Journal of Pharmaceutical & Biomedical Analysis
Vol. 2, Nos 3/4, pp. 381–394, 1984
Printed in Great Britain

0731–7085/84 $3.00 + 0.00

Chemical and statistical considerations in the determination of partition coefficients of weakly ionizable drugs and poisons

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Abstract: Equations have been developed that relate the concentration (or a parameter directly proportional to concentration, such as optical absorbance) of a weakly ionizable solute in a water-immiscible phase, in equilibrium with an aqueous phase, to the pH of the aqueous phase, the partition coefficient of the unionized solute and the phase volume ratio. These relationships have been used in the design of experimental methods for determining partition coefficients, which require measurement of solute concentration in only one phase. Data obtained in this way permit ready recognition of deviations from assumptions made in the development of the model; these assumptions include insolubility of the ionized solute in the water-immiscible phase and lack of interaction between buffer components and solute. Conditions for optimal liquid–liquid extraction of weakly ionizable solutes are more easily recognized. With these techniques, the negative logarithm of the acid dissociation constant (pK'_a) and the logarithm of the octanol–water partition coefficient (log P) have been measured for warfarin ($pK'_a = 5.15 \pm 0.04$; log $P = 2.82 \pm 0.06$), strychnine ($pK'_a = 8.29 \pm 0.02$; log $P = 2.23 \pm 0.04$), phenol ($pK'_a = 9.88 \pm 0.02$; log $P = 1.75 \pm 0.05$), procaine ($pK'_a = 8.11 \pm 0.04$; log $P = 1.10 \pm 0.08$), and ephedrine ($pK'_a = 9.92 \pm 0.01$; log $P = 1.65 \pm 0.04$) at 21°C.

Keywords: *Octanol–water partition coefficient; acid dissociation constant; experimental design; warfarin; strychnine; phenol; procaine; ephedrine.*

Introduction

The *n*-octanol–water partition coefficient of a drug or toxicologically important substance is a thermodynamic parameter widely used to predict the chemical properties and biological fate of that substance [1–5]. Partition coefficients are used to model environmental pollution [3, 6] and to predict the pharmacological or toxicological activity of a substance [1, 2, 7]. Partition coefficients are linearly related to physiological absorption processes [8, 9], to the ease of separation of mixed solutes by countercurrent

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distribution [10, 11] and to solute retention time in liquid chromatography [12, 13]. Partition coefficient measurements as a function of pH have also been used to determine dissociation constants for weak acids and bases [1, 14]. Simple, reliable experimental methods to measure partition coefficients would therefore be useful to scientists in many fields.

The *n*-octanol–water partition coefficients of the conjugate acid and base forms of weakly ionizable drugs or poisons differ appreciably. In the absence of ion-pairing agents, the ionized form usually has very limited solubility in the organic phase. The contribution of the ionized form to the solute concentration in the organic phase is therefore frequently neglected [15]. The fundamental relationships between the apparent octanol–water partition coefficient, the true partition coefficient of the ionized form of the solute, its acid dissociation constant and the pH of the aqueous medium are well known [10, 16–18]. The assumption that the ionized form does not contribute to the partitioning process requires experimental verification [19]; corrections for ion-pair formation between the ionized solute and other salts present are frequently required [20–22].

The practical consequences of the pH-dependence of the apparent partition coefficient for weakly ionizable drugs and poisons are widely misunderstood. For example, analysts have been advised [15, 23–24] to adjust the pH of the aqueous phase in a two-phase system to a value 2–3 units removed from the pK_a' of the drug or poison to ensure maximum extraction into one of the phases. The correct choice of pH depends on the lipophilicity of the solute, the phase volume ratio, and the acidity of the solute considered jointly.

The objective of the present study was to develop and test a procedure for determining the extraction ratios, which are the experimentally measurable forms of partition coefficients, for weakly ionizable solutes. The method that has been developed requires measurement of the solute concentration in only one phase and should also permit deviations from ideal behavior to be readily recognized.

The method has been evaluated by determining the acid dissociation constants and extraction ratios for warfarin and phenol, both weakly acidic drugs, and for strychnine, ephedrine and procaine, all weakly basic drugs.

