should be calculated as the ratio of the un-ionized form in the organic solvents to that of the un-ionized form in water. This may be stated in equation form.

$$\log P \text{ (neutral, solvent 1)} \quad b \log P \text{ (neutral, solvent 2)} + a \tag{2}$$

In further derivations log P or P refers to partitioning of the neutral form of the drug between some nonaqueous solvent and water.

Model 1. In this set of models there is one aqueous compartment in equilibrium with a biologically inert nonaqueous compartment. There is also a second equilibrium between the aqueous (or, equivalently, nonaqueous) and the receptor compartments. This model would probably apply to many in vitro assays except those in which a covalent bond is formed and some antibacterial systems. It may also apply to in vivo assays when the drug is administered by continuous infusion and the concentrations in the various tissues are constant.

The extrathermodynamic assumptions are that the equilibrium constants, for the concentration of the neutral form of drug, between the inert nonaqueous and the aqueous compartment [K(biol)] and that between the receptor and aqueous compartment [K(rec)] are related to log P by the following equations.

$$\log K(\text{biol}) = \log (d') + c' \log P \tag{3}$$

$$\log K(\text{rec}) = \log (a') + b \log P \tag{4}$$

Based on the algebra in the appendix, the equation which relates potency $(1/C)$ to log P and log $(1 - \alpha)$ when the neutral form of the drug reacts with the receptor is

$$\log (1/C) = \log \left[\frac{1}{1 + dP^c + \dfrac{a}{P^b(1 - \alpha)}} \right] + X \tag{5}$$

In certain cases both an ionic and hydrophobic interaction may occur between a small molecule and a protein. An example is the binding of lipophilic anions by serum albumin[18] in which it has been shown that although the anionic group is a prerequisite for binding, the free energy of the interaction is proportional to its hydrophobicity. Hence it is possible that the ionic form of a drug would

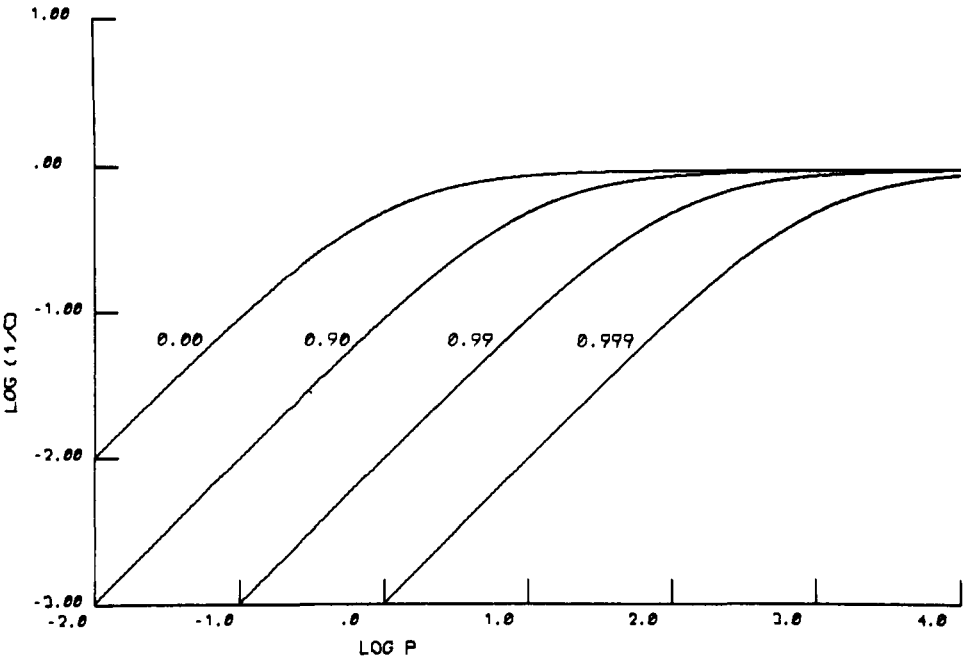


Figure 1. Drug equilibrated in one aqueous and one nonaqueous compartment, and the neutral form reacts with the receptor (eq 5). The constants are $a = 1$, $b = 1$, $c = 0$, $X = 0$, and $d = 0.01$. The fraction ionized in the aqueous compartment is indicated on each line.

