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Interlaboratory study of log *P* determination by shake-flask and potentiometric methods

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Abstract

The pK_a and log *P* values of 23 structurally diverse compounds, including well known drugs and two pharmacons under development, were determined by potentiometry. Also, the log *P* data were measured by the shake-flask method. Many of the samples were investigated at both of the participating laboratories in order to evaluate the reproducibility of the pH-metric log *P* technique. The interlaboratory evaluation of pK_a and log *P* data obtained by potentiometry showed excellent agreement (average $\Delta pK_a = \pm 0.02$ and $\Delta \log P = \pm 0.07$). The log *P* values obtained by the two different methods, ranging from -1.84 to 5.80 (nearly eight orders of magnitude), were in very good concordance, as shown by the linear regression analysis: $\log P_{\text{pH-metric}} = 0.9794 \log P_{\text{shake-flask}} - 0.0397$ ($r = 0.9987$, $s = \pm 0.091$, $F = 8153$). The advantages of potentiometric log *P* determination are discussed.

Keywords: Log *P*; pK_a; pH-metric log *P* determination; Shake-flask method; Validation; Dual-phase titration

1. Introduction

The logarithm of the octanol–water partition coefficient (log *P*), as the best measure of lipophilicity of drugs, has been an extensively used parameter in the complex process of drug development, from rational drug design (e.g. QSAR analysis), through drug-formulation and drug-delivery studies, up to regulatory registration [1]. Reliable and accurate log *P* values are required with increasing frequency for pharmaceutical and environmental protection studies. Although the generally accepted standard method for log *P* measurement is still the shake-flask technique introduced by Leo et al. [2], several other direct log *P* measurement approaches have been developed to overcome the well known limitations of the traditional method. These include the filter probe [3], filter chamber [4,5] and stir-flask [5,6] techniques, centrifugal partition chromatography [7,8], dual-phase potentiometry [9,10] and liquid–liquid segmented flow extraction [11]. (Note that conventional chromatographic techniques are not mentioned, because HPLC, TLC and GC do not yield a partition coefficient, but rather a hydrophobicity parameter; however, numerous studies

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[1] Contribution No. 9 in the pH-metric log *P* series from Sirius.

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have shown that the retention index correlates well with the partition coefficient.) Of these alternative direct methods, the pH-metric log P determination technique is growing in usage, being a fast, simple and easily automated approach which requires small amounts of sample.

Recently, in a series of papers, Avdeef and co-workers summarized their activity in the further development of potentiometric log P measurement. They have described (a) methods for estimating log P values from Bjerrum 'difference' plots [12], (b) refinement of log P values of multiprotic substances by a general non-linear least-squares approach [13] and (c) some special applications of the pH-metric technique, such as micro-log P determination of niflumic acid [14] and a study of ion-pair partitioning of prostaglandins [15].

Although in the evaluation of a new method comparison with formerly used methods is very important, only a few validation studies have been published so far, comparing the log P values determined by potentiometry and by the traditional shake-flask method [16,17]. In these investigations, the log P data obtained by the pH-metric technique under well-defined experimental conditions (e.g. standard temperature, ionic strength, inert gas atmosphere) were compared with literature values from different sources, where the experimental conditions were in some cases incompletely specified or were considerably varied. Dearden and Bresnen, in an excellent review [18], pointed out that the accuracy of partition coefficient determination by the shake-flask method can be affected by many factors (e.g. temperature, mutual phase saturation, ionic strength, solute and solvent purity and non-equilibrium conditions). Thus, the validation studies using literature shake-flask log P data are useful for overall comparison; however, they may not reveal occasional tendential differences between methods. To do so, one needs to perform investigations in which log P values are measured by a given method under similar experimental conditions and with high precision.

The present study was aimed at carrying out such a validation of potentiometric log P measurement. The log P values of 23 selected compounds of different structure were determined by automated dual-phase potentiometric titrations and by the shake-flask method optimized according to the recommendations of Dearden and Bresnen [18]. A further purpose of this study was to assess the comparability of the results obtained at two different laboratories, using the same type of apparatus, the recently developed pK_a and log P analyser (Sirius PCA101), but with slightly different experimental set-ups. The compounds tested included 21 known drugs (Table 1) and two molecules under development (see structures in Fig. 1).