Theory

A solute S which can dissolve in each of two immiscible solvents will distribute between them when a solution of S in one solvent is shaken with the other [10, 16–18]. Usually one solvent is an organic solvent whereas the other is an aqueous salt solution. The ratio of $[S]_o$, the concentration of S in the organic phase, to $[S]_w$, the concentration of S in the aqueous phase, is the extraction ratio:

$$E^{o/w} = \frac{[S]_o}{[S]_w}. \tag{1}$$

$E^{o/w}$ is a constant for each solvent pair at a given temperature. It is related to the true thermodynamic partition coefficient $P^{o/w}$ by:

$$E^{o/w} = \frac{\gamma_w}{\gamma_o} P^{o/w}. \tag{2}$$

In equation (2), γ_o and γ_w are the thermodynamic activity coefficients for S in the organic and aqueous phases respectively.

For a weak acid HA, partitioning behaviour is complicated by the dissociation of the acid to its conjugate base A^- and hydrogen ions in the aqueous phase. For purposes of discussion, it is assumed that HA is unionized and can partition between the two phases, whereas A^- is ionic and has negligible solubility in the organic phase. It is also assumed that dissociation in the organic phase does not occur, and that specific interactions with salts or buffer components, such as ion-pair formation, do not occur. The apparent extraction ratio $E_{app}^{o/w}$ is given as a function of hydrogen ion activity a_{H^+} by:

$$E_{app}^{o/w} = \frac{a_{H^+}}{K'_a + a_{H^+}} E^{o/w}. \tag{3A}$$

For a weak base B, partitioning behaviour is complicated by the association of the base with hydrogen ions to form its conjugate acid BH^+ in the aqueous phase. Again, it is assumed that B is unionized and can partition between the two phases, whereas BH^+ is ionic and has neglible solubility in the organic phase. It is also assumed that association in the organic phase does not occur, and that specific interactions with salts or buffer components do not occur. In this case, the apparent extraction ratio $E_{app}^{o/w}$ is given as a function of hydrogen ion activity a_{H^+} by:

$$E_{app}^{o/w} = \frac{K'_a}{K'_a + a_{H^+}} E^{o/w}. \tag{3B}$$

In both equations (3A) and (3B), K'_a is the concentration-modified acid dissociation constant given by:

$$K'_a = \frac{a_{H^+}[A^-]}{[HA]} = \frac{a_{H^+}[B]}{[BH^+]}. \tag{4}$$

If HA is initially dissolved in a volume of the organic phase V_o, to yield an initial concentration $[HA]_{init}$, and is then equilibrated with a volume of the aqueous phase, V_w, the fraction remaining in the organic phase after equilibration is given by:

$$\frac{[HA]_o V_o}{[HA]_{init} V_o} = \frac{E_{app}^{o/w} V_o}{V_w + E_{app}^{o/w} V_o}. \tag{5A}$$

Similarly, if B is initially dissolved in a volume of the organic phase V_o, to yield an initial concentration $[B]_{init}$, the fraction remaining in the organic phase after equilibration with a volume V_w of the aqueous phase is:

$$\frac{[B]_o V_o}{[B]_{init} V_o} = \frac{E_{app}^{o/w} V_o}{V_w + E_{app}^{o/w} V_o}. \tag{5B}$$

By substituting the volume ratio $V_r = V_o/V_w$ and the expression for $E_{app}^{o/w}$ from equation (3A) or (3B) into equation (5), it follows that:

$$\frac{[HA]_o}{[HA]_{init}} = \frac{\frac{1}{K'_a + a_{H^+}} E^{o/w} V_r}{1 + \frac{a_{H^+}}{K'_a + a_{H^+}} E^{o/w} V_r} \tag{6A}$$

$$\frac{[B]_o}{[B]_{init}} = \frac{\frac{1}{K'_a + a_{H^+}} E^{o/w} V_r}{1 + \frac{K'_a}{K'_a + a_{H^+}} E^{o/w} V_r} . \tag{6B}$$

Since $K_w = a_{H^+} a_{OH^-}$ and if K'_b is defined as $K'_b = K_w/K'_a$, then equation (6B) may be rewritten as:

$$\frac{[B]_o}{[B]_{init}} = \frac{\frac{1}{K'_b + a_{OH^-}} E^{o/w} V_r}{1 + \frac{a_{OH^-}}{K'_b + a_{OH^-}} E^{o/w} V_r} . \tag{6C}$$

Equation (6A) may be rearranged to yield:

$$[HA]_o = \frac{\frac{1}{1 + E^{o/w} V_r} [HA]_{init} a_{H^+}}{\frac{K'_a}{1 + E^{o/w} V_r} + a_{H^+}} . \tag{7A}$$

