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Determination of lipophilicity by means of reversed-phase thin-layer chromatography

I. Basic aspects and relationship between slope and intercept of TLC equations

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ABSTRACT

The main aspects of the authors' chromatographic work are reviewed. The determination of lipophilicity by means of TLC techniques is mainly based on the linear relationship between R_M values and organic solvent concentrations in the mobile phase, as described by the TLC equation. The very good correlation between experimental and extrapolated R_M values supports the validity of the extrapolation technique. Another interesting aspect is that the nature of the organic solvent does not affect the measurement of lipophilicity. However, the main purpose of this paper was to re-examine all the TLC equations in order to assess whether the relationship between intercepts and slopes is a basic feature of the chromatographic determination of lipophilicity. The analysis of more than 700 TLC equations showed that the above relationship holds only when dealing with series of strictly congeneric compounds. The structural meaning of chromatographic congenerity is discussed.

INTRODUCTION

Lipophilic character often seems to be the most important physico-chemical parameter in accounting for the variations of biological activity within series of chemical agents. As an expression of the lipophilic character of a given compound, its partition coefficient, P, between an aqueous and a non-aqueous phase, can be used. The partitioning between water and *n*-octanol, as proposed by Hansch, has been established as the reference system, with log P defined as the logarithm of the ratio of concentration of a neutral, non-ionized substance, in *n*-octanol to that in water [1]. Boyce and Milborrow [2] suggested using the chromatographic R_M value in order to avoid the practical difficulties that often arise in the direct determination of the partition coefficient. The R_M value can be shown to be related to the logarithm of the partition coefficient between the polar mobile phase and the non-polar stationary phase of a TLC system [3]. In our laboratory we have been using the R_M values as measured by means of a reversed-phase TLC system, where the mobile phase is represented by aqueous buffers alone or in various proportions with acetone, acetonitrile or methanol, and the non-polar stationary phase is a silica

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gel G layer impregnated with silicone oil [4,5].

The determination of lipophilicity by means of the chromatographic technique is mainly based on the linear relationship between the R_M values and the organic solvent concentrations in the mobile phase. In fact, from the linear equations a theoretical R_M value at 0% organic solvent in the mobile phase can be calculated even for those compounds which do not migrate with an aqueous buffer alone. For the more hydrophilic compounds, which migrate even at 0% organic solvent, one can measure an experimental R_M value and calculate an extrapolated value. The very good correlation between experimental and extrapolated R_M values supports the validity of the extrapolation technique [4].

Another interesting point is that the nature of the organic solvent does not affect the measurement of the lipophilic character. In fact, the extrapolated R_M values are the same whether the organic solvent of the mobile phase is acetone, acetonitrile or methanol [4]. The above features of the TLC technique contributed to establishing the reliability of the R_M values as lipophilicity parameters. Their usefulness was finally supported by the very good correlations between R_M and log P values [4,5]. Similar relationships between log k' and log P values have been shown in reversed-phase HPLC [4,6–11]. In fact, log k' values are currently used as a measure of lipophilicity [12]. Attention has been drawn by several workers [13–19] to the relationship between intercepts and slopes of the equations relating the capacity factor (log k') to the composition of the mobile phase (percentage of organic modifier). As regards the TLC system, this point was taken into consideration by Kuchar and co-workers [20,21] and Cserháti [22].

The purpose of this paper is to review the main aspects of our chromatographic work and re-examine all our TLC equations in order to assess whether the relationship between intercepts and slopes is a basic feature of the chromatographic determination of lipophilicity. For the present study, the data provided by recent chromatographic investigations on series of naphthalenes and quinolines, 4-nitropyrazoles and 1,4-dihydropyridines and some unpublished data on prostaglandins were also used.

