Considerations on the Potentiometric Log P Determination

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INTRODUCTION

The titration of an ionizable compound in a biphasic system like octanol/water or liposomes/water at various volume ratios r permits the calculation of the true partition coefficients (P) (1) of all its ionization species (2,3). With the introduction of high precision potentiometric titrators equipped with sophisticated calculation programs (PCA 101, GLpKa and GLPhD from Sirius Analytical Instruments Ltd., United Kingdom), potentiometric log P determination is gaining interest in the applied as well as fundamental sciences. Interpreting the results obtained by calculation with the program pKaLOGP which comes with the Sirius titrators is not always straightforward. In this note we present a complementary approach to the existing calculation program to calculate log P values from the titrated aqueous pK_a and the apparent pK_a values (pK_a^{app}) in biphasic systems. Log P values are fitted from the r-dependent differences between the pK_a^{app} and the aqueous pK_a values, which were determined using the Sirius titrator PCA 101 and calculated by the program pKaLOGP.

The presented data analysis method can be used for all kinds of protonable and deprotonable molecules like monoprotic and multiprotic acids and basis, mixed functional, and zwitterion forming compounds. It permits an accurate evaluation of the results, which is not always evident when using the complex calculation program delivered with the instruments.

THEORY

The partitioning behavior of an ionizable compound in a biphasic system is given by the dissociation equilibria in the two phases and the partition equilibria of all ionization species. The dissociation equilibria in the aqueous phase are expressed by the pK_a constants of the molecule; the partition equilibria are described by the log P values of the single ionization species. The overall dissociation equilibria regarding the whole system are described by the apparent pKa (pK_a^{app}); they are r-dependent. The overall partitioning of the whole compound, despite of its ionization degree, is given by the apparent partition coefficient between the lipophilic and the aqueous phase (D, distribution coefficient), which is pH-dependent.

As described by (3) the following relations between the equilibria can be derived from the mass balance.

$$10^{(pK_a^{app}{}_n - pK_{a_n})} = \frac{P_{XH_n} \cdot r + 1}{P_{XH_{n-1}} \cdot r + 1} \quad (1)$$

P_{XH_n} and $P_{XH_{n-1}}$ are the true partition coefficients, i.e. the concentration ratios between the lipophilic and the aqueous phase at equilibrium, of the ionization species XH_n and XH_{n-1}, respectively. X stands for the fully deprotonated compound. pK_{a_n} and $pK_{a_n}^{app}$ are the aqueous pK_a for the deprotonation of XH_n and the correspondent apparent pK_a in the biphasic system, respectively. For multiprotic compounds the series of equations is therefore

$$10^{(pK_a^{app}{}_n - pK_{a_n})} = \frac{P_{XH_n} \cdot r + 1}{P_{XH_{n-1}} \cdot r + 1} \quad (1)$$

$$10^{(pK_a^{app}{}_{n-1} - pK_{a_{n-1}})} = \frac{P_{XH_{n-1}} \cdot r + 1}{P_{XH_{n-2}} \cdot r + 1} \quad (2)$$

$$10^{(pK_a^{app}{}_{n-2} - pK_{a_{n-2}})} = \frac{P_{XH_{n-2}} \cdot r + 1}{P_{XH_{n-3}} \cdot r + 1} \quad (3)$$

etc.

The pK_a^{app} and pK_a values can be determined by titration of the compound in the biphasic system and in the aqueous phase. Based on the above functions the P values are determinable from titrations at several different volume ratios r by curve fitting, as was proposed by (3). But especially for molecules, where the experimental derived $10^{(pK_a^{app} - pK_a)}$ values are spread over several magnitudes, log P values cannot be accurately fit without weighting the $10^{(pK_a^{app} - pK_a)}$ values at the lower end of the scale. This is often the case for multiprotic compounds since ($pK_a^{app} - pK_a$) can be positive for one protonable group and negative for another one, resulting in a wide range of $10^{(pK_a^{app} - pK_a)}$ values. Therefore we propose to use the logarithmic functions for curve fitting

$$pK_{a_n}^{app} - pK_{a_n} = \log(P_{XH_n} \cdot r + 1) - \log(P_{XH_{n-1}} \cdot r + 1) \quad (4)$$

The logarithmic function allows to fit the curve without weighting. Fig. 1 shows the plotted functions for a molecule, which has a higher P_{XH_n} than $P_{XH_{n-1}}$ (A), and (B) with a higher $P_{XH_{n-1}}$ than P_{XH_n}. In the octanol/water partition system acids mostly behave like (A), bases like (B), since the neutral compound is normally more lipophilic than the ionized one. In the case of a diprotic molecule containing an acidic and a basic group, the graph looks similar, but only three different P values exist, i.e. P_{XH_2}, P_{XH} and P_X. P_{XH} appears in both functions (see eq. 1 and 2).

