OECD GUIDELINES FOR THE TESTING OF CHEMICALS

PROPOSAL FOR A NEW GUIDELINE 122

Partition Coefficient (n-Octanol/Water), pH-Metric Method for Ionisable Substances

INTRODUCTION

1. Several methods are available for determining n-octanol/water partition **coefficients (P_{ow} values)**. **P_{ow}** values in the logarithmic range -2 to 4 (occasionally up to 5) can be experimentally determined by the shake-flask method set out in OECD Guideline 107. P_{OW} values in the range log P_{OW} between 0 and 6 can be estimated using high performance liquid chromatography as set out in OECD Guideline 117. Whilst both these methods can be applied to ionisable compounds buffered to a pH at which the compound is unionised, they are of limited applicability to multiprotic substances. The method set out in this guideline allows ~~for~~ **the pH-metric** determination of log P_{ow} values ~~of~~ **for** ionisable and multiprotic compounds in the range -2 to 7. This guideline is base**d** on a proposal submitted by the Department of the Environment, **Transport and the Regions** of the United Kingdom. The proposal was **further** developed ~~also based~~ **on the basis of** existing methods described in literature (1)(2)(3)(4)(5)(6).

INITIAL CONSIDERATIONS

2. The n-octanol/water partition coefficient is a measure of lipophilicity and a key parameter in the prediction of the environmental fate of organic chemicals. However, for ionisable substances the partition coefficient of the neutral form of the substance may lead to a wrong appreciation of the environmental partitioning **and changes of ionisation and partitioning with pH need to be understood**. For instance, the ionised species of an organic acid is generally adsorbed by sediments to a much lesser degree than is the neutral form because of its high water solubility and low lipophilicity. The fate of ~~polyprotic~~ **multiprotic** molecules may depend on the ambient pH. Thus, a single value of log P_{ow} is an incomplete description of the lipophilicity of ionisable substances. **The pH-metric method provides a much faster method for establishing the pH-dependency of partitioning than other methods.**

PRINCIPLE OF THE METHOD

3. The pH-metric technique consists of two linked potentiometric titrations. The pK_a of the test substance is determined by **acid-base** titration **of an aqueous solution of the substance** using potentiometric measurement of pH (OECD Guideline 112). **To measure the log P_{OW}, one or more additional titrations are done. In these additional titrations, n-octanol is added to a solution of the substance in water and the apparent pK_a of the substance in the two-phase system is measured. This apparent pK_a value is denoted by the term p_oK_a. The pK_a values derived from the aqueous and the two-phase titration curves are different.** ~~At the completion of the first titration, n-octanol is added~~

~~and a second titration is performed, returning the well-mixed two-phase system to the starting pH. The second titration yields an apparent pK_a, denoted by p_eK_a. As the substance partitions into the n-octanol rich phase, the pK_a values derived from the two titration curves are different~~. The difference is related to the value of log P_{ow}. Using established equations, an estimation of the partition coefficient is obtained.

INFORMATION ON THE TEST SUBSTANCE

4. **It should be noted that pK_a and log P_{OW} are physicochemical parameters which refer to the properties of single, pure substances, and that it is not valid to attempt to use these terms to describe the properties of mixtures of substances. Therefore, the samples chosen for measurements must be single, pure substances. Before measurement, and in order to facilitate the design of experiments, it is useful to estimate the number and nature of possible ionisations. However it may be possible to discover this by trial and error after the titrations have been done unless information on ionisation of the test substance is available, using suitable computing facilities.** ~~The number and nature of possible ionisations should be known beforehand. These can be discovered by trial and error when the titrations have been done, using suitable computing facilities~~. Information on solubility and hydrolysis characteristics is useful.

5. The method does not apply to surface active, volatile and light sensitive compounds.

REPEATABILITY AND ACCURACY

~~5~~**6**. In order to increase the confidence in the measurement, ~~several~~ **at least three** determinations should be made and the log P_{ow} values derived from the different measurements should fall within a range of ± 0.1 log units.

REFERENCE SUBSTANCES AND VALIDATION

~~6~~**7**. Reference substances need not ~~to~~ be used in all investigations but they are useful to calibrate the method from time to time and to compare with other methods. Recommended reference compounds and their pK_a and log P_{ow} values (non-ionised form) are listed in Table 1.