2. Experimental

2.1. Materials

Samples of pharmacopoeial substances (all of Pharm. Hung. VII grade) were purchased from Reanal (Budapest, Hungary), and some other compounds were generously supplied by Chinoin Pharmaceutical Works (Budapest, Hungary) (KHL-8430, pF-deprenyl, flumequine, ofloxacin), Alkaloida Pharmaceutical Works (Tiszavasvári, Hungary) (A-2545, buspirone), Gedeon Richter Chemical Works (Budapest, Hungary) (niflumic acid) and EGIS Pharmaceutical Works (Budapest, Hungary) (atenolol), and were used without further purification. n-Octanol was of HPLC grade (Aldrich) and methanol was of spectroscopic grade (Fluka). The preparation and standardization of 0.5 M HCl (Fisons) and 0.5 M NaOH and KOH (Volucon, Rhône-Poulenc) have been described elsewhere [12,13,19]. All other reagents were of analytical grade.

2.2. Apparatus

The details of the instrument used (PCA101; Sirius, Forest Row, UK) for potentiometric pK_a and log P determination were described earlier [12,13].

2.3. Potentiometric determination of protonation constants

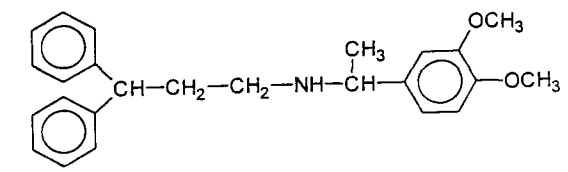
Typically, 10 ml of 0.5–10 mM solutions of the samples were pre-acidified to pH 1.8–2.0 with 0.5

Table 1
pK_a values of model compounds

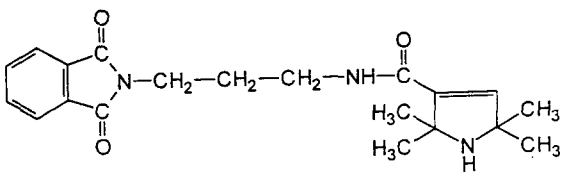
Compound	$pK_a \pm SD$ (n)			Literature pK_a
	Semmelweis lab.	Sirius lab.	Δ	
Bases				
KHL-8430	10.60 ± 0.05[a] (5)	–	–	–
Atenolol	9.58 ± 0.01 (3)	9.58 ± 0.01[c] (3)	0	9.60[e]
Ephedrine	9.64 ± 0.03 (7)	9.65 ± 0.01[c] (3)	0.1	9.63[f]
Chloropromazine	–	9.24 ± 0.02[a] (10)	–	9.3[f]
Procaine	9.04 ± 0.01 (3)	–	–	8.97[e]
A-2545	8.63 ± 0.02 (5)	–	–	–
Codeine	8.25 ± 0.01 (3)	8.22 ± .0.01[c] (3)	0.03	8.21[f]
Buspirone	7.60 ± 0.01 (3)	–	–	–
pF-deprenyl	7.38 ± 0.01 (5)	7.42 ± 0.01[c] (3)	0.04	–
Pilocarpine	7.08 ± 0.02 (3)	–	–	7.05[f]
Papaverine	6.38 ± 0.03 (3)	6.39 ± 0.01[c]	0.01	6.4[f]
Aminophenazone	5.06 ± 0.01 (3)	–	–	5.0[f]
Acids				
Salicyclic acid	2.83 ± 0.03 (3)	2.88 ± 0.01[c] (3)	0.05	2.97[f]
ASA	3.47 ± 0.01 (3)	3.50 ± 0.01[c] (3)	0.03	3.50[f]
Benzoic acid	3.98 ± 0.01 (3)	3.99 ± 0.01[d] (10)	0.01	4.01[e]
Ascorbic acid	4.05 ± 0.01 (3)	–	–	4.17[f]
	11.62 ± 0.04 (3)			11.57[f]
Flumequine	6.38 ± 0.04[a] (8)	6.27 ± 0.01[b] (3)	0.11	–
Phenobarbital	7.43 ± 0.05 (6)	7.49 ± 0.02[d] (3)	0.06	7.41[f]
Paracetamol	9.63 ± 0.01 (5)	–	–	9.5[f]
Ampholytes				
Morphine	9.34 ± 0.01 (5)	9.26 ± 0.01[j] (5)	0.08	9.51[e]
	8.81 ± 0.01 (5)	8.18 ± 0.01[j] (5)	0	8.31[e]
Pyridoxine	8.89 ± 0.01 (5)	8.87 ± 0.01[c] (5)	0.02	9.04[h]
	4.87 ± 0.01 (5)	4.84 ± 0.01[c] (5)	0.03	4.84[h]
Niflumic acid	–	4.44 ± 0.03[a,b] (5)	–	5.14[g]
		2.26 ± 0.08[a,b] (5)		2.11[g]
Ofloxacin	8.31 ± 0.01 (3)	8.31 ± 0.01[b] (3)	0	8.22[i]
	6.08 ± 0.01 (3)	6.09 ± 0.01[b] (3)	0.01	6.05[i]