From Fig. 1, it is obvious that titrations should include phase ratios r, which equal the inverse P values in order to provide useful pK_a^{app} values to fit accurate P values. If only r around $r = P^{-1}$ at the lower r scale is studied, the higher P value only can be fitted, which for monoprotic molecules is mostly the one of the neutral form. This is found for very hydrophilic solutes. Very lipophilic molecules, in contrast, do not allow titrations at sufficiently low r values to get data around the lower $r = P^{-1}$. But since pK_a^{app} at indefinitely low r equals

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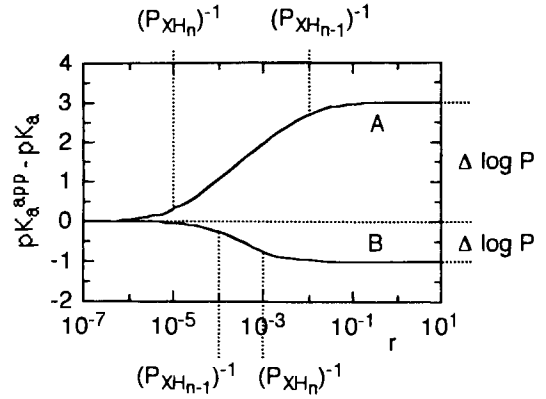

Fig. 1. $pK_a^{app} - pK_a$ as a function of the volume ratio r of the lipophilic phase to the water phase in a biphasic system. (A) represents a molecule with a more lipophilic ionization species XH than X, which is typical of acids in the octanol/water system, (B) is typical of a base with a more lipophilic ionization species X than XH. The P values are A, $P_{XH} = 10^5$, $P_X = 10^2$; B, $P_{XH} = 10^3$, $P_X = 10^4$. ($pK_a^{app} - pK_a$) changes most between the two r values equal to P^{-1}. At high r (r higher than upper $r = P^{-1}$) $|pK_a^{app} - pK_a|$ equals $|\log P_{XH} - \log Px|$ (Δ log P). At low r (r smaller than the lower $r = P^{-1}$) pK_a^{app} equals pK_a.

pK_a both log P can be fitted from the data between the two $r = P^{-1}$ and at r values higher than the upper $r = P^{-1}$. If all determined pK_a^{app} values are equal, but different from pK_a, only the difference between the two log P values of two different ionization species can be determined. The higher P would be superior to the inverse value of the lowest experimental r value.

MATERIALS AND METHODS

Chemicals

Amiodarone was synthesised by Sanofi, metoprolol and atenolol provided by Sigma, ramipril was kindly given by Hoechst. 1-Octanol, #820931, was purchased from Merck. For titrations methanol of HPLC grade was used. All other chemicals were of analytical grade.

Titrations

To determine aqueous pK_a and pK_a^{app} values, titrations of 4 to 100 μmol compound in 20 ml 0.15 M KCl, 20 ml methanol/0.15 M KCl mixtures and in 20 to 90 ml of the biphasic system n-octanol/0.15 M KCl were performed on the PCA 101 titrator (Sirius Analytical Instruments Ltd., United Kingdom) at 25°C for amiodarone, metoprolol and ramipril and at 37°C for atenolol. The pK_a values of metoprolol, atenolol and ramipril were determined in 0.15 M KCl. For the extrapolation of the aqueous pK_a of amiodarone we used the Yasuda-Shedlovsky plot (4). Apparent pK_a values (+ log $[H_2O]$) in the methanol/0.15 M KCl mixtures at ratios between 47 and 71%(w/w) methanol were plotted against the inverse dielectric constant of the mixtures and the pK_a of the molecule in 100% aqueous solution was extrapolated by linear regression. In Log P experiments r (n-octanol/0.15 M KCl) was between 0.0025 and 18. The aqueous volume was ≥ 5 ml. n-Octanol was saturated with 0.15 M KCl prior to dose. r was not corrected for the partial solubility of n-octanol in water. The solubility of n-octanol in water is about 0.04% (v/v) at 37°C (5). This corresponds to 16% of the n-octanol volume at lowest r. pK_a and pK_a^{app} values were fitted from the titration curves using the pKaLOGP program from Sirius Analytical Instruments Ltd..