Table 1: Recommended reference compounds

Reference substance	Log P_{OW} (7)	**PK_a**	
		Measured at zero ionic strength	**Converted to 0.15M (11a)**
Acetic acid *****	**-0.17**	**4.75 (8)**	**4.51**
Aniline ******	0.9	**4.87 (8)**	**4.87**
4-Methoxyphenol	**1.34**	**10.21 (8)**	**9.97**
Phenoxyacetic acid	**1.34**	**3.17 (8)**	**2.93**
Phenol	**1.46**	**9.99 (8)**	**9.75**
2,4-Dinitrophenol	**1.67**	**4.07 (8)**	**3.83**
2-Nitrophenol	**1.79**	**7.23 (8)**	**6.99**
4-Chloroaniline	**1.88**	**3.98 (8)**	**3.98**
Benzoic acid	**1.87**	**4.21 (8)**	**3.97**
4-Methylphenol	**1.94**	**10.26 (8)**	**10.02**
4-Chlorophenol	**2.39**	**9.41 (8)**	**9.17**
3-Chlorobenzoic acid	**2.68**	**3.84 (8)**	**3.60**
1-Naphth**h**ol	**2.84**	**9.39 (8)**	**9.15**
4-Phenylphenol	**3.20**	**9.55 (8)**	**9.31**
2,4,6-Trichlorophenol	**3.69**	**6.23 (9)**	**5.99**
Pentachlorophenol	**5.12**	**4.69 (10)**	**4.45**
2-Aminobenzoic acid	**1.26 (12)**		**2.15, 4.75 (12)**
Nicotine	**1.32 (12)**		**3.17, 8.11 (12)**
Quinine **	**LogP(X^0) 3.47 (12)** **LogP(XH^+) 0.87**		**4.24, 8.55 (12)**

*** use of sodium salt recommended**
**** use of hydrochloride salt recommended**

Several validation studies of the pH-metric technique for log P_{OW} measurement have been published (10) (13) (14).

DESCRIPTION OF THE TEST METHOD

Preliminary estimate of the partition coefficient

~~7~~**8**. **Before measurement, the** ~~The~~ partition coefficient of the test substance **may be** ~~is~~ estimated**,** preferably by using a calculation method (OECD Guideline 117)**,** or from the ratio between the solubilities of the compound in the pure solvents. Alternatively, it may be roughly determined by the method itself using trial and error. ~~to achieve the correct range of n-octanol/water ratios.~~ **The existence of an estimated value will facilitate the choice of optimum ratios of volumes of n-octanol/water to use in titrations**.

Solvents

~~8~~**9**. Double-distilled water or water purified by reverse osmosis should be **used to make up all aqueous solutions used in the titrations.** The ~~water~~ **solutions** should not stand in contact with air to avoid absorption of carbon dioxide. ~~Titrations are usually done using solutions of relatively high ionic strength, e.g. 0.15 M potassium chloride.~~ **It is advantageous to do the titrations in conditions which approach as nearly as possible to constant ionic strength. This is because pK_a values change slightly as a function of ionic strength. To minimise changes in ionic strength, samples may be prepared in "ionic-strength-adjusted water" (a strong solution of unreactive electrolyte such as 0.15 M potassium chloride), and titrations done with strong titrants (e.g. 0.5 M hydrochloric acid and potassium hydroxide).** For all partitions, analytical grade n-octanol previously saturated with **ionic-strength-adjusted** water ~~adjusted to the ionic strength~~ is used.

Apparatus

~~9~~**10**. The apparatus consists of an automatic titrator with an efficient stirrer and a research-grade semi-micro combination pH electrode, attached to a pH meter. It ~~would~~ **may** ~~normally~~ incorporate a dedicated computer capable of fully automatic data collection together with the appropriate software for calculation of the initial estimates of pK_a, p_oK_a and log P_{ow} and for subsequent refinement of these values taking into account the many variables associated with the titrations. Further information concerning these computations is given in the annex.

~~10~~**11**. ~~Following~~ **The following** practical considerations apply:

- the dispenser resolution of the titrator should be **as far as possible** 0.0005 ml or better;
- the pH electrode ~~resolution~~ **reading** should be **displayed to** 0.001 pH units, **with a resolution of 0.005 pH** or better;
- the pH electrode should be standardised over a wide rang**e**, ~~like 1.8 to 12.2; using only buffers of pH 4.7 and 10 does not allow calibration of the electrode for non-linear effects associated with measurements.~~ **e.g. from pH 1.8 to 12.2; a titrimetric method of standardisation is recommended which will allow for non-ideal electrode behaviour at low and high pH (see Annex).**

Test conditions

~~11~~**12**. The temperature of the titration vessel should be kept constant at ± 1 °C and lie in the range 20-25 °C.

~~12~~**13**. **Absorption of carbon dioxide causes difficulties in calculation of results because carbon dioxide has two pK_as at about 6.1 and 9.9, and its log P_{OW} value is about +0.5.** The test solution should **therefore** be protected from air with an inert gas blanket. This is especially important at high pH ~~and~~ **or** when n-octanol is present **because carbon dioxide is readily absorbed from air under these conditions**. Alternatively corrections must be made for carbon dioxide ~~present in the solution~~ during ~~the~~ refinement of results **(see Annex).**

Test substance

~~13~~**14**. The test and reference substances should be as pure and dry as possible. ~~**although**~~ **The effect of some** impurities **(e.g. water, solvent or salts)** can be corrected for during the refinement of the results.

