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RELATIONSHIPS BETWEEN HYDROPHOBIC (LIPOPHILIC) PROPERTIES OF BASES AND THEIR RETENTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY USING AQUEOUS METHANOL MOBILE PHASES

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SUMMARY

Relationships between reversed-phase liquid chromatographic capacity factors, and octan-1-ol–buffer distribution coefficients, and aqueous solubilities of 30 "strongly" basic drugs (aqueous $pK_a > 7.5$) have been examined. Two different solute reference states have been used, namely that of the solute under mobile phase conditions and that of the unionized solute. Employing both models, and semiempirical corrections for solute ionization, good correlations are found between logarithmic capacity factors obtained using aqueous methanol mobile phases and octan-1-ol–buffer distribution coefficients, while aqueous solubilities can be adequately described by multiple linear combinations of logarithmic capacity factors and logarithms of solute melting points (although some significant outliers were identified). It is suggested that the relationships obtained using a semiempirical ion correction are applicable for estimation of the hydrophobic (lipophilic) properties of the bases.

INTRODUCTION

The evaluation of retention data of "strong' organic bases (with pK_a in water $>$ *ca.* 7) applicable as solvophobic–lipophilic solute parameters can be considered as a Cinderella of the non-analytical applications of alkyl-bonded reversed-phase liquid chromatography (RPLC). This is due to problems in defining an unambiguous physicochemical reference state for such solutes for which dynamic (RPLC) and static (*e.g.*, octan-1-ol–water distribution, aqueous solubility) parameters are comparable. For ionizable compounds the unionized state is generally preferred as the reference state. Since in alkyl-bonded RPLC the use of mobile phases is restricted to pH values

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below *ca.* 8, it follows that strong bases (*i.e.*, many compounds of pharmaceutical interest) cannot be chromatographed in their unionized form.

Upon correction for the effects of solute ionization on retention, complications may arise due to: (a) effects of organic modifiers on pK_a values of solutes and mobile phase buffer components, and (b) solutes chromatographed as ion-pair complexes with buffer anions. Furthermore, stationary phase silanol groups may exert a (not necessarily unambiguous) influence on retention. The latter two complications have been examined and discussed by Unger and Chiang[1] in a study on the relationships between RPLC retention and octan-1-ol–buffer distribution coefficients for a variety of basic drugs. In that study silanol effects were found to be eliminated upon addition of a sufficient concentration of N,N-dimethylaminooctane (acting as a silanol masker) to the mobile phase. When studying ion-pairing effects by using the same buffers for static and dynamic experiments, the authors observed that the effects of variations in anion concentrations on the results of both methods differed considerably, indicating a difficulty in unambiguously correcting for ion-pair complexation. This complication is enhanced by additional solvent effects occurring when modified aqueous mobile phases are used.

The problem of correcting for partial ionization and ion-pair formation also plays a rôle in determinations of distribution coefficients, K_d, and aqueous solubilities, S_w on a molar basis, X_w on a mole fraction basis, of very hydrophobic ionizable compounds. Measurement of K_d or S_w values at a pH close to the solute pK_a ($|pH - pK_a| <$ *ca.* 2) combined with the proper correction for ionization offers a possibility for obtaining K_d^0 or $S_w^0(X_w^0)$, *i.e.*, the corresponding parameter of the unionized solute (with superscript 0 referring to the unionized state).

In this paper the behaviour of 30 strongly basic drugs in RPLC using aqueous methanol mobile phases has been examined in relation to solute electronic (pK_a) and hydrophobic (X_w), or hydrophobic–lipophilic ($K_{d,oct}$, the octan-1-ol–water distribution coefficient) properties.

EXPERIMENTAL

Materials

All solutes were of the highest available purity and were obtained from various sources. The following compounds were kindly donated by pharmaceutical companies: chlorpheniramine maleate, chlorpromazine hydrochloride, prochlorperazine dimaleate (Smith, Kline & French Research, Welwyn Garden City, U.K.); chlorprothixene (Hoffmann-La Roche, Mijdrecht, The Netherlands); cyclizine hydrochloride (Wellcome Nederland, Weesp, The Netherlands); haloperidol (Janssen Pharmaceutica, Beerse, Belgium); nalorphine hydrochloride (Diosynth, Apeldoorn, The Netherlands); procaine hydrochloride (Hoechst Holland, Amsterdam, The Netherlands) and propanolol hydrochloride (ICI-Farma, Rotterdam, The Netherlands). The stationary phase was Hypersil ODS (5 μm; Shandon Southern, Runcorn, U.K.). Mobile phases were made up volumetrically from combinations of analytical grade methanol (Baker, Deventer, The Netherlands) and (i) a pH 7.00 ammonium phosphate buffer, (ii) a pH 4.00 ammonium phosphate solution or (iii) a pH 4.00 ammonium acetate solution, all containing 80 mmol dm^{-3} NH_4^+ and 0.8 mmol dm^{-3} N,N-dimethylaminododecane (DMAD). N,N-Dimethylaminododecane was obtained from Fluka

(Buchs, Switzerland) and was distilled *in vacuo* before use (b.p. at 3 mmHg = 98.5–100.2°C). In all cases the volume fraction of methanol in the mobile phase before mixing, φ, was 0.50. Potassium dihydrogen phosphate, ammonium chloride and chloroform were all of analytical grade purity (Merck, Amsterdam, The Netherlands.)