Data Analysis

The Sigma Plot program from Jandel Scientific was used for the curve fitting. For multiprotic molecules the curves were fitted to the multiple functions in one step (see Theory). In order to directly fit the log P values and their standard deviations s the function (eq. 4) was transformed to

$$pK_{a_n}^{app} - pK_{a_n} = \log (10^{(\log P_{XH_n})} \cdot r + 1) - \log (10^{(\log P_{XH_{n-1}})} \cdot r + 1) \quad (5)$$

RESULTS AND DISCUSSION

To show the possibilities of the presented data analysis method as described in the Theory section, and of the potentiometric log P determination, we chose amiodarone, atenolol, metoprolol and ramipril. pK_a and pK_a^{app} were determined as described under Materials and Methods and all log P values were fitted as described in the Theory section and under Data Analysis. We studied amiodarone, the most lipophilic reference compound for potentiometric log P determination used by Sirius Analytical Instruments Ltd.. Fig. 2A shows that only the very high r end of the sigma curve (Fig. 1) could be examined because of the miscibility of the two phases at lower r. As described in the Theory section, these data allow one to fit the two log P values of the protonated and neutral base (Table I). The high standard deviations of the log P values for amiodarone are due to the small difference, i.e. 0.26, between the experimentally determined pK_a^{app}-pK_a values at the lowest accesible r and the highest r. This is also reflected in the relatively strong scattering of the values in Fig. 2A. Atenolol is an example for hydrophilic compounds, of which only log P of the neutral species can accurately be determined. The plateau of the curve at higher r values in Fig. 2B is not defined by the experimental data. Therefore log P_{XH^+} shows a very high standard deviation (Table I). The log P value of all ionization species can be best fit if the whole sigma curve $pK_a^{app} - pK_a$ versus r is described by experimental data. This is almost the case for metoprolol (Fig. 2C). Log P is 1.95 for the neutral and −0.60 for the protonated molecule. The standard deviations of the log P values are relatively small. Ramipril is a diprotic molecule with an acidic (pK_a 3.3) and a basic group (pK_a 5.7). Experimental data are shown in Fig. 2D. The macroscopic ionization species $XH^{+/-}$, which corresponds to the zwitterion, has the highest log P value, i.e. 1.07. All ionization species are relatively hydrophilic, so the higher end of the r scale is not very well defined. This is reflected in the relatively high standard deviations of log P of the two ionization species XH_2^+ and X^-. Log $P_{XH^{+/-}}$, i.e. log P of the zwitterion, is best defined, since it is the highest value and therefore derived from a well defined r range (see Fig. 2D, r around $(P_{XH^{+/-}})^{-1}$). From the high log P value of the zwitterion, it can be assumed that the two charges interact and neutralize each other, which increases the lipophilicity of the molecule. The solid lines in Fig. 2 represent the fitted functions (eq. 5) from which the log P values are derived.

