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A REVERSED-PHASE THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF RELATIVE PARTITION COEFFICIENTS OF VERY LIPOPHILIC COMPOUNDS

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SUMMARY

A reversed-phase thin-layer chromatographic method has been developed for the determination of partition coefficients. A support phase has been chosen, following investigation of the lack of adsorptive properties, which has a minimal effect on the pH of the buffer system. A stationary phase has been chosen to give ΔR_M values of the same magnitude as Hansch π values for a series of phenothiazines. The method can be applied to molecules of a wide range of lipophilicity following preliminary investigations of suitable phase-volume ratios and of the pH and composition of the binary mobile phase, providing adsorption on the support phase is excluded.

INTRODUCTION

The importance of the use of partition coefficients in quantitative structure–activity relationships (QSAR)[1] is now well established. The measurement of partition coefficients by equilibration methods is frequently made difficult or even impossible by the impurity or instability of the compound, by a strong preference of the compound for one of the two phases of the system or by the formation of stable emulsions after shaking. When measuring the partition coefficients of phenothiazines in 1-octanol–water[2] it was necessary to maintain a sufficiently high pH in the aqueous solution, in order to exclude the possibility of ion-pair extraction. This means that, due to the extreme hydrophobicity of these compounds, large amounts of drug are needed to obtain a concentration large enough for measurement in the aqueous solution. Impurities that are extracted to a much lesser degree than the compound itself can thus seriously influence the measurements (mostly ultraviolet absorption). Another problem that is met with the phenothiazines is their instability in aqueous solution.

These difficulties can be overcome by the use of partition chromatography. In a number of investigations (see, for example, refs. 3–7), reversed-phase thin-layer chromatography (TLC) has been applied in the measurement of the lipophilicity of compounds, using an adsorbent (Kieselgel, Kieselguhr, cellulose) as support for a non-aqueous stationary phase. R_M values obtained from partition chromatography

have been shown to be useful in QSAR studies[4,5,8,9]. However, there has been little or no agreement among investigators as to the most preferable chromatographic system of support, stationary phase and mobile phase. Amongst others, silicon oil[4,5,9], ethyl oleate[3], 1-octanol[8,10] and liquid paraffin[8] have been used as the stationary phase. The mobile phase normally consists of water, or a mixture of water with an organic solvent such as methanol or acetone.

The aim of the present work was the development of a reversed-phase TLC method in which no adsorption of the compounds on the support takes place, and which can be used even for highly lipophilic compounds, such as the phenothiazines, to yield ΔR_M values of the same magnitude as Hansch π values[11].

THEORY

Martin and Synge[12] and Consden *et al.*[13] derived a relationship between the R_F value in partition chromatography and the partition coefficient:

$$ {}_sP = \frac{V_M}{V_S} \cdot \left(\frac{1}{R_F} - 1\right) \tag{1} $$

where ${}_sP$ = partition coefficient* [= the concentration in the stationary phase (in mol/l) divided by the contration in the mobile phase (in mol/l)], V_M = volume of the mobile phase and V_S = volume of the stationary phase. Bate-Smith and Westall[14] introduced the symbol R_M:

$$ R_M = \log\left(\frac{1}{R_F} - 1\right) \tag{2} $$

On substituting eqn. 2 into eqn. 1 and rearranging, we obtain

$$ R_M = \log({}_sP) + \log r \tag{3} $$

where r is the phase-volume ratio, V_S/V_M, which is a constant for a given chromatographic system.

In reversed-phase chromatography, R_F values can be influenced by the pH* of the mobile phase**, when dealing with dissociable compounds. If, in the case of monoprotic organic bases, only the free non-protonated form of the base partitions between the mobile and stationary phases, and dissociation or association equilibria other than acid dissociation in the mobile phase are absent, then the apparent partition coefficient, ${}_sP_a$ (defined as the ratio of the molar concentrations of the compound in the stationary and in the mobile phase), is given in terms of the true partition coefficient[2], ${}_sP$, representing the ratio of the molar concentrations of the non-protonated

* The subscript indicates that a mixed solvent, such as a methanol–water, is involved. ${}_sP$ is thus the partition coefficient in, for instance, the system oleyl alcohol and a methanol–water mixture. If the polar phase is a pure solvent such as water, the subscript s is omitted.

** pH-meter readouts of measurements in methanol–water mixtures, after standardizing the meter against a methanol–water buffer solution of the same methanol content, are denoted by the symbol pH*.

base in both phases by ${}_sP_a = {}_sP \cdot$(fraction of drug as base); the value of (fraction of drug as base) is equal to ${}_sK_a^c/({}_sK_a^c + [H^+]_s)$, where ${}_sK_a^c$ is the concentration-dependent acid-dissociation constant (defined in terms of molal concentration) and $[H^+]_s$ is the molal concentration of protonated solvent. Thus, we obtain

$$ {}_sP_a = \frac{{}_sK_a^c}{{}_sK_a^c + [H^+]_s} \cdot {}_sP \tag{4} $$

When acid dissociation occurs, eqn. 3 should be written as

$$ R_M = \log({}_sP_a) + \log r \tag{5} $$

Combination of eqns. 4 and 5 gives

$$ R_M = \log({}_sP) + \log({}_sf) + \log r \tag{6} $$

where ${}_sf = {}_sK_a^c/({}_sK_a^c + [H^+]_s)$, the fraction of the drug present as the free base.

