Estimation of Distribution Coefficients from the Partition Coefficient and pK_a

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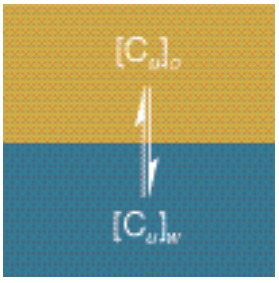

The authors discuss the relationship between the partition coefficient and the distribution coefficient and relate the two with the use of equations. To accurately explain this relationship, the authors consider the partitioning of both the ionized and un-ionized species. The presence of both species in the oil phase necessitates a dissociative equilibrium among these species such as that in the water phase. Despite this fact, a survey of octanol–water coefficients for small, monovalent molecules showed that a simple relationship among the distribution coefficient, partition coefficient, and dissociation constant is adequate for most purposes.

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The use of partition coefficients has received much attention in the assessment of relative lipophilicity and hydrophilicity of a compound. Recent advances in computational chemistry have enabled scientists to estimate partition coefficients for neutral species very easily. For ionizable species, the distribution coefficient is a more relevant parameter; however, less effort has been applied to its assessment. Knowledge of the distribution coefficient and pK_a is important for the basic characterization of a compound (1), particularly when assessing the compound's potential to penetrate biological or lipid barriers (2). In addition, the majority of compounds are passively absorbed and a large number of new chemical entities fail in the development stages because of related pharmacokinetic reasons (3). Assessing a compound's relative lipophilicity dictates a formulation strategy that will ensure absorption or tissue penetration, which ultimately will lead to delivery of the drug to the site of action. In recognition of the value of knowing the extent of a drug's gastrointestinal permeation along with other necessary parameters, FDA has issued guidance for a waiver of in vivo bioavailability and bioequivalence testing for immediate-release solid oral dosage forms. This guidance is based on the Biopharmaceutics Classification System, which ranks drugs according to aqueous solubility, intestinal permeability, and drug product dissolution (4). Although distribution coefficients are not entirely representative of permeability through gastrointestinal tissue, skin, or other tissues, they are related—the distribution coefficient is a key factor in determining the permeability of drugs through lipid barriers.

Methods of estimating the distribution coefficient vary from simple approaches to those that are highly complex and can be measured or calculated (5–10). A few methods consider the partitioning of the ionized species in the oil phase. For example, ACD/LogD Suite (Advanced Chemistry Development, Inc., Toronto, Canada) is a computational chemistry software package that includes the presence of the ionized species in the oil phase. In most cases, it may seem unreasonable that the ionized species can exist at any appreciable amount in the oil phase (octanol); however, this can be explained by the fact that octanol, when it comes into contact with water such as during the

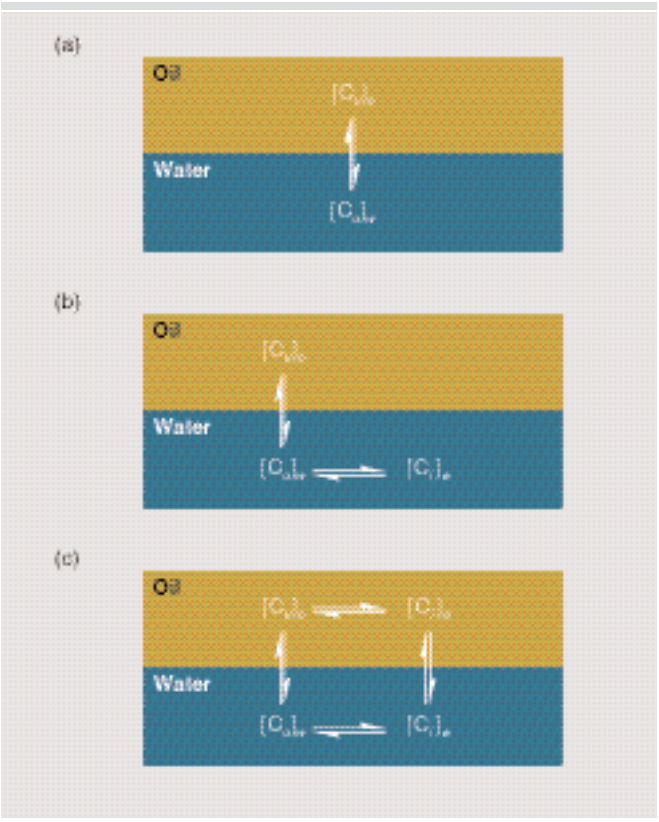

Figure 1: Schematic depictions of the partitioning of (a) un-ionized species between oil and water (see Equation 1); (b) un-ionized species between oil and water with ionized species in water only (see Equation 2); and (c) un-ionized and ionized species between oil and water (see Equation 5).