Determination of aqueous solubilities

Where necessary, aqueous solubilities of the unionized bases were determined using the turbidity-titration method described by Büchi *et al.*[2] and by Thoma and Albert[3]. The equipment used consisted of a Radiometer Type 26 pH meter (Radiometer, Copenhagen, Denmark), a Dosimat automated titrator with a 10-ml burette and either an EA 121 or an EA 125 combination electrode (all from Metrohm, Herisau, Switzerland).

Titrations were performed under a nitrogen atmosphere in a jacketed vessel kept at 20.0 ± 0.5°C using a Thermomix 1441 circulating water-bath (Salm & Kipp, Breukelen, The Netherlands). In cases were the solutes were only available as free bases, these were converted into their cationic forms using an appropriate amount of an aqueous HCl solution. The pH meter was calibrated using the following buffers: (i) 0.050 mol dm^{-3} potassium hydrogenphthalate, pH 4.00; (ii) titrisol, pH 7.00 (Merck) and (iii) 0.010 mol dm^{-3} sodium tetraborate, pH 9.23. Water was doubly distilled and decarbonated prior to use by purging with a stream of nitrogen. All solubility values were determined at least in triplicate and were found to be of good reproducibility, with relative standard deviations of 0.3–2.9% except in the case of chlorprothixene, where a relative standard standard deviation of 8.5% was found.

Determination of pK_a in methanol–water

All pK_a values were determined at 20.0 ± 0.5°C using the equipment and buffers described above by setting the second derivative of a recorded titration curve to zero, after correction for dilution due to the titrant added. Such titration curves were obtained by stepwise addition of 0.10 ml of a potassium hydroxide solution in the mixed solvent to 50 ml of the solute salt (generally the chloride) solution, and recording the pH value 15 sec after addition. The pK_a values of $H_2PO_4^-$ and NH_4^+ were determined in quadruplicate by titration of a 41.4 mmol dm^{-3} solution of potassium dihydrogenphosphate or ammonium chloride with a 1.00 mol dm^{-3} potassium hydroxide solution in the mixed solvent. The concentration of 41.4 mmol dm^{-3} equals the maximum concentration of NH_4^+ in the mobile phase (after correction for volume contraction upon mixing, which is 3.42% at 20.0°C for $\varphi = 0.50$), assuming the DMAD present to behave indistinguishably from NH_4^+.

pK_a Values of solutes were determined in triplicate by titration of a 1.00 mmol dm^{-3} solution in 50 mmol dm^{-3} potassium chloride in the mixed solvent, except for chlorprothixene and thioridazine, where a 0.50 mmol dm^{-3} solution was used, since at a concentration of 1.00 mmol dm^{-3} precipitation of the free base occurred upon titration.

Prochlorperazine was titrated as the dimesylate, whereas chlorpheniramine maleate was converted into the free base by extraction from an aqueous ammonia solution using chloroform as extracting solvent, drying of the extract and subsequent evaporation of the solvent.

Depending on the solute studied, titrants were: (i) 25 mmol dm^{-3} potassium

hydroxide + 25 mmol dm^{-3} potassium chloride solution or (ii) 50 mmol dm^{-3} potassium hydroxide solution, both in methanol–water (φ = 0.50).

Measured pK_a values were corrected for the presence of methanol using a correction term of −0.10[4] and were found to be well reproducible (total standard deviations for triplicate determinations $\leqslant$0.08).

Mobile phase pH and ionic strength

The mobile phase pH was obtained by direct measurement using the equipment described previously, and was subsequently corrected for the presence of methanol (−0.10), resulting in a value of 7.82. This value was combined with the measured pK_a values of $H_2PO_4^-$ and NH_4^+ in methanol–water, 7.84 and 9.03 respectively, and the maximum concentration of NH_4^+ (41.4 mmol dm^{-3}, again assuming DMAD to behave indistinguishably from NH_4^+), to calculate the ionic strength, I, of the mobile phase. For I a value of 0.052 was found. Since the value differs somewhat from the ionic strength at which the pK_a values of $H_2PO_4^-$ and NH_4^+ were determined, these were redetermined at the appropriate ionic strength. The new pK_a values found (7.83 for $H_2PO_4^-$, 9.07 for NH_4^+) had negligible influence on the calculated mobile phase ionic strength.

Chromatography

Chromatographic equipment consisted of an Altex 110 A single-piston pump (Altex, Berkeley, CA, U.S.A.) with additional damping, a Model 7125 injection valve equipped with a 10-μl sample loop (Rheodyne, Berkeley, CA, U.S.A.) and a variable-wavelength detector (Type 2151, LKB; Bromma, Sweden) and a refractive index detector (R 401; Waters, Etten-Leur, The Netherlands) arranged in tandem. Peak recording was achieved using a Kipp BD 41 flat-bed potentiometric recorder (Kipp & Zonen, Delft, The Netherlands).