~~**Performance of the test**~~
PERFORMANCE OF THE TEST

Titration

~~14~~**15**. If the accurate pK_a of the test substance is known, **the following procedure should be used to measure the log P_{OW} (If the accurate value for the pK_a of the substance is not known beforehand, it must first be measured. The technique is similar to that described hereafter, but without n-octanol.). A** ~~a~~ sample is weighed into the titration vessel. **If the sample is expected to dissolve easily in water, i**~~I~~onic-strength adjusted water is added to give a solution with a concentration between 0.0005 and 0.005 M. The appropriate volume of n-octanol is added and the mixture is stirred vigorously for at least 30 seconds to achieve thorough mixing. **Alternatively, if the sample is poorly soluble in water but dissolves easily in n-octanol, the appropriate volume of n-octanol is added and the sample is dissolved before the water is added. In either method of sample preparation, it is essential that the sample is completely dissolved, whether in the aqueous phase or in n-octanol, before the pH is adjusted.**

16. The pH is adjusted to the required starting point with standardised acid or base, with at least 10 seconds of vigorous stirring after each incremental addition and before measuring pH. **The titration is done with standardised base or acid, again with vigorous stirring for 10 seconds before measuring pH.** Equilibrium must be obtained, however long this takes, before any pH measurement is made. ~~The titration is done with standardised base or acid, again with vigorous stirring for 10 seconds between points.~~ An approximate value for p_oK_a is then obtained as the pH at half neutralisation and this is the basis for further computation. **Table 2 gives suggested volumes of n-octanol and water to use in titration experiments. In general, larger values of r are required for measurement of lower log P_{OW} values, in order to bring about an adequate difference between pK_a and p_oK_a. Volumes may be scaled in proportion to the suggestions below according to the capacity of the titration vessel.**

Table 2: Recommended volume ratio of n-octanol and water

Expected log P_{OW} value	Volume of octanol (ml)	Volume of water (ml)	r (volume ratio, $V_{n\text{-octanol}} / V_{water}$)
Below –1	**≥ 75**	**≤ 5**	**>10.0**
Below 0	**15**	**5**	**3.0**
0 to 1	**1**	**10**	**0.1**
1 to 2	**1**	**15**	**0.067**
2 to 3	**0.5**	**15**	**0.033**
Above 3	**< 0.5 (but above 0.01)**	**20**	**<0.025**

~~15. If an accurate value for the pK_a of the substance is not known beforehand, a separate determination without the addition of n-octanol should be done in the same way and under the same conditions.~~

Number of titrations required

17. **The number of titrations required to measure log P_{OW} depends on the type of sample (monoprotic, multiprotic) and also on the accuracy of result required. Table 3 provides relevant information.**

Table 3: Required minimum number of titrations

	Minimum number of titrations required to make measurement		
	pK_a (water-soluble sample)	**Log P_{OW}, neutral species only**	**Log P_{OW}, neutral plus ionised species**
Monoprotic (one pK_a)	**one**	**One (use the smallest possible volume of n-octanol that will create an observable difference between pK_a and p_oK_a)**	**Two (use two different ratios of n-octanol to water, to create a large difference between the two p_oK_a values)**
Multiprotic (two or more pK_as)	**one**	**One (use the smallest possible volume of n-octanol that will create an observable difference between pK_a and p_oK_a)**	**One (use a large ratio of n-octanol to water, to create a large difference between the p_oK_a values)**

~~16~~**18**. All determinations should be done three times or more and preferably with different n-octanol/water ratios to identify any ion-pair partitioning that may occur. **In titrations where the volume of n-octanol is very small (r < 0.01), corrections must be made in the refinement of results for the quantity of n-octanol dissolved in the aqueous phase, which was not pre-saturated with n-octanol.**

CALCULATION OF RESULTS

19. **Results for log P_{OW} may be calculated from pK_a and p_oK_a values together with n-octanol/water volume ratios using the equations below. However, a refinement procedure is required to calculate pK_a and p_oK_a values from titration data. Refinement is described in the annex.**

Monoprotic compounds, neutral species partitioning only

~~17~~**20**. ~~The first estimation of the partition coefficient for an acid is calculated from the equation.~~ **The P_{OW} value for the neutral species of a monoprotic compound is calculated using the following equation.**

$$P_{ow} = \frac{10^{|poKa - pKa|} - 1}{r}$$

where **$|p_oK_a - pK_a|$ is the absolute difference between p_oK_a and pK_a (expressed as a positive number) and r** is the volume ratio $V_{n\text{-octanol}} / V_{water}$.

~~18 Equations linking P_{ow} with (p_eK_a – pK_a) and r also exist for multiprotic substances, and these are applicable even if the separation of pK_a values is small. (1). Corrections must be made in the refinement of results for the quantity of n-octanol dissolved in the aqueous phase, which was not pre-saturated with n-octanol.~~

DATA AND REPORTING

Test report

~~19~~**21**. The following should be included in the report:

- test substance: purity**, structural formula, CAS number** and form introduced (e.g. salt);
- description of the equipment;
- method and pH range of calibration of the electrode and standardisation of titrants;
- temperature of determinations;
- ionic strength of aquatic solution, **and chemical(s) used for maintaining the desired ionic strength** (mean value should be given as the ionic strength will change during the course of the titration);
- weight of the sample used, volume of ionic-strength adjusted water and volume of n-octanol;
- **typical titration curve(s)**, pK_a in aqueous solution and how obtained;
- all determined values of P_{ow} and their standard deviations about the mean;
- the mean of all determinations expressed as its logarithm (base 10).

