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Potentiometric determination of octanol–water and liposome–water partition coefficients (log *P*) of ionizable organic compounds

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Abstract—The logarithm of the octanol–water partition coefficient, log *P*, is a key physicochemical property for both pharmaceutical drugs and agrochemicals. It is also required by legislation as part of the physicochemical properties profile for high volume production chemicals. This Letter describes a simple method for determining log *P* values (over a wide range from −0.8 to 5.3) for 12 organic weak acids and bases using potentiometric titrations, with octanol or phosphatidyl choline liposomes as the partitioning medium. Such titrations take comparatively little time (about 30–45 min per titration), are easy to implement, and can be carried out with an inexpensive laboratory titrator.

For a non-ionizable compound, the partition coefficient, *P*, is defined as the ratio of the concentration of the compound in two immiscible liquid phases such as octanol and water. For practical purposes, the logarithm of the partition coefficient, log *P*, is most commonly used. Octanol is the preferred partitioning solvent for most studies since, with its hydrophobic tail and polar head group, this liquid alcohol serves as a simplified, but well-established, membrane model.[1,2] Moreover, it is not too expensive, with a litre of octanol ($\geqslant$99.5% purity) costing approximately £16 ($30). More recently, phospholipid liposomes have been identified as superior models of a cell membrane.[2] Despite this, the vast amount of literature data available for the octanol–water system is likely to ensure that octanol remains important in future evaluations of log *P*.

Most pharmaceutical drugs are nowadays designed to have log *P* values in the range of 1–4. This log *P* range has been found to correlate well with the ability of a drug to pass through cell membranes using the 'transcellular' mechanism.[3] It is therefore not surprising to find that log *P* is a key parameter in quantitative structure–activity relationship studies (QSAR).[4] These pharmaceutical concepts can similarly be applied to agrochemicals.[5] In addition, partition coefficient measurements are increasingly being demanded by regulatory authorities to predict the environmental fate of an organic chemical, since compounds that exhibit large bioconcentration factors (e.g., DDT, PCBs) tend to also have high log *P* values between 5 and 7.[6] Although many *in silico* methods have been developed to predict partition coefficients, these methods are largely limited to neutral compounds of similar structure.[7]

While the ionization constant, pK_a, of a weak acid is routinely obtained from a straightforward acid–base titration, the shake-flask method is still the standard method for determining log *P*.[8] In the case of an ionizable compound, the log *P* measurement has to be repeated in various pH buffers to account for the pH-dependence of partitioning.[9] Potentiometric titrations conducted in the presence of a partitioning medium provide an alternative approach for determining log *P*.[10] The scope of potentiometric methods is emphasized by

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the fact that over 60% of all known pharmaceutical drugs are weak acids or bases.[2] Physicochemical properties such as lipophilicity, solubility, and permeability through membranes are often pH-dependent, and these factors have to be routinely optimized during the development of a new drug or agrochemical. To deal with this, the pharmaceutical industry has invested in the use of automated titrators, together with specialized software, for pK_a and $\log P$ measurements of ionizable lead compounds in drug development.[2] Unfortunately, the relatively high cost of the hardware has, to date, precluded a more widespread application of the potentiometric method for determining $\log P$. It is recognized that potentiometric $\log P$ determinations would also be of great interest to many laboratories that do not have this specialized equipment but nevertheless have a need to measure $\log P$ experimentally.

The aim of this Letter is to provide a simple procedure for deriving $\log P$ values readily and reproducibly by carrying out several linked acid–base titrations with an ordinary laboratory titrator, which is a standard analytical instrument available in most laboratories. The analysis of titration data is easily achieved with the help of simple spreadsheets, via curve fitting of the titration curve (mL titrant added vs pH)[11] or the difference curve (degree of protonation vs pH).[12] The procedure described here requires comparatively little financial investment or training, and it gives access to the $\log P$ of an ionizable compound within a few hours. The versatility of potentiometric $\log P$ determinations is illustrated for 12 ionizable organic compounds (comprising carboxylic acids, phenols, sulfonamides, amines and a quinoline derivative), with a $\log P$ range from −0.8 to +5.3. The selection includes weak acids such as acetic acid, benzoic acid, sulfanilamide, sulfathiazole (an antimicrobial sulfonamide of historical interest), ibuprofen and paracetamol (two pain-killers), 2,4-dichlorophenoxyacetic acid (2,4-D, a herbicide) and pentachlorophenol (a wood preservative). Examples of weak bases were chlorpromazine (a tranquilizer), procaine (a local anaesthetic) and propranolol (a β-blocker). Quinine (an antimalarial drug) served as an example of a diprotic compound.