The columns used were 50 × 4.6 mm. The injection valve and main column were preceded by a 30 × 4.6 mm precolumn packed with Hypersil ODS. The precolumn, injection valve and main column were kept at 20.0 ± 0.1°C by immersion in a thermostat water-bath (Hetotherm 0.2 PT 623; Heto, Birkeröd, Denmark). The mobile phase reached the precolumn through a *ca.* 1-ml coil immersed in the water-bath. All other chromatographic procedures have been described previously[5,6].

RESULTS AND DISCUSSION

Relevant physicochemical data for the compounds studied are presented in Table I, and comprise their melting points, T_m[7,8], measured methanol–water p$K_{a,mix}$ values (φ = 0.50, I = 0.05), logarithmic capacity factors κ, measured using mobile phases made up with an aqueous part either of pH 4.00 ($\kappa_{4.00}$) or of pH 7.00 ($\kappa_{7.00}$), selected literature values of thermodynamic aqueous pK_a values (p$K^0_{a,w}$) (20–25°C, refs. 9–19) and values of log $K^0_{d,oct}$ and −log X^0_w (pX^0_w). Selected values of log $K^0_{d,oct}$ were (i) measured values reported in the literature[10,17], (ii) calculated from measured literature log $K_{d,oct}$ values for various octan-1-ol–phosphate buffer systems[10] using ion-correction terms or (iii) calculated using fragment constants[10] (cyclizine, opipramol, prochlorperazine), or estimated from values for related compounds using a group contribution approach (chlorprothixene, orphenadrine).

TABLE I

PHYSICOCHEMICAL PROPERTIES OF SOLUTES

	Compound	T_m(°C)	$pK^0_{a,w}$*	log $K^0_{d,oct}$*	pX^0_w*	$\kappa_{4.00}$**	$\kappa_{7.00}$**	$pK_{a,mix}$***
1	Alprenolol	58§	9.65	3.10	4.19§§	0.241	0.626	9.27
2	Amitriptyline	–	9.46	4.98	6.19	0.759	1.471	8.79
3	Atropine	115	9.85	1.83	3.85	−0.93	−0.342	9.43
4	Chlorpheniramine	–	9.26	3.25	4.38§§	0.089	0.619	8.69
5	Chlorpromazine	58	9.36	5.34	6.84	1.031	1.710	8.65
6	Chlorprothixene	98	8.83	5.62§§§	7.17§§	1.053	1.938	8.52
7	Clonidine	130	8.05	1.59	<3.27§§	< −1.50	−0.291	8.23
8	Cocaine	98	8.60	2.28	4.35	−0.615	0.508	8.42
9	Codeine	155	8.21	1.14	3.31	–†	0.078	7.81
10	Cyclizine	108	8.16	3.25§§§	5.24	0.349	1.247	7.87
11	Cyproheptadine	113	9.12	4.84	6.32	0.671	1.556	8.37
12	Diphenhydramine	–	9.02	3.27	4.44§§	0.189	0.849	8.72
13	Droperidol	146	7.64	3.50	5.74§§	0.069	1.365	7.06
14	Haloperidol	149	8.66	4.02	6.11§§	0.390	1.083	8.44
15	Imipramine	–	9.58	4.70	5.92	0.672	1.284	8.93
16	Lidocaine	69	7.86	2.26	3.55§§	−0.606	0.963	7.50
17	Methadone	78	10.12	4.78	5.91§§	0.642	0.862	9.60
18	Metoprolol	51§	9.68	2.04	<3.05§§	−0.561	−0.143	9.28
19	Nalorphine	209	7.88	1.86	–††	–†	0.570	7.41
20	Naloxone	184	7.94	2.09	5.13	–†	0.696	7.67
21	Opipramol	100	8.02	3.46§§§	5.30	0.597	1.295	7.66
22	Orphenadrine	–	8.91	3.77§§§	4.71§§	0.476	1.103	8.70
23	Pethidine	–	8.50	2.72	3.56§§	−0.300	0.560	8.16
24	Procaine	61	8.96	1.87	3.43	–†	−0.186	8.52
25	Prochlorperazine	–	8.10	4.74§§§	6.14	1.323	2.044	7.67
26	Promazine	–	9.42	4.55	6.05	0.634	1.254	8.80
27	Promethazine	60	9.10	4.59	6.00	0.544	1.475	8.31
28	Propranolol	96	9.60	3.48	4.53	0.225	0.592	9.29
29	Strychnine	268	8.27	1.68	5.10	−0.774	0.145	7.83
30	Thioridazine	73	9.50	5.90	7.57	1.204	1.851	8.90

* At 20–25°C.
** At 20°C.
*** At I = 0.05 and 20°C.
§ From ref. 8.
§§ This study.
§§§ Estimated value.
† k = 0.
†† Decomposed during titration.

pX^0_w Values were (i) determined in this study, (ii) taken from the literature[3,12,14] or (iii) calculated from solubility data for water[7,11] by using a correction for solute "auto-ionization". All literature values of pK_a, log $K_{d,oct}$ and aqueous solubility used in this study were determined at 20–25°C, although, where possible, data obtained at 20°C have been used. The missing pX^0_w values of clonidine and metoprolol are due to their high solubilities ($> 3 \cdot 10^{-2}$ and $> 10^{-2}$ mol dm^{-3}, respectively), while nalorphine was found to decompose during titration in water.