Similarly, equation (6C) may be rearranged to yield:

$$[B]_o = \frac{\frac{1}{1 + E^{o/w} V_r} [B]_{init} a_{OH^-}}{\frac{K'_b}{1 + E^{o/w} V_r} + a_{OH^-}} . \tag{7B}$$

Equations (7A) and (7B) are both rectangular hyperbolae (Fig. 1) of the form

$$[HA]_o = \frac{k_1 a_{H^+}}{k_2 + a_{H^+}} \tag{8A}$$

or

$$[B]_o = \frac{k_1 a_{OH^-}}{k_2 + a_{OH^-}} . \tag{8B}$$

Hence in principle, if V_r and $[HA]_{init}$ or $[B]_{init}$ are known, both K'_a (for weak acids) or K'_b (for weak bases) and $E^{o/w}$ can be determined simultaneously by measuring the

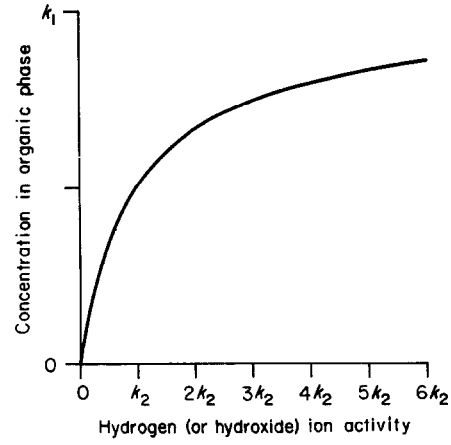


Figure 1
Concentration of the unionized form of a weak acid (———) in the organic phase as a function of the hydrogen ion activity in the aqueous phase. For a weak base, hydrogen ion activity is replaced by hydroxide ion activity.

concentration of the unionized form in the organic phase as a function of hydrogen ion (or hydroxide ion) activity and then fitting the experimental data to equation (8A) or (8B).

In practice, a problem is encountered if the product $E^{o/w}V_r$ is either very large or very small. The product $E^{o/w}V_r$ is identical to the *capacity factor* of chromatographic theory [17]. If the capacity factor is large, k_1 in equation (8A) or (8B) is statistically indistinguishable from the initial concentration of the unionized solute in the organic phase, so that one is unable to estimate both K_a' (or K_b') and $E^{o/w}$ simultaneously. It is evident from inspection of equation (7A) or (7B) that V_r must be chosen to be equal to the reciprocal of $E^{o/w}$, if both K_a' (or K_b') and $E^{o/w}$ are to be determined simultaneously. This follows because $E^{o/w}$ must first be estimated from k_1, $[HA]_{init}$ (or $[B]_{init}$) and V_r before K_a' (or K_b') can be computed. Since

$$E^{o/w} = \frac{k_1}{V_r([HA]_{init} - k_1)} \tag{9A}$$

or

$$E^{o/w} = \frac{k_1}{V_r([B]_{init} - k_1)} \tag{9B}$$

the difference between k_1 and $[HA]_{init}$ (or $[B]_{init}$) must be as large as possible, while k_1 is also kept as large as possible in order to minimize the effects of measurement errors in the determination of k_1 on the precision with which $E^{o/w}$ is estimated. Clearly, this occurs when k_1 is equal to one-half of the initial solute concentration, that is when $V_r = 1/E^{o/w}$. Workers wishing to determine both the partition coefficient and the dissociation constant from pH-partition data must choose the phase volume ratio carefully.

If the capacity factor $E^{o/w}V_r$ is very small, k_1 in equation (8A) or (8B) is also very small and measurement of both k_1 and k_2 may be extremely difficult. Even if the capacity factor $E^{o/w}V_r$ is too large to permit precise determination of k_1 in equation (8A) or (8B), k_2 may still be determined precisely by conventional least-squares curve-fitting [25] or by

use of the direct linear plot technique [26, 27]. If only k_2 is to be determined, the optimal experimental design requires measurement of the concentration of the unionized solute in the organic phase after equilibration with at least two buffers, the pH of which are symmetrically disposed about the logarithm of k_2 [26]. Pilot studies must therefore be conducted over a wide pH range in order to provide an initial estimate for k_2. Once k_2 has been determined, either the dissociation constant or the partition coefficient can be calculated if the other is known, since for a weak acid