n = Number of parallel measurements.
[a] Extrapolated from methanol–water p_sK_a values (see Table 2).
[b] $I = 0.15$ M NaCl.
[c] $I = 0.15$ M KCl.
[d] $I = 0.10$ M KNO_3.
[e] Ref. [22].
[f] Ref. [23].
[g] Ref. [24].
[h] Ref. [25].
[i] Ref. [26].
[j] Sulphate salt, in 0.15 M KCl.

M HCl, and were then titrated alkalimetrically to some appropriate high pH (maximum 12.0). The titrations were carried out at 25.0 ± 0.1°C, at constant ionic strength and under an inert gas atmosphere. The initial estimates of pK_a values were obtained from Bjerrum difference plots ($\bar{n}_H$



KHL-8430



A-2545

Fig. 1. Structures of model compounds KHL-8430 and A-2545.

vs. pH) and then were refined by a weighted non-linear least-squares procedure [12,13]. For each molecule a minimum of three and occasionally five or more separate titrations were performed, and the average pK_a values along with the standard deviations were calculated (Table 1).

In the case of molecules sparingly soluble in water, the ionization constants were determined by a mixed-solvent method. A series of semi-aqueous solutions of the samples containing 3–60% (w/w) methanol were titrated. From these titrations, the p_sK_a values (the apparent ionization constants in methanol–water solvent) were obtained (Table 2), and the Yasuda–Shedlovsky procedure was applied to estimate the aqueous pK_a values [19].

The four-parameter procedure was used for electrode standardization in both aqueous and semi-aqueous solutions [12,19].

2.4. Potentiometric determination of paritition coefficients

Typically, 5–20 ml of 0.5–10 mM solutions of samples were titrated under the same conditions as in pK_a determinations but in the presence of various amounts of the partitioning solvent, water-saturated octanol. The phase ratio applied was varied from 20 ml water–0.1 ml octanol to 5 ml water–15 ml octanol, depending on the expected log P value of the compound. From the octanol-containing titrations the p_0K_a (the apparent ionization constant in the presence of octanol) and then log P values were estimated and refined by a weighted non-linear least-squares procedure, where the aqueous pK_a values (taken from aqueous titrations) were used as unrefined contributions.

For each compound a minimum of three titrations at different phase volume ratios were measured in each laboratory, and the respective average log P values were calculated. The ion-pair

Table 2
Apparent dissociation constants (p_sK_a) in methanol–water mixtures and aqueous pK_a values obtained by Yashuda–Shedlovsky extrapolation

KHL-8430		Chlorpromazine		Flumequine		Niflumic acid		
MeOH (% w/w)	p_sK_a	MeOH (% w/w)	p_sK_a	MeOH (% w/w)	p_sK_a	MeOH (% w/w)	p_sK_{a1}	p_sK_{a2}
				3.2	6.37	29.8	4.40	2.23
29.4	9.07	34.5	8.65	20.0	6.70	34.0	4.38	2.39
33.9	8.80	42.2	8.45	25.3	6.80	39.0	4.33	2.29
38.8	8.34	49.6	8.30	34.5	6.99	43.3	4.31	2.27
				44.1	7.16	53.5	4.31	2.31
p$K_{a(aq)}$ = 10.60 ± 0.05		p$K_{a(aq)}$ = 9.24 ± 0.02		p$K_{a(aq)}$ = 6.38 ± 0.04		pK_{a1aq} = 4.44 ± 0.03 p$K_{a2(aq)}$ = 2.26 ± 0.08		
r^2 = 0.9935		r^2 = 0.9975		r^2 = 0.9682		r^2 = 0.9606	r^2 = 0.6243	
n = 3		n = 3		n = 5		n = 5	n = 5	