It is often necessary in reversed-phase chromatography to use mixtures of water and an organic solvent, *e.g.*, methanol or acetone, in order to obtain measurable R_F values. In the present work methanol–water mixtures were used. The $\log({}_sP)$ value of a compound in a ternary system of a non-aqueous stationary phase and a methanol–water mixture as the mobile phase is a linear function of the volume fractions of the two components in the mobile phase[15,16]. This function can be represented by

$$ \log({}_sP) = a + bC \tag{7} $$

where a and b are constants, and C is the methanol concentration. ${}_sP$ should be expressed here as the quotient of the mole fractions of the drug in the two phases, and C as the mole fraction of methanol in the two-component phase. Soczewiński and Matysik[17] discussed deviations from the $\log({}_sP)$–solvent composition relationship when $\log({}_sP)$ is defined as the quotient of the molar concentrations and when C is expressed in volume fractions. The ratio of the molar volumes of methanol and water is 2.25. Deviations from linearity due to the introduction into eqn. 7 of partition coefficients based on molar concentrations are considered negligible by Soczewiński and Matysik[17]. Expressing C in volume fractions (or volume percentages) instead of in mole fractions will cause more pronounced deviations from linearity. These are, however, often compensated by deviations from ideality of the mixed phase[5,15,17].

In the present work the volume percentage scale gives good results (see Fig. 4). Substitution of eqn. 7 in eqn. 6 yields

$$ R_M = a + bC + \log({}_sf) + \log r, $$

and, since at $C = 0$, $\log({}_sP) \equiv \log P = a$ (from eqn. 7), we obtain

$$ R_M = \log P + \log r + \log({}_sf) + bC \tag{8} $$

Eqn. 8, derived as above, shows how the R_M value of a basic compound is determined by its true partition coefficient, P, in the stationary phase–water system, by the phase-volume ratio, r^*, by the fraction, ${}_sf$, of the drug present as the free base and by the methanol concentration, C, in the mobile phase. It should be noted that eqn. 8 is valid only if a chromatographic system is used in which partitioning is the sole process. In the development of a chromatographic system suitable for the determination of relative partition coefficients, it should be determined that there is no adsorption on the support. Graphs of $R_M - \log({}_sf)$ against the methanol concentration, C, will result in a straight line of gradient b and intercept $\log P + \log r$. When $C = 0$, we can write

$$R_{M_w} = \log P + \log r \tag{9}$$

where R_{M_w} is the R_M value at zero methanol concentration under conditions where the compound exists only in the non-protonated form (high pH). ΔR_{M_w} values are thus equal to the $\Delta \log P$ values of the compounds in the system stationary phase–water.

EXPERIMENTAL

Materials

The hydrochlorides of promazine, chlorpromazine and triflupromazine were obtained from several commercial sources and recrystallised three times from isopropanol. The free bases of chlorpromazine and triflupromazine were obtained by adding alkali to aqueous solutions of the hydrochlorides, shaking the solution with freshly distilled dichloromethane (DCM), washing the DCM layer twice with water and evaporating the DCM layer under reduced pressure. Methylpromazine hydrochloride (2-methylpromazine), methopromazine maleate (2-methoxypromazine) and cyanopromazine base (2-cyanopromazine) were gifts from Rhone-Poulenc (Paris, France), and were used as supplied. Oleyl alcohol (Schuchardt, München, G.F.R.), containing 92–96% of *cis*-9-octadecen-1-ol, was distilled (135–140°; 0.05 mmHg) and passed through a column of basic aluminium oxide (Merck, Darmstadt, G.F.R.). The resulting product was colourless and odourless; the density at 25° was 0.845 g/ml.

Chemically pure methanol was treated with both silver nitrate and sodium hydroxide, then distilled twice. Liquid paraffin (26–35 cP, Brocacef, Maarsen, The Netherlands) was used without further purification (density, 0.858 g/ml at 25°). Chemically pure dioxane was freed from acid by passing it through a column of basic aluminium oxide (Merck). Light petroleum, chemically pure, b.p. 40–60°, was used as such. Distilled water was used throughout. Kieselguhr G (Merck), MN Kieselguhr N (Machery, Nagel & Co., Düren, G.F.R.), cellulose MN 300 (Machery, Nagel & Co.), cellulose AC (Machery, Nagel & Co.), aluminium oxide G (Merck) and Polyamid DC 11AC (Machery, Nagel & Co.) were used as supplied by the manufacturers. All of the other materials were reagent grade.

pH measurements were made with a Metrohm Präzisions pH meter (E 510)

* It is assumed that log r remains constant at different methanol concentrations, or changes linearly with the methanol concentration. In the latter case the value of the gradient, b, will change.

using a Metrohm (EA 121) combination glass electrode. The pH meter was standardized against methanol–water buffer solutions[18,19], containing the same amount of methanol as in the mobile phase.

TLC experiments

A non-aqueous stationary phase was obtained by impregnating the support either directly (Kieselguhr G) or indirectly (Kieselguhr G, MN Kieselguhr N, cellulose MN 300 and cellulose AC).

Direct impregnating method[20,21]. 24 g of Kieselguhr G were shaken during 90 sec with a mixture of x % (v/v) of oleyl alcohol or liquid paraffin, 7 ml of acetone and diluted with dioxane to 60 ml. Glass plates (20 × 20 cm) were coated with a 0.25-mm layer using standard equipment. The volatile components of the solvent were allowed to evaporate at room temperature for at least 16 h.