Determination of the $\log P$ of an ionizable compound by an acid–base titration requires knowledge of the compound's aqueous pK_a. If this value is taken from the literature, it is important to know whether or not the literature-reported data took the ionic strength dependence of pK_a into account.[13] Otherwise, aqueous pK_a values can be readily determined by a single titration if the compound is soluble in water throughout the whole pH range of interest (e.g., acetic acid, benzoic acid, paracetamol, quinine, sulfanilamide). Compounds such as propranolol or chlorpromazine that are poorly soluble in water in their neutral form (at concentrations of ~5 mM) can be titrated in mixtures of 0.15 M aqueous KCl and a miscible organic co-solvent, followed by extrapolation towards 0% co-solvent. Examples of such Yasuda–Shedlovsky extrapolations (Table 1)[14,15] are shown in the Supplementary data.

Table 1. Results of pK_a and octanol–water $\log P$ measurements (25 °C, ionic strength 0.15 M)[a]

Compound	Aqueous pK_a	$\log P_{XH}$	$\log P_X$
Acetic acid	4.47	-0.3 ± 0.1	<-2[b]
Benzoic acid	4.08	1.7 ± 0.1	<-2[b]
Chlorpromazine	9.28 ± 0.18[c]	1.3 ± 0.1	5.0 ± 0.1
2,4-D[d]	2.58 ± 0.03[c]	2.8 ± 0.1	-1.0 ± 0.3
Ibuprofen	4.50 ± 0.03[c]	3.9 ± 0.1	0.2 ± 0.3
Paracetamol	9.50	0.4 ± 0.1	<-2[b]
Pentachlorophenol	4.73 ± 0.02[c]	5.3 ± 0.1	1.8 ± 0.1
Procaine	9.04 ± 0.04[c]	-1.1 ± 0.2	2.1 ± 0.1
Propranolol	9.68 ± 0.01[c]	0.1 ± 0.1	3.4 ± 0.1
Quinine	8.53 ± 0.03	0.8 ± 0.1	3.2 ± 0.1
	4.38 ± 0.05		
Sulfanilamide	10.54 ± 0.05	-0.8 ± 0.3	<-2[b]
Sulfathiazole	7.23 ± 0.02[c]	0.3 ± 0.1	<-1[b]

[a] Standard deviation of residuals given.
[b] Value could not be quantified.
[c] Determined by a Yasuda–Shedlovsky extrapolation using 0.15 M aqueous KCl–methanol mixtures.
[d] 2,4-Dichlorophenoxyacetic acid.

With the aqueous pK_a of an organic acid being known, $\log P$ could be determined by carrying out one or more titrations in water–octanol mixtures. The pK_a^{oct} of a weak acid in the presence of octanol is shifted relative to its aqueous pK_a. The difference in pK_a in the presence and absence of a partitioning medium, $\Delta pK_a = pK_a^{oct} - pK_a$, depends on both the size of $\log P$ and the octanol–water volume ratio, r (Eqs. 1 and 2)[13]

$$r = \frac{V_{oct}}{V_{aq}} \tag{1}$$

$$\log P = \log\left(\frac{10^{|\Delta pK_a|} - 1}{r}\right) \tag{2}$$

In this Letter, a protonated base was treated like an acid, and all equations are applied equally to both acids and bases. It should be noted, though, that the direction of the pK_a shift for a protonated weak base ($pK_a^{oct} <$ aqueous pK_a) is opposite to that of a weak acid ($pK_a^{oct} >$ aqueous pK_a). Hence, a protonated weak base becomes more acidic, whereas a weak acid becomes a weaker acid, in the presence of octanol.

Generally, it was advantageous to start a $\log P$ determination at an octanol-to-water ratio, r, of 1. Eq. 2 then provided an initial and, in many cases, quite accurate estimate of the $\log P$ of the neutral form of the compound (Fig. 1). Subsequent titrations conducted at different octanol-to-water ratios served to reduce the overall error in $\log P$ as well as to identify the extent to which partitioning of the ionized species took place. The $\log P$ values determined were thus scattered more or less around an average as long as the ionic species did not partition into octanol. This was clearly the case for acetic acid (Fig. 1a) for which the only outlier was, not surprisingly, found at the lowest octanol-to-water ratio.