execution of a partitioning measurement, actually contains a considerable amount of water. The solubility of water in octanol is 26.4 mole% or 4.73% w/w (11), which is possible because water exists in an inverse micelle as a result of the amphiphilic nature of octanol (12). It has been suggested that the solubility of water in octanol is ideal for representing biological barriers. Although biological barriers to diffusion are generally considered lipophilic, they actually exist as bilayers or other similar structures composed of lipophilic materials as well as water. It has also been proposed that this is the reason that octanol–water partition coefficients reasonably correlate with the biological behavior of drugs.

In this article, the authors demonstrate that for a monovalent species, a simple calculation that assumes no partitioning of the ionized species in the oil phase typically provides an accurate estimate of the distribution coefficient in the pH range of physiologic interest. The authors also provide guidance in cases where a simple equation is not adequate and inclusion of the partitioning of the ionized species in the oil phase is necessary.

Theoretical

The partition coefficient of a neutral species, p, is defined as

$$p = \frac{[C_u]_o}{[C_u]_w} \quad [1]$$

in which $[C_u]_o$ and $[C_u]_w$ are the concentrations of the un-ionized species in oil and aqueous phases, respectively (see Figure 1a). To simplify, concentration units are used throughout this article instead of the more-correct activity units.

The partition coefficient applies to neutral species, whereas the distribution coefficient applies to ionizable species. If the ionized species has an appreciable solubility only in the aqueous phase, then the distribution coefficient, D, can be defined as

$$D = \frac{[C_u]_o}{[C_u]_w + [C_i]_w} \quad [2]$$

in which $[C_i]_w$ is the concentration of the ionized species in the aqueous phase (see Figure 1b).

Using the Henderson-Hasselbalch equation, the distribution coefficient for monovalent acids and bases can also be expressed as

$$D_{acid} = \frac{p}{1 + K_a/H^+} \quad [3a]$$

$$D_{base} = \frac{p}{1 + H^+/K_a} \quad [3b]$$

in which K_a is the dissociation constant for the acid or base.

In the case in which the ionized species is appreciably soluble in the oil phase, the distribution coefficient is expressed as

$$D' = \frac{[C_u]_o + [C_i]_o}{[C_u]_w + [C_i]_w} \quad [4]$$

Substitution of the Henderson-Hasselbalch equation into Equation 4 yields the following:

$$D'_{acid} = \frac{p}{1 + K_a/H^+} + \frac{p'}{1 + H^+/K_a} \quad [5a]$$

$$D'_{base} = \frac{p}{1 + H^+/K_a} + \frac{p'}{1 + K_a/H^+} \quad [5b]$$

in which p' is the partition coefficient of the ionized species

$$p' = \frac{[C_i]_o}{[C_i]_w} \quad [6]$$

and $[C_i]_o$ is the concentration of the ionized species in the oil phase (see Figure 1c). More-sophisticated treatments exist (10); however, the preceding equations are usually adequate for practical use.

Existence of the ionized and un-ionized species in the oil phase dictates an equilibrium of these species, and the same is true in the aqueous phase. A survey of acids for which the dissociation constant was available in both water and oil (13) shows that the acid dissociation constant in the oil phase is usually five to six orders of magnitude smaller than the aqueous dissociation constant ($pK_{a0} >> pK_{aw}$). Equations 5a and 5b do not contain the oil-phase dissociation constant or the oil-phase H^+ concentration. Through examination of the ratio of the partition coefficients and use of the Henderson-Hasselbalch equation for both phases, the following relationship can be derived for acids or bases:

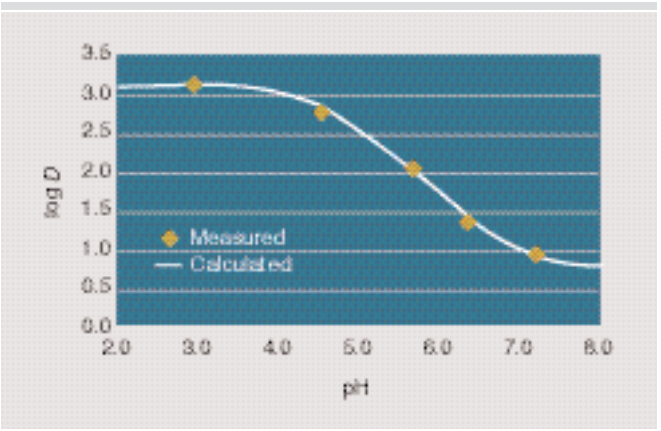

Figure 2: Distribution coefficient as a function of pH for ketoprofen using Equation 5a.