Indirect impregnating method. Glass plates (20 × 20 cm) were covered with a 0.25-mm layer of an aqueous slurry of the support. After drying for at least 16 h at room temperature, the support was impregnated by placing the plates in chromatographic chambers and developing with a solution of oleyl alcohol or liquid paraffin in light petroleum. 30 min after the solution had reached the top of the plates, the volatile components of the solution were allowed to evaporate at room temperature for at least 1 h. 0.3 % solutions of the compounds in methanol were prepared. 1 μl of the solutions was spotted on to the plates, in varying order, on a line 2 cm from the lower edge of the plate, at 1.5-cm intervals. Each phenothiazine was spotted twice on to the same plate. A migration of 10 cm on all of the plates was obtained by cutting the layer at 12 cm from the lower edge. Each plate was placed in a chromatographic chamber that had been equilibrated for 16 h with the mobile phase. The temperature was maintained at 25° throughout. The mobile phases were methanol–water buffer solutions, saturated with oleyl alcohol.

After development, the plates were sprayed with FPN reagent[22] (5 ml of 5% iron trichloride, 45 ml of 20 % perchloric acid and 50 ml of 50 % nitric acid). In each series of experiments (5–7 plates), one plate was included on which spots of cyanopromazine were applied at 2-cm intervals along a diagonal line, starting in one corner at the lower edge of the plate and ending in the opposite corner at the top of the plate. If the conditions during development do not change, then the spots resulting after development are situated on a straight line.

Choice of support

The support should have as weakly adsorbing properties as possible. Furthermore, when buffers of a certain, well defined, pH must be used as the mobile phase, the support should not markedly influence the pH of these buffers. In most investigations in reversed-phase TLC, silica gel has been used as the support. However, in a number of cases, as pointed out by Mercier[20], silica gel exerts disturbingly strong adsorption activity towards the compounds investigated. In addition, Bird and Marshall[10] also pointed out that silica gel can alter the pH of buffers. For these reasons, silica gel was excluded from the list of possible supports in the current investigations.

Six supports (Table I) were tested as to their influence on the pH* of three different buffer solutions. 3 g of support were shaken with 20 ml of buffer solution.

TABLE I

ΔpH* AFTER SHAKING IMPREGNATED AND NON-IMPREGNATED SUPPORTS WITH VARIOUS BUFFER SOLUTIONS

Buffers: a = 0.1 *m* NH_4Cl and NH_3 in 50% (w/w) methanol (pH* = 8.12); b = a three-fold dilution of buffer a (pH* = 8.19); c = 0.025 *m* Na_2HPO_4 and 0.025 *m* KH_2PO_4 in water (pH = 6.88); d = 0.1 *m* NH_4Cl and NH_3 in 50% (w/w) methanol (pH* = 7.00); e = 0.1 *m* NH_4Cl and NH_3 in 10% (w/w) methanol (pH* = 8.00); f = 0.1 *m* NH_4Cl and NH_3 in 10% (w/w) methanol (pH* = 9.00). Buffers d and e have the lowest capacity of the series.

Support	*a*	*b*	*c*	*d*	*e*	*f*
Cellulose MN 300	−0.12	−0.18	−0.10			
+ 5% oleyl alcohol	−0.10	−0.15				
+ 10% oleyl alcohol	−0.08			−0.20	−0.12	−0.05
+ 5% paraffin	−0.09					
+ 10% paraffin	−0.08			−0.43	−0.16	−0.05
Cellulose AC	−0.09	−0.17	−0.07			
+ 5% oleyl alcohol	−0.10	−0.12				
+ 10% oleyl alcohol	−0.10			−0.40	−0.15	−0.07
+ 5% paraffin	−0.11					
+ 10% paraffin	−0.08			−0.35	−0.21	
Kieselguhr G	−0.02	0.04	−1.33			
+ 5% oleyl alcohol	−0.01	−0.01		0.04		
+ 10% oleyl alcohol	−0.06	−0.05		0.06	0.00	0.00
+ 5% paraffin	−0.01	0.00				
+ 10% paraffin	−0.02			0.00	0.00	
MN Kieselguhr N	−0.02	0.01	0.07			
+ 5% oleyl alcohol	−0.01	0.00		−0.03		
+ 10% oleyl alcohol	−0.07	−0.04		−0.02	0.00	0.00
+ 5% paraffin	−0.01					
+ 10% paraffin	0.00			0.05	0.00	
Aluminium oxide G	0.20					
Polyamid DC 11AC	−0.37					

After standing for 24 h and centrifugation, the pH* of the supernatant liquid was measured. The ΔpH* values (pH* before shaking minus pH* after shaking) are shown in Table I. Both types of Kieselguhr seemed to be most suitable, when phosphate buffers are excluded. The two types of cellulose also seemed suitable for further evaluation. Layers of directly impregnated Kieselguhr G plates [5 or 10% (v/v) of oleyl alcohol or liquid paraffin] and of indirectly impregnated plates [Cellulose MN 300, Cellulose AC, MN Kieselguhr N, also with 5 or 10% (v/v) of oleyl alcohol or liquid paraffin] were scraped off the plates and 3–6 g of the layer was shaken vigorously for 2 min with 8–11 ml of a buffer solution. After centrifugation, the pH* of the supernatant liquid was measured. The ΔpH* values are also shown in Table I.

Obviously, for experiments in which the pH* of the mobile phase should be constant during the chromatographic process, Kieselguhr G and MN Kieselguhr N are the most suitable supports. Two restrictions must be made: a minimum buffer capacity is required, and the support should be impregnated with less than 10% (v/v) of oleyl alcohol in the impregnating mixture.

The absence of adsorption of the phenothiazines on the two types of Kieselguhr was checked by spotting 1-μl amounts of solutions of the phenothiazines on plates (covered with a 0.25-mm layer of non-impregnated Kieselguhr) and developing

the plates with methanol–water mixtures of pH* = 8–11 and methanol concentrations of 30–50% (w/w). R_F values were always higher than 0.8 and usually exceeded 0.9, indicating only weak adsorption of the phenothiazines on Kieselguhr. More evidence for the minimal adsorption is given below.