The number of parallel measurements for each methanol–water mixture is $n = 3$. The SD of p_sK_a values is less than 0.03.

partitioning of charged species was characterized, also from the titrations of different phase ratios. The relevant relationships between log P, pK_a and p_0K_a for mono- and multiprotic substances, including cases of ion-pair formation, have been described in detail earlier [12].

Some slight differences in experiments between the two laboratories in pH-metric pK_a and log P determinations are the following:

	Titrant	Ionic strength	Inert gas
Semmelweis lab.	0.5 M NaOH	0.10 M (NaCl)	Nitrogen
Sirius lab.	0.5 M KOH	0.15 M (NaCl) 0.15 M (KCl) 0.10 M (KNO_3)	Argon

2.5. Shake-flask determination of partition coefficients

The apparent partition coefficients (log P_{app}) were measured using the shake-flask technique, as described previously [14,20,21]. The two phases were mutually saturated by shaking in a thermostated water bath (Lauda, M20S) at 25.0 ± 0.1°C for 3 h. The phases were allowed to separate on standing and were then filtered (aqueous phase on analytical filter-paper and octanol on a G_4 glass filter under vacuum). The mass balance and the time of equilibration were monitored in the partitioning experiments. Generally, 1 h of intensive shaking at constant temperature was enough to reach the partitioning equilibrium of the solute. The Britton–Robinson buffers (acetic, phosphoric and boric acids, each at 0.04 M, treated with various amounts of 0.2 M NaOH) were used as the aqueous phase for the pH range 2–12 with the exception of pyridoxine and morphine, where Sörensen buffers (potassium dihydrogenphosphate and sodium phosphate dihydrate, each at 0.067 M) were applied in order to avoid complex formation with the borate anion. For pH 0 and 1, 1 and 0.1 M HCl served as the aqueous phase. No additional ionic strength adjuster was added. The pH of the aqueous phase was chosen so that the ionization of the molecule was minimal (generally, pH ≈ pK_a − 2 for acids and pH ≈ pK_a + 2 for bases). In the case of some very lipophilic molecules (e.g. KHL-8430, chlorpromazine, pF-deprenyl) we had to deviate from this principle and the log P_{app} values were measured at several pH values with higher proportions of the compound in the ionized state (but less than 50%). The log P_{app} data of amphoteric compounds were determined over a wide pH range, including the isoelectric point, as a part of another study [21]. After separation of the equilibrated phases (in a centrifuge at 730g for 10 min) the concentration of the solute was determined in the aqueous phase by UV spectrophotometry (Hewlett-Packard 8452A) at λ_{max} above 230 of each compound.

Each log P value is an average of a minimum of six or more replicate measurements (n and SD values are given in Table 3.

3. Results and discussion

Determination of the partition coefficients of ionizable compounds requires the knowledge of the pK_a values. In the pH-metric technique the measurement of the aqueous pK_a is part of the method, while in the shake-flask determination the pK_a value is needed for the calculation of the true partition coefficient of the non-ionized form, using the experimentally obtained apparent partition coefficient.

Thus, first the aqueous pK_a values were measured by potentiometric titrations. The data are summarized in Table 1, where the compounds are classified into three groups: bases, acids ampholytes. The measured pK_a values span a wide range, from 2.18 to 11.62. The average standard deviation is ± 0.01 log unit, indicating the very good reproducibility of the potentiometric titration using this automated approach.