$$\text{pH} = \text{p}k_2 = \log (1 + E^{\text{o/w}}V_r) + \text{p}K_a' \tag{10}$$

when $[\text{HA}]_\text{o}$ is plotted against pH, and for a weak base

$$\text{pOH} = \text{p}k_2 = \log (1 + E^{\text{o/w}}V_r) + \text{p}K_b' \tag{11}$$

when $[\text{B}]_\text{o}$ is plotted against pOH. Such plots are sigmoidal curves [26] with the inflection point at $\text{pH} = \text{p}k_2$ (for a weak acid) or at $\text{pOH} = \text{p}k_2$ (for a weak base). It is sometimes convenient to express the inflection point in the plot of $[\text{B}]_\text{o}$ versus pOH on a pH scale instead. This occurs when

$$\text{pH} = \text{p}K_a' - \log (1 + E^{\text{o/w}}V_r). \tag{12}$$

In either of the preceding examples, it is unnecessary to plot the concentration of the unionized solute in the organic phase itself; any experimentally measured parameter which is known to be directly proportional to the concentration (such as optical absorbance or chromatographic peak area) may be used instead. The concentration in the organic phase may also be computed from the concentration of both ionized and unionized forms in the aqueous phase and a knowledge of the phase volumes and total quantity of solute present in the system. In any instance, the concentration in only *one* phase is required.

In the experimental portion of this study, conditions were chosen so that the capacity factor $E^{\text{o/w}}V_r$ was large. K_a' was determined spectrophotometrically [28].

If it is convenient to adjust V_r to equal $1/E^{\text{o/w}}$, both K_a' (or K_b') and $E^{\text{o/w}}$ can be determined simultaneously. The optimum experimental design in this case [26] requires measurement of the concentration of the unionized solute in the organic phase, both at a pH where essentially as much of the solute is in the organic phase as possible, that is (for a weak acid)

$$\text{pH} < \log (1 + E^{\text{o/w}}V_r) + \text{p}K_a' - 2 \tag{13}$$

or (for a weak base)

$$\text{pH} > 2 + \text{p}K_a' - \log (1 + E^{\text{o/w}}V_r) \tag{14}$$

as well as at approximately (for a weak acid)

$$\text{pH} \approx \log (1 + E^{\text{o/w}}V_r) + \text{p}K_a' + 1 \tag{15}$$

or (for a weak base)

$$\mathrm{pH} \approx \mathrm{p}K_a' - \log (1 + E^{\mathrm{o/w}}V_r) - 1. \quad (16)$$

Measurements of the concentration of the unionized solute should also be made at one or more intermediate pH values. Intermediate values are used mainly to judge the goodness-of-fit of the data to the model (equation 8A or 8B). The use of several intermediate values of pH and replication of the whole experiment several times should enable interactions of buffers with the solute to be detected; such interactions would result in statistical lack of fit for the experimental measurements for that particular buffer, relative to the model determined by the remainder of the observations.

Essentially complete (99%) extraction of a weak acid from the organic phase into the aqueous phase occurs when

$$\mathrm{pH} \geq \mathrm{p}K_a' + \log (1 + E^{\mathrm{o/w}}V_r) + 2. \quad (17)$$

Retention in the organic phase (99%) occurs when

$$\mathrm{pH} \leq \mathrm{p}K_a' + \log (1 + E^{\mathrm{o/w}}V_r) - 2. \quad (18)$$

For a weak base, essentially complete (99%) extraction from the organic phase into the aqueous phase occurs when

$$\mathrm{pH} \leq \mathrm{p}K_a' - \log (1 + E^{\mathrm{o/w}}V_r) - 2. \quad (19)$$

Retention in the organic phase (99%) occurs when

$$\mathrm{pH} \geq \mathrm{p}K_a' - \log (1 + E^{\mathrm{o/w}}V_r) + 2. \quad (20)$$

