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STUDY OF THE LIPOPHILICITY OF ORGANIC BASES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH ALKALINE ELUENTS

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SUMMARY

The correlation between reversed-phase liquid chromatographic (RPLC) capacity ratios and *n*-octanol–water partition coefficients can often allow RPLC to be used for simple and rapid estimation of partition coefficients. However, there are still difficulties involved in the evaluation of retention data of organic bases with pK_a in water greater than about 7. For ionisable compounds the non-ionised state is generally preferred as the reference state. The use of mobile phases is restricted to pH values below *ca.* 8 for the alkyl-bonded silicas commonly used in RPLC. Therefore until now RPLC derived lipophilicity parameters of non-ionised organic bases have had to be obtained by applying corrections for solute ionisation to retention data measured at pH ca. 7.

In the present study, the feasibility was examined of using the styrene–divinylbenzene copolymeric stationary phase PRP-1 for the direct measurement of retention data of non-ionised organic bases suitable for the estimation of their lipophilic properties. A correlation coefficient of 0.906 was obtained for the correlation of the logs of the capacity ratios on PRP-1 with experimentally determined partition coefficients for non-ionised bases.

Graphitised carbon was also evaluated. This material appeared not to retain organic bases by a simple partition mechanism.

INTRODUCTION

The *n*-octanol–water partition coefficient (log *P*) is generally accepted as being a useful parameter in structure activity relationship studies for correlation with biological or pharmacological activity of compounds. It is by now almost equally accepted that the correlation between reversed-phase liquid chromatographic (RPLC) capacity ratios [$k' = (t_R - t_0)/t_0$] and *n*-octanol–water partition coefficients can often allow RPLC to be used for simple and rapid estimation of partition coefficients. The advantages of the RPLC method over the traditional bulk phase shake-flask method have been enumerated by several authors[1–3].

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However, there are still difficulties involved in the evaluation of retention data of organic bases with pK_a in water greater than about 7. Amongst these, the problem of the influence on retention exerted by "uncapped" silanol groups on the RPLC stationary phase has been addressed by Unger and Chiang[4]. Hafkenscheid and Tomlinson[5] have highlighted the problem of defining an unambiguous physicochemical reference state for such basic solutes for which dynamic (RPLC) and static (*n*-octanol–water distribution) parameters are comparable. For instance, in making comparisons at physiological pH (*ca.* 7.4) at which bases are partially ionised, complications may arise from (i) effects of organic modifiers on pK_a values of solutes and the effective pH of the mobile phase, (ii) chromatography of solutes as ion-pair complexes with buffer anions and (iii) the fact that variations in anion concentration on the results of both methods (static and dynamic) differ considerably[4]. The reference state which is generally preferred for ionisable compounds is the non-ionised state. However, the use of mobile phases is restricted to pH values below *ca.* 8 for the alkyl-bonded silicas commonly used in RPLC. Hafkenscheid and Tomlinson[5] therefore employed a semi-empirical ion correction for solute ionisation on data determined under such conditions to predict retention data for the non-ionised bases.

In the present study, the feasibility was examined of using the styrene–divinylbenzene copolymeric stationary phase PRP-1 for the direct measurement of retention data of non-ionised organic bases suitable for the estimation of their lipophilic properties. This phase was chosen because (i) it is stable over a wide range of mobile phase pH, (ii) it contains no silanol groups, and (iii) it is said[6] to be especially well-suited for reversed-phase separations.

EXPERIMENTAL

The chromatographic system consisted of a Water Assoc. (Milford, MA, U.S.A.) M6000A dual-piston, reciprocating pump, a Rheodyne (Kotati, CA, U.S.A.) 7125 injection valve fitted with a 20-μl sample loop, a Schoeffel (Div. of Kratos, Westwood, NJ, U.S.A.) 770 variable-wavelength UV detector fitted with a 12-μl flow cell and operated at 230 nm, and a Shimadzu (Tokyo, Japan) Chromatopac CR1A computing integrator. Columns used were a 150 × 4.6 mm I.D. stainless-steel column packed with PRP-1 (Hamilton, Reno, NV, U.S.A.) and a 50 × 4.6 mm I.D. stainless-steel column packed with a spherical, totally porous, graphitised carbon microparticulate material (kindly donated by Professor J. H. Knox, Edinburgh University, U.K.). Mobile phases were made up by volumetrically mixing HPLC-grade methanol (Rathburn Chemical, U.K.) and 0.5 *M* sodium hydroxide solution, prepared from sodium hydroxide pellets, grade AR (Fisons, Loughborough, U.K.) and water, distilled and deionised before use. The pH of the aqueous component was measured at 13.24. This was chosen so that, even for the mobile phase with the highest proportion of methanol (90%) the effective mobile phase pH would still be well above the condition required [*i.e.* (pH − pK_a) > 2] for the solutes to be chromatographed principally in their nonionised form (the highest aqueous solute pK_a being 9.65 for alprenolol). A flow-rate of 2.0 ml/min was used throughout.