In the case of 13 molecules the determinations were carried out at both the Semmelweis and Sirius laboratories, using the same experimental technique, but with slight differences in background salt concentrations, as noted in Table 1. The pK_a values obtained at the two laboratories show ex-

Table 3
Log P values of model compounds obtained by shake-flask and pH-metric methods

Compound	Log $P_{\text{shake-flask}} \pm$ SD (n)	Log $P_{\text{pH-metric}} \pm$ SD (n)			Literature log P
		Semmelweis lab.	Sirius lab.	Δ	
Bases					
KHL-8430	5.80 ± 0.05 (12)	5.74 ± 0.01 (9)			–
Chlorpromazine	5.13 ± 0.10 (18)	5.34 ± 0.04 (3)	5.40 ± 0.03 (10)	0.06	5.19[a]
pF-deprenyl	3.00 ± 0.02 (12)	3.06 ± 0.01 (3)	3.06 ± 0.01 (5)	0	–
Papaverine	2.90 ± 0.06 (12)	2.91 ± 0.08 (3)	2.95 ± 0.01 (3)	0.04	–
Buspirone	2.63 ± 0.02 (18)	2.78 ± 0.05 (5)			–
Procaine	1.92 ± 0.01 (12)	2.03 ± 0.02 (3)			1.92[a]
A-2545	1.58 ± 0.04 (18)	1.63 ± 0.03 (5)			–
Codeine	1.19 ± 0.01 (12)	1.26 ± 0.01 (3)	1.19 ± 0.01 (3)	0.07	1.14[b]
Ephedrine	1.00 ± 0.02 (12)	1.10 ± 0.01 (3)	1.17 ± 0.01 (3)	0.07	0.93[b]
Aminophenazone	0.81 ± 0.03 (12)	0.85 ± 0.01 (3)			1.00[a]
Pilocarpine	0.16 ± 0.03 (12)	0.20 ± 0.01 (3)			0.12[c]
Atenolol	0.10 ± 0.02 (12)	0.15 ± 0.02 (3)	0.29 ± 0.03 (3)	0.14	0.16[b]
Acids					
Salicylic acid	2.30 ± 0.01 (12)	2.34 ± 0.01 (3)			2.26[b]
Benzoic acid	1.97 ± 0.05 (12)	1.95 ± 0.02 (3)	1.96 ± 0.02 (10)	0.01	1.87[b]
Flumequine	1.60 ± 0.17 (24)	1.72 ± 0.01 (3)	1.72 ± 0.01 (3)	0	–
Phenobarbital	1.41 ± 0.04 (6)	1.53 ± 0.01 (3)	1.53 ± 0.03 (3)	0	1.47[b]
ASA	1.17 ± 0.02 (12)	1.27 ± 0.04 (5)			0.81[b]
Paracetamol	0.31 ± 0.02 (6)	0.20 ± 0.01 (3)			0.46[c]
Ascorbic acid	−1.84 ± 0.04 (6)	−1.85 ± 0.01 (3)			−1.64[a]
Ampholytes					
Morphine	1.22 ± 0.05 (84)	1.25 ± 0.01 (5)	0.93 ± 0.01 (10)	0.32	0.76[b,d]
Pyridoxine	0.33 ± 0.08 (84)	0.43 ± 0.02 (10)	0.40 ± 0.02 (10)	0.03	−0.77[c,d]
Niflumic acid	4.81 ± 0.14 (94)	5.14 ± 0.08 (5)	5.14 ± 0.04 (5)	0	–
Ofloxacin	0.35 ± 0.03 (84)	0.56 ± 0.02 (5)	0.39 ± 0.02 (3)	0.17	−0.39[a,d]

[a] Ref. [28].
[b] Ref. [29].
[c] Ref. [30].
[d] log P_{app} values which are in agreement with our log P_{app} data measured at the isoelectronic point pHs: morphine, 0.90; pyridoxine, −0.73 [21]; ofloxacin, −0.39 [20].

cellent agreement; the differences are within the experimental error or very close to it, and are not significant according to t-test analysis ($t = 0.003$).

The pK_a values of the compounds measured in this work are in acceptable agreement with the literature data (Table 1). Closer agreement cannot be expected since in many cases the exact experimental conditions are unknown, and may be very different in temperature and ionic strength.