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ANNEX

Refinement of pK_a and p_oK_a results by computation

Ionization and partitioning equilibria

Figure 1 depicts a "Four-equation partition model" for the ionisation and partitioning of a monoprotic base in the octanol-water system. Similar models could be constructed for ionisation and partitioning of acids, and of multi-protic molecules.
The equation for the ionisation equilibrium of the base in aqueous solution is represented by:

$$X^0 + H^+ = XH^+$$

and the "aqueous pK_a" value is

$$pK_a = \log \frac{[XH^+]}{[H^+][X^0]} \qquad [1]$$

The equation for the partitioning of the neutral species X^0 between water and octanol is

$$X^0_{aq} \leftrightarrow X^0_{oct}$$

which leads to equation [2] for the log P value for the neutral species. This is the value which is normally quoted as the official "log P_{OW}" of any substance. In this example the number of protons associated with the neutral species = 0, and its partition coefficient may therefore be written as P^0.

$$\log P^0 = \log \frac{[X^0]_{oct}}{[X^0]_{aq}} \qquad [2]$$

Note: $[X]_{oct}$ represents the concentration of X in terms of moles dissolved per litre of octanol.

The equation for the partitioning of the cationic species XH^+ into octanol is;

$$XH^+_{aq} \leftrightarrow XH^+_{oct}$$

It is assumed that the cation XH^+ partitions as an ion-pair with a suitable anion from the aqueous solution. The value of the log P of the ionized species therefore varies according to the concentration of background electrolyte. In this example the number of protons associated with the cationic species = 1, and its partition coefficient may therefore be written as P^1. Thus,

$$\log P^1 = \log \frac{[XH^+]_{oct}}{[XH^+]_{aq}} \qquad [3]$$

The equation for the ionization of the substance in octanol is

$$X^0_{oct} + H^+ = XH^+_{oct}$$

This equation leads to equation [4] for the apparent pKa in water-saturated octanol (1).

$$pK_a^{oct} = \log \frac{[XH^+]_{oct}}{[H^+][X^0]_{oct}} \qquad \textbf{[4]}$$

pH-metric technique: pK_a measurement

In a typical pH-metric pK_a measurement of a water-soluble sample, a weighed sample of pure substance is dissolved in an inert electrolyte solution (typically 0.001M sample in 15mL KCl solution). Typically, the solution is acidified with standardized 0.5M HCl solution until its pH is at least 1-2 units lower than any expected pK_a, and titrated under argon at 20-25°C (± 1°C) with standardised 0.5M KOH solution until the pH is at least 1-2 units higher than the pK_a. (Alternatively, the solution may be raised to high pH and titrated with acid). The pH of the solution is monitored throughout the titration with a standardised pH electrode. A table of data points (pH values vs. mLs of titrant) is saved for further study. Note that pK_as can be measured in titrations which start of finish within 1-2 pH units from the pK_a, but accuracy may be compromised.

The structure of the molecule is studied to ascertain the number of ionizable groups. An equation is proposed for each ionization (e.g. $X^0 + H^+ = XH^+$, $XH^+ + H^+ = XH_2^{++}$, etc.). The pK_a values are then estimated using a Bjerrum Difference curve (Figure 2).

These estimated pK_a values are improved in a non-linear least-squares process known as Refinement. In this process, a simulated titration curve is first constructed in a suitable computer program. The points in this titration curve consist of pH vs. mLs of titrant. Each pH value is calculated from a polynomial equation of the form $A[H]^n + B[H]^{n-1}\ldots = 0$, which may be derived from equations based on the following information:

- **Experimental pH/mL data**
- **Concentration of sample**
- **Ionic strength of sample solution, concentrations of titrants**
- **Ionic product of water**
- **Mass balance for sample**
- **Charge balance for all solution components**
- **pK_a/s for sample (initially, the values estimated from the Bjerrum Difference curve are used)**
- ***Mass balance for CO_2 (often present as impurity)**
- ***Estimated concentration of CO_2**
- ***pK_a/s for CO_2 under the temperature and ionic-strength conditions of the experiment (these values may be computed from published values)**

***If carbon dioxide cannot be fully excluded from the solution during titration, it is necessary for the computation to determine the amount of CO_2 in solution and correct the data for its presence.**

Note: the equations used in these non-linear least squares procedures (2)(3)(4) must be based on a complete mass balance of all relevant components in solution (5).

Note: the exact form of the polynomial equations varies according to whether the sample is an acid, base or ampholyte, mono or multi-protic. Although the polynomial has n solutions for [H], only the solution which corresponds to the estimated pK_a value/s need be considered.