Sample solutions were prepared at a concentration of *ca.* 0.1 mg/ml by dissolving the bases in mobile phase. The sample solutions also contained sodium nitrite, grade AR (Fisons) at a concentration of *ca.* 0.03 mg/ml to act as a marker for the

retention time (t_0) of an unretained peak. All solutes were obtained from Sigma (St. Louis, MO, U.S.A.). Those obtained as a salt were converted to the free base by extraction, followed by evaporation of the organic (dichloromethane, HPLC-grade) layer. Log *P* data for the non-ionised bases were determined experimentally, using *n*-octanol and 0.5 *M* sodium hydroxide solution, by a modification of the method of Rummens *et al.*[7]. The modification involved measurement of the absorbance of the organic rather than the aqueous layer. Linear regression analysis was carried out by the programme Curve Fit on an Apple IIE microcomputer programmed in BASIC.

RESULTS AND DISCUSSION

Because PRP-1 is more retentive than most typical alkyl-bonded silicas[8] and the free bases are more lipophilic than the partially ionised forms which are more usually chromatographed, a mobile phase with a very high percentage of the organic component, *i.e.* methanol–0.5 *M* aqueous sodium hydroxide (90:10) was needed to elute all twelve bases. Although the samples were injected as the free base, it was demonstrated that identical capacity ratios were obtained when sample solutions, prepared by dissolving the salt form in mobile phase (and thus converting to the non-ionised base), were injected. There were no apparent deleterious effects on equipment arising from the use of this mobile phase. The column's performance was unimpaired by its use in this study.

The retention data of the free bases under these conditions were first correlated with the log *P* data at pH 14, calculated by Hafkenscheid and Tomlinson[5] (Fig. 1).

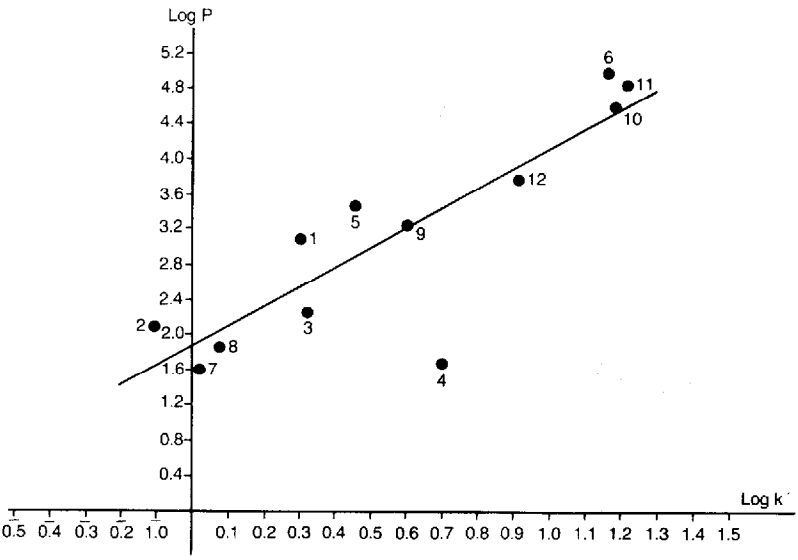

Fig. 1. "Ion-corrected" partition coefficients, from the literature[5], *vs.* logarithmic capacity ratios, log *k'*, on PRP-1 [mobile phase, methanol–0.5 *M* NaOH solution (90:10)] for the non-ionised bases. The solutes are (1) alprenolol, (2) naloxone, (3) lidocaine, (4) strychnine, (5) 1-propranolol, (6) amitriptyline, (7) clonidine, (8) procaine, (9) chlorpheniramine, (10) promethazine, (11) cyproheptadine, (12) orphenadrine. Correlation coefficient = 0.854, on omission of strychnine[4] = 0.957.

A correlation coefficient of 0.854 was obtained. In studies of this nature it has been common practice[5,9] to designate some points as outliers on a statistical basis. Omission of strychnine would improve the correlation coefficient to 0.957. However, elimination of outliers is of no value in the use of retention data as an indicator of partition coefficients, unless a method for predicting what type of compound would give rise to an anomalous result is available.

Because of the relatively small number of compounds used in this study, it was practicable to determine all the partition coefficients of the non-ionised bases. Correlation of the PRP-1 retention data with these experimentally determined partition coefficients (Fig. 2) was better than with the "ion-corrected" partition coefficients. The correlation coefficient was 0.906, and strychnine was no longer an obvious outlier.

Table I shows the data used to plot Figs. 1 and 2. Because of experimental differences between the determinations of the partition coefficients of the non-ionised bases in this study and the determinations of the partition coefficients which were subsequently "ion-corrected"[5], the absolute values of the experimental partition coefficients were different from the "ion-corrected" partition coefficients. However, importantly, both methods show a similar order of lipophilicities.

