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THE DETERMINATION OF DISSOCIATION CONSTANTS AND PARTITION COEFFICIENTS OF PHENOTHIAZINE DERIVATIVES

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SUMMARY

A method of determining the dissociation constants of lipophilic weak acids or bases of low aqueous solubility based on the measurement of the pH dependency of partition coefficients is described. The measurements, although subject to the possibility of error due to the partitioning of impurities, are free from the errors of extrapolation involved in mixed solvent titrimetric or solubility methods for the determination described in the literature. Precise spectrophotometry is essential, in the method described, but this coupled with a large number of data points can lead to a precision of $< \pm 0.03$ in pKa.The cyclohexane/water partition coefficients of 3 phenothiazines measured during the course of this work are discussed in terms of published affinity constants for plasma proteins and support the view of the hydrophobic nature of drug-binding to plasma.

INTRODUCTION

The usual methods for determination of dissociation constants (Perrin, 1965) are readily applicable only to substances with a reasonable solubility in water in all states of ionization. They are thus not useful for phenothiazines, which in the unionized state are very insoluble in water (Green, 1967). Dissociation constants can be obtained from titration curves in mixed aqueous solvents, but the necessary extrapolation of the results to 100% water introduces errors which are often large (Chatten and Harris, 1962). The solubility dependence on pH has been used for phenothiazines and tricyclic drugs by Green (1967), but there are discrepancies of up to 0.9 pH units in some of the results from these two methods. Such discrepancies can cause difficulties in correlating pKa with structure and especially in attempts to relate the effects of substituent groups with dissociation of the drug species.

The method described in this paper has shown that the discrepancies in the literature are due mainly to systematic errors in the titration method arising from the deviation from the linear dependence of apparent pKa on the content of organic solvent. Systematic errors in the solubility method are much smaller, though measurable, and arise from the variation of supersaturation with pH. In this paper a method is described for the determination of dissociation constants, based on measurement of partition coefficient at a range of pH values. The partition coefficients which we obtained for 8 phenothiazines are used in attempts to correlate binding to proteins and other molecules with the lipophilic characteristics of the drugs.

THEORY

If the drug as pure base is dissolved in the oil phase (o) (cyclohexane) and equilibrated with an aqueous buffer (w) of known pH, the concentration of the unionized species in the aqueous phase is c_{uw}. If the pH of the aqueous phase is within 2 units of the pKa, then the aqueous molar concentration of the ionized species is given by

$$c_{iw} = c_{uw}(10^{pKa-pH}) \tag{1}$$

If c_1 and c_2 are the analytically determined concentrations in the aqueous phase after equilibration

$$c_1 = c_{uw_1}(1 + 10^{pKa-pH_1}) \tag{2}$$

and similarly

$$c_2 = c_{uw_2}(1 + 10^{pKa-pH_2}) \tag{3}$$

If the partition coefficients of ionized species and unionized species are P_i and P_u respectively, the concentrations of the species in the oil phase (c_o) are related to the aqueous phase concentrations by the following equations

$$c_{uo} = P_u c_{uw} \tag{4}$$

$$c_{io} = P_i c_{uw}(1 + 10^{pKa-pH}) \tag{5}$$

The experiment is arranged by trial and error, by preparing and using a selection of buffers with pH values near the pKa of the compound under study. With most phenothiazine derivatives, $P_i \approx 10^{-3.6} P_u$ hence (Hansch et al., 1971)

$$c_o = c_{uo} + c_{io} \approx P_u c_{uw} \tag{6}$$

If the experiment is carried out at two different pH values we can write from Eqns 2 and 3

$$\frac{c_1}{c_2} = \frac{c_{uw_1}(1 + 10^{pKa-pH_1})}{c_{uw_2}(1 + 10^{pKa-pH_2})} = \frac{c_{o_1}}{c_{o_2}} \frac{(1 + 10^{pKa-pH_1})}{(1 + 10^{pKa-pH_2})}$$

c_{o_1} and c_{o_2} are obtained by difference between the accurately known initial concentrations and the values of c_1 and c_2 respectively. These compounds do not dimerize, apparently, in the oil phase, (Hansch et al., 1971). The effects of dimerization on the results, however, are negligible if $c_{o_1} \approx c_{o_2}$, as was the case in this work. If is not necessary to know P if the same solution is used at both pH values as the values of pKa are determined from the analytically determined values of c_1 and c_2. It is more convenient, rather than to solve the two equations, to interpolate values of pKa from tabulated data of c_1/c_2 for given values of pKa between pH_1 and pH_2.

The method is the converse of that of Kaufmann et al. (1975) for the deriviation of log P values from changes in apparent pKa in the presence of oil phases.