interact with the receptor but that the free energy of the interaction would be a linear function of its hydrophobicity. In such a case the equation is

$$\log (1/C) = \log \left[\frac{\alpha/(1-\alpha)}{\frac{\alpha}{1-\alpha} + dP^c + \frac{a}{P^b(1-\alpha)}} \right] + X \quad (6)$$

When both forms interact with the receptor it is

$$\log (1/C) = \log \left[\frac{1 + Z[\alpha/(1-\alpha)]}{1 + Z\frac{\alpha}{1-\alpha} + dP^c + \frac{a}{P^b(1-\alpha)}} \right] + X \quad (7)$$

The adjustable coefficients to be fit, a, b, c, d, X, and Z, are directly defined by the model; see the Appendix. The important ones are b, the slope of the log K (rec) vs. log P relationship, and Z, the relative affinity of the ionic vs. the neutral form for the receptor.

Typical relationships between log P, log $(1/C)$, and α are shown in Figures 1–4. For all three equations the slope of the log $(1/C)$ vs. log P curve is b at low log P and $-c$ at high log P. Hence a successful fit of data to one of the equations can provide a hypothesis as to the possibility of hydrophobic bonding of the drugs to the receptor; only if there is hydrophobic bonding between the drug and the receptor will there be a positive slope of log $(1/C)$ vs. log P.

If the partitioning to the receptor and to the inert nonaqueous compartments change the same amount with changes in log P, that is, if the inert and receptor compartments are of approximately the same polarity, then the slope of the curves is b at low log P and zero at high log P. An example of this kind of relationship is shown in Figure 1 for eq 5 and Figure 2 for eq 6. Note the different influences of ionization for the two cases.

For the curve to resemble a parabola, the dependence of the log K(biol) on log P must be twice that of the dependence of log K(rec) on log P, that is, when $c = b$ or $c' = 2b$. From the correlations reported by Leo et al.,[5] this would be the case if the inert nonaqueous compartment is substantially more lipophilic than the receptor compartment. Such curves are shown in Figures 3 and 4.

For all cases for which there is an optimum log P, for example, when b and $c > 0$, the optimum log P is not constant but varies with log $(1-\alpha)$. The equation for the optimum log P is

$$\log P(\text{opt}) = \left[\frac{1}{b+c} \right] \log \left[\frac{ab}{cd(1-\alpha)} \right]$$

The log $(1/C)$ at the optimum for eq 5 is

$$\log (1/C, \text{opt}) = -\log \left[1 + dP^c + \frac{cdP^c}{b} \right]$$

If the ion is the active form it is

$$\log (1/C, \text{opt}) = -\log \left[1 + dP^c + \frac{cdP^c(1-\alpha)}{b(\alpha)} \right]$$

Model 2. Next, we will consider the more general model, that for which the observed biological activity is dependent on equilibration of drug in the receptor compartment, in one or more aqueous compartments in which it is ionized to a different extent, and in one or more nonaqueous biologically inert compartments.

The example with two aqueous and one nonaqueous phase is probably a realistic one for the in vitro antibacterial activity of many substances.[19-21] The different degrees of ionization may result from a simple difference in pH in the two compartments or from the net result of a complex electrochemical potential gradient. The latter is more reasonable for bacteria;[19] the resulting equations are identical.

In this derivation it is assumed that the concentration of the neutral form is the same in all aqueous phases. In these equations there are m inert nonaqueous compartments and n aqueous compartments, and the drug at the receptor is in equilibrium with drug in aqueous compartment n. The subscripted coefficients are defined by