The method of impregnation

When using the same methanol–water buffers as mobile phase, directly impregnated plates of Kieselguhr G yielded reproducible R_F values. Spots of cyanopromazine, applied on a diagonal line, always lay on a perfectly straight line after development. Differences in the R_M values of the phenothiazines were the same for all of the directly and indirectly impregnated plates. However, cyanopromazine spots applied diagonally on indirectly impregnated plates sometimes did not lie on a straight line after development. Consequently, directly impregnated plates of Kieselguhr G were used for all further experiments. Impregnation of the plates by immersion was not tried, since the thin layer is reported to be damaged by this method[23].

The choice of the stationary phase

Hansch and his co-workers strongly recommend[24–28] the use of the 1-octanol–water system for the determination of partition coefficients to be used in QSAR studies. It is the system which is most often used, and with which the largest number of determinations containing the widest selection of functional groups have been made. However, 1-octanol is not always suitable as the stationary phase in liquid–liquid partition chromatography. Many drugs are so lipophilic, that when plates impregnated with 1-octanol are developed with aqueous solutions the applied compounds hardly move from the starting point. The normal procedure is to add an amount of organic solvent (methanol, acetone) to the mobile phase. By measuring the R_F values (and R_M) at different concentrations of the organic solvent, the R_{M_W} values, directly comparable with log P, can be obtained by extrapolation (see eqns. 8 and 9). However, an appreciable amount of 1-octanol will be dissolved in methanol–water mixtures containing *ca.* 30% (v/v) or more of methanol. Therefore, 1-octanol can only be used as the stationary phase when dealing with relatively hydrophilic drugs. The phenothiazines are very lipophilic, so that 1-octanol cannot be used. Collander[29] showed the relationship between the partition coefficient in two solvent systems to be

$$\log P_2 = a \cdot \log P_1 + b \tag{10}$$

This relationship holds very well, even when hydrogen-donor and hydrogen-acceptor solutes[30] are included, if similar solvent systems such as isobutanol–water, isopentanol–water, 1-octanol–water and oleyl alcohol–water are compared. Oleyl alcohol is a more apolar solvent than 1-octanol; it is less miscible with methanol–water mixtures than 1-octanol [concentrations as high as 60% (w/w) of methanol can be used without causing dissolution of large amounts of oleyl alcohol] and the relationship between log $P_{\text{oleyl alcohol}}$ and log P_{octanol} is expressed by[30]

$$\log P_{\text{oleyl alcohol}} = 0.999 \cdot \log P_{\text{octanol}} - 0.575$$

TABLE II

$1/R_F$ AND R_M VALUES FOR 0.5–5% (v/v) OLEYL ALCOHOL IN THE IMPREGNATING MIXTURE

Compound	*Oleyl alcohol (%, v/v)*													
	0.5		*1*		*1.5*		*1.75*		*2.25*		*3*		*5*	
	$1/R_F$	R_M	$1/R_F$	R_M	$1/R_F$	R_M	$1/R_F$	R_M	$1/R_F$	R_M	$1/R_F$	R_M	$1/R_F$	R_M
Cyanopromazine	1.22	−0.644	1.33	−0.492	1.46	−0.338	1.52	−0.283	1.62	−0.209	1.84	−0.078	2.50	0.174
Promazine	1.29	−0.543	1.42	−0.377	1.55	−0.262	1.72	−0.141	1.80	−0.098	2.11	0.046	2.95	0.290
Chlorpromazine	1.72	−0.140	2.30	0.111	2.75	0.240	3.22	0.345	3.62	0.418	4.54	0.549	7.13	0.787

Thus, $\Delta\log P_{\text{oleyl alcohol}}$ (and ΔR_{M_W}) values should be practically the same as $\Delta\log P_{\text{octanol}}$ (π) values. Oleyl alcohol was consequently chosen as the stationary phase in all further experiments.

Concentration and nature of the applied compounds

No change in R_F values occurred when the amount of applied substance was increased three-fold. Also the salt and free base gave similar R_F values.

RESULTS AND DISCUSSION

With the support, the stationary phase, the method of impregnation and the nature of the mobile phase fixed, R_F values are still dependent on the phase-volume ratio, r, and on the pH* and methanol concentration in the mobile phase (see eqn. 8). As already stated, R_{M_W} values of phenothiazines had to be obtained by measuring R_F values at different methanol concentrations in the mobile phase, and by extrapolating the plots of $R_M - \log(_sf)$ against C to zero methanol concentration. The accuracy with which R_{M_W} values can be calculated is best when the methanol concentrations are as near as possible to zero. Therefore the phase-volume ratio, r, and the pH* of the mobile phase should be as low as possible. The influence of both factors on R_F and R_M was investigated further.