In contrast, the ionic forms of chlorpromazine, pentachlorophenol and propranolol were found to partition into octanol to a considerable extent, particularly at lar-

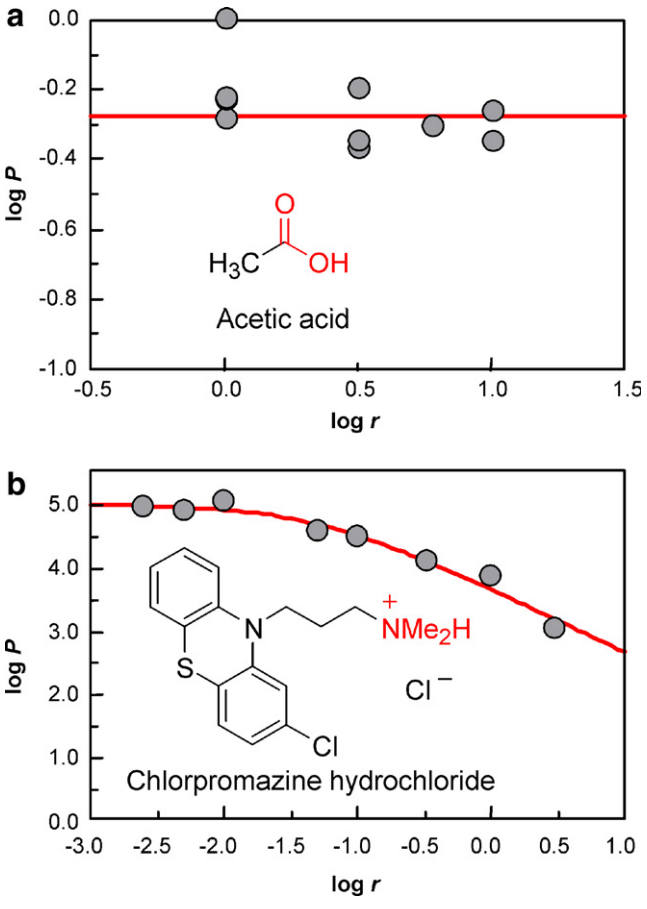

Figure 1. log P of the neutral species of (a) acetic acid and (b) chlorpromazine calculated from experimental ΔpK_a using Eq. 2 (solid circles) at various octanol-to-water ratios, r.

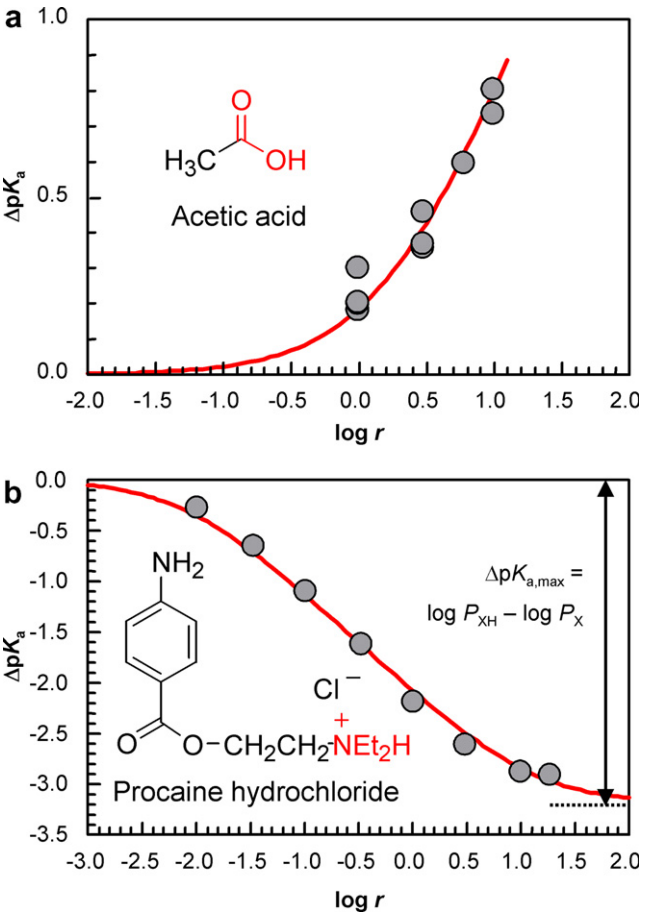

Figure 2. Plot of ΔpK_a versus log r for (a) acetic acid and (b) procaine in octanol/0.15 M aqueous KCl mixtures at 25 °C. Solid circles represent experimental data points. The curve drawn represents the best fit to Eq. 3 in each case. In the case of procaine, as expected for a base, the deprotonated species, X, is the more lipophilic species, and ΔpK_a becomes negative.

ger octanol-to-water ratios. The log P values for these compounds, when calculated with Eq. 2, appeared to become 'smaller' with increasing log r with the downward trend starting around the point where log r equals $-\log P$ of the ionic species (Fig. 1b). For more accurate measurements of the partition coefficients of neutral and ionic species, the experimental ΔpK_a versus log r data were fitted to Eq. 3. This equation depends on two partition coefficients, one for the protonated (P_{XH}) and the other for the deprotonated form (P_X) of the compound.[16] The partition coefficient of the neutral species ($\log P_{XH}$ for a weak acid or $\log P_X$ for a weak base) could, in all cases, be readily obtained—even for quite hydrophilic compounds such as acetic acid or sulfanilamide. Figure 2 shows that, despite occasional minor variations in reproducing pK_a^{oct} values, the overall error averaged so that the majority of the data points lay close to the curve described by Eq. 3