EXPERIMENTAL

The reversed-phase TLC technique was described previously [23]. The details of the chromatographic determination of the R_M values for the recently investigated series of naphthalenes and quinolines, 4-nitropyrazoles and 1,4-dihydropyridines will be described in a further paper. In any case, for these classes of compounds and for all others listed in Table I a non-polar stationary phase was obtained by impregnating a silica gel GF_{254} layer (E. Merck, Darmstadt, F.R.G.) with silicone DC 200 (350 cSt) from Applied Science Labs. (State College, PA, USA). The choice of silicone oil as impregnating agent was initially made because most organic substances can be detected on siliconized silica G by charring with an alkaline solution of potassium permanganate. This offers the possibility of detecting very different classes of compounds without the need for any specific reagent [23]. The impregnation was carried out by developing the plates in a 5% silicone solution in diethyl ether. The mobile phases, saturated with silicone, were water or aqueous buffers alone or mixed with various amounts of acetone, acetonitrile or methanol. The pH of the mobile phase was chosen on the basis of the ionization profiles of the test compounds, in order to measure, whenever possible, the R_M value of their non-ionized form. The compositions of the mobile phases used for the determination of the R_M values of the compounds taken into consideration are reported in Table I. The general formulae for the chemical series listed in Table I are reported in Fig. 1.

RESULTS

TLC equations

In a typical reversed-phase TLC experiment, the detection of the compounds on the developed plate results in the appearance of round spots at different distances from the starting line. For the more hydrophilic compounds a reliable R_F value, *i.e.*, the ratio between the distance travelled by the compound on the TLC plate and the distance travelled by the solvent front, can be measured even when the mobile phase is

TABLE I

COMPOSITION OF THE TLC MOBILE PHASES FOR MEASUREMENT OF R_M VALUES

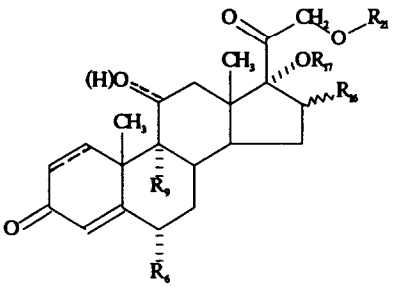
Chemical class	Compound No.	TLC mobile phase		Ref.
		Organic solvent	Aqueous component	
Steroids	88	Acetone	Water	24
Naphthalenes and quinolines	57	Acetone	Glycine (pH 9.0)[a]	25
		Methanol	Glycine (pH 9.0)[a]	25
Nitroimidazo-thiazoles	47	Acetone	Glycine (pH 9.0)	26
β-Carbolines	15	Acetone	Glycine (pH 9.0)	27
Penicillins	18	Acetone	Sodium acetate–veronal (pH 7.0)	28
		Acetone	Glycine (pH 1.2)	28
Benzodiazepines	39	Acetone	Water	29
Phenols	28	Acetone	Sodium acetate–veronal (pH 7.4)	30
Triazines	20	Acetone	Sodium acetate–veronal (pH 7.0)	31
		Acetonitrile	Sodium acetate–veronal (pH 7.0)	32
		Methanol	Sodium acetate–veronal (pH 7.0)	31
4-Nitropyrazoles	32	Acetone	Sodium acetate–veronal (pH 7.4)	25
		Acetone	Glycine (pH 1.2)	25
Prostaglandins	12	Acetone	Sodium acetate–veronal (pH 7.0)	33
		Methanol	Sodium acetate–veronal (pH 7.0)	33
		Acetonitrile	Sodium acetate–veronal (pH 7.0)	25
1,4-Dihydropyridines	53	Acetone	Sodium acetate–veronal (pH 7.4)	25
Cardiac glycosides	41	Acetone	Sodium acetate–veronal (pH 7.2)	34
Xanthones	41	Methanol	Glycine (pH 9.0)	35
Xanthines and adenosines	44	Acetone	Sodium acetate–veronal (pH 7.0)[b]	36
Cephalosporins	20	Acetone	Sodium acetate–veronal (pH 7.0)	28
		Acetone	Glycine (pH 1.2)	28
5-Nitroimidazoles	22	Methanol	Ammonium chloride (pH 9.0)[c]	37
Dermorphins	23	Acetone	Sodium acetate–veronal (pH 7.0)	38
		Methanol	Sodium acetate–veronal (pH 7.0)	38

[a] For the hydroxy derivatives, sodium acetate–veronal (pH 7.4) was used.
[b] For some derivatives, glycine buffer (pH 1.2) was used.
[c] For one compound, sodium acetate–veronal (pH 3.6) was used.