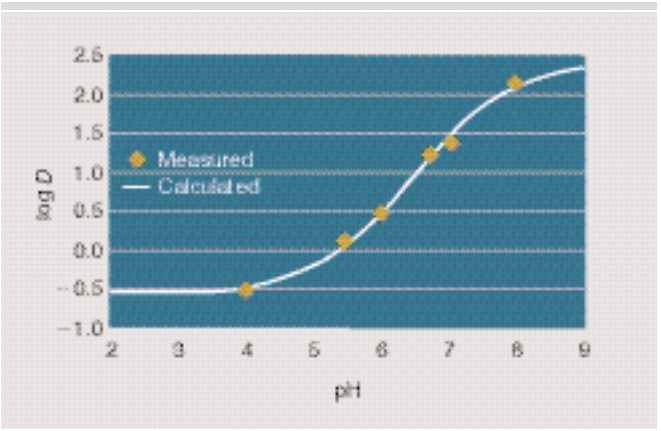

Figure 3: Distribution coefficient as a function of pH for lidocaine using Equation 5b.

$$\frac{[H^+]_o}{[H^+]_w} = \frac{K_{aw}p'}{K_{ao}p} \qquad [7a]$$

or, alternatively,

$$\Delta pH = \Delta pK_a - \log\left(\frac{p'}{p}\right) \qquad [7b]$$

in which ΔpH and ΔpK_a refer to the differences between the oil and water phases for the respective values.

These relationships show that the ratio of the hydrogen ion concentrations (or the differences in pH) of the two phases is a constant for a given compound. This is true for the entire pH range. Equations 5a and 5b could have been similarly derived using K_{ao} and $[H^+]_o$ with equivalent results instead of using K_{aw}. K_{aw} and K_{ao} are not the dissociation constants for the respective solvents; they are the dissociation constants for the compound in the respective solvents.

Discussion

Equations 5a and 5b describe the distribution coefficient for the entire pH range using only three well-defined parameters: pK_a, p, and p'. The literature provides pK_a and p for numerous molecules. The values for p' are less plentiful; however, they can be easily determined by measuring the partition coefficient when the molecule is in its fully ionized state. In the following discussion, all measured or experimental partition and distribution coefficients are based on octanol–water systems.

In Figure 2, experimental distribution coefficients (which were derived from internally generated data) are provided for the monovalent weak acid, ketoprofen. Figure 3 shows the same coefficients for the monovalent weak base, lidocaine (14). The solid lines are the least-squares best fit of Equations 5a (acid) and 5b (base) to the experimental data.

The coefficients are generated by the following calculation: Equations 5a and 5b include three parameters: p, p', and pK_a. The values of these three parameters is guessed initially. The difference between the experimental values of log D and the calculated values of log D using the three parameters is squared, and the sum of these squares is minimized by varying the three parameters with a quasi-Newton method of Microsoft Excel's Solver function. The parameters yielding the best fit for ketoprofen as well as parameters for other nonsteroidal anti-inflammatory drugs (NSAIDs) applying the same least-squares fit method are shown in Table I. The partition coefficients are in good agreement with experimental and calculated values.

Table I: Distribution equation parameters for NSAIDs.

	Best Fit*		Experimental**			Calculated†	
Substance	**log *p***	**log *p′***	**pK_a**	**log *p***	**pK_a**	**log *p***	**pK_a**
Diclofenac	4.34	0.75	3.98	4.40	4.00	4.37	4.18
Diflunisal	4.48	0.57	2.74	4.44	3.00	3.42	2.94
Fluphenamic acid	5.27	1.94	3.36	—	—	—	—
Ibuprofen	3.65	−0.41	4.55	3.65	4.55	4.10	4.41
Indomethacin	4.21	0.31	4.02	4.27	4.50	2.60	4.17
Indoprofen	2.77	−1.12	4.16	2.77	5.80	3.12	4.39
Ketoprofen	3.11	0.67	4.54	3.12	4.60	3.46	4.23
Mefenamic acid	4.91	1.45	4.32	5.12	4.20	4.66	3.69
Naproxen	3.28	0.20	4.13	3.18	4.20	2.99	4.40
Phenbutene	3.41	0.13	4.40	—	—	—	—
Salicylic acid	2.30	−0.99	2.97	2.19	3.00	1.46	3.01
Sulindac	3.34	−1.16	3.88	3.42	4.50	3.48	4.24
Tolmetin	2.77	−1.14	3.69	2.79	3.50	1.90	4.46

*Results obtained from a least-squares best fit of Equation 5a to experimental data. Data taken from A. Tsantili-Kakoulidou et al. (9), except for ibuprofen (Hadgraft and Valenta [14]) and ketoprofen (internally generated).