The phase-volume ratio, r

Glass plates were coated in the usual direct way with Kieselguhr G suspended in impregnating mixtures containing varying amounts of oleyl alcohol. The following oleyl alcohol concentrations in the impregnating mixtures were used: 0.5, 1, 1.5, 1.75, 2.25, 3 and 5% (v/v). 4–8 plates of each concentration were developed with 0.1 *m* ammonium chloride and ammonia in 50% (w/w) methanol–water (pH* = 7.76). The

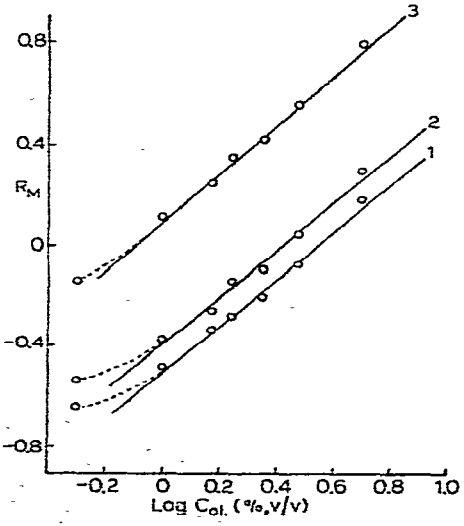

Fig. 1. Effect of the concentration of oleyl alcohol ($C_{ol.}$) in the impregnating mixture on R_M values of cyanopromazine (1), promazine (2) and chlorpromazine (3).

resulting mean values for $1/R_F$ and R_M are shown in Table II for three of the phenothiazines at each concentration of oleyl alcohol. It is a reasonable assumption that the phase-volume ratio, r, is a linear function of the concentration of oleyl alcohol $C_{ol.}$, in the impregnating mixture:

$$r = k \cdot C_{ol.} \tag{11}$$

Combination of eqns. 11 and 6 gives:

$$R_M = \log({}_sP) + \log({}_sf) + \log k + \log C_{ol.} \tag{12}$$

Thus, when the R_M values of a certain compound are plotted against $\log C_{ol.}$ a straight line with a slope equal to 1 and an intercept of $\log({}_sP) + \log({}_sf) + \log k$ should result. As can be seen (Fig. 1), straight lines were obtained for oleyl alcohol concentrations between 1 and 5% (v/v). 0.5% (v/v) of oleyl alcohol in the impregnating mixture caused R_M values of two of the compounds to be too high. It was concluded that the amount of oleyl alcohol in the impregnating mixture should be at least 1% (v/v). In all further experiments a concentration of 1.25% (v/v) of oleyl alcohol was used.

From eqn. 12, we obtain

$$\frac{1}{R_F} = {}_sP \cdot {}_sf \cdot k \cdot C_{ol.} + 1 \tag{13}$$

If the theory, as it is presented above, is valid, then only a partitioning process takes place and graphs of $1/R_F$ against $C_{ol.}$ should be straight lines with intercepts of one and slopes of ${}_sP \cdot {}_sf \cdot k$. Graphs for three phenothiazines are shown in Fig. 2. In Table III the slopes and intercepts are given for the graphs according to eqns. 12 and 13. From the fact that the slopes of the graphs in Fig. 2 are close to 1, and the intercepts

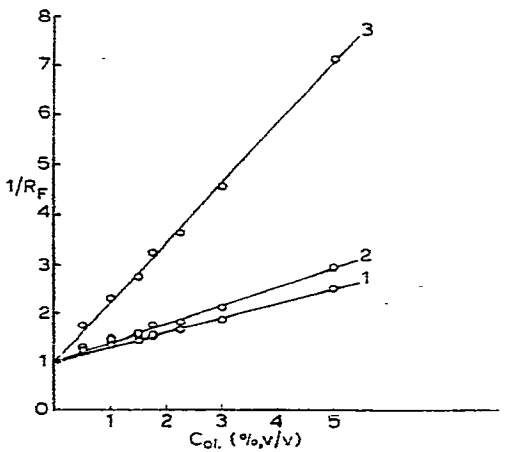

Fig. 2. Effect of the concentration of oleyl alcohol ($C_{ol.}$) in the impregnating mixture on $1/R_F$ values. For details see Fig. 1.

TABLE III

CALCULATED VALUES OF SLOPES AND INTERCEPTS OF THE GRAPHS IN FIGS. 1 AND 2

n is the number of determinations; S_a and S_b are the standard deviations of a and b respectively.

Compound	*General equation:* $R_M = a + b \cdot \log C_{ol}$					*General equation:* $1/R_F = a + b \cdot C_{ol}$			
	a	S_a	b	S_b	n	a	S_a	b	S_b
Cyanopromazine	−0.510	0.008	0.943	0.018	84	0.999	0.018	0.294	0.006
Chlorpromazine	0.091	0.007	0.976	0.015	86	0.971	0.051	1.219	0.017
Promazine	−0.403	0.008	0.964	0.018	82	0.988	0.020	0.388	0.007

of the graphs in Fig. 3 are practically equal to 1, it is concluded that, under the prevailing conditions, only partitioning of the compounds between the stationary phase and the mobile phase takes place, without any adsorption at the support.

The mobile phase

Since R_F values of the phenothiazines are not measurable when aqueous buffer solutions are used as the mobile phase, an organic solvent had to be added. Methanol was chosen because a large number of reliable physical constants have been collected for methanol–water mixtures. In order to check the influence of the nature of the buffer, 50% (w/w) methanol–water buffer solutions were prepared, containing ammonium chloride, bromide, nitrate and sulphate, respectively, all with ionic strength 0.1 m and adjusted to pH* = 7.90 with ammonia. Use of these buffer solutions as the mobile phase always yielded small round spots of the phenothiazines after detection. R_F values for the same compound did not change when different anions were present in the mobile phase. When a borax buffer of the same methanol concentration, pH* and ionic strength was used, the resulting spots were elongated and the R_F values were too high. A 0.1 m solution of ammonium bicarbonate in

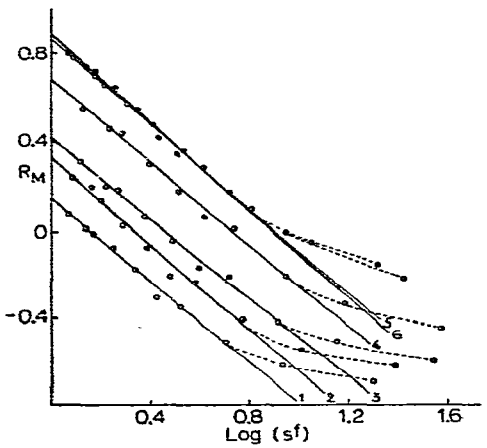

Fig. 3. Effect of pH* and ${}_sK_A^c$ on R_M values of cyanopromazine (1), methopromazine (2), promazine (3), methylpromazine (4), chlorpromazine (5) and triflupromazine (6, ●).