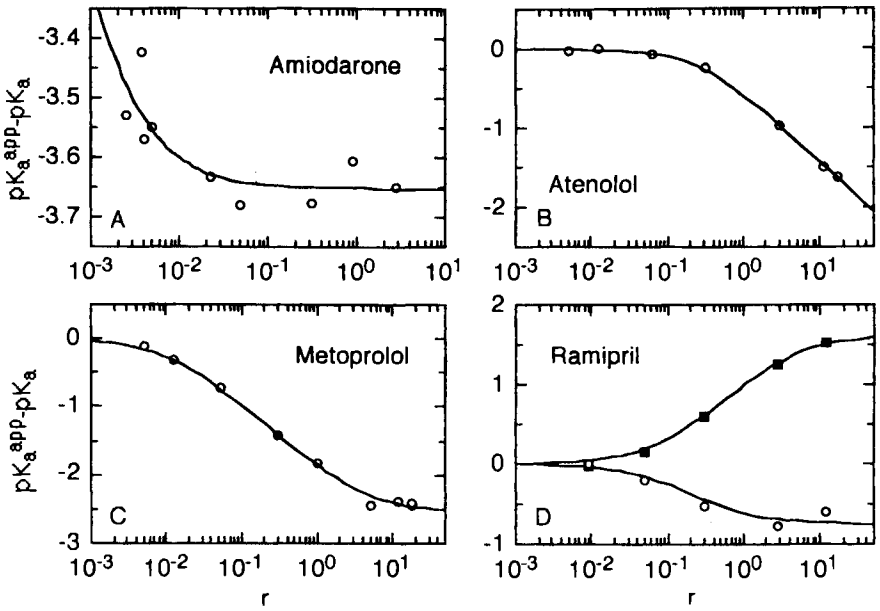

Fig. 2. Differences between the apparent pK_a (pK_a^{app}) values in the n-octanol/0.15 M KCl system and the aqueous pK_a as plotted against the volume ratios r (n-octanol/0.15 M KCl). pK_a and pK_a^{app} of amiodarone, atenolol, metoprolol and ramipril were determined by titrations using the PCA 101 and the pKaLOGP program from Sirius. Fig. 2D, ramipril, (○) $pK_{a_n}^{app}$, i.e. acidic function; (■) $pK_{a_{n-1}}^{app}$, i.e. basic function. Solid lines are the fitted functions as described under Theory. pK_a and fitted log P values are listed in Table I.

Table I. pK_a and Fitted Log P Values of Amiodarone, Metoprolol, Atenolol and Ramipril in n-octanol/0.15 M KCl

Compound	pK_a(25°C)	Ionization state	Fitted log P±s acc. to eq. 5	Fitted log P±s using pKaLOGP	n for log P
Amiodarone	8.7 ± 0.2 [a] (B)	XH^+	2.91 ± 0.14	2.99 ± 0.054	9
		X	6.56 ± 0.13	6.63 ± 0.051	
Metoprolol	9.55 ± 0.00[b] (B)	XH^+	−0.60 ± 0.07	−0.62 ± 0.035	8
		X	1.95 ± 0.04	1.97 ± 0.018	
Atenolol	9.56 ± 0.00[b] (B)	XH^+	−2.20 ± 0.39	−2.18 ± 0.107	7
	9.26 ± 0.00[b] (37°C)	X	0.46 ± 0.03	0.461 ± 0.006	
Ramipril	3.30 ± 0.01[b] (A)	XH_2^+	0.32 ± 0.11	0.30 ± 0.023	5
	5.75 ± 0.00[b] (B)	$XH^{+/-}$	1.07 ± 0.08	1.03 ± 0.016	
		X^-	0.55 ± 0.15	−0.61 ± 0.028	

[a] As extrapolated from titrations in methanol/0.15 M KCl mixtures (Yasuda-Shedlovsky plot) using the PCA 101 titrator and the pKaLOGP program from Sirius (see Materials and Methods). n = 11.
[b] As titrated in 0.15 M KCl using the PCA 101 and calculated with the program pKaLOGP. n = 3. (A) Acidic function, (B) basic function.

Titrated pK_a and fitted log P values of the molecules shown in Fig. 2 are listed in Table I. The results obtained with the described method are comparable with those obtained with the same data using the program pKaLOGP from Sirius Analytical Instruments Ltd. (see Material and Methods). However, the standard deviations resulting from the presented fit method are higher than those obtained from the pKaLOGP program.

CONCLUSIONS

The logarithmic form of the fit function described in (3) allows one to calculate the log P values and to evaluate the titration data. At r around P^{-1} and between the two values P^{-1}, the highest changes in $pK_a^{app} - pK_a$ occur with the variation of r. The experimental r values should be chosen depending on the expected P values. For the determination of all log P values at least one r that is higher than the upper $r = P^{-1}$ and one r between the two $r = P^{-1}$ should be measured beside the aqueous pK_a. With these three values per ionizable group of the molecule all log P values can be fitted.

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