**Hansch (16).

†Partition coefficients calculated using Molecular Modeling Pro, Revision 2.1.1, WindowChem Software, Inc. (Fairfield, CA). Dissociation constants calculated by ACD/pK_a calculator, version 3.5, Advanced Chemistry Development, Inc.

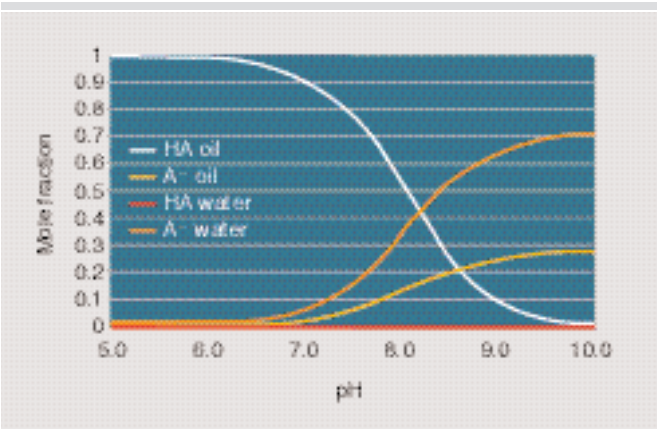

Figure 4: Relative composition of oil and water phases calculated for ibuprofen using Equation 5a and the Henderson-Hasselbalch equation.

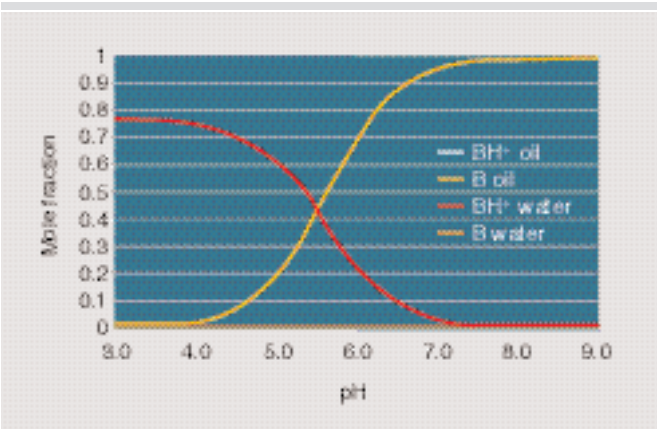

Figure 5: Relative composition of oil and water phases calculated for lidocaine using Equation 5b and the Henderson-Hasselbalch equation.

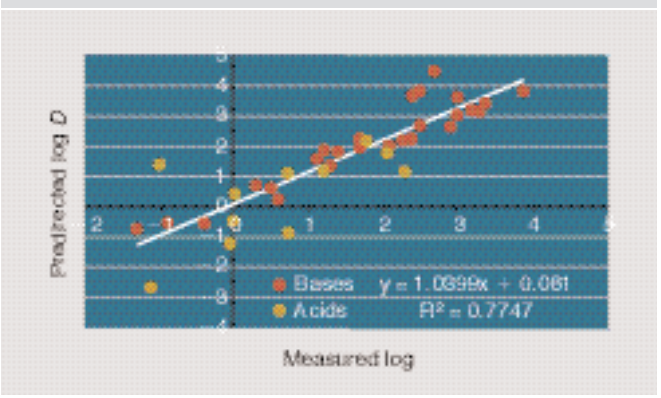

Figure 6: Calculated versus experimental distribution coefficients using Equations 3a and 3b.