Corrections for ionization

Reversed-phase liquid chromatography. A comprehensive equation for the RPLC retention of monoprotic basic solutes as a function of solute and mobile phase properties may be derived in an analogous fashion to that given by Van de Venne[4] for monoprotic acidic solutes, *viz.*

$$k = \left(1 + \frac{a_{H^+}}{K_a}\right)^{-1} k^0 + \left(1 + \frac{K_a}{a_{H^+}}\right)^{-1} (k^+ + a_{X^-}\, k_{RH^+X^-}) \quad (1)$$

where k is the solute capacity factor, a_{H^+} and a_{X^-} are the activities in the mobile phase of hydrogen ions and buffer anions, respectively, and k^0, k^+ and $k_{RH^+X^-}$ are the capacity factors of the unionized and fully ionized solute and of the ion pair(s) formed by solute cation and buffer anions, respectively. Making the assumptions that (a) $a_{H^+} = [H^+]$, the hydrogen-ion concentration in the mobile phase, and (b) the ion-pairing term $a_{X^-}\, k_{RH^+X^-}$ may be taken as approximately equal to zero by appropriate choice of buffer anions (low ion-pairing capacities and low concentration, *i.e.*, high buffering capacities), then eqn. 1 will reduce to

$$k = [1 + 10^{(pK_a - pH)_{mob}}]^{-1}\, k^0 + [1 + 10^{(pH - pK_a)_{mob}}]^{-1}\, k^+ \quad (2)$$

where the subscript mob refers to the mobile phase.

Aqueous solubility and liquid–liquid distribution. As mentioned previously, determination of K_d or S_w values at a pH close to the solute pK_a and the use of a correction for ionization effects offers a possibility for obtaining K_d^0 and S_w^0. For monoprotic basic solutes the following corrections are widely used[2,3,12,18]

$$K_d^0 = K_d[1 + 10^{(pK_a - pH)_{aq}}] \quad (3)$$

and

$$S_w^0 = S_w[1 + 10^{(pK_a - pH)}] \quad (4)$$

with the subscript aq referring to the aqueous phase of the distribution system. In eqns. 3 and 4 the contributions of the ionized species are assumed to be negligible. Since, for the solutes studied, the aqueous solubilities of the cationic species are usually very much larger than those of the free bases, *e.g.*, refs. 7, 20, such an assumption is indeed valid for aqueous solubilities. The validity of results obtained using eqn. 3 is dependent upon the assumption that only unionized solutes will distribute between an aqueous and a non-aqueous phase, which is in turn greatly dependent upon the nature of the "non-aqueous" phase. Because of the high solubility of water in octan-1-ol (0.22 mole fraction units at 20°C), this assumption may not hold for octan-1-ol–aqueous buffer phase systems, since eqn. 3 does not account for (i) ionization in the octan-1-ol phase and (ii) possible distribution of ion pairs that may be formed between solute cations and buffer anions. Application of eqn. 3 will therefore result in approximate values for $K_{d,oct}^0$.

Relations between RPLC retention, $K_{d,oct}$ and X_w

Because of the use of aqueous methanol mobile phases for the determination

of solute k values, there are two possibilities for a suitable solute reference state, namely either that of the solute under mobile phase conditions (φ, pH, I) or that of the unionized solute. Use of the former reference state requires the correction of log $K^0_{d,oct}$ and pX^0_w values to mobile phase conditions, which implies the reverse application of eqns. 3 and 4 using $pK_{a,mix}$ values and a pH value of 7.82.

In order to investigate the possibility of using $\kappa_{7.00}$ data for bases as a measure of their hydrophobic–lipophilic balance, or their hydrophobicity, the resulting values for log $K^c_{d,oct}$ and pX^c_w (Table II) have been related to their corresponding κ values using the equations

$$\log K_{d,oct} = a_1 + b_1\kappa \tag{5}$$

and

$$pX_w = a_2 + b_2\kappa + c_2 \log \frac{T_m}{T} \tag{6}$$

Such equations have been successfully applied for neutral, acidic and weakly basic compounds[5,6,21], *i.e.*, for compounds that can be chromatographed in their unionized states. The following results have been obtained

$$\log K^c_{d,oct} = 0.970(0.096) + 1.905(0.086)\kappa_{7.00} \tag{7}$$

$$n = 29,\ R = 0.974,\ F = 491,\ s = 0.310$$

$$pX^c_w = 2.214(0.143) + 1.831(0.095)\kappa_{7.00} + 7.061(0.870)\log \frac{T_m}{T} \tag{8}$$