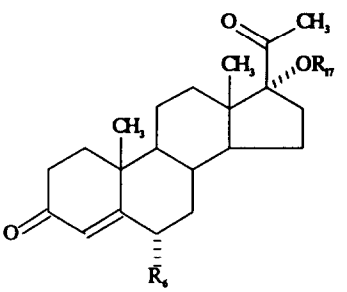
water or an aqueous buffer. The addition of an organic solvent to the mobile phase induces longer migrations of the compound and therefore higher R_F values. On the other hand, when the mobile phase is represented by an aqueous buffer only, the more lipophilic compounds tend not to move from the starting line ($R_F = 0$), and the addition of an organic solvent is necessary in order to obtain a reliable R_F value, *i.e.*, $0 < R_F < 1$. At higher organic solvent concentrations all the compounds tend to move with the solvent front and therefore to yield R_F values close to 1.

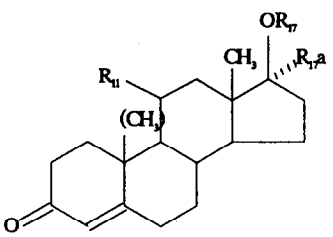
Because of the relationship $R_M = \log(1/R_F - 1)$ from lower or higher R_F values higher or lower R_M values, respectively, are obtained. Since the R_F values range from 0 to 1, R_M values ranging from $+\infty$ to $-\infty$ could theoretically be obtained. However, in the practice of TLC, when the compounds tend not to move from the starting line or to move with the solvent front, the lowest and highest R_F values which can be reliably measured are about 0.03 or 0.97, respectively, corresponding to R_M values of about 1.5 or −1.5.

Steroids:

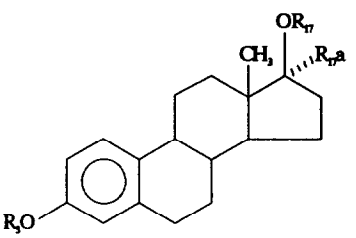


pregnane derivatives



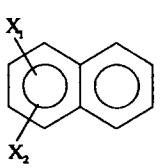


androstane derivatives

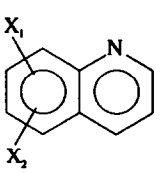


estrane derivatives

Naphthalenes & Quinolines:

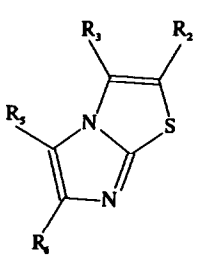


naphthalenes



quinolines

Nitroimidazothiazoles:



β-carbolines:

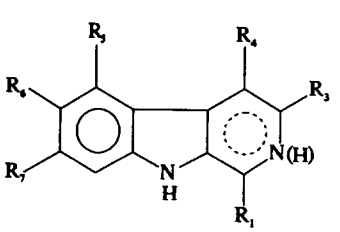
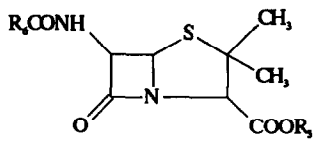
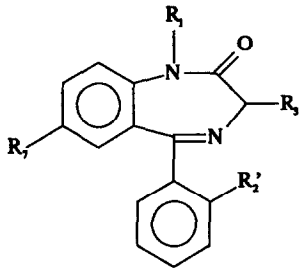


Fig. 1.