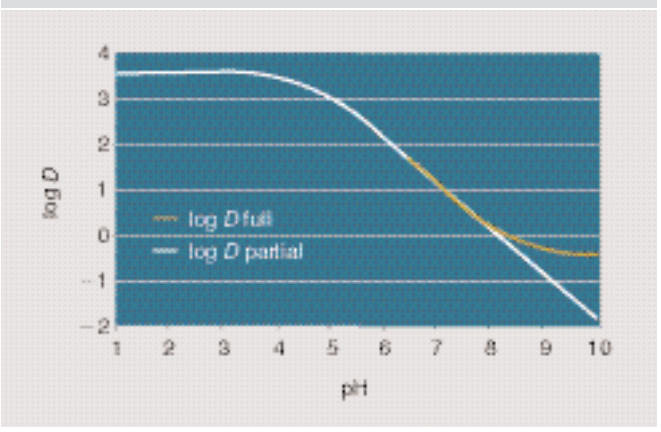

Figure 7: Comparison of two methods for calculating the distribution coefficient of ibuprofen using Equation 3a (partial) and Equation 5a (full).

The ionized partition coefficients are much lower than the un-ionized values, which is expected because these reflect partitioning of the ionized species in the oil phase. Values for log(p') are three to four units below the log(p) values, which might be expected for the deprotonation of a carboxylic acid to a carboxylate anion. The pK_a values are also in reasonable agreement with experimentally measured and calculated values.

Figure 4 shows the molar fractions of the individual species of ibuprofen. These have been calculated from the distribution coefficient (see Equation 5a) and the K_a in the aqueous phase. As expected, the un-ionized form is contained almost exclusively in the oil phase at low pH, whereas the ionized form is predominately in the aqueous phase at high pH. Interestingly, for this example, more of the ionized species exists in the oil phase at high pH than the un-ionized species in the water phase at low pH. Hence, the presence of ionized species in the oil phase should not be neglected at certain pH values. Similarly, Figure 5 shows the distribution of the various species for the weak base, lidocaine.

To make use of equations 5a and 5b, one should know the value of p'. Because p' is not always readily available or accurately known for many compounds in the pH range of physiological interest, Equations 3a and 3b can be used ignoring the contribution of the ionized species in the oil phase.

Table II contains literature values, including pK_a, p, and D, for various compounds—usually in the pH range 7–7.5 (15–16). These compounds were selected because they possess pK_a values that are at least one pH unit different from the pH that was determined for the distribution coefficient in each case. This ensured that the distribution coefficient was measured with at least 90% of the ionized species in the aqueous phase. The distribution coefficients were calculated using the partition coefficient and Equations 3a and 3b. This enables a comparison of the calculated D (on the basis of p and pK_a) with the measured D. A plot of calculated D versus measured D is shown in Figure 6. The correlation is reasonably good ($R^2 = 0.77$), in which the slope is unity and the intercept is nearly zero. This demonstrates that D can be reasonably calculated from a measured p and pK_a using the simple equations that do not include the partitioning of the ionized species in the oil phase. Likewise, p can be calculated from a measured D. These relationships are expected to have greater error when the pH is significantly further away from the pK_a (i.e., only the ionized species are present) and/or the ratio p'/p is not small. It appears that the simple

Table II: Partition and distribution coefficients.

Drug	pH*	pK_a†	log *D**	log *p***	
Aspirin	7.4	3.5	−1.1	1.19	Acid
Diclofenac	6.6	4.0	2.07	4.4	Acid
Fenoprofen	7.4	4.5	2.3	4.05	Acid
Ibuprofen	7.0	4.55	1.21	3.65	Acid
Indomethacin	7.4	4.5	−1.0	4.27	Acid
Indomethacin	6.6††	4.5	1.76††	4.27	Acid
Ketoprofen	7.4	4.6	0.0	3.12	Acid
Ketoprofen	6.6††	4.6	0.72††	3.12	Acid
Ketorolac	6.6	3.5	−0.05	1.88	Acid
Piroxicam	6.6	5.5	0.72	0.26	Acid
Warfarin	8.0	5.0	0.0	2.52	Acid
Acepromazine	7.4	9.3	2.3	4.1	Base
Alprenolol	7.0	9.5	0.5	3.1	Base
Amantidine	7.4	10.4	−0.4	2.44	Base
Amitriptyline	7.4	9.42	3.0	5.04	Base
Chlorpromazine	7.4	9.3	3.4	5.35	Base
Chlorprothixene	7.0	7.6	2.7	5.18	Base
Clomipramine	7.4	9.38	3.3	5.19	Base
Codeine	7.4	8.21	0.6	1.14	Base
Cyclazocine	7.4	9.38	1.3	3.31	Base
Cyprohetadine	7.4	8.87	3.2	4.69	Base
Desipramine	7.4	10.44	1.4	4.9	Base
Dibenzepin	7.4	8.25	1.7	3.14	Base
Doxepin	7.4	9.0	2.4	3.88	Base
Flupenthixol	7.0	7.8	3.0	4.51	Base
Imipramine	7.4	9.5	2.5	4.8	Base
Levorphanol	7.4	9.2	1.1	3.4	Base
Methadone	7.4	8.3	2.1	2.97	Base
Nortriptyline	7.4	9.7	1.7	4.32	Base
Pindolol	7.4	9.7	−0.9	1.75	Base
Practolol	8.0	9.5	−1.3	0.79	Base
Prochlorperazine	7.0	9.5	2.4	6.15	Base
Promazine	7.4	8.1	2.5	4.55	Base
Promethazine	7.4	9.4	2.9	4.65	Base
Propranolol	7.4	9.1	1.2	3.56	Base
Thebaine	7.5	8.2	0.3	1.48	Base
Trifluoperazine	7.0	8.1	3.9	5.0	Base