Experimental

Materials

(±)-Warfarin (Sigma Chemical Co., St Louis, MO, USA) was recrystallized from 70% aqueous acetone. Strychnine sulphate pentahydrate N. F. and phenol (Mallinkrodt Chemical Works, St Louis, MO, USA), procaine hydrochloride (Sigma Chemical Co., St Louis, MO, USA), and ephedrine sulphate (Merck, Sharp & Dohme, West Point, PA, USA) were used without further purification. *n*-Octanol (Eastman Organic Chemicals, Rochester, NY, USA) was extracted twice with 0.1 M NaOH, twice with distilled water, and was then distilled twice. Each time the fraction boiling at 192–194°C was collected. PIPES [piperazine-*N*,*N*-bis(2-ethanesulphonic acid), 1.5 sodium salt monohydrate], HEPES [*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid], AMPSO [3-N-(α,α,-dimethylhydroxyethyl)-amino-2-hydroxypropane sulphonic acid], and CAPS [cyclohexylaminopropane sulphonic acid] (Research Organics, Inc., Cleveland, OH, USA), glycine and glutamic acid (Sigma Chemical Co., St Louis, MO, USA), and 6-aminocaproic acid (Aldrich Chemical Co., Milwaukee, WI, USA) were used as obtained. Deionized, distilled water (ASTM Type II) was used to prepare all reagents. All other chemicals used were reagent grade.

Buffers

Buffers were prepared in water saturated with *n*-octanol to give a final ionic strength of 30 mM. For pH 2, 3.28 g of glycine and 30 ml of 1 M HCl were diluted to 1 l. For pH 3, 12.55 g of glycine and 30 ml of 1 M HCl were diluted to 1 l. For pH 4, 12.26 g of glutamic acid and 30 ml of 1 M HCl were diluted to 1 l. For pH 5, 4.98 g of 6-aminocaproic acid and 30 ml of 1 M HCl were diluted to 1 l. For pH 6, 6.47 g of PIPES and 6.67 ml of 1 M HCl were diluted to 1 l. For pH 7, 2.81 g of PIPES and 0.9 ml of 1 M NaOH were diluted to 1 l. For pH 8, 9.68 g of HEPES and 30 ml of 1 M NaOH were diluted to 1 l. For pH 9, 11.20 g of AMPSO and 30 ml of 1 M NaOH were diluted to 1 l. For pH 10, 22.05 g of CAPS and 30 ml of 1 M NaOH were diluted to 1 l. For pH 11, 8.20 g of 6-aminocaproic acid and 30 ml of 1 M NaOH were diluted to 1 l. The final pH of each buffer was measured using a pH meter (Digital Ionanalyzer 501, Orion Research Inc., Cambridge, MA, USA) equipped with a combination glass electrode with a Ag/AgCl reference half-cell (Orion Research Inc., Cambridge, MA, USA). The pH electrode was standardized immediately before use against 0.1 M phosphate buffer (pH 6.88) and either 0.1 M phthalate buffer (pH 4.00) or 0.1 M borate buffer (pH 9.22) [29].

Methods

Determination of pK_a'. Saturated solutions of warfarin, phenol, strychnine, procaine and ephedrine were prepared in water saturated with *n*-octanol. The appropriate buffer solution (pH 2, 5 and 7 for warfarin; pH 7, 10 and 13 for phenol; pH 6, 8 and 10 for strychnine; pH 6, 10 and 13 for ephedrine; and pH 6.8 and 10 for procaine) was mixed with the drug solution (1:2, v/v). The wavelength for the maximum difference in absorbance between the conjugate acid and base forms of each drug (311 nm for warfarin, 269 nm for phenol, 290 nm for strychnine, 258 nm for ephedrine and 266 nm for procaine) was determined by scanning absorbance as a function of wavelength for a solution of low pH (pH 2 for warfarin, pH 7 for phenol and ephedrine, and pH 6 for strychnine and procaine), using as reference a solution of high pH (pH 7 for warfarin, pH 13 for phenol and ephedrine, and pH 10 for strychnine and procaine), using a double-beam UV-visible spectrophotometer (Model 559, Perkin–Elmer Corp., Norwalk, CN, USA). Measurements of absorbance of drug–buffer mixtures (prepared as described) against buffer blanks prepared from a mixture of buffer solution and water (saturated with *n*-octanol) (1:2, v/v) were made at each pH described above. The temperature was maintained at 21°C and the pK_a' values were calculated as described by Connors [28].