Graphitised carbon is another material used as a RPLC stationary phase which is stable at basic pH. The relationship between retention data on a graphitised carbon phase and partition coefficients of the non-ionised bases was also studied. The optimum mobile phase for reasonably short retention times was, as on the PRP-1 phase, methanol–0.5 *M* aqueous sodium hydroxide (90:10). Correlation of the capacity factors under these conditions with the experimentally determined partition coefficients was very poor (correlation coefficient, 0.216). It was thought that, for this stationary

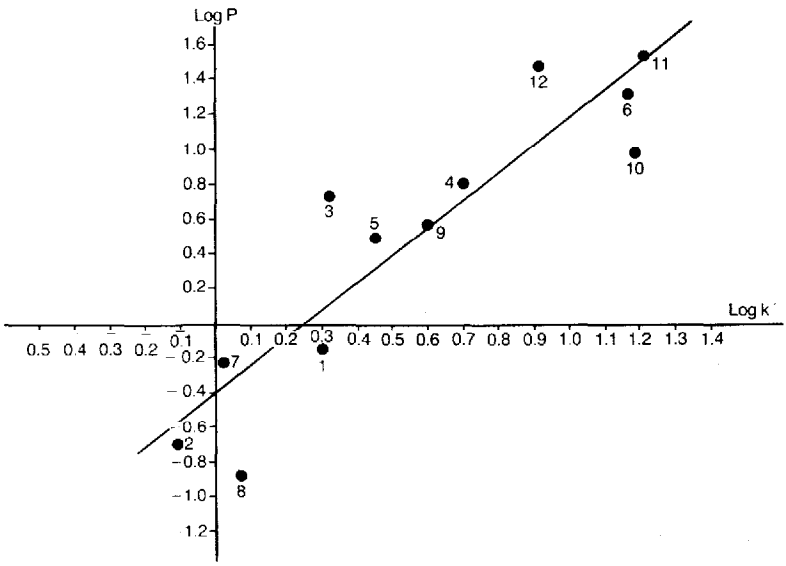

Fig. 2. Experimentally determined partition coefficients *vs.* logarithmic capacity ratios, log *k'*, on PRP-1 [mobile phase, methanol–0.5 *M* NaOH (90:10)] for the non-ionised bases. The solutes are numbered as in Fig. 1. Correlation coefficient = 0.906.

TABLE I

PHYSICOCHEMICAL PROPERTIES OF SOLUTES

Compound	*log k'**	$K_{d,oct}$**	$K_{d,oct}$***
Alprenolol	0.30	3.10	−0.150
Naloxone	−0.11	2.09	−0.700
Lidocaine	0.32	2.26	0.732
Strychnine	0.70	1.68	0.800
1-Propranolol	0.45	3.48	0.492
Amytryptiline	1.16	4.98	1.315
Clonidine	0.02	1.59	−0.223
Procaine	0.07	1.87	−0.880
Chlorpheniramine	0.60	3.25	0.570
Promethazine	1.18	4.59	0.965
Cyproheptadine	1.21	4.84	1.525
Orphenidrine	0.91	3.77	1.467

* On PRP-1 [mobile phase, methanol–0.5 *M* aqueous sodium hydroxide (90:10)].
** "Ion-corrected" partition coefficients.
*** Experimentally determined partition coefficients for the non-ionised form.

phase, the use of a more aqueous mobile phase might provide a closer model of an octanol–water partition system. However, the correlation did not improve up to an aqueous content in the mobile phase of 40%. At this point, retention times were unacceptably long. The correlation did not improve, even when the capacity factors were extrapolated to the 100% aqueous component mobile phase.

The conclusions that can be drawn from this study are as follows:

(i) graphitised carbon does not retain strong organic bases by a simple partition mechanism;

(ii) bearing in mind that lipophilicity parameters, once determined are usually subsequently correlated with biological data which are often subject to a relatively large error, the degree of correlation of the PRP-1 retention data with both the experimental and ion-corrected partition coefficients of non-ionised bases confirms that the semi-empirical corrections of Hafkenscheid and Tomlinson[5] can be used in assessing relative lipophilicities of the non-ionised form of bases;

(iii) most importantly, even on the basis of the limited number of compounds studied, it can be said that the determination of capacity factors on PRP-1 at very basic mobile phase pH values shows great promise as a simple, direct, and rapid method for the estimation of lipophilicity parameters of non-ionised organic bases.

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REFERENCES

1 J. L. G. Thus and J. C. Kraak, *J. Chromatogr.*, 320 (1985) 271.
2 N. El Tayar, H. van de Waterbeemd and B. Testa, *J. Chromatogr.*, 320 (1985) 305.
3 R. Kaliszan, *J. Chromatogr. Sci.*, 22 (1984) 362.
4 S. H. Unger and G. H. Chiang, *J. Med. Chem.*, 24 (1981) 262.
5 T. L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 292 (1984) 305.
6 Hamilton Bonaduz A.G., Switzerland, advertising literature (1981).
7 F. H. A. Rummens, R. F. Bujaczek, M. Peters and R. D. Green, *J. Pharm. Sci.*, 73 (1984) 231.
8 V. de Biasi, W. J. Lough and M. B. Evans, in preparation.
9 B. Rittich, M. Polster and O. Králik, *J. Chromatogr.*, 197 (1980) 43.