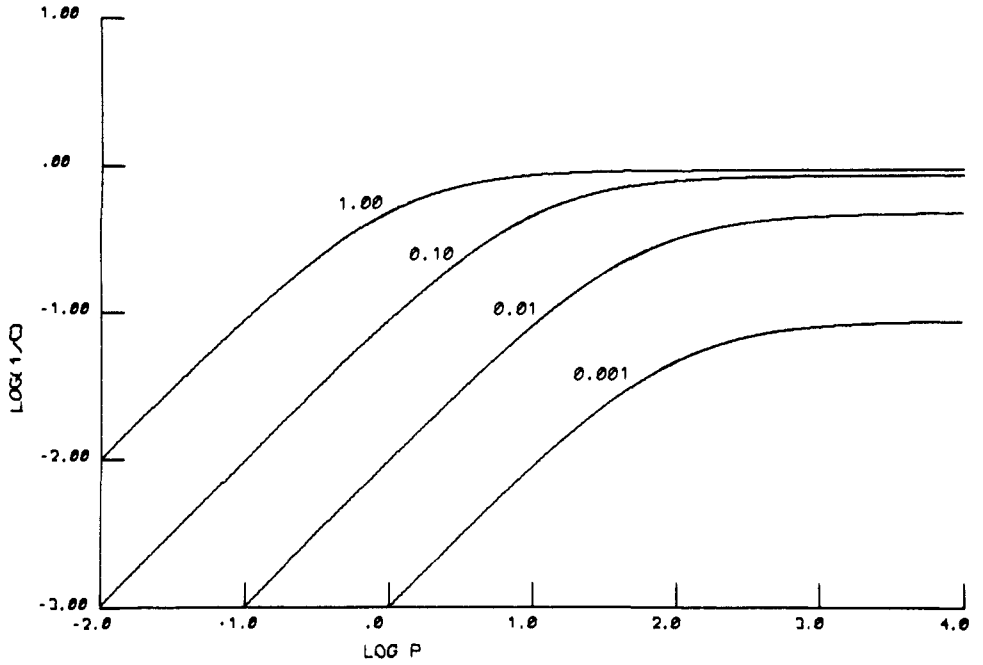


Figure 2. Drug equilibrated in one aqueous and one nonaqueous compartment, and the ionic form reacts with the receptor (eq 6). The constants are $a = 1$, $b = 1$, $c = 0$, $X = 0$, and $d = 0.01$. The fraction ionized in the aqueous compartment is indicated on each line.

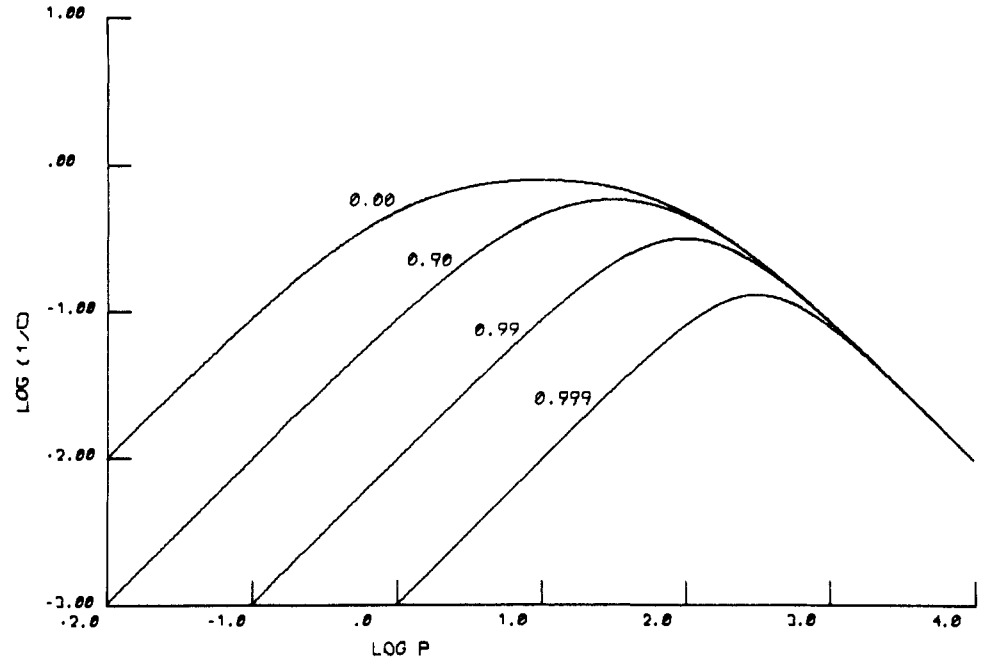


Figure 3. Drug equilibrated in one aqueous and one nonaqueous compartment, and the neutral form reacts with the receptor (eq 5). The constants are $a = 1$, $b = 1$, $c = 1$, $X = 0$, and $d = 0.01$. The fraction ionized in the aqueous compartment is indicated on each line.

analogy to those for model 1 above.