Determination of partition behaviour as a function of pH. Mixtures of 2 ml of buffer, 4 ml of a saturated solution of drug in water (saturated with *n*-octanol) and 10 ml of *n*-octanol (saturated with water) were mixed in 20 ml culture tubes with Teflon-lined caps. The tubes were shaken horizontally at low speed for 1 h and then centrifuged at 600 ***g*** for 45 min. The absorbance of the octanol layer was measured (at 280.9 nm for warfarin, 269.0 nm for phenol, 283.4 nm for strychnine, 258.0 nm for ephedrine and at 266.0 nm for procaine) against a reagent blank prepared by equilibrating 10 ml of *n*-octanol (saturated with water) with 4 ml of water (saturated with *n*-octanol) and 2 ml of buffer. The temperature was maintained at 21°C throughout. After each measurement, the spectrum of the drug in the octanol phase was measured from 200 to 400 nm in order to verify that only the unionized form of the drug was present; the ionized and unionized forms have distinctive spectra in the aqueous phase.

Data analysis. The partition data were fitted to equation (8A) or (8B) by nonlinear least-squares regression analysis [25]. Extraction ratios were calculated from equation (10) or (11).

Results

The pK_a' value (expressed as mean ± standard error (SE)) for each analyte in water (saturated with *n*-octanol) was as follows: for warfarin, 5.15 ± 0.04 ($n = 5$); for phenol, 9.88 ± 0.02 ($n = 3$); for strychnine 8.29 ± 0.02 ($n = 3$); for procaine, 8.11 ± 0.04 ($n = 3$); for ephedrine, 9.92 ± 0.01 ($n = 3$).

Plots of absorbance of the octanol phase at the analytical wavelength for each drug against extraction buffer pH displayed inflection points at pH = 8.20 ± 0.03 (mean ± SE) for warfarin (Fig. 2) and at pOH = 8.17 ± 0.02 for strychnine (Fig. 3). Similar plots

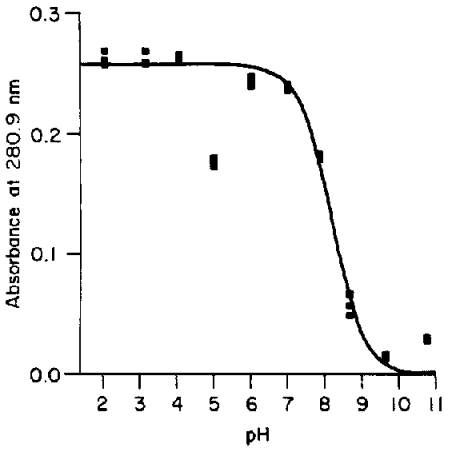


Figure 2
Absorbance of warfarin (■) in the octanol phase as a function of pH. The model (———) fitted to equation (8A) had $k_1 = 0.258$ and $k_2 = 6.375 \times 10^{-9}$.

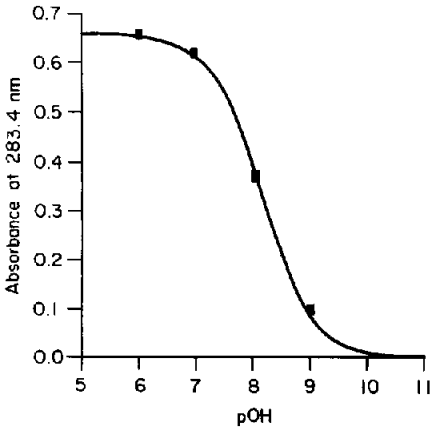


Figure 3
Absorbance of strychine (■) in the octanol phase as a function of pOH. The model (———) fitted to equation (8B) had $k_1 = 0.660$ and $k_2 = 6.806 \times 10^{-9}$ M.

featured inflection points at pH = 11.85 ± 0.03 for phenol, at pH = 7.23 ± 0.04 for procaine, and at pH = 5.95 ± 0.03 for ephedrine.

Using as volume ratio V_r = 1% and equation (10) or (12), the logarithm (expressed as mean ± SE) of the *n*-octanol–water extraction ratio each analyte was: for warfarin, 2.82 ± 0.06 (n = 21); for phenol, 1.75 ± 0.05 (n = 12); for strychnine, 2.23 ± 0.04 (n = 12); for procaine, 1.10 ± 0.08 (n = 12); and for ephedrine, 1.65 ± 0.04 (n = 9). No measurable partitioning of the ionized form of either drug into the octanol phase was observed.