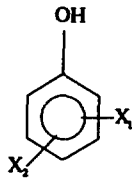
Penicillins:



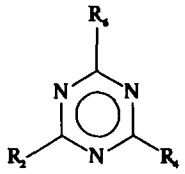
Benzodiazepines:



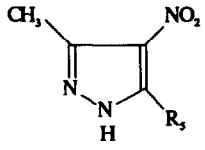
Phenols:



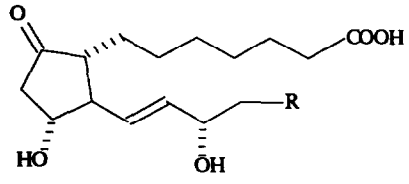
Triazines:



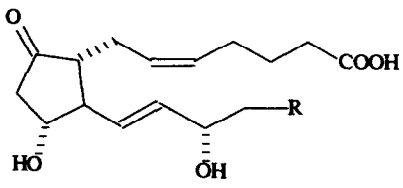
4-nitropyrazoles:



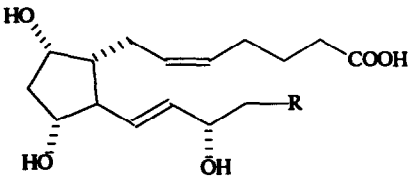
Prostaglandins:



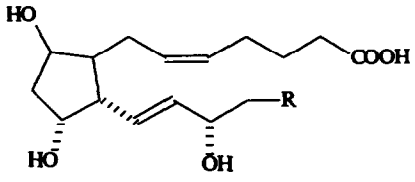
PGE_1 derivatives



PGE_2 derivatives



$PGF_{2\alpha}$ derivatives



$PGF_{2\beta}$ derivatives

1,4-dihydropyridines:

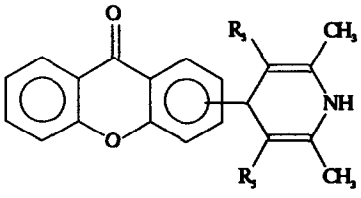
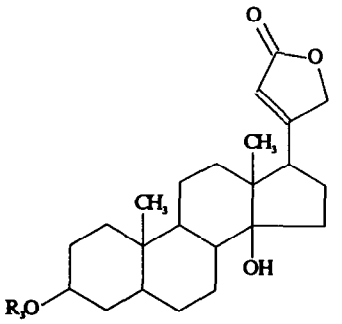


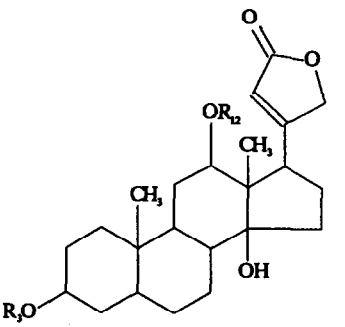
Fig. 1.

(*Continued on p. 346*)

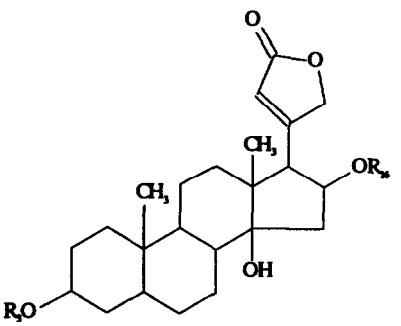
Cardiac glycosides:



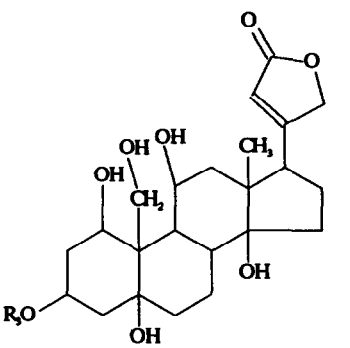
digitoxigenin derivatives



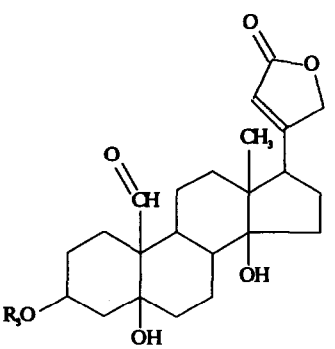
digoxigenin derivatives



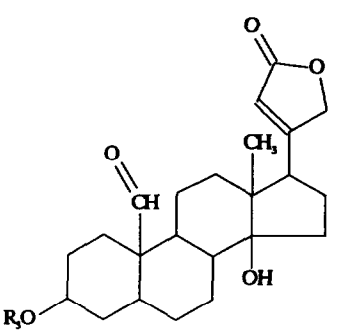
gitoxigenin derivatives



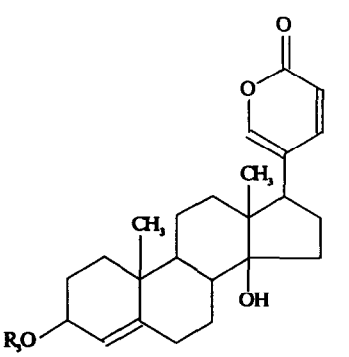
ouabagenin derivatives



strophanthidin derivatives



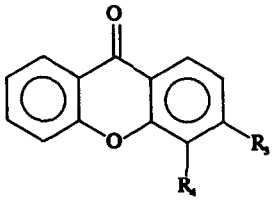
cannogenin derivatives



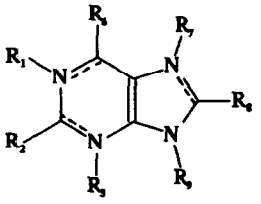
scillarenin derivatives

Fig. 1. (*continued*)

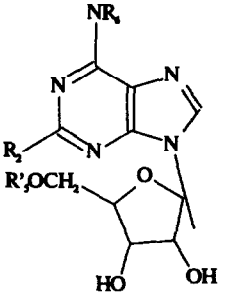
Xanthones:



Xanthines & Adenosines:

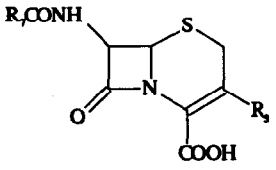


xanthine derivatives

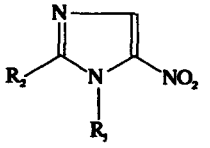


adenosine derivatives

Cephalosporins:



5-nitroimidazoles:



Dermorphins:

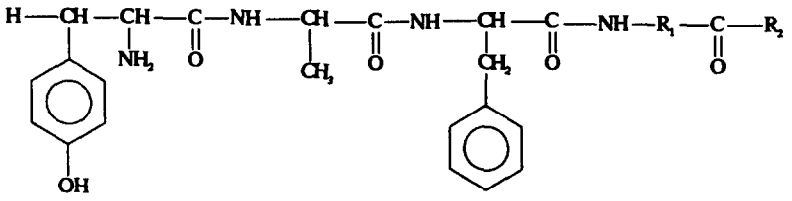


Fig. 1. General formulae for the series of compounds listed in Table I.

In agreement with the first observation of Boyce and Milborrow [2], our chromatographic work has shown that for each compound in all the chemical series taken into consideration there is a range of linear relationship between the R_M values and the organic solvent concentrations in the mobile phase. It is important to note that such a linear relationship has been shown also in reversed-phase HPLC [6,39–42]. In Fig. 2, some examples are reported describing the relationship between the R_M values of selected compounds from the newly investigated series and the composition of the mobile phase in a reversed-phase TLC system. The more lipophilic compounds such as AP 838 (a dihydropyridine derivative) at lower acetone concentrations tend not to move from the starting line (R_M values between 1.2 and 1.3), whereas at higher concentrations they tend to move with the solvent front (R_M values between −1.2 and −1.3). The extrapolation from the linear part of the curve yields the theoretical R_M values at 0% acetone in the mobile phase. This chromatographic behaviour, over the full range of acetone concentrations, was previously described as an S-shaped curve [6]. Werkhoven-Goewie *et al.* [43] showed very similar S-shaped curves describing the relationship between log k' values and the composition of the mobile phase in an HPLC system. On the other hand, the more hydrophilic compounds such as P_3 (a 4-nitropyrazole derivative) show deviations from linearity only at higher acetone concentrations. In fact, even at 0% acetone experimental R_M values can be determined. The first part of the curve can be fitted by a straight line and a theoretical R_M value at 0% can be calculated and compared with the experimental R_M value. A particular case is represented by very hydrophilic compounds, which tend to move with the solvent front even at 0% organic solvent in the mobile phase. For such compounds it is difficult to calculate a TLC equation. In fact, the slope tends to be close to 0. An example of a very hydrophilic compound is 6-aminopenicillanic acid: at pH 1.2 and 0% acetone it shows an R_M value of −0.99 [28]. In Fig. 2 it is shown that any addition of acetone cannot further increase the migration of the compound. The linear part of the curves reported in Fig. 2 is described by the following TLC equations, where % Me_2CO indicates the acetone concentration in the mobile phase:

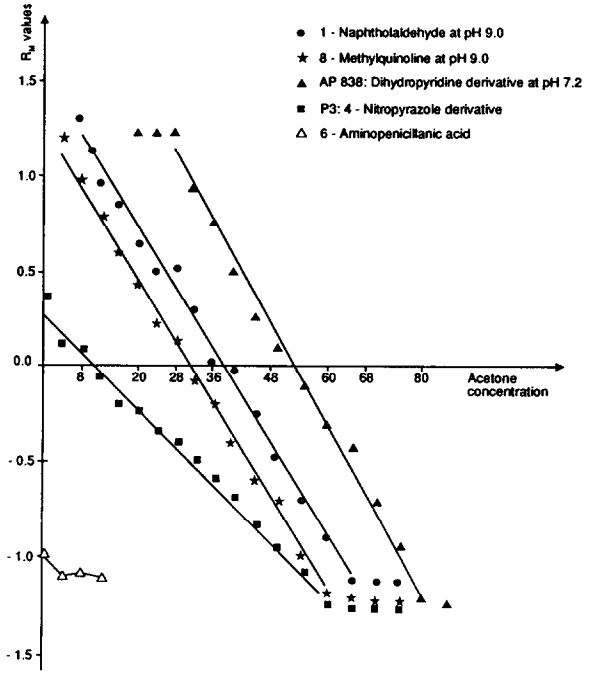


Fig. 2. Relationship between R_M values and acetone concentrations in the mobile phase for some selected compounds.

AP 838 (dihydropyridine derivative):

$$R_M = 2.343(\pm 0.073) - 0.044(\pm 0.001)(\%Me_2CO) \quad (n = 11;\ r = 0.996;\ s = 0.072) \quad (1)$$

1-naphtholaldehyde:

$$R_M = 1.528(\pm 0.038) - 0.040(\pm 0.001)(\%Me_2CO) \quad (n = 14;\ r = 0.996;\ s = 0.064) \quad (2)$$

8-methylquinoline:

$$R_M = 1.246(\pm 0.040) - 0.040(\pm 0.001)(\%Me_2CO) \quad (n = 14;\ r = 0.995;\ s = 0.073) \quad (3)$$

P_3 (4-nitropyrazole derivative):

$$R_M = 0.270(\pm 0.018) - 0.025(\pm 0.001)(\%Me_2CO) \quad (4)$$
$$(n = 15;\ r = 0.997;\ s = 0.037)$$

The intercepts of eqns. 1–4 represent the theoretical R_M values at 0% organic solvent in the mobile phase, *i.e.*, in a standard system, where all the compounds can be compared on the basis of their lipophilicity. In particular, if the assumption is made that reversed-phase TLC is true partition chromatography, the intercepts of the TLC equations can be considered as a measure of the partitioning of compounds between silicone oil (stationary phase) and water or an aqueous buffer (mobile phase at 0% organic solvent). The negative slope of the TLC equation indicates the increase in migration per unit increase in organic solvent concentration, *i.e.*, the rate at which the solubility of the compound increases in the mobile phase.