EXPERIMENTAL

Methods and materials

The phenothiazine derivatives used were obtained as follows: fluphenazine hydrochloride and triflupromazine hydrochloride from E.R. Squibb (Moreton, U.K.); pericyazine, trifluperazine, prochlorperazine methanesulphonate, chlorpromazine hydrochloride and promethazine hydrochloride from May and Baker (Dagenham, U.K.); and thioridazine from Sandoz-Warner (Leeds, U.K.).

Phenothiazine bases (prepared in the case where salts were supplied by ether extraction from alkaline solutions and washing) were dissolved in A.R. grade cyclohexane (chosen for its low density, the relatively low log P values obtained when it is used and lack of polarity or tendency to emulsify) and equilibrated with aqueous buffers by shaking in 'Quickfit' separatory funnels of either 50 or 250 ml for a few minutes. It was observed that a final state was reached after 200–300 inversions, or mechanical shaking for 2 min, amplitude 20 cm, at 2–3 Hz. The temperature was maintained at 20 ± 0.5°C. Aqueous phases were analyzed for total drug content with a Perkin Elmer 124 scanning spectrophotometer, with either 1 cm or 5 cm silica cells. Content of oil phases was determined by difference. Buffers were standardised by least squares linear fitting to standard pH borax solution, Wellcome Soloid buffer tablets pH 4.0, 7.0 and 9.0, phosphate and phthalate standard solutions and Corning standard buffer, pH 7.02, using Pye model 291 and Corning EEL pH meters. The buffers used in the pKa determinations were borate buffer, pH 10.00, 9.00, 8.87, 8.20, 8.04, 8.02 without added KCl and phosphate buffer, pH 7.82.

Three ionic strengths of each buffer at each pH were prepared; equal to, approximately double, and approximately quadruple, those of the buffers cited. Determinations of $\log_{10}$ P and pKa were distributed evenly among these buffers, and means calculated.

RESULTS AND DISCUSSION

Table 1 gives the pKa values determined by the method described and by titration (Chatten and Harris, 1962 unless otherwise stated) and by solubility (Green, 1967). The uncertainties in c_1 and c_2 arising from assumed errors of 0.02 in pH_1 and pH_2 and log P values are also given. The error in log P approximates to ΔpKa, the standard error of pKa.

TABLE 1

pKa VALUES OF PHENOTHIAZINES AND PARTITION COEFFICIENTS BETWEEN CYCLOHEXANE AND BUFFER SOLUTIONS

Base	pH_1	pH_2	pKa titration	pKa solubility	pKa this work	N	ΔpKa	$\log_{10}Pu$	Pu
Promethazine	10.00	9.00	9.09	9.1	8.99	16	0.031	4.41	2.57×10^4
Chlorpromazine	10.00	9.00	9.26	9.3	9.40	7	0.045	5.24	17.39×10^4
Triflupromazine	10.00	9.00	9.41	9.2	9.29	13	0.039	5.42	26.30×10^4
Pecazine	10.00	9.00	–	9.7	9.48	14	0.030	4.80	6.31×10^4
Thioridazine	10.00	9.00	9.16	9.5	9.62	16	0.035	5.81	64.57×10^4
Prochlorperazine	9.00	8.02	7.54	8.1	8.14	6	0.06	4.28	1.905×10^4
Trifluperazine	9.00	8.04	8.40	8.1	8.05	16	0.043	4.53	3.39×10^4
Fluphenazine	8.87 8.20	7.82	8.05 [a]	–	7.98	39	0.027	3.28	0.19×10^4

[a] Flory (1971).

The method applies to pure compounds only. Aminoalkylphenothiazines form oxidative and photodegradative products which have low partition coefficients and similar spectrophotometric properties. The presence of one part in 10^5 of a photodegradation product of typical lipophilicity (i.e. $\log_{10}P \approx 0$) can produce large systematic errors in the measured $\log_{10}P$ and probably also in pKa. These more soluble products are formed in aqueous phases, rapidly at high pH and also in some unsaturated oily phases. Insufficient consideration of this source of error has resulted in several low values of partition coefficients in the literature. For example, occasional systematic errors in $\log_{10}P$ of 2.0 are apparent in Baur's (1971) data. Degradation rates of the phenothiazine in cyclohexane were observed to be very low, ($<10^{-5}$ per day). Such impurities in most of the samples were removed by successive washings with volumes of buffers approximately double that of the oil phase, and monitoring the asymptotic approach of the absorbances of the washing solutions to a final value, that of the pure base, after which the oil phase content of the base was redetermined. Most bases prepared from the salts require only 4–6 washings with double volumes of buffers of pH about 1–2 units below pKa. Triflupromazine and promethazine, however, required 9 successive washings with quadruple volumes of M/5 boric acid, and 11 times with quadruple volumes of phosphate buffer, pH 7.8, respectively.