If it is the neutral form which reacts with the receptor the equation is

$$\log (1/C) = \log \left[\frac{1}{1 + \sum^{m} d_i P^{c_i} + \sum^{n} \frac{a_j}{P^{b}(1 - \alpha_j)}} \right] + X \quad (8)$$

It is necessary to consider a particular aqueous compartment in this equation only if the term corresponding to it contributes significantly to the value of log $(1/C)$. Since the volume of aqueous compartment number 1 is usually the largest, if the cut-off point is 1%, then aqueous compartment j can be ignored if

$$a_j/(1 - \alpha_j) < 0.01 a_1/(1 - a_1)$$

or, equivalently

$$V_j/(1 - \alpha_j) < 0.01 V_1/(1 - \alpha_1)$$

Thus both the volume and the fraction un-ionized or pK_a determine if it is necessary to use a several compartment model in any particular case for which the nonionized species is the only one which reacts with the receptor.

For example, in the growth-rate type of antibacterial assay, the maximum bacterial concentration is 10^9/ml.[21] This represents a ratio of V_1/V_2 of approximately 250. However, stationary phase occurs at 10^{10} bacteria/ml or

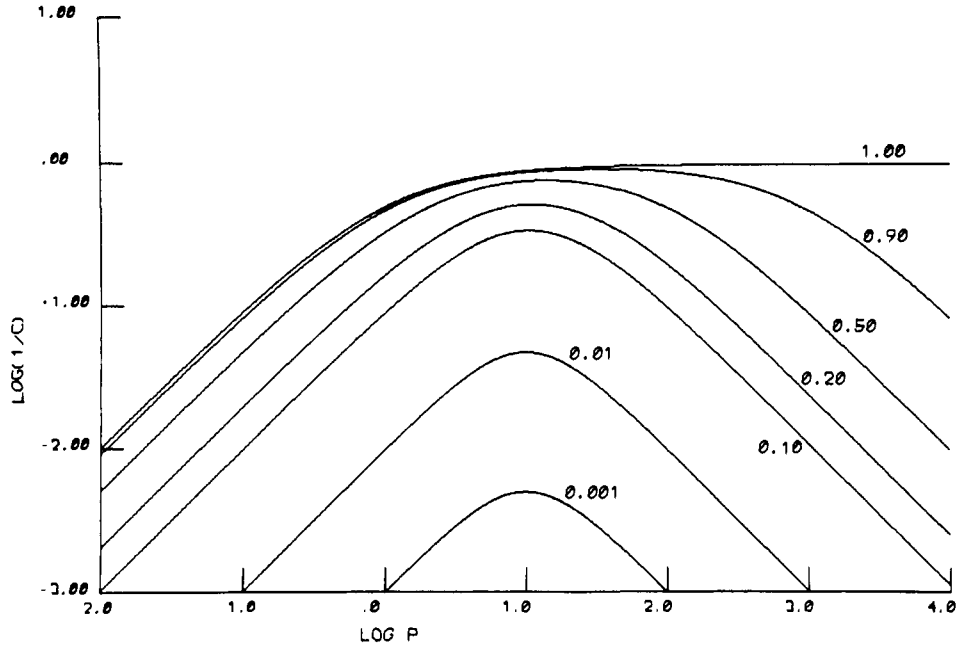


Figure 4. Drug equilibrated in one aqueous and one nonaqueous compartment, and the ionic form reacts with the receptor (eq 6). The constants are $a = 1$, $b = 1$, $c = 1$, $X = 0$, and $d = 0.01$. The fraction ionized in the aqueous compartment is indicated on each line.

higher. Thus for the minimum inhibitory concentration type of antibacterial assays, in which the end point of drug activity is measured at stationary phase, V_1/V_2 might be 25 or less. Clearly, one is more likely to need to consider the second aqueous compartment for the latter type of assay.

The inclusion of terms contributing to K(biol) greater than $m = 1$ might be considered where the log $(1/C)$ vs. log P plot shows nonlinearity beyond an optimum or bend.