$$n = 24,\ R = 0.974,\ F = 192,\ s = 0.289$$

where n is the number of solutes, R the multiple correlation coefficient, F the variance ratio, s the standard deviation of regression and figures in parentheses represent the standard deviations of the regression coefficients. In eqn. 8 the log T_m/T values of liquid solutes have been parameterized as zero. The above correlations have been improved by omission of significant outliers, which were identified using the criterion that calculated log $K^c_{d,oct}$ and pX^c_w values should lie outside the 95% probability area of the regression. This implies that the absolute difference between the calculated and observed log $K^c_{d,oct}$ or pX^c_w values should exceed $\hat{t}s$, with $\hat{t}$ being the Student t value corresponding to the 95% confidence level and the appropriate number of degrees of freedom. Lidocaine was identified as being an outlier from eqn. 7, while lidocaine, propranolol and alprenolol were found to be outliers from eqn. 8.

Figs. 1 and 2 are graphical representations of eqns. 7 and 8, with Fig. 2 being a plot of estimated ($pX^c_{w,e}$) *versus* observed ($pX^c_{w,o}$) solubility values. It can be seen from these equations and figures that (a) a good correlation exists between $\kappa_{7.00}$ and corrected log $K_{d,oct}$ values of strong bases, and (b) after omission of three outliers, reliable estimations of aqueous solubilities can be obtained using eqn. 8.

The use of the unionized solute as the reference state requires the application of eqn. 2 to obtain k^0, the capacity factor of the unionized solute. For this purpose the retentions of the fully ionized solutes have been measured using a pH 4.00 phos-

TABLE II

CALCULATED SOLUTE PARAMETERS

	Compound	log K_a^{c*}	pX_w^{c*}	κ^0	ΔpK_a^{**}
1	Alprenolol	1.63	2.72	1.87	−0.52
2	Amitriptyline	3.97	5.18	2.40	−0.81
3	Atropine	0.21	2.13	1.15	−0.56
4	Chlorpheniramine	2.33	3.46	1.41	−0.71
5	Chlorpromazine	4.45	5.95	2.51	−0.85
6	Chlorprothixene	4.84	6.39	2.67	−0.45
7	Clonidine	1.04	<2.72	0.25	0.04
8	Cocaine	1.58	3.65	1.18	−0.32
9	Codeine	0.84	3.01	0.37	−0.54
10	Cyclizine	2.92	4.91	1.54	−0.43
11	Cyproheptadine	4.18	5.66	2.17	−0.89
12	Diphenhydramine	2.32	3.49	1.71	−0.44
13	Droperidol	3.43	5.67	1.43	−0.72
14	Haloperidol	3.31	5.40	1.72	−0.36
15	Imipramine	3.56	4.78	2.31	−0.79
16	Lidocaine	2.09	3.38	1.13	−0.50
17	Methadone	2.99	4.12	2.26	−0.66
18	Metoprolol	0.57	<1.58	1.13	−0.54
19	Nalorphine	1.72	–	0.71	−0.61
20	Naloxone	1.86	4.90	0.93	−0.41
21	Opipramol	3.23	5.07	1.49	−0.50
22	Orphenadrine	2.84	3.78	1.94	−0.35
23	Pethidine	2.22	3.06	1.02	−0.48
24	Procaine	1.09	2.65	0.59	−0.58
25	Prochlorperazine	4.51	5.91	2.24	−0.57
26	Promazine	3.53	5.03	2.17	−0.76
27	Promethazine	3.98	5.39	2.05	−0.93
28	Propranolol	2.00	3.05	1.84	−0.45
29	Strychnine	1.37	4.79	0.42	−0.58
30	Thioridazine	4.79	6.46	2.86	−0.74

* Under mobile phase conditions.
** At $I = 0$ using eqn. 12.

phate solution as the aqueous part of the mobile phase. A pH value of 4.00 has been chosen so as to avoid multiple ionization effects that would occur for some compounds (4, 10, 21, 25 in Table I) at lower pH. The resulting calculated k^0 values (given in their logarithmic form as κ^0 in Table II) have been examined as descriptors of solute hydrophobic (lipophilic) behaviour, by relating them to log $K_{d,oct}^0$ and pX_w^0, respectively. Eqns. 9 and 10 and Figs. 3 and 4 show the results, the latter again being a plot of observed *versus* estimated pX_w values:

$$\log K_{d,oct}^0 = 0.597(0.136) + 1.819(0.078)\kappa^0 \quad (9)$$

$$n = 28,\ r = 0.977,\ F = 543,\ s = 0.298$$

$$pX_w^0 = 1.887(0.205) + 1.710(0.092)\kappa^0 + 8.292(0.910)\log\frac{T_m}{T} \quad (10)$$