The pH values in this simulated titration curve are then systematically varied in a computerised calculation in which the values used for the pKa/s, acidity error, sample concentration and CO_2 concentration are changed iteratively (the ability to refine the apparent substance-concentration factor and the net acidity error in addition to pK_a values is necessary (5) since test substances are rarely 100 % pure). These iterations affect the terms A, B etc. in the polynomial equation, and the pH values thus calculated are compared with the experimental pH values. The best calculated value for the sample pK_a/s occurs when the sum of the squares of the differences between the calculated and measured pH values (divided by a "variance" term whose value increases with the slope of the titration curve) converges to a minimum value. The "variance" term derives from a "Weighting Scheme", in which each point (volume of titrant, pH) in the data has an error assigned to it, based on sound experimental criteria. The error is based on both uncertainties in titrant volume dispensed and uncertainties in pH measurement (5)(6). Given an experimentally verifiable weighting scheme, the criteria for convergence must be: χ_V^2 (reduced-chi-squared (2)) less than 3; and no calculated parameter shift greater than 0.01 times its calculated standard deviation, derived from the diagonal terms of the variance-covariance matrix (7). Without a weighting scheme, refinement of pK_a values of multiprotic molecules can be very unreliable, especially at low concentration.

Expressed more simply, the simulated titration curve is compared point by point with the experimental titration curve. The pK_a values required to achieve the best fit are assumed to be the correct measured pK_a values.

<u>pH-metric technique: log P_{ow} measurement</u>

The pH-metric technique (first described by Dyrssen in 1952 (8)) provides a method of determining $logP_{ow}$ directly from acid-base titration in a dual-phase water-partition solvent system. To use this method, the pK_a value(s) must be known. In a typical pH-metric measurement of log P, a weighed sample is dissolved in 0.15M KCl solution plus a measured volume of partition solvent, and the pKa/s are measured pH-metrically. When an ionizable substance is titrated in a two-phase system, its Bjerrum difference curve is displaced from the aqueous curve because some titratable sample "disappears" by partitioning into the non-aqueous phase. For monoprotic acids the pK_a shifts to a higher value, while for bases it shifts to a lower value. These "shifted" pK_a values are called p_oK_a values.

A series of equations (9) relate pK_a and p_oK_a values to partition coefficient (P). Equations for mono, di, tri and tetraprotic acids, bases and ampholytes are detailed in Table 1 for partition of single species and for contiguous species (those existng in adjoining ionization states); other equations may be readily derived. In the two simplest cases, P(XH) for a monoprotic acid is calculated from:

$$P(XH) = \frac{10^{p_oK_a - pK_a} - 1}{r} \qquad \textbf{[5]}$$

and P(X) for a monoprotic base is calculated from:

$$P(X) = \frac{10^{-(p_oK_a - pK_a)} - 1}{r} \qquad \textbf{[6]}$$

where $r = \frac{\text{volume of partition solvent}}{\text{volume of aqueous phase}}$ **[7]**

The structure of the molecule is studied to ascertain the likely partitioning equations. Thus for a monoprotic acid HX^0, an equation such as $HX^0_{aq} = HX^0_{oct}$ would be expected. Log P value/s are estimated for the proposed equations. Using these initial/estimated log P values together with the p_oK_a values and the aqueous pK_a values, a similar refinement process to that described for pK_a measurement is used to determine the correct measured log P values.

TABLE 1 **Partition coefficient (P) expression as a function of δ (p_oK_a-pK_a) and r ($V_{octanol}/V_{water}$)[a]**

[a] $\delta i = \log_o K_i - \log K_i$, where $K_i = [XH_i] / [H][XH_{i-1}]$.

Case 1: Monoprotic weak acid or base			
P(HX)		**P(X)**	
One component in the organic phase (2 possible solutions)			
$(10^{+\delta 1}-1)/r$			
		$(10^{-\delta 1}-1)/r$	
Case 2: Diprotic weak acid or base			
$P(XH_2)$	**P(HX)**	**P(X)**	
One component in the organic phase (3 possible solutions)			
$(10^{+\delta 2}-1)/r$			
	$(10^{-\delta 2}-1)/r = (10^{+\delta 1}-1)/r$		
		$(10^{-\delta 1}-1)/r$	
Two components in organic phase (2 possible contiguous solutions)			
$(10^{+(\delta 1+\delta 2)}-1)/r$	$(10^{+\delta 1}-1)/r$		
	$(10^{-\delta 2}-1)/r$	$(10^{-(\delta 2+\delta 1)}-1)/r$	
Case 3: Triprotic weak acid or base			
$P(XH_3)$	**$P(XH_2)$**	**P(HX)**	**P(X)**
One component in the organic phase (4 possible solutions)			
$(10^{+\delta 3}-1)/r$			
	$(10^{-\delta 3}-1)/r = (10^{+\delta 2}-1)/r$		
		$(10^{-\delta 2}-1)/r = (10^{+\delta 1}-1)/r$	
			$(10^{-\delta 1}-1)/r$
Two components in organic phase (3 possible contiguous solutions)			
$(10^{+(\delta 2+\delta 3)}-1)/r$	$(10^{+\delta 2}-1)/r$		
	$(10^{-\delta 3}-1)/r = (10^{+(\delta 1+\delta 2)}-1)/r$	$(10^{-(\delta 3+\delta 2)}-1)/r = (10^{+\delta 1}-1)/r$	
		$(10^{-\delta 2}-1)/r$	$(10^{-(\delta 2+\delta 1)}-1)/r$
Three components in organic phase (2 possible solutions)			
$(10^{+(\delta 1+\delta 2+\delta 3)}-1)/r$	$(10^{+(\delta 1+\delta 2)}-1)/r$	$(10^{+\delta 1}-1)/r$	
	$(10^{-\delta 3}-1)/r$	$(10^{-(\delta 3+\delta 2)}-1)/r$	$(10^{-(\delta 3+\delta 2+\delta 1)}-1)/r$