If it is the ionic form which reacts with the receptor, the equation is

$$\log (1/C) = \log \left[\frac{\alpha_n/(1-\alpha_n)}{\frac{\alpha_n}{1-\alpha_n} + \sum^{m} d_i P^{ci} + \sum^{n} \frac{a_j}{P^b(1-\alpha_j)}} \right] + X \quad (9)$$

Because of the term $\alpha_n/(1-\alpha_n)$ it will always be necessary to specifically consider the pH of the nth aqueous phase. If this pH is not known but the pK_a's of the compounds are known, then the pH can be fit in the regression analysis as a factor of d_i and a_j. As with eq 8, depending on its volume and pH, it might or it might not be necessary to consider more than one aqueous compartment in the summation.

The generalized equation if both forms interact with the receptor is

$$\log (1/C) = \log \left[\frac{1 + Z[\alpha_n/(1-\alpha_n)]}{1 + Z\frac{\alpha_n}{1-\alpha_n} + \sum^{m} d_i P^{ci} + \sum^{n} \frac{a_j}{P^b(1-\alpha_j)}} \right] + X \quad (10)$$

The log $(1/C)$ vs. log P dependencies of these equations are similar to those for eq 5 and 6. The slope at low log P is always b; the slope of the log K(rec) vs. log P relationship. If there is only one inert nonaqueous compartment then the slope at high log P is $-c$. Again, the parameter Z is a measure of the relative affinity of the ionic and neutral forms of the drug for the receptor.

Models Which Include Other Physical Properties.

Model 3. If the potency is a function not only of the concentration in the receptor phase but also steric (E_s) and electronic (σ) effects, then one may simply add the following to the equation to be fit: $\rho\sigma + \delta E_s$. Equation 5 would then be

$$\log (1/C) = \log \left[\frac{1}{1 + dP^c + \frac{a}{P^b(1-\alpha)}} \right] + \rho\sigma + \delta E_s + X \quad (11)$$

Model 4. If the *partitioning* to the receptor is a function of log P, σ, and E_s, then

$$\log K(\text{rec}) = \log (a') + b \log P + \rho\sigma + \delta E_s$$

Then eq 5, for example, becomes

$$\log (1/C) = \log \left[\frac{1}{1 + dP^c + \frac{a}{10^{\rho\sigma}10^{\delta E_s}P^b(1-\alpha)}} \right] + X \quad (12)$$

Model 5. If, however, it is the equilibrium constant to the inert nonaqueous compartment which is a function of these properties, then eq 5 becomes

$$\log (1/C) = \log \left[\frac{1}{1 + 10^{\rho\sigma}10^{\delta E_s}dP^c + \frac{a}{P^b(1-\alpha)}} \right] + X \quad (13)$$

Models 4 and 5 can be combined while corresponding changes can be made to eq 6–10.

Discussion

It is now possible to compare the model-based equations with the traditional parabolic ones. Two forms of the