$$n = 23,\ R = 0.972,\ F = 172,\ s = 0.283$$

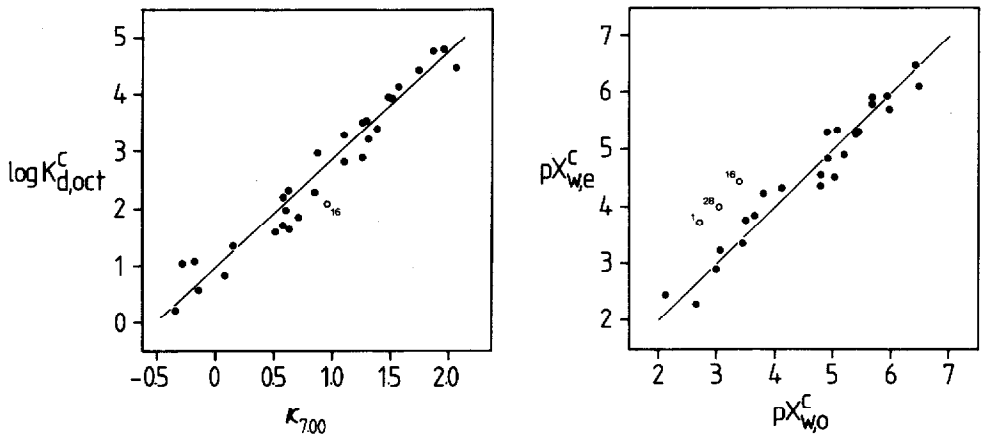

Fig. 1. Relationship between isocratic logarithmic capacity factors obtained using a mobile phase pH of 7.82 with a methanol volume fraction of 0.50 ($\kappa_{7.00}$) and logarithmic octan-1-ol–buffer distribution coefficients of basic drugs, corrected for ionization in the mobile phase. Open circles are outliers (see text) denoted by their compound numbers (Tables I and II). The solid line is the regression line according to eqn. 7.

Fig. 2. Relationship between negative logarithmic mole fraction aqueous solubilities of bases ($pX^c_{w,o}$), corrected for mobile phase conditions, and negative logarithmic solubilities ($pX^c_{w,e}$) estimated using isocratic logarithmic capacity factors, $\kappa_{7.00}$, *i.e.*, using eqn. 8. Key for outlier notation as in Fig. 1. The solid line is the ideal regression line between observed and estimated (log) solubility values.

Using the previously described criterion, alprenolol and atropine were found to be outliers from eqn. 9, while in addition lidocaine and propranolol were identified as outliers from eqn. 10. It is found that, after omission of these outliers from regression,

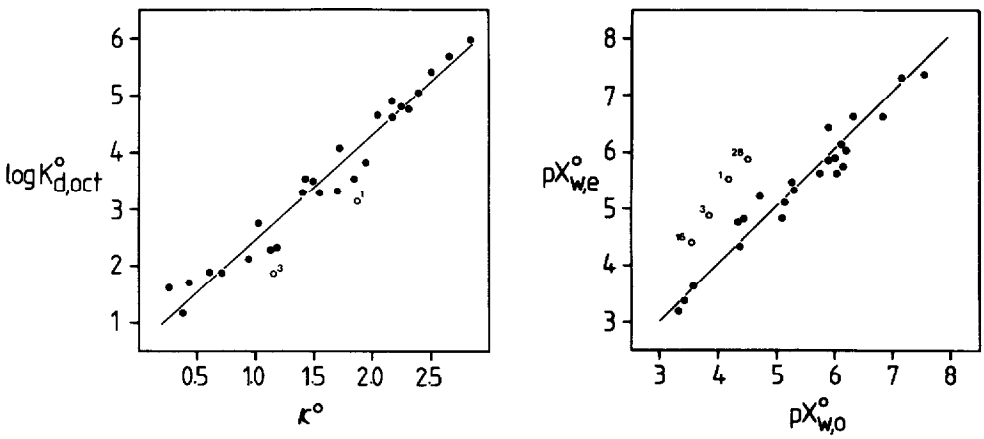

Fig. 3. Relationship between isocratic logarithmic capacity factors of unionized bases calculated using eqn. 2 (κ^0) and logarithmic octan-1-ol–buffer distribution coefficients of the unionized solutes. Key for outlier notation as in Fig. 1. The solid line represents the regression line according to eqn. 9.

Fig. 4. Relationship between observed negative logarithmic mole fraction aqueous solubilities of unionized bases ($pX^0_{w,o}$) and negative logarithmic solubilities ($pX^0_{w,e}$) estimated using calculated isocratic logarithmic capacity factors, κ^0, *i.e.*, using eqn. 10. Keys as for Fig. 2.

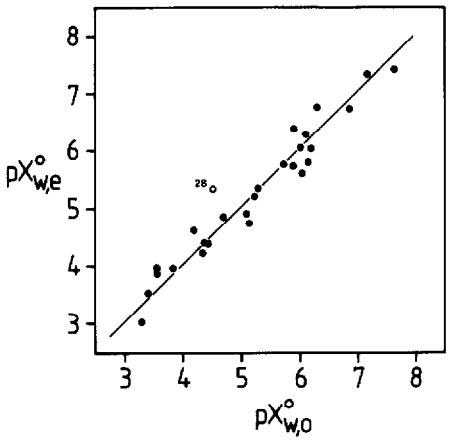

Fig. 5. Relationship between observed negative logarithmic mole fraction aqueous solubilities of unionized bases ($pX^0_{w,o}$) and negative logarithmic solubilities ($pX^0_{w,e}$) estimated using logarithmic octan-1-ol–buffer distribution coefficients of the unionized solutes, log $K^0_{d,oct}$, *i.e.*, using eqn. 11. Keys as for Fig. 2.