Discussion

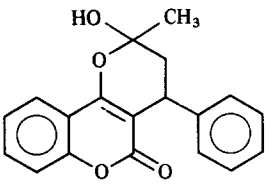
The pK'_a for strychnine in water has been reported as 8.26 at 25°C by potentiometric titration [30]. The value (8.29 ± 0.02) in the present work is slightly higher; this is consistent with the higher ionic strength of the aqueous medium employed. The logarithm of the octanol–water partition coefficient for strychnine has been reported to be 1.93 [31], a value substantially lower than the result (2.24 ± 0.03) in the present experiments. Details of the earlier measurement [31] are, however, not available.

The pK'_a for phenol has been reported as 9.99 at 25°C by potentiometric titration [32]. The value (9.88 ± 0.02) reported here is in agreement with the predicted value (9.86), after correction for the higher ionic strength of the aqueous medium used in the present work. The logarithm of the octanol–water partition coefficient for phenol has been reported to be 1.46 [33], a value substantially lower than the result of the present experiments (1.75 ± 0.05). However, no attempt was made to control ionization in the earlier experiment; any ionization, if present, was ignored.

The pK'_a for procaine has been reported as 8.05 at 15°C by colorimetry [34] or as 8.91 at 25°C by potentiometric titration [35]. The value (8.11 ± 0.04) in the present work was considerably lower than 8.91, but agreed closely with the value obtained colorimetrically using an indicator dye, after allowing for the higher ionic strength in the present experiments. The octanol–water partition coefficient for procaine has been reported as 27.8 at 23°C [36] corresponding to log P = 1.44. In the present work log P was 1.10 ± 0.08. The discrepancy may reflect the large difference in pK'_a values used in calculating log P, since the reported value for log P was derived using pK'_a = 8.91.

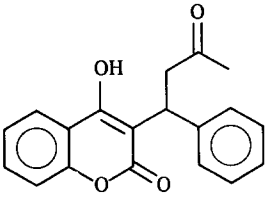
The pK'_a for ephedrine has been reported as 9.68 at 20°C by potentiometric titration after correction for concentration and ionic strength [37]. The value (9.92 ± 0.01) in the present work is higher (by 0.12 pH units) than that predicted from the literature value, after allowance for the higher ionic strength employed here. The logarithm of the octanol–water partition coefficient for ephedrine has been reported to be 0.93 [31], a value substantially lower than the result (1.65 ± 0.04) in the present experiments. Again, details of the earlier measurement are not available; the differences are larger than can be accounted for by the slightly higher value for pK'_a in the present work.

The pK'_a for warfarin has been reported as 5.05 [38]. The value (5.15 ± 0.04) in the present work is higher. The different ionic strength of the aqueous medium used in the earlier study [38] should have led to a lower value in the present work. However, warfarin exists as a mixture of three major tautomeric forms in chloroform [39], dimethylsulfoxide and acetonitrile solutions [40]. The major tautomeric form in aqueous solution at acidic pH is the hemiketal **1** [41]:



1

The acidity of warfarin solutions in water is most probably attributable to small amounts of the open chain tautomer **2** which contains an unsaturated enol structure. This tautomer behaves like a vinylogous carboxylic acid:



2

The conjugate base of warfarin in aqueous solution is the enolate anion of **2** [41] so that small amounts of tautomer **2** must exist at least as an intermediate in the formation of tautomer **1** when solutions of sodium warfarin are acidified. Differences in the pK'_a of warfarin in water and in water saturated with *n*-octanol may result from a change in the equilibrium between open-chain (**2**) and ring (**1**) tautomers of warfarin due to the presence of octanol. The existence of this discrepancy points out the need to measure the pK'_a of a weakly ionizable solute in precisely the same medium as that used as the aqueous phase in partition experiments (e.g. in water saturated with *n*-octanol and with buffer salts present at the same ionic strength) when such data are used to compute the partition coefficient of the unionized form of the solute.

The *n*-octanol–water partition coefficient of warfarin has not, so far as the authors are aware, been previously reported. Muller [15] reported that the extraction ratios for warfarin in diethyl ether/water and in chloroform/water systems exceeded 99 (i.e. log $P^{o/w} \geq 2$). The *n*-octanol–water partition coefficient of warfarin can be estimated by the fragment contribution method of Hansch *et al.* [7] in which the contributions due to each molecular fragment are added [1]. The logarithm of the *n*-octanol–water partition coefficient estimated in this way for the open-chain tautomer (**2**) is 2.46, whereas that for the ring tautomer (**1**) is 2.76. The latter agrees more closely with the experimentally determined extraction ratio (2.82 ± 0.06).