Four of the compounds, KHL-8430, chlorpromazine, flumequine and niflumic acid, are very slightly soluble in water and could not be measured directly in aqueous solution by potentiometry. Conventional spectrophotometry was suitable for the pK_a measurement of flumequine only (p$K_a = 6.35 \pm 0.05$, $n = 12$), because KHL-8430 and chlorpromazine have no appreciable pH-dependent UV spectrum. In the case of niflumic acid the pK_{a_1} (ionization constant of COOH group) can be measured spectrophotometrically but the protonation of pyridine nitrogen (pK_{a_2}) does not cause a measurable shift in the UV spectrum. Therefore, for the pK_a determination of the four sparingly soluble compounds the mixed-solvent

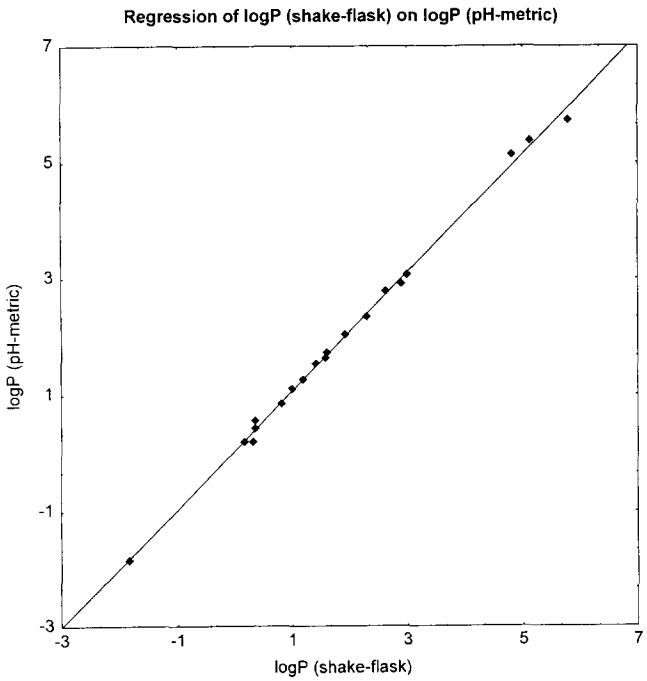


Fig. 2. Linear regression curve of log P values obtained by shake-flask and pH-metric methods.

method was applied. The pK_as were extrapolated from p_sK_a values obtained in different methanol–water mixtures. Table 2 lists the p_sK_a values obtained from 48 separate titrations in mixtures of 3–60% (w/w) methanol, the pK_a values extrapolated to zero methanol content, together with the statistical data from the Yasuda–Shedlovsky linear regression equation $(p_sK_a + \log[H_2O] = a(1/\epsilon) + b$, where ϵ is the dielectric constant of the solvent mixture). For compounds of very low solubility the p_sK_a values are measured in the methanol-rich region (above 30%, w/w); the error in such 'long-distance' extrapolation is expected to be ± 0.1 log unit [19]. The reliability of the co-solvent method is suggested (a) by comparison of the result with that obtained from spectrophotometry ($\Delta = 0.03$) and (b) the interlaboratory agreement of the constants ($\Delta = 0.11$).

The log P data obtained by the two different methods are summarized in Table 3. The data shown are true partition coefficient values, which were calculated in the case of shake-flask technique from the experimental log P_{app} values using the well known relationships for acids and bases: $\log P = \log P_{app} + \log(1 + 10^{pH - pK_a})$ and $\log P = \log P_{app} + \log(1 + 10^{pK_a - pH})$, respectively. The pH-metric log P determination method provides directly the lipophilicity data of the non-ionized form (given in Table 3) and that of the charged species (not shown). For the diprotic, amphoteric molecules examined, again the true partition coefficient values are given in Table 3 according to the recently published concept [21] that the lipophilicity of ampholytes must be expressed by the true partition coefficient (log P) or the micro-log P value [14]. This term was defined as the concentration ratio of the non-ionized microspecies [XH^0] in octanol and water. The non-ionized form is the predominantly partitioning species from the four microspecies (anion (X^-), zwitterion ($XH^\pm$), non-ionized (XH^0), cation (XH_2^+)) co-existing in the aqueous phase. The calculation of the micro-log P requires the knowledge of protonation microconstants (e.g. k_1^0, k_2^0, $k_2^\pm$) as shown in the relationship between log P and log P_{app} derived by Takács-Novák et al. [21]:

$$\log P = \log P_{app} + \log\left(1 + \frac{1}{k_1^0[H^+]} + \frac{k_2^0}{k_2^\pm} + k_2^0[H^+]\right) \quad (1)$$

The experimentally measured log P_{app} values [21] and the protonation microconstants [27] have been published elsewhere; only the calculated true partition data are collected here for comparison with potentiometric data. The macro-log P values of ampholytes determined by potentiometry were converted [14] into micro-log P values of non-ionized micro-species (XH^0):

$$\log p_{XH^0} = \log P_{XH} + \log K_1 - \log k_1^0 \quad (2)$$

Consequently, the data in Table 3 for ampholytes in both of the methods are the true or micro-log P values suitable for the expression and comparison of the lipophilicity of these molecules.