The chromatographic work carried out over many years in our laboratory provided 734 TLC equations for the chemical classes and under the mobile phase conditions listed in Table I. Most of these equations were published previously. The present analysis of our original chromatographic data resulted in the recalculation of 48 TLC equations, yielding new values for intercepts and/or slopes. In particular, the new TLC equations were 1 out of 22 for the 5-nitroimidazoles, 2 out of 20 for the triazines, 3 out of 47 for the nitroimidazothiazoles, 7 out of 40 for the cardiac glycosides, 2 out of 88 for the steroids, 6 out of 30 for the penicillins and 27 out of 46 for the dermorphins. This means that only 6.5% of the TLC equations had to be recalculated. Moreover, most of these new TLC equations, *i.e.*, 27 out of 48, were calculated for dermorphin-related derivatives. On excluding this class of compounds, the above percentage decreases to 3.0, *i.e.*, only 21 new equations out of 688.

Relationship between experimental and extrapolated R_M values

As the more hydrophilic compounds can migrate in a reliable way even at 0% organic solvent, the equations describing the correlation between their experimental and extrapolated R_M values can be calculated (eqns. 5–18, Table II). In fact, in all the chemical classes listed in Table II there were several hydrophilic compounds, for which it was possible to measure an experimental R_M at 0% organic solvent. The intercepts and slopes of eqns. 5–18 are very close to 0 and 1, respectively, and can support the validity of the extrapolation technique. The only exception is represented by the dermorphin-related derivatives (eqns. 15 and 16). In any case eqns. 5–18 were combined into eqn. 19, which shows that the very good correlation between experimental and extrapolated R_M values does not depend on the structure of compounds and the composition of the mobile phase.

$$R_{M\,\mathrm{exptl}} = 0.031(\pm 0.009) + 1.000(\pm 0.011)R_{M\,\mathrm{extrap}} \quad (19)$$
$$(n = 240;\ r = 0.986;\ s = 0.080;\ F = 8592;\ P < 0.005)$$

TLC equations from different solvent systems

Another interesting point arising from our previous work is the very close overlap of the extrapolated R_M values obtained with different organic solvents in the mobile phase. If the extrapolated R_M values represented the partitioning of the compounds between the silicone oil of the stationary phase and a mobile phase constituted only by water, we would expect the same extrapolated R_M values whether the organic solvent of the mobile phase was acetone, acetonitrile or methanol. This aspect is illustrated by means of five classes of compounds listed in Table III. Eqns. 20–27 (Table III) show very good correlations between the extrapolated R_M values at 0% acetone, acetonitrile or methanol in the mobile phase. Again, only the dermorphin-related derivatives deviate slightly. The slopes and intercepts close to 1 and 0, respectively, show the overlapping of the R_M values extrapolated from different solvent systems. Again, eqn. 20 shows a small difference for the dermorphin-related derivatives. In any case eqns. 20, 21, 24 and 25 were combined into eqn. 28.

TABLE II

CORRELATION BETWEEN EXPERIMENTAL AND EXTRAPOLATED R_M VALUES

Chemical class	TLC system		$R_{M\,\mathrm{exptl}} = a + bR_{M\,\mathrm{extrap}}$					Eqn. No.	Ref.
	Organic solvent	pH	a	b	n	r	s		
Xanthines and adenosines	Acetone	7.0	0.036	0.972	33	0.997	0.042	5	36
Cardiac glycosides	Acetone	7.2	0.038	1.033	6	0.960	0.065	6	34
Penicillins and cephalosporins	Acetone	1.2	0.036	0.978	27	0.997	0.032	7	28
	Acetone	7.0	0.023	1.021	28	0.996	0.044	8	28
Quinolines and naphthalenes	Acetone	9.0	0.076	0.958	23	0.970	0.062	9	25
	Methanol	9.0	0.066	0.959	23	0.951	0.079	10	25
Triazines	Acetone	7.0	0.010	1.017	5	0.997	0.022	11	31
	Methanol	7.0	0.063	0.940	5	0.985	0.050	12	31
	Acetonitrile	7.0	0.008	0.985	6	0.999	0.015	13	32
5-Nitroimidazoles	Methanol	9.0	−0.002	0.990	22	0.999	0.024	14	37
Dermorphins	Acetone	7.0	0.355	1.026	5	0.986	0.041	15	38
	Methanol	7.0	0.322	0.866	5	0.981	0.048	16	38
4-Nitropyrazoles	Acetone	1.2	0.049	0.996	26	0.991	0.052	17	25
	Acetone	7.0	0.045	0.958	26	0.965	0.099	18	25