TABLE IV

R_M AND $\log(_sf)$ AT DIFFERENT pH* VALUES OF THE MOBILE PHASE [50% (w/w) METHANOL–WATER], AND THE CALCULATED SLOPES AND INTERCEPTS OF THE GRAPHS IN FIG. 3

a and b are the intercept and slope, respectively, of the general equation: $R_M = a + b \cdot \log(_sf)$; S_a and S_b are the standard deviations of a and b respectively; n is the number of determinations.

Compound	*pH**									
	6.99		*7.39*		*7.66*		*7.90*		*8.05*	
	R_M	$\log(_sf)$	R_M	$\log(_sf)$	R_M	$\log(_sf)$	R_M	$\log(_sf)$	R_M	$\log(_sf)$
Cyanopromazine	−0.695	−1.304	−0.617	−0.936	−0.513	−0.709	−0.344	−0.528	−0.302	−0.248
Methopromazine	−0.615	−1.387	−0.544	−1.013	−0.406	−0.775	−0.233	−0.586	−0.197	−0.480
Promazine	−0.599	−1.545	−0.511	−1.163	−0.416	−0.921	−0.206	−0.717	−0.160	−0.600
Methylpromazine	−0.445	−1.574	−0.330	−1.191	−0.198	−0.947	0.015	−0.742	0.068	−0.623
Chlorpromazine	−0.210	−1.418	−0.045	−1.043	0.106	−0.808	0.292	−0.615	0.345	−0.507
Trifluprom azine	−0.145	−1.319	0.003	−0.950	0.181	−0.717	0.366	−0.535	0.419	−0.434

50% (w/w) methanol–water (pH* = 8.05) gave lower R_F values for the phenothiazines than a 0.1 *m* solution of ammonium chloride and ammonia of the same methanol concentration and pH*; the resulting spots were small and round in both cases.

Apparently a certain amount of free ammonia in the mobile phase is needed to obtain round spots and consistent R_F values, whereas the nature of the counter-ions does not seem to influence the results. Murthy and Zografi[2] assumed that apparent partition coefficients for chlorpromazine in the system 1-octanol–water (at pH* = 6.6 and higher pH* values) are due essentially to the free base. At pH* = 6.6 in an aqueous buffer solution, 0.14% of the drug is present in the unprotonated form. At pH* = 7.9 in 50% (w/w) methanol–water, 24% of the drug is present as the free base. The fact that partitioning in the present experimental conditions did not depend on the counter-ion supports the statements of Murthy and Zografi[2]. In all further experiments, 0.1 *m* buffer solutions of ammonium chloride and ammonia in methanol–water mixtures were used.

The pH of the mobile phase*

The pH* range for which eqn. 6 holds was determined, using plates coated

TABLE V

R_M AND $\log(_sf)$ AT DIFFERENT METHANOL CONCENTRATIONS AND pH* OF THE MOBILE PHASE

Compound	*Percentage (v/v) methanol (pH*)*									
	36.20	*(7.85)*	*39.04*	*(7.83)*	*41.85*	*(7.81)*	*44.62*	*(7.79)*	*47.36*	*(7.77)*
	R_M	$\log(_sf)$	R_M	$\log(_sf)$	R_M	$\log(_sf)$	R_M	$\log(_sf)$	R_M	$\log(_sf)$
Cyanopromazine	0.263	−0.970	0.142	−0.908	0.039	−0.870	−0.072	−0.827	−0.135	−0.798
Methopromazine	0.421	−0.988	0.285	−0.952	0.176	−0.913	0.082	−0.887	−0.017	−0.873
Promazine	0.398	−1.189	0.290	−1.134	0.181	−1.102	0.062	−1.066	−0.012	−1.041
Methylpromazine	0.699	−1.171	0.579	−1.125	0.471	−1.093	0.330	−1.057	0.242	−1.046
Chlorpromazine							0.672	−0.940	0.571	−0.906
Trifluprom azine							0.808	−0.827	0.703	−0.798

8.20		8.38		8.59		8.70		9.02						
R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	a	S_a	b	S_b	n
−0.171	−0.340	−0.073	−0.252	−0.007	−0.171	0.014	−0.139	0.077	−0.072	0.147	0.007	0.943	0.017	72
−0.068	−0.385	0.033	−0.289	0.140	−0.199	0.205	−0.162	0.245	−0.085	0.327	0.008	0.972	0.019	72
−0.041	−0.493	0.068	−0.380	0.187	−0.270	0.205	−0.222	0.313	−0.121	0.416	0.008	0.905	0.014	71
0.181	−0.514	0.305	−0.397	0.439	−0.284	0.459	−0.235	0.551	−0.128	0.678	0.009	0.929	0.016	72
0.478	−0.409	0.569	−0.309	0.659	−0.214	0.705	−0.175	0.785	−0.092	0.865	0.007	0.953	0.016	72
0.544	−0.346	0.649	−0.256	0.718	−0.175	0.746	−0.141	0.798	−0.073	0.882	0.007	0.982	0.016	72