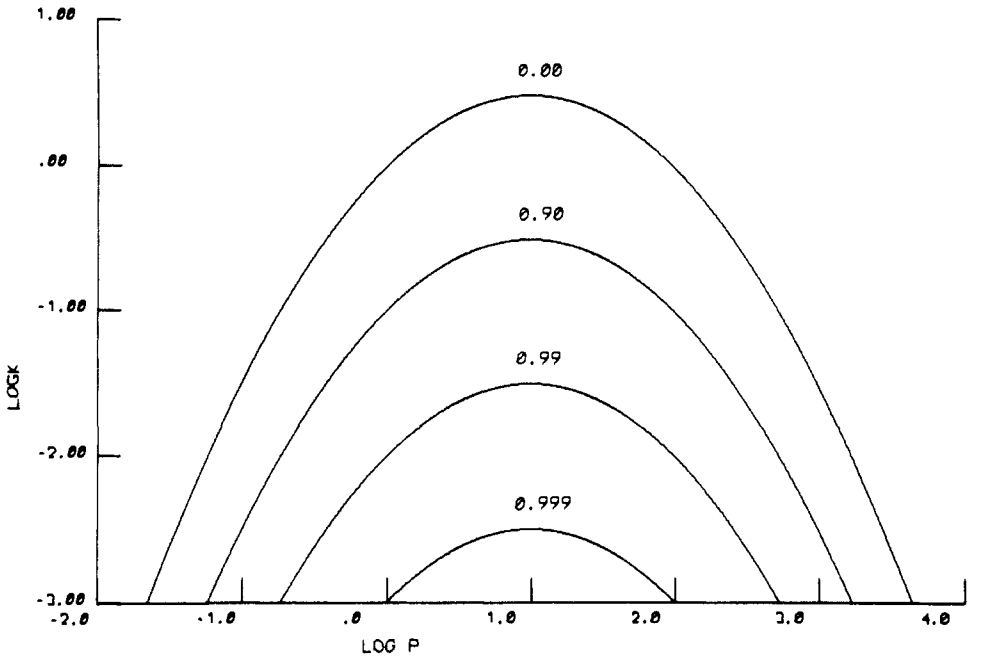


Figure 5. A plot of eq 14. The constants are $a = 1.0$, $b = 0.5$. The fraction ionized in the aqueous compartment is indicated on each line.

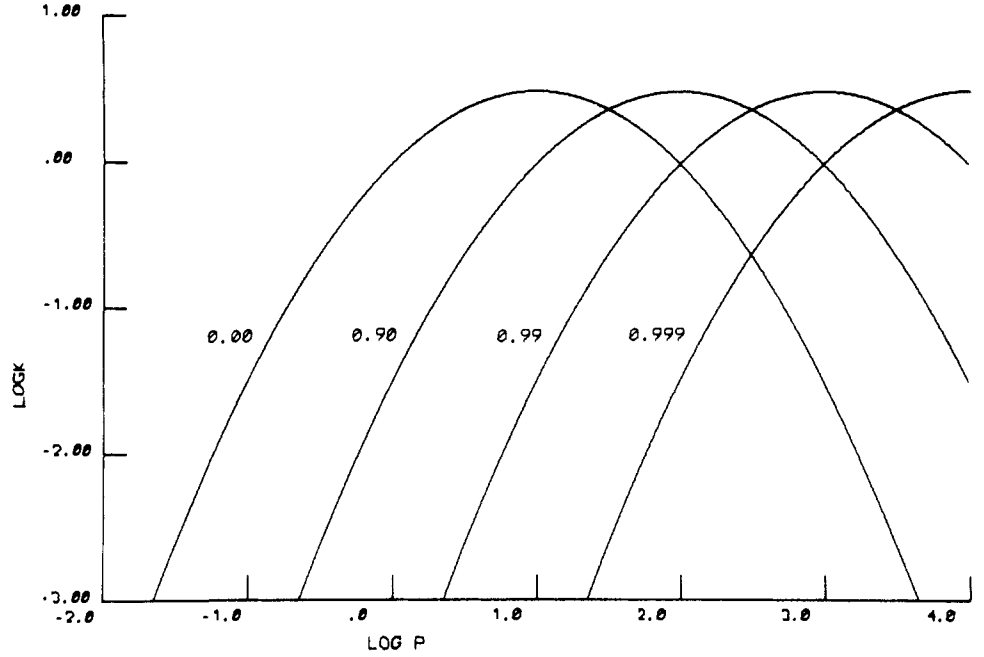


Figure 6. A plot of eq 15. The constants are $a = 1.0$ and $b = 0.5$. The fraction ionized in the aqueous compartment is indicated on each line.

parabola have been used to include the effects of ionization. The first "corrects" the potency to that of the neutral form.

$$\log (1/C) - \log (1 - \alpha) = a \log P - b(\log P)^2 + X \quad (14)$$

The second correlates potency with observed log P.

$$\log (1/C) = a \log [P(1 - \alpha)] - b [\log [P(1 - \alpha)]]^2 + X \quad (15)$$

Figures 5 and 6 are plots of these equations for various degrees of ionization. It is obvious that the shapes of these functions and the possible influence of ionization are much more limited than is true for eq 5–9 above.

It should also be noted that the requirements for the models above are similar to those chosen by Penniston et al.[22] to show that empirically a parabolic equation fits the data generated from a random walk model. Similarly, McFarland[23] derived a function hyperbolic in log P for the probability of a molecule reaching the receptor. Neither work considered the influence of ionization on the function, and both considered all partitioning to be of the same proportionality to log P. Although this assumption may be true in certain cases, there is no a priori reason to expect it to be true in every case.