The experimental error of the log P data in Table 3 is low. For the shake-flask method the standard deviations are within the generally accepted ± 0.05 log unit, with the exception of

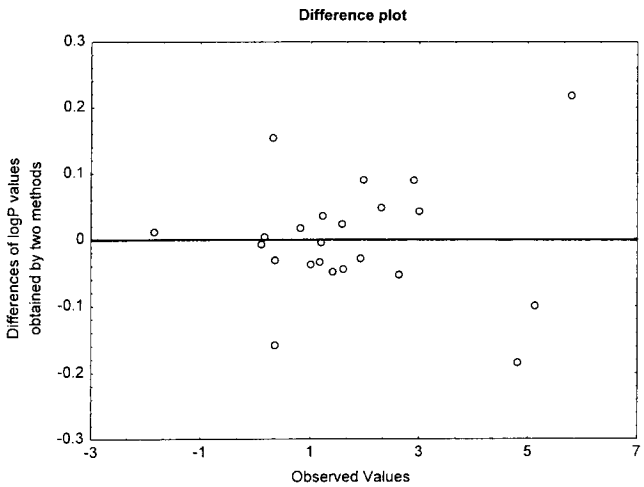


Fig. 3. Differences between log P values obtained by the two methods.

very lipophilic chlorpromazine, niflumic acid and flumequine, the last being a molecule of low solubility in both octanol and water. For these samples the shake-flask experiments were carried out in saturated solutions. The average standard deviation of the pH-metric log P data is ± 0.02, which shows better reproducibility of the method relative to shake-flask technique.

Comparison of the pH-metric log P values measured at the two laboratories, using the t-test shows no significant differences ($t = 0.033$). The average Δ log P is 0.07; the highest deviation was found in micro-log P of morphine (0.32).

Fig. 2. represents the results of linear regression analysis using a standard statistical program [31] for the evaluation of log P data obtained by the shake-flask and pH-metric techniques. Good agreement of the data was found. The parameters of the linear regression equation, slope = 0.9754 and intercept = -0.0397, are close to the ideal values 1 and 0, indicating that there is no tendential deviation between the data. The correlation coefficient $r = 0.9987$ and standard deviation $s = \pm 0.091$ are better than those published in a previous validation study by Slater et al. [17], possibly owing to the optimized experimental conditions in shake-flask measurements and the improvements in the experimental design of the potentiometric titrations. The difference plot in Fig. 3 clearly shows the higher uncertainty of the data above log $P = 5$. It is possible to obtain log P values as high as 7 and even 8 with the potentiometric technique, but it is not always easy.

4. Conclusions

The results presented here show that pH-metric log P measurement provides reliable and accurate experimental partition coefficients. This method has several advantages over the traditional technique, The most important ones are: (1) time saving (e.g. the determinations of the log P values of the 23 compounds examined by the shake-flask method took 3 months, whereas pH-metric measurements were completed within 2 weeks); (2) applicability when a compound has no appreciable chromophore; (3) suitability for recognition of ion-pair partitioning (e.g. we determined the octanol–water log P of prostaglandins E_1 and E_2 and examined the ion-pair partitioning of these molecules in the presence of N-methyl-D-glucamine [15]); and (4) a good tool for indication of impurity, instability and precipitation (using the Bjerrum difference curve).

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References

[1] P.J. Taylor, in C. Hansch, P.G. Sammes and J.B. Taylor (Eds.), Comprehensive Medicinal Chemistry, Vol IV, Pergamon Press, Oxford, 1990, pp. 241–294.
[2] A. Leo, C, Hansch and D. Elkins, Chem. Rev., 71 (1971) 525–616.
[3] E. Tomlinson, S.S. Davis, G.D. Parr, M. James, N. Farraj, J.R.M. Kinkel, D. Gaisser and H.J. Wynne, in W.J. Dunn, J.H. Block and R.S. Pearlman (Eds.), Partition Coefficient Determination and Estimation, Pergamon Press, Oxford, 1986, pp. 83–99.
[4] E. Tomlinson, J. Pharm. Sci., 71 (1982) 602–604.