$$\Delta pK_a = \log \frac{1 + r \cdot P_{XH}}{1 + r \cdot P_X} \qquad (3)$$

More lipophilic compounds were studied over a wider range of octanol-to-water ratios, selected in such a manner that they were equally spaced on a logarithmic scale. There was a lower practical limit for r since, eventually, the amount of octanol became so small that ΔpK_a was <0.2 and, therefore, close to the error for the pK_a^{oct} measurement (typically less than 0.1 pK_a units). It should be noted that the partition coefficient for the ionic species could only be determined with reasonable accuracy when ΔpK_a actually reached a maximum at high log r. However, the values of $r \geqslant 10$ (or $\log r \geqslant 1$) were again problematic from a practical point of view since, in the presence of large amounts of octanol, the glass electrode would struggle to give an accurate pH reading due to the small volume of aqueous solution present under these conditions.

Table 1 summarizes our results based on this procedure. In general, there is good agreement between literature values and our own recorded measurements (see Supplementary data). Occasional problems in determining log P by potentiometric titrations in octanol–water mixtures usually resulted from either: (i) inefficient mixing causing poor reproducibility at extreme values of log r; (ii) a limited range of log r values, as small ΔpK_a values at low octanol-to-water ratios and scatter in the electrode response at higher ratios ($r \geqslant 10$) tended to complicate the analysis of titration data; or (iii) low solubility of a compound in both octanol and water.

Table 2. Selected results of liposome–water partition coefficient measurements (25 °C, ionic strength 0.15 M)[a]

Compound	$\log P_{XH}$	$\log P_{X}$
Chlorpromazine	3.1 ± 0.2	5.1 ± 0.1
2,4-D[b]	3.6 ± 0.1	1.7 ± 0.3
Procaine	0.7 ± 0.4	2.2 ± 0.1
Propranolol	2.6 ± 0.1	3.4 ± 0.1
Quinine	1.9 ± 0.1	2.7 ± 0.1

[a] Using liposomes made by sonication of egg yolk phosphatidyl choline (97.5%) in 0.15 M aqueous KCl. Standard deviation of residuals are given.

[b] 2,4-Dichlorophenoxyacetic acid.

The potentiometric method for determining $\log P$ was found to work equally well for liposomes as the partitioning medium. Phospholipid liposomes are much better mimics of a cell membrane than octanol but, in contrast to octanol, no bulk phase separation occurs between aqueous and liposome phases which prevents their use in the shake-flask method.[17] Table 2 summarizes our results after evaluating ΔpK_a in the presence and absence of liposomes as a function of $\log r$. Most notably, the ionic species contributed significantly to partitioning, promoted by ionic interactions between the attractive charges on the drug and the phosphatidyl choline head group in the liposomes. Here, $\log P$ measurements were, however, somewhat limited to more lipophilic compounds. This was due to the fact that the $\log r$ range was significantly narrowed, with the highest accessible liposome-to-water ratio being $r = 0.1$, corresponding to a 10 wt % dispersion of phosphatidyl choline in 0.15 M aqueous KCl.

This Letter outlines how octanol–water (and liposome–water) partition coefficients can be evaluated for ionizable compounds without the need to resort to expensive, specialized equipment or software. This approach will be valuable for many research laboratories where experimental $\log P$ values are required only occasionally and only a standard laboratory titrator is available. There are just a few practical, albeit not fundamental, limitations: (i) the method works well for monoprotic and diprotic ionizable compounds with pK_a values in the range of 3–10, which are easier to analyse than compounds with more extreme pK_a values; and (ii) compounds have to be soluble in aqueous KCl or octanol at millimolar concentrations throughout the whole pH range. Each titration required about 0.13–0.15 mmol of weak acid (or protonated base). Between one and five titrations were necessary to obtain the aqueous pK_a value as reference, whereas 4–10 titrations were required in the presence of octanol (or liposomes) to determine the $\log P$ value of the neutral form of an ionizable organic compound. The $\log P$ of the ionized species could also be estimated for more lipophilic compounds and when liposomes were used as partitioning medium. The potentiometric measurement of octanol–water partition coefficients proved a reproducible and convenient method for obtaining $\log P$. The formalism for data analysis was straightforward, and demanded little time (1–3 min per titration or $\log P$ analysis) or training. The method should find wide applicability as demonstrated by the extensive range of $\log P$ values (−0.8 to 5.3) covered.

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Supplementary data

Derivation of Eqs. 2 and 3, details of experimental procedures, comparison with literature data, calculation of distribution coefficients, plots of ΔpK_a versus $\log r$ for all studied examples, and example spreadsheets are included. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.03.085.

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