with Kieselguhr G and 1.25% (v/v) of oleyl alcohol. In the first series of experiments the mobile phase consisted of 0.1 *m* solutions of ammonium chloride and ammonia in 50% (w/w) methanol–water mixtures of pH* values varying between 7.0 and 9.0. 4–6 plates were developed with each mobile phase. A graph of R_M *versus* $\log({}_sf)$ should, according to eqn. 6, result in a straight line of slope equal to 1 and intercept of $\log({}_sP) + \log r$. ${}_sK_a^c$ values for the six phenothiazines were determined previously[31]. $[H^+]_s$ values were derived from the pH* using activity coefficients calculated from the extended Debye-Hückel equation[32], with the necessary constants from refs. 33–35. $\text{Log}({}_sf)$ values and the mean R_M values are shown in Table IV. Graphs of R_M *versus* $-\log({}_sf)$ for the six phenothiazines are shown in Fig. 3. For $\log({}_sf)$ values, belonging to pH* values higher than 7.6, the graphs were straight lines. The values for the slopes and intercepts are also shown in Table IV. In a second series of experiments, a number of plates on which cyanopromazine was spotted were developed with 0.1 *m* solutions of ammonium chloride and ammonia in 30% (w/w) methanol–water mixtures with pH* values between 7.4 and 8.4. In this case, the graph of R_M against $\log({}_sf)$ was a straight line for $\log({}_sf)$ values belonging to pH* values of 7.8 and higher.

50.06	(7.76)	52.73	(7.74)	55.37	(7.71)	57.98	(7.70)	60.53	(7.69)	63.03	(7.68)
R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$	R_M	$\log({}_sf)$
−0.225	−0.751	−0.321	−0.729	−0.443	−0.709	−0.512	−0.675				
−0.108	−0.826	−0.221	−0.803	−0.331	−0.783	−0.438	−0.745	−0.551	−0.714		
−0.106	−0.992	−0.198	−0.959	−0.315	−0.929	−0.411	−0.883	−0.533	−0.839		
0.139	−0.992	0.028	−0.968	−0.097	−0.947	−0.201	−0.909	−0.322	−0.882	−0.391	−0.845
0.446	−0.860	0.324	−0.837	0.175	−0.817	0.099	−0.772	−0.029	−0.747	−0.121	−0.719
0.563	−0.751	0.435	−0.729	0.271	−0.717	0.199	−0.688	0.047	−0.659	−0.060	−0.633

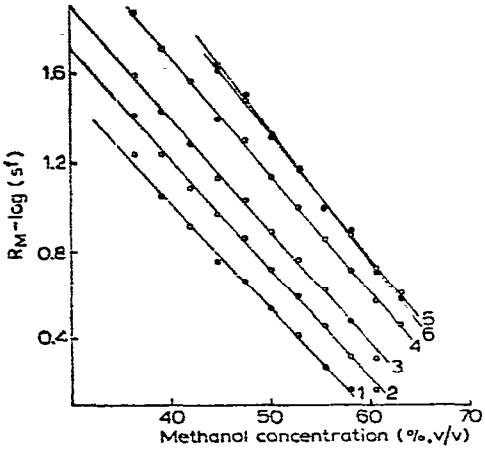

Fig. 4. Effect of methanol concentration in the mobile phase on $R_M - \log({}_sf)$. For details see Fig. 3

Determination of R_{M_w} and $\Delta\log P$ values

Mobile phases with varying methanol concentrations and pH* values were selected in the following manner. From the preceding section it follows that the pH* of a mobile phase containing 57.98% (v/v) (*i.e.*, 50%, w/w) of methanol should be 7.6 or higher, and the pH* of a mobile phase containing 36.20% (v/v) (*i.e.*, 30%, w/w) of methanol should be 7.8 or higher. pH* values of 7.70 and 7.85 were used for mobile phases containing 57.98 and 36.20% (v/v) of methanol respectively. Promazine was chosen as the reference substance. If $\log({}_sf)$ for promazine varies linearly with the methanol concentration of the mobile phase according to

$$\log({}_sf) = a + bC \tag{14}$$

TABLE VI

CALCULATED SLOPES AND INTERCEPTS OF THE GRAPHS IN FIG. 4, AND THE DERIVED ΔR_{M_w} VALUES

a and b are the intercept and slope, respectively, of the general equation: $R_M - \log({}_sf) = a + bC$; $a = R_{M_w} = \log P + \log r$ (eqn. 8). S_a and S_b are the standard deviations of a and b respectively; n is the number of determinations.

Compound	a	S_a	b	S_b	n	ΔR_{M_w}	$\log P_{octanol}$*	π**
Cyanopromazine	2.944	0.026	−0.0483	0.0005	118	−0.48	4.18	−0.57
Methopromazine	3.194	0.026	−0.0497	0.0005	128	−0.23	4.73	−0.02
Promazine	3.427	0.025	−0.0509	0.0005	128	0	4.75	0
Methylpromazine	3.778	0.020	−0.0529	0.0004	134	0.35	5.31	0.56
Chlorpromazine	4.115	0.035	−0.0560	0.0007	76	0.69	5.46	0.71
Triflupromazine	4.238	0.038	−0.0582	0.0007	76	0.81	5.63	0.88

* $\log P_{octanol}$ values were calculated, from the $\log P_{octanol}$ value for chlorpromazine as calculated by Leo *et al.*[30].