TABLE 1 (Cont.) Partition coefficient (P) expression as a function of δ (p_oK_a-pK_a) and r ($V_{octanol}/V_{water}$)[a]

Case 4: Tetraprotic weak acid or base				
$P(XH_4)$	$P(XH_3)$	$P(XH_2)$	$P(HX)$	$P(X)$
One component in the organic phase (5 possible solutions)				
$(10^{+\delta 4}-1)/r$				
	$(10^{-\delta 4}-1)/r$ = $(10^{+\delta 3}-1)/r$			
		$(10^{-\delta 3}-1)/r$ = $(10^{+\delta 2}-1)/r$		
			$(10^{-\delta 2}-1)/r$ = $(10^{+\delta 1}-1)/r$	
				$(10^{-\delta 1}-1)/r$
Two components in organic phase (4 possible contiguous solutions)				
$(10^{+(\delta 3+\delta 4)}-1)/r$	$(10^{+\delta 3}-1)/r$			
	$(10^{-\delta 4}-1)/r$ =$(10^{+(\delta 2+\delta 3)}-1)/r$	$(10^{-(\delta 4+\delta 3)}-1)/r$ =$(10^{+\delta 2}-1)/r$		
		$(10^{-\delta 3}-1)/r$ =$(10^{+(\delta 1+\delta 2)}-1)/r$	$(10^{-(\delta 3+\delta 2)}-1)/r$ =$(10^{+\delta 1}-1)/r$	
			$(10^{-\delta 2}-1)/r$	$(10^{-(\delta 2+\delta 1)}-1)/r$
Three components in organic phase (3 possible solutions)				
$(10^{+(\delta 2+\delta 3+\delta 4)}-1)/r$	$(10^{+(\delta 2+\delta 3)}-1)/r$	$(10^{+\delta 2}-1)/r$		
	$(10^{+(\delta 1+\delta 2+\delta 3)}-1)/r$ =$(10^{-\delta 4}-1)/r$	$(10^{+(\delta 1+\delta 2)}-1)/r$ =$(10^{-(\delta 4+\delta 3)}-1)/r$	$(10^{+\delta 1}-1)/r$ =$(10^{-(\delta 4+\delta 3+\delta 2}-1)/r$	
		$(10^{-\delta 3}-1)/r$	$(10^{-(\delta 3+\delta 2)}-1)/r$	$(10^{-(\delta 3+\delta 2+\delta 1)}-1)/r$
Four components in organic phase (2 possible solutions)				
$(10^{+(\delta 1+\delta 2+\delta 3+\delta 4)}-1)/r$	$(10^{+(\delta 1+\delta 2+\delta 3)}-1)/r$	$(10^{+(\delta 1+\delta 2)}-1)/r$	$(10^{+\delta 1}-1)/r$	
	$(10^{-\delta 4}-1)/r$	$(10^{-(\delta 4+\delta 3)}-1)/r$	$(10^{-(\delta 4+\delta 3+\delta 2)}-1)/r$	$(10^{-(\delta 4+\delta 3+\delta 2+\delta 1)}-1)/r$

Calibration of the pH electrode

The pH electrodes used for pK_a and logP measurement are preferably calibrated by a titrimetric procedure called a "Blank titrtion". In this procedure the electrode is first read in a traceable buffer solution, and this pH is used as a fixed point to calibrate the electrode on the standard operational scale. Then a measured volume of inert electrolyte solution (e.g. 20 mL of 0.15M KCl) is acidified (or made basic) and titrated over a pH range from pH 1.8 to pH 12.2 using standardised acid or base titrant. The KCl maintains ionic strength at a more or less constant value during the experiment. At constant ionic strength the activity coefficient of hydrogen ions is constant, and hence their activity is proportional to their concentration. This allows the pH to be expressed on a "concentration scale" (10), which is important because pK_as are calculated from concentrations of substances, not activities. To convert the operational pH readings to their equivalent concenration p[H] values, the experimental titration curve is fitted to a theoretical curve by a Refinement procedure similar to that for pK_a, using equation [8] to obtain the calculated p[H] values:

$$pH = \propto + S\ p[H^+] + j_H\ [H^+] + j_{OH}\ Kw/[H^+] \qquad [8]$$

The terms ∝, S, j_H and j_{OH} are varied iteratively during the refinement, together with the concentration factor of the acid or base used for pH adjustment and the CO_2, until the sum of the squares of the differences between the calculated and measured pH values (divided by a "variance" term whose value increases with the slope of the titration curve) tends to a minimum value. The term ∝ represents the activity coefficient of hydrogen ions at the ionic strength of the experiment, S represents the slope of the electrode (in mV/pH units), while j_H and j_{OH}, represent, respectively, the liquid junction potentials of the electrode at low and high pH. Values for p[H] are calculated from pH by applying the computed values of ∝, S, j_H and j_{OH} in the equation above after refinement.