(a) log $K^0_{d,oct}$ and κ^0 are well correlated and (b) reliable estimations of aqueous solubilities can be made using eqn. 10.

Interestingly, there appears to be a tendency for overestimation of log $K_{d,oct}$ or pX_w of the outliers from eqns. 7–10, implying that both measured and calculated κ values of these solutes are relatively too high as compared to their bulk hydrophobic (lipophilic) properties. This is partly confirmed upon relating pX^0_w and log $K^0_{d,oct}$ (Fig. 5) using an equation of the form of eqn. 6:

$$pX^0_w = 1.363(0.199) + 0.927(0.044)\log K^0_{d,oct} + 7.368(0.860)\log \frac{T_m}{T} \qquad (11)$$

$$n = 26,\ R = 0.975,\ F = 221,\ s = 0.278$$

With only propranolol identified as being an outlier, the statistical significance of eqn. 11 can be seen to be at least equivalent to that of eqn. 10. This is in contradistinction to earlier findings for neutral, acidic and weakly basic compounds for which the use of RPLC κ values in model solubility equations generally yields better estimates of pX^0_w compared to use of solute log $K_{d,oct}$ values[6,21].

The applicability of RPLC retention data obtained using, *e.g.*, aqueous methanol mobile phases as descriptors of hydrophobic (lipophilic) properties of strong bases is dependent upon the knowledge of solute pK_a values in the mixed solvent used as the "basis" for the mobile phase. This is due to the requirement that, for example, static and dynamic parameters should be compared in essentially the same reference state. Although these pK_a values may be determined using titration methods, these require tens of milligrams of pure solute (in this study triplicate pK_a determinations required 40–84 mg of solute salts). Other possibilities may be found in the application of general correction terms for the presence of methanol to aqueous solute pK_a values that are either known from the literature or can be estimated from values of related compounds.

Empirical values of correction terms ΔpK_a ($= pK_{a,mix} - pK_{a,w}$) for basic solutes may be obtained from known $pK_{a,mix}$ and $pK_{a,w}$ values at a reference ionic strength. In order to examine the data used in this study for general trends in pK_a shifts, measured $pK_{a,mix}$ values (at $I = 0.05$) were first converted into thermodynamic values, $pK^0_{a,mix}$ ($pK_{a,mix}$ at $I = 0$), by applying a modified form of the extended Debye–Hückel equation[22], *viz.*

$$pK^0_{a,mix} - pK_{a,mix} = -\frac{A(2Z - 1)I^{1/2}}{1 + BI^{1/2}} \tag{12}$$

where Z is the ionic charge of the acid, and A and B are coefficients with magnitudes depending on the temperature, mean ion-size parameter and solvent properties (density, dielectric constant). A and B were calculated according to Hulshoff and Perrin[16], using values of 3.1 Å for the mean ion-size parameter[22], 0.9255 kg dm^{-3} for the solvent density at 20°C (this study) and 59.6 for the solvent dielectric constant at 20°C[23]. The resulting values of A and B were 0.762 and 1.12, respectively.

The subsequently obtained $pK^0_{a,mix}$ values were used to calculate values of ΔpK_a (Table II). For the solutes studied an average ΔpK_a value of -0.59 ± 0.17 is found when the ΔpK_a value of clonidine ($+0.04$) is not taken into account. The averaged ΔpK_a values may be combined with aqueous pK^0_a values to calculate solute $pK^0_{a,mix}$, which can be converted (eqn. 12) into $pK_{a,mix}$ values at the mobile phase ionic strength. This results in a general empirical relationship between $pK_{a,mix}$ ($I = 0.05$) and $pK^0_{a,w}$, *viz.*:

$$pK_{a,mix} = pK^0_{a,w} - 0.45 \tag{13}$$

Combinations of eqn. 13 and eqns. 3 and 4 can be used to evaluate a general correction term for $\log K^0_{d,oct}$ and pX^0_w values to mobile phase conditions (aqueous methanol, $\varphi = 0.50$, pH = 7.82, $I = 0.05$). The resulting values of $\log K^c_{d,oct}$ and pX^c_w may subsequently be related to $\kappa_{7.00}$ through expressions of the form of eqns. 5 and 6. Using a mobile phase pH value of 7.82, the following relationships are found:

$$\log K^0_{d,oct} - \log (1 + 10pK^{0\,-8.27}_{a,w}) = 0.950(0.082) + 1.892(0.073)\kappa_{7.00} \tag{14}$$

$n = 28$, $r = 0.981$, $F = 678$, $s = 0.247$

$$pX^0_w - \log (1 + 10pK^{0\,-8.27}_{a,w}) = 2.257(0.118) + 1.748(0.078)\kappa_{7.00} + \\ + 7.377(0.717) \log \frac{T_m}{T} \tag{15}$$

$n = 24$, $R = 0.980$, F 261, $s = 0.239$

Outliers from eqn. 14 were clonidine and lidocaine, while alprenolol, lidocaine and propranolol were identified as outliers from eqn. 15.