*Moffat (15), unless otherwise noted.
**Hansch (16).
†(15,16).
††Cordero (6).

Equations 3a and 3b are reasonable for predicting the distribution coefficient with the ionized species present in the water phase at a level as high as 90%. This is true because the p'/p ratio typically is small. Figure 7 exemplifies this by comparing the distribution coefficient calculated from the full equation (Equation 5a) with the partial equation (Equation 3a) for ibuprofen. Equations 5a and 3a differ substantially only at values above pH 8.5–9.

The relative contribution of ionized and un-ionized terms in Equation 5a and 5b for acid is

$$\log\left(\frac{D_i}{D_u}\right) = \log\left(\frac{p'}{p}\right) + \mathrm{pH} - \mathrm{p}K_a \quad [8a]$$

and for base

$$\log\left(\frac{D_i}{D_u}\right) = \log\left(\frac{p'}{p}\right) - \mathrm{pH} + \mathrm{p}K_a \quad [8b]$$

in which D_i is the second term on the right-hand side of Equations 5a and 5b (the ionized contribution) and D_u is the first term (the un-ionized contribution). The value of $\log(D_i/D_u)$ as a function of the difference of pH and pK_a for various $\log(p'/p)$ values is shown in Figure 8.

For a value of p'/p of 0.001, which is at the high end of the range for carboxylic acids, the relative contribution of the two D terms becomes similar when the pH is 3 units greater than the pK_a. Even at a pH that is 3 units away from the pK_a, the difference between Equations 3 and 5 is only a factor of ~2. A factor of 2 difference for D is only a log(2) difference in $\log(D)$. Simply stated, the value of $\log(D)$ would be off by 0.3 units, which likely is within the realm of experimental error.

Conclusion

In this article, the authors have presented two methods of evaluating the distribution coefficient as a function of pH. The complete method using Equations 5a and 5b requires knowledge of three parameters: pK_a, p, and p'. The benefit of this approach is that the distribution coefficient can be evaluated at any pH. Alternatively, if the distribution coefficient is known as a function of pH, all three parameters can be determined.

In the case in which p' is not known, the simplified form (Equations 3a and 3b) requires knowledge of only pK_a and p. The problem with this method is that one is restricted to the pH range that is dependent on the assumed ratio of partition coefficients and the pK_a. For many common acids and bases, in which the pK_a values are 4–5 or 8–9, respectively, and with a typical $\log(p/p')$ of 3–4, this method is appropriate in the pH range of physiological interest.

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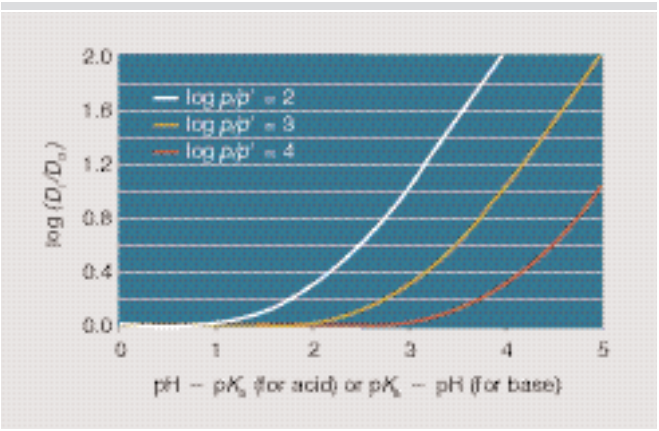

Figure 8: Log (D_i / D_u) as a function of pH–pK_a and p/p' using Equations 8a and 8b.

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