<u>Lipophilicity Profiles</u>

Lipophilicity profiles are graphs which show how lipophilicity changes with respect to pH. Lipophilicity profiles are calculated from data which may include partition coefficients, log D values, ionization constants and pH values. Figure 3 shows the lipophilicity profile for a monoprotic base. Lipophilicity is expressed as log D, where the Distribution Coefficient D represents the ratio of concentrations of the drug dissolved in each of the two phases at equilibrium. Because the ratio of concentration of ionized and neutral species changes with pH, the log D term is pH-dependent. Note that D = P at pH values where only the unionised species is present.

The term D for a monoprotic base is defined as:

$$D = \frac{[X^0]_{oct} + [XH^+]_{oct}}{[X^0]_{aq} + [XH^+]_{aq}} \qquad [9]$$

By combining equation [9] with [1], [2] and [3] above, equation [10] may be derived, which may be used to plot the lipophilicity profile of a monoprotic base.

$$D = \frac{P^0 + P^1[H^+]K_a}{1 + [H^+]K_a} \qquad [10]$$

The argument above can be extended to cover monoprotic acids, and then to include multiprotic acids and bases. This equation may be extended to a general form (11) (equation [11]) to calculate lipophilicity profiles for a single compound with any number of acidic or basic ionizable groups.

$$D = \frac{P^0 + [H^+]\beta_1 P^1 + [H^+]^2 \beta_2 P^2 + ...}{1 + [H^+]\beta_1 + [H^+]^2 \beta_2 + ...} \qquad [11]$$

In equations [10] and [11], superscripts represent the number of protons associated with each partition coefficient in the equation, and the β terms represent stability constants. Thus, for the first proton added the ionization constant is K_{a1}, for the second K_{a2} and for the third K_{a3}, and the corresponding stability constants are:

$X^0 + H^+ = XH^+$	K_{a1}	$K_{a1} = \beta_1$
$XH^+ + H^+ = XH_2^{++}$	K_{a2}	$K_{a1}K_{a2} = \beta_2$
$XH_2^{++} + H^+ = XH_3^{+++}$	K_{a3}	$K_{a1}K_{a2}K_{a3} = \beta_3$, etc

In order to draw lipophilicity profiles, it is necessary to know values for $[H^+]$ (which may be derived from pH), ionization constants (K_{a1}, K_{a2} etc.), and the partition coefficients of each species (P^0, P^1, etc.). Equation [11] will also correctly depict the lipophilicity profile for ampholytes whose basic pK_a is more than 3 pH units below the acidic pK_a, though not for ampholytes with a significant zwitterionic component.

A partial lipophilicity profile may be drawn without knowing all the information required for the full profile, provided it is assumed that ion-pair partitioning does not occur. For example, a partial profile for a monoprotic substance can be drawn if the values of log P of the neutral species and the pK_a are known, by applying equation [12] or [13]. If the log D at a particular pH and the pK_a are known, a profile can also be drawn after first calculating P^0 or P^1 from equation [12] or [13].

The partial lipophilicity profile for a monoprotic acid is expressed by:

$$D = \frac{[H^+]K_a P^1}{1+[H^+]K_a} \qquad \textbf{[12]}$$

The partial lipophilicity profile for a monoprotic base is expressed by:

$$D = \frac{P^0}{1+[H^+]K_a} \qquad \textbf{[13]}$$

Software

Software which runs on a PC and is suitable for the calculations described above has been developed for sale. A well-known program is called pKaLOGP, and is supplied by Sirius Analytical Instruments Ltd, RH18 5DW, UK.

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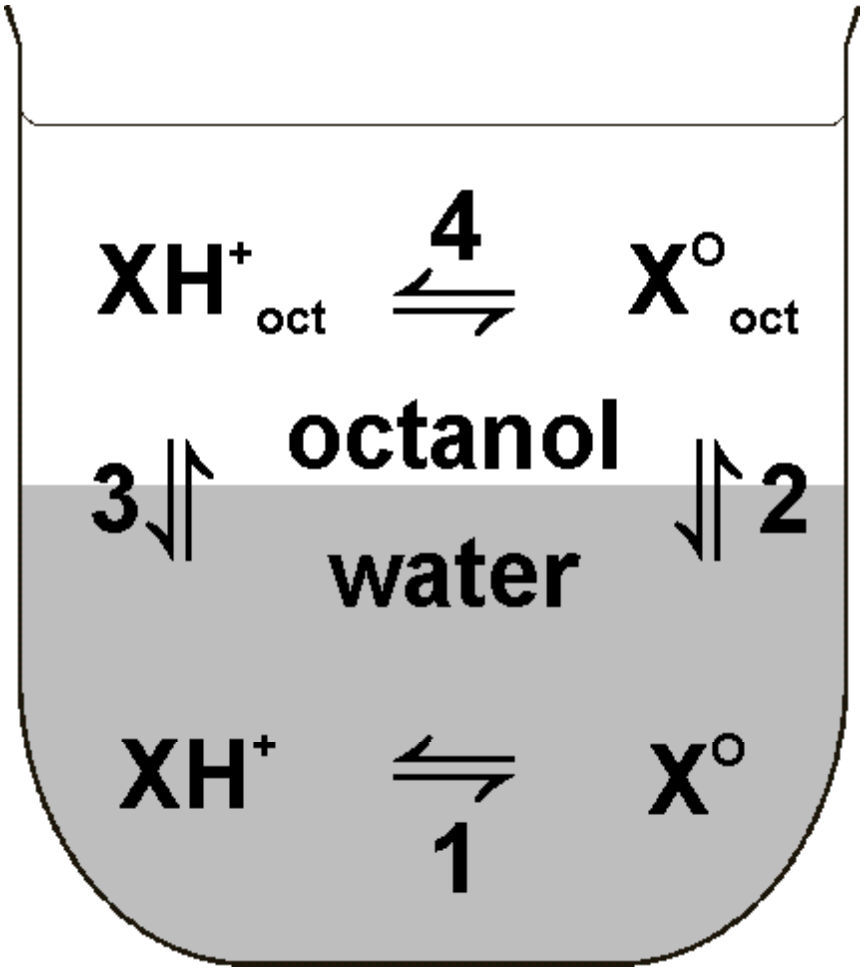
Figure 1:

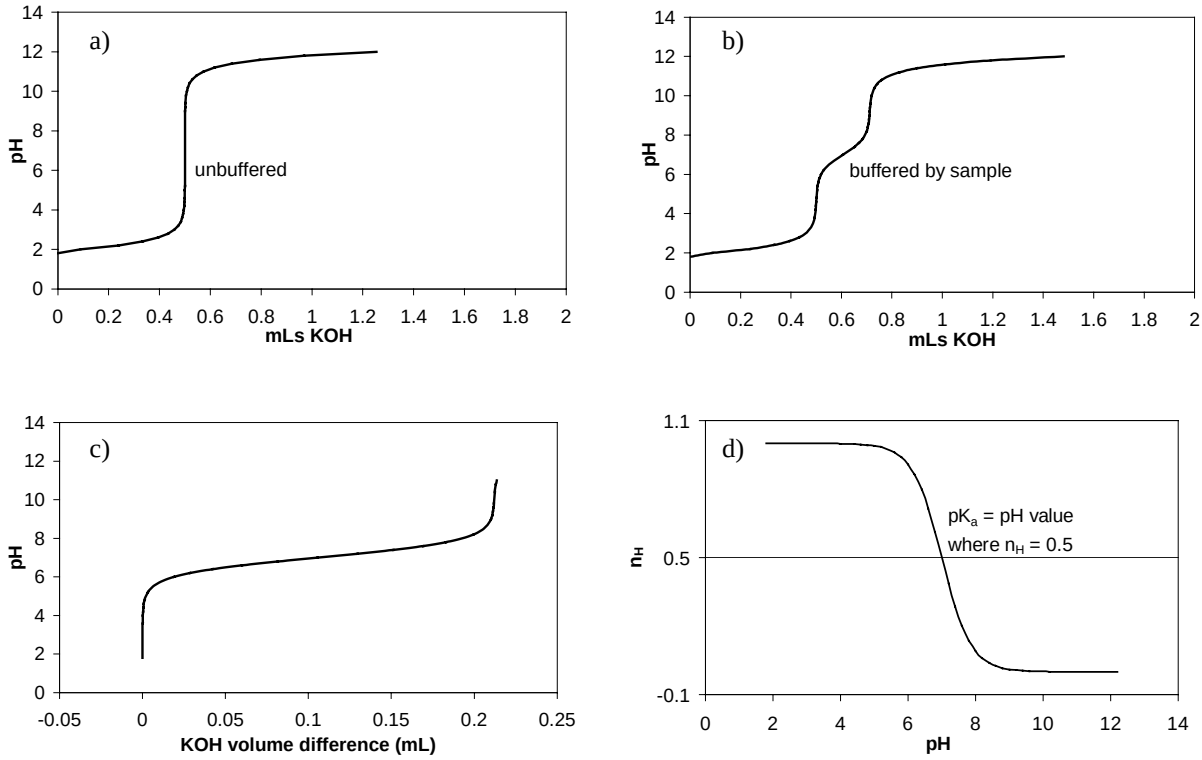
"Four equation" partition model for ionization and partitioning of a weak base (X).



Note species XH^+ is expected to partition as an ion-pair with an anion from the water layer.

Figure 2:

The Bjerrum difference curve



a): "Blank" titration of a strong acid with a base (no sample present)
b): Titration of strong acid with a base, with sample of weak acid or base also present
c): Curve a is subtracted from curve b to provide a volume difference, plotted as a function of pH
d): Approximation to Bjerrum curve produced by rotating curve c through 90° and rescaling Y-axis to represent average number of bound protons per molecule of sample. The pK_a is equal to the pH where $\bar{n}_H = 0.5$ (for monoprotic molecule), 1.5, 2.5 etc.

Figure 3:

Lipophilicity profile for a monoprotic base