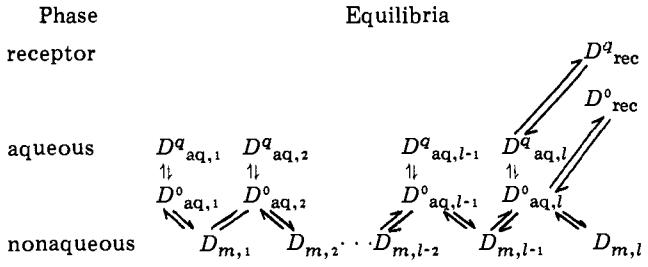
The equations derived above are clearly different from those previously used for quantitative structure–activity analysis of drugs. They are more cumbersome to fit because simple linear regression analysis cannot be used; iterative nonlinear regression analysis is necessary. In

addition, compounds on both sides of the optimum are needed since the upward and downward slopes are independently fit. On the other hand, by fitting equations which are based on models, much insight into the properties of the model and biological system can be gained. For a series of compounds of varying degrees of ionization explicit attention can be paid to the separate influences of ionization on partitioning to nonaqueous phases and to the receptor.

It must be recognized that the equations derived in this paper will be reliable representatives of reality only if the models on which they are based are reliable representations. Thus, careful workers will consider the relevance of the model assumptions to their assay before they fit data to the equations. In particular, attention may easily be paid to the assumption that the system is at equilibrium.

Appendix. Derivation of General Equation

A. Model



(The superscripts refer to the charge on the molecule and the subscripts refer to the phase in which it is found.)

B. Assumptions

(1) Extrathermodynamic assumptions re equilibrium constants. (a) Equilibrium between the last aqueous phase and the receptor:

$$K(\text{rec},0) = ([D^0_{rec}]/[D^0_{aq,l}]) = a_0' P^b$$

$$K(\text{rec},q) = ([D^q_{rec}]/[D^q_{aq,l}]) = a_q' P^b$$

(b) Equilibrium between each aqueous phase and a nonaqueous phase:

$$K(\text{biol},k) = ([D_{m,k}]/[D^0_{aq,k}] = d_k' P^{c_k}$$

in which $k = 1, 2, \ldots, l$.

(2) Activity coefficients of the neutral form in all aqueous phases are equal to 1.0:

$$[D^0_{aq,1}] = [D^0_{aq,2}] = \ldots = [D^0_{aq,l}] = [D^0_{aq}]$$

(3) Material balance, i.e., no metabolism.

(4) Same pK_a in all phases.

C. Equations

$$\text{potency} = k\left[\frac{\text{amt of drug at receptor}}{\text{total amt of drug in system}}\right]$$

From material balance

$$\text{potency} = k\left[\frac{V_r([D^0_{rec}] + [D^q_{rec}])}{[V_r([D^0_{rec}] + [D^q_{rec}]) + \sum^{l} V_{aq,k}([D^0_{aq,k}] + [D^q_{aq,k}]) + \sum^{l} V_{m,k}[D_{m,k}]]}\right]$$

in which V_r = volume of the receptor phase, $V_{aq,k}$ = volume of aqueous phase k, and $V_{m,k}$ = volume of nonaqueous phase k. Substituting in the extrathermodynamic assumptions we have

$$\text{potency} = k\left[\frac{V_r a_0' P^b (1 + a_q'(\alpha_l)/(a_0'(1 - \alpha_l)))}{[V_r a_0' P^b (1 + a_q'(\alpha_l)/(a_0'(1 - \alpha_l))) + \sum^{l} V_{aq,k}/(1 - \alpha_k) + \sum^{l} V_{m,k} d_k' P^{c_k'}]}\right]$$

If the following new constants are defined

$$Z = a_q'/a_0'$$

$$a_k = V_{aq,k}/a_0' V_r$$

$$d_k = d_k' V_{m,k}/a_0' V_r$$

$$c_k = c_k' - b$$

the resulting equation is

$$\text{potency} = k\left[\frac{1 + Z(\alpha_l)/(1 - \alpha_l)}{[1 + Z(\alpha_l)/(1 - \alpha_l) + \sum^{l} d_k P^{c_k} + \sum^{l} (a_k/(1 - \alpha_k)) P^b)]}\right]$$

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