** Values for π are taken from ref. 11.

then inserting in eqn. 14 the proper values for ${}_sf$ at $C = 57.98$ and 36.20% (v/v) yields two equations, from which a and b were solved to give

$$\log({}_sf) = -1.698 + 0.0140 \cdot C \quad (15)$$

With the use of eqn. 15, pH* values were calculated at each methanol concentration. 4–8 plates [1.25% (v/v) of oleyl alcohol] were developed with each mobile phase. Mean R_M values and $\log({}_sf)$ values for the six phenothiazines are shown in Table V. Graphs of $R_M - \log({}_sf)$ against C (see eqn. 8) for the six phenothiazines, yielding straight lines with slopes of b and intercepts of $R_{M_W} = \log P + \log r$, are shown in Fig. 4. Values of the slopes and intercepts are shown in Table VI. ΔR_{M_W} values, obtained by subtracting the R_{M_W} value for promazine from the R_{M_W} value of each phenothiazine, represent the changes in $\log P$ (in the system oleyl alcohol–water) due to the substituents at position 2 of the phenothiazine ring structure. These ΔR_{M_W} values were compared with the hydrophobic substituent constants, π, as defined by Hansch *et al.*[11]. In Table VI values are shown for ΔR_{M_W}, π and the calculated values for $\log P$ (1-octanol–water). The correlations are given by

$$\pi = 1.034 \cdot \Delta R_{M_W} + 0.076 \quad (n = 5, r = 0.977, s = 0.147)$$

$$\log P_{\text{octanol}} = 1.036 \cdot R_{M_W} + 1.263 \quad (n = 6, r = 0.977, s = 0.132$$

where n is the number of compounds, r is the correlation coefficient and s is the standard deviation. The slopes are in good agreement with the value of 1.0 given by Leo *et al.*[30].

The method developed here has also been used successfully for measuring ΔR_{M_W} values for a series of barbiturates and benzodiazepines.

REFERENCES

1 C. Hansch and W. J. Dunn, *J. Pharm. Sci.*, 61 (1972) 1.
2 K. S. Murthy and G. Zografi, *J. Pharm. Sci.*, 59 (1970) 1281.
3 L. S. Bark and R. J. T. Graham, *J. Chromatogr.*, 23 (1966) 417.
4 G. L. Biagi, A. M. Barbaro, M. C. Guerra, G. C. Forti and M. E. Fracasso, *J. Med. Chem.*, 17 (1974) 28.
5 J. D. Turnball, G. L. Biagi, A. J. Merola and D. G. Cornwell, *Biochem. Pharmacol.*, 20 (1971) 1383.
6 M. C. Bonjean, J. Alary and M. C. Luu Duc, *Chim. Ther.*, (1973) 93.
7 J. C. Dearden, A. M. Patel and J. H. Tubby, *J. Pharm. Pharmacol.*, 26 (1974) 74P.
8 J. C. Dearden and E. Tomlinson, *J. Pharm. Pharmacol.*, 24 (1972) 115P.
9 G. L. Biagi, M. C. Guerra and A. M. Barbaro, *Farmaco, Ed. Sci.*, 25 (1970) 755.
10 A. E. Bird and A. C. Marshall, *J. Chromatogr.*, 63 (1971) 313.
11 C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani and E. J. Lien, *J. Med. Chem.*, 16 (1973) 1207.
12 A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358.
13 R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, 38 (1944) 224.
14 E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, 4 (1950) 427.
15 E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
16 W. Kemula and H. Buchowski, *Rocz. Chem.*, 29 (1955) 718; *C.A.*, 50 (1956) 6147e.
17 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 32 (1968) 458.

18 R. G. Bates, *Determination of pH*, Wiley, New York, 2nd ed., 1973, p. 249.
19 R. G. Bates, M. Paabo and R. A. Robinson, *J. Phys. Chem.*, 67 (1963) 1833.
20 M. Mercier, *Complexes par transport de charge et coefficients de partage des Phenothiazines*, University Press of Louvain (Belgium).
21 M. Mercier and P. Dumont, *Int. Symp. V of Chromatography Electrophoresis, September 1968*, Brussels, Presses Académiques Européennes, Brussels, 1969, p. 491.
22 L. S. Forrest and F. M. Forrest, *Clin. Chem.*, 6 (1960) 11.
23 G. L. Biagi, A. M. Barbaro, M. F. Gamba and M. C. Guerra, *J. Chromatogr.*, 41 (1969) 371.
24 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*, 86 (1964) 5175.
25 J. Iwasa, T. Fujita and C. Hansch, *J. Med. Chem.*, 8 (1965) 150.
26 C. Hansch, A. R. Steward, S. M. Andersen and D. Bentley, *J. Med. Chem.*, 11 (1968) 1.
27 A. Leo and C. Hansch, *J. Org. Chem.*, 36 (1971) 1539.
28 R. N. Smith, C. Hansch and M. M. Ames, *J. Pharm. Sci.*, 64 (1975) 599.
29 R. Collander, *Acta Chem. Scand.*, 5 (1951) 774.
30 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
31 A. Hulshoff and J. H. Perrin, *Pharm. Acta Helv.*, in press.
32 R. G. Bates, *Determination of pH*, Wiley, New York, 2nd ed., 1973, p. 216.
33 *Tabellenboekje ten Dienste van Laboratoria*, D.B. Centen, Hilversum, 18th Impression, 1962, p. 351.
34 P. S. Albright and L. J. Gosting, *J. Amer. Chem. Soc.*, 68 (1946) 1061.
35 A. N. Martin, J. Swarbrick and A. Cammarata, *Physical Pharmacy*, Lea & Febiger, Philadelphia, 2nd ed , 1969, p. 186.