The values of $\log_{10}P$ and pKa obtained did not depend on the ionic strengths of the buffers. Ion-pairing effects (Murthy and Zografi, 1970) would obtrude only at $\text{pKa} - \text{pH} > 2.8$, and then very little with trivalent ion buffers.

The upper limit to P_u affording a given precision in pKa varies in this method approximately directly with specific absorbance (ϵ) and inversely with absorbance band width. For phenothiazines generally, the limiting value of $\log_{10}P_u$ for which $\Delta\text{pKa} = 0.04$ is possible, seems to be about 6.0. The experiment is most accurate when $\text{pH}_1 \approx \text{pKa}$ and $\text{pK}_2 - \text{pKa} > 0.4$; if pKa is not known approximately, the experiment must be repeated at a number of pH values in order to obtain this state of affairs, so avoiding ill-conditioning in pKa in Eqn. 7.

Correlations of P with serum albumin binding

Affinity constants (log K) have been determined for phenothiazines and related compounds, with bovine serum albumin at pH 7.0 by Nambu and Nagai (1972) and human serum albumin at pH 6.5 by Sharples (1975, 1976). Perrin and Hulshof (1976) have raised some objections to Sharples' conclusions of a specific binding mechanism not hydrophobic in character.

The availability from the present work of an internally consistent system of pKa and P_u values permits determinations of linear correlations between a measure of hydrophobic character in P_u and binding parameters.

N is the number of pairs of data correlated, r the sample correlation coefficient and s the degree of significance of r (not quoted where $s \geqslant 0.1$), assuming a null hypothesis for r. Using the affinity constant (K) for BSA from Nambu and Nagai (1972) one obtains the relationship

$$\log P_u = 1.371 \log K - 0.3186 \quad (N = 5, r = 0.699) \tag{8}$$

When the affinity constant is recalculated to account for binding of the non-ionized species only (K_v) one obtains

$$\log P_u = 0.700 \log K_v + 0.9320 \ (N = 5, r = 0.950, s < 0.01) \quad (9)$$

Using Sharples' (1976) affinity data for HSA the following relationships are obtained

$$\log P_u = 0.028 \log K + 4.47 \ (N = 4, r = 0.017) \quad (10)$$

$$\log P_u = 0.800 \log K_v - 0.634 \ (N = 4, r = 0.927, s < 0.1) \quad (11)$$

Sharples' R_m values where $R_m = \log (1/R_f - 1)$, do not correlate with either log P or solubility data as criteria of lipophilicity and, although affinity constants uncorrected for ionization do not correlate with $\log_{10}P_u$ (Eqns. 8 and 10), those based upon binding of non-ionized forms alone correlate significantly, though data are sparse (Eqns. 9 and 11).

Although the correlation of binding of non-ionized phenothiazines with riboflavine (Sharples, 1976) with log P_u is not significant ($r = 0.828$), the affinity coefficients of riboflavine and HSA are high ($\log K_v = 0.765 \log K_{vc} + 2.86$; $N = 8$, $r = 0.890$; $\log K = 2.168 \log K_c - 0.203$; $N = 8$, $r = 0.984$). The difference, which is significant ($s < 0.02$), may be due to the appearance of another binding mechanism occurring at low pH, where there is nearly complete ionization of the drug substance. The high correlations obtained despite the paucity of data, suggest a lipophilic, non-specific protein binding mechanism at pH 6.5–7.0.

Thoma and Arnig (1976) find a linear relation between albumin binding and apparent log P, uncorrected for ionization. There is no correlation, in this study, between partition data for octanol/acetate buffer, pH 4.3 and for the two separate systems arachis oil/acetate buffer, pH 4.3 and 5.3. Correcting Thoma and Arnig's partition data using our pKa values to give partition coefficients for unionized drug gives $r = 0.986$ and 0.994, respectively ($N = 5$) for the octanol and arachis oil data. Values of correlation of log P_u (Table 1) with these three systems are 0.965, 0.929 and 0.961, respectively ($N = 5$). Values of K^+ derived by these authors from the equation of Scholtan (1962) and corrected for ionization are not the same at pH 4.3 and 5.3, though correlating significantly (0.898 and 0.912; $s < 0.05$). This may suggest again that a different binding mechanism, specific and not hydrophobic in origin, predominates at low pH. Further protein binding experiments at a range of higher pHs are indicated. Binding with receptors in the brain, however, appears to be specific, and electronic: for example, the effective doses of prochlorperazine and trifluperazine are larger than that of fluphenazine, despite larger partition coefficients, corrected or uncorrected for ionization.

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