These results indicate that eqns. 14 and 15, being combinations of a semiempirical ion-correction term and (multiple) linear regression equations may yield predictions of octan-1-ol–buffer distribution coefficients and aqueous solubilities of unionized strong bases. Moreover, the use of these equations does not require knowledge of aqueous methanol pK_a values, and is therefore practically preferable over the use of model-based relationships such as eqns. 7–10.

CONCLUSIONS

It has been found that for 30 "strongly" basic drugs (aqueous pK_a > 7.5) general relationships can be established between their aqueous solubilities, octan-1-ol–buffer distribution coefficients and the RPLC capacity factors of the partially ionized solutes obtained using aqueous methanol mobile phases. When ignoring possible ion-pairing effects between solute cations and buffer anions, two solute reference states can be defined, *i.e.*, that of the solute under mobile phase conditions and that of the unionized solute. The use of either reference state requires knowledge of the mobile phase pH and solute pK_a values (both aqueous and under mobile phase conditions) in order to correct for ionization effects. For both reference states good correlations are observed between κ and log $K_{d,oct}$, while pX_w can be reliably estimated from a multiple linear combination of κ and the logarithm of the solute melting point (although some significant outliers can be identified, the occurrence of which appears to be due to high RPLC retention parameter values).

A major disadvantage in the practical application of such relationships is the required knowledge of pK_a values under mobile phase conditions, practical determination of which consumes tens of milligrams of pure compounds. Therefore a semiempirical relationship between mobile phase and aqueous pK_a values is applied to circumvent the required determinations of pK_a values onder mobile phase conditions. It is observed that, when using this relationship to correct for solute ionization under mobile phase conditions, also good correlations are found between κ and log $K_{d,oct}$, and that pX_w can again be adequately predicted from a multiple linear combination of κ and log T_m/T.

These findings suggest that such combinations of semiempirical ion-correction and (multiple) linear regression equations may be used to predict the hydrophobic (lipophilic) behaviour of strong bases.

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REFERENCES

1 S. H. Unger and G. C. Chiang, *J. Med. Chem.*, 24 (1981) 262–270.
2 J. Büchi, X. Perlia and S. P. Studach, *Arzneim.-Forsch.*, 17 (1967) 1012–1021.
3 K. Thoma and K. Albert, *Pharm. Acta Helv.*, 56 (1981) 69–77.
4 J. L. M. van de Venne, *Ph.D. Thesis*, Eindhoven University of Technology, 1979.
5 T. L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 218 (1981) 409–425.
6 T. L. Hafkenscheid and E. Tomlinson, *Int. J. Pharm.*, 17 (1983) 1–21.

7 M. Windholz (Editor), *The Merck Index*, Merck & Co, Rahway, 9th ed., 1976.
8 N. Schouten, Astra Pharmaceutica B.V., Rijswijk, The Netherlands, personal communication, 1983.
9 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965, Suppl. 1972.
10 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979, and Supplements of the Pomona College Medicinal Chemistry Project Data File.
11 J. E. F. Reynolds (Editor), *Martindale, The Extra Pharmacopoeia*, The Pharmaceutical Press, London, 28th ed., 1982.
13 A. M. de Roos, R. F. Rekker and W. Th. Nauta, *Arzneim.-Forsch.*, 20 (1970) 1763–1765.
14 R. H. Levy and M. Rowland, *J. Pharm. Sci.*, 60 (1971) 1155–1159.
15 J. J. Kaufman, N. M. Semo and W. S. Koski, *J. Med. Chem.*, 18 (1975) 647–655.
16 A. Hulshoff and J. H. Perrin, *Pharm. Acta Helv.*, 51 (1976) 65–71.
17 P. B. M. W. M. Timmermans and P. A. van Zwieten, *Arzneim.-Forsch.*, 28 (1978) 1676–1681.
18 H. H. van Rooy, *Ph.D. Thesis*, University of Amsterdam, 1980.
19 K. Thoma and K. Albert, *Pharm. Acta Helv.*, 55 (1980) 8–12.
20 *The United States Pharmacopeia*, United States Pharmacopeial Convention, Rockville, XXth ed., 1980.
21 T. L. Hafkenscheid, *Ph.D. Thesis*, University of Amsterdam, 1984.
22 A. N. Martin, J. Swarbrick and A. Cammarata, *Physical Pharmacy*, Lea & Febiger, Philadelphia, PA, 2nd ed., 1969.
23 J. Timmermans, *Physicochemical Constants of Binary Systems in Concentrated Solution*, Vol. IV, Interscience, New York, 1960.