[5] A. Hersey, A.P. Hill, R.M. Hyde and D.J. Livingstone, Quant. Struct.-Act. Relat., 8 (1989) 288–296.
[6] D. Brooke, I. Nielsen, J. De Bruijn and J. Hermens, Chemosphere, 21 (1990) 119–133.
[7] H. Terada, Y. Kosuge, N. Nakaya, W. Murayama, Y. Nunogaki and K. Nunogaki, Chem. Pharm. Bull., 35 (1987) 5010–5014.
[8] N. El Tayar, R.-S. Tsai, P. Vallat, C. Altomare and B. Testa, J. Chromatogr., 556 (1991) 181–194.
[9] D. Dyrssen, Sven. Kem. Tidskr., 64 (1952) 213–224.
[10] P. Seiler, Eur. J. Med. Chem. Chim. Ther., 9 (1974) 665–666.
[11] L.-R. Danielsson and Z. Yu-Hui, J. Pharm. Biomed. Anal., 12 (1994) 1475–1481.
[12] A. Avdeef, Quant. Struct.-Act. Relat., 11 (1992) 510–517.
[13] A. Avdeef, J. Pharm. Sci., 82 (1993) 183–190.
[14] K. Takács-Novák, A. Avdeef, K.J. Box, B. Podányi and Gy. Szász, J. Pharm. Biomed. Anal., 12 (1994) 1369–1377.
[15] A. Avdeef, K.J. Box and K. Takács-Novák, J. Pharm. Sci., 84 (1995) 523–529.
[16] A. Avdeef in C. Silipo and A. Vittoria (Eds.), QSAR: Rational Approach to the Design of Bioactive Compounds, Elsevier, Amsterdam, 1991, pp. 119–222.
[17] B. Slater, A. McCormack, A. Avdeef and J.E.A. Comer, J. Pharm. Sci., 83 (1994) 1280–1283.
[18] J.C. Dearden and G.M. Bresnen, Quant. Struct.-Act. Relat., 7 (1988) 133–144.
[19] A. Avdeef, J.E.A. Comer and S.J. Thomson, Anal. Chem., 65 (1993) 42–49.
[20] K. Takács-Novák, M. Józan, I. Hermecz and Gy. Szász, Int. J. Pharm., 79 (1992) 89–96.
[21] K. Takács-Novák, M. Józan and Gy. Szász, Int. J. Pharm., 113 (1995) 47–55.
[22] C. Hansch, P.G. Sammes and J.B. Taylor (Eds.), Comprehensive Medicinal Chemistry, Vol. VI, Pergamon Press, Oxford, 1990.
[23] D.W. Newton and R.B. Kluza, in W.O. Foye (Ed.), Principles of Medicinal Chemistry, Lea and Febiger, Philadelphia, 1981, pp. 899–910.
[24] A.G. Asuero, Int. J. Pharm., 52 (1989) 129–137.
[25] J.M. Sanchez-Ruiz, J. Llor and M. Cortijo, J. Chem. Soc., Perkin. Trans. 2, (1984) 2047–2051.
[26] D.L. Ross and C.M. Riley, J. Pharm. Biomed. Anal., 12 (1994) 1325–1331.
[27] K. Takács-Novák, M. Tőkés-Kövesdi, Gy. Szász and B. Noszál, Magy. Kém. Foly., 100 (1994) 538–545.
[28] C. Hansch, A. Leo and D. Hoekman, Exploring QSAR. Hydrophobic, Electronic and Steric Constants (ACS Professional Reference Book), American Chemical Society, Washington, DC, 1995.
[29] C. Hansch and A. Leo, MEDCHEM Project, Issue No. 26, Pomona College, Claremont, CA, 1985.
[30] J. Sangster, Log KOW Databank, Sangster Research Laboratories, Montreal, 1994.
[31] STATISTICA for Windows, Release 4.5, StatSoft, Tulsa, OK, 1993.