An important advantage of the present experimental approach is that the concentration of the solute (or a parameter directly proportional to concentration, such as absorbance) need only be measured in *one* phase. Even knowledge of the initial concentration is not needed if K'_a is measured experimentally. This approach may therefore be used where one phase is analytically inaccessible, provided that its volume is known. For example, crude petroleum–sea water partition coefficients or liposome–water partition coefficients could be measured in this way.

A second advantage of the present technique is that interactions of buffer components

with the partitioning solute can be readily detected as deviations from the behaviour predicted by equation (8A) or (8B), provided that a sufficient number of other buffers are used to achieve a good fit of the experimental data to the model. This check of validity by internal consistency is not possible when the traditional, single-buffer shake-flask method is used [1]. For example, the absorbance of the octanol phase in the partition experiment with warfarin was significantly lower than that predicted by equation (8A) at pH 5 and significantly higher than that predicted at pH 11. Consequently, experimental measurements obtained using these buffers were omitted from the final calculation.

Buffers should be carefully selected to minimize pH changes due to extraction of buffer components into the organic phase. The use of zwitterionic buffers, such as those used in the present work, minimizes such problems. Nevertheless, ion-pair formation between a buffer component and the ionized form of the solute may still occur; the presence of such phenomena are indicated by a failure of the data obtained with a particular buffer to conform to behaviour predicted by a model based on data obtained with other buffers.

Finally, the use of equations (17)–(20) leads to a greater appreciation of the effects of changing phase volume ratios and pH on the extractability of weakly ionizable solutes into aqueous buffers from organic solvents. For example, if a mixture of warfarin and strychnine in *n*-octanol were to be separated by liquid–liquid extraction, equations (17) and (20) could be solved by graphical methods to determine the minimum extraction buffer pH and phase volume ratio to ensure that at least 99% of the warfarin would be extracted from the *n*-octanol into the aqueous buffer, while more than 99% of the strychnine would be retained in the organic phase. The minimum pH (9.01) occurs when the volume of buffer is 9.30 times that of *n*-octanol. If the volume ratio is altered, the pH of the aqueous phase must be made more alkaline to achieve the same degree of separation (Fig. 4) This example clearly shows that the familiar advice to operate at a pH

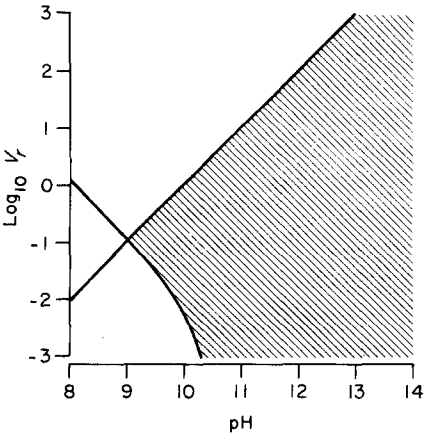


Figure 4
Simultaneous solution of equations (17) and (20) for the system: *n*-octanol (saturated with water)/water (saturated with *n*-octanol), ionic strength 10 mM. The shaded region indicates the values for the logarithm of the phase volume ratio and aqueous phase pH that will yield at least 99% recovery of warfarin in the aqueous phase, with less than 1% contamination by strychnine after phase equilibration.

2–3 units removed from the pK_a' of the drug will not be satisfactory when the partition coefficient is greatly different from 1; the phase volume ratio is an important variable to consider in the design of liquid–liquid extraction schemes for separating weak acids and bases.

Conclusions

The theoretical behaviour of weakly ionizable substances partitioning between a phase in which they can dissociate, and a phase in which they do not, has been used to design experimental methods for determining extraction ratios from measurements made in only one phase. The proposed method permits ready detection of aqueous buffer interactions with the partitioning solute when the results for one buffer are compared with the predicted behaviour deduced from results obtained with other buffers. The model equations that have been developed more clearly demonstrate the effects of changes in extraction ratios and phase volume ratios on the pH-dependent liquid–liquid extraction behaviour of weakly ionizable solutes.

Acknowledgements: This work was supported in part by the University of Wisconsin Graduate School Project No. 110277 and a grant from Bell Laboratories, Inc., Madison, WI. The authors thank Dr P. Mukerjee and Dr K. A. Connors for helpful discussions.

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[*First received for review 28 February 1983; revised manuscript received 7 May 1984*]