$$R_{M\,\mathrm{acetone}} = 0.046(\pm 0.047) + 0.926(\pm 0.025)R_{M\,\mathrm{methanol}} \quad (28)$$

$$(n = 99;\quad r = 0.967;\quad s = 0.185;\quad F = 1413;\quad P < 0.005)$$

This shows that the linear relationship between R_M values and the mobile phase composition yields extrapolated R_M values that are not dependent on the nature of the organic solvent. In other words, the extrapolated R_M values are referred to a standard system represented only by water and silicone oil.

TABLE III

CORRELATIONS BETWEEN EXTRAPOLATED R_M VALUES FROM DIFFERENT TLC SYSTEMS

Chemical class	Organic solvent		$R_{M\,\mathrm{I}} = a + b\,R_{M\,\mathrm{II}}$					Eqn. No.	Ref.
	I	II	a	b	n	r	s		
Dermorphins	Acetone	Methanol	−0.252	0.993	23	0.980	0.192	20	38
Prostaglandins	Acetone	Methanol	−0.025	0.958	12	0.967	0.139	21	33
	Acetone	Acetonitrile	−0.197	1.141	12	0.973	0.126	22	25
	Methanol	Acetonitrile	−0.021	1.113	12	0.940	0.187	23	25
Quinolines and naphthalenes	Acetone	Methanol	0.043	0.969	44	0.965	0.178	24	25
Triazines	Acetone	Methanol	0.021	0.961	20	0.981	0.074	25	32
	Acetone	Acetonitrile	−0.013	0.971	20	0.974	0.087	26	32
	Methanol	Acetonitrile	−0.019	0.999	20	0.982	0.075	27	32

Relationship between slopes and intercepts of the TLC equations

As already pointed out, for any chemical agent there is a range of linear relationship between R_M values and organic solvent concentration in the mobile phase, unless it is too hydrophilic. The straight lines describing such linear relationships for a series of twelve prostaglandin derivatives and their TLC equations are reported in Fig. 3 [33]. The plot of Fig. 3b shows that there is a linear relationship between the slopes and the intercepts of the TLC equations, *i.e.*, the extrapolated R_M values. The very good correlation is described by eqn. 37 in Table IV. The correlation is not affected by the lower slope of the TLC equation of compound 9. The plots in Fig. 3a and the negative slope of eqn. 37 mean that with increasing methanol concentration the R_M values of more lipophilic compounds decrease faster than those of less lipophilic derivatives. In other words, lipophilic compounds are more sensitive to variations of the polarity of the mobile phase.

In a similar way, the linear relationships between the slopes and intercepts of the TLC equations for several chemical series were calculated and are reported in Table IV. All the members of the steroid, 5-nitroimidazole, nitroimidazothiazole, phenol, triazine and dermorphin series fitted the respective straight lines, the only exception being prednisolone palmitate. The deviation of this derivative is due to its very high and probably unreliable R_M value (9.15). Therefore, eqn. 29 was calculated without this compound. Although their chromatographic studies have not yet been published, three other series of compounds, 4-nitropyrazoles, 1,4-dihydropyridines and naphthalenes and quinolines, are considered in Table IV. In all three series no deviation was observed from the linear relationships between the intercepts and slopes of the TLC equations. In the case of naphthalenes and quinolines, eqn. 46 was calculated including fifteen naphthols for which the TLC data were available and already published [44].

However, a very interesting aspect is that in several instances not all the members of a chemical series fit the same straight line. The TLC equations reported in Fig. 4a for fifteen β-carbolines show that the chromatographic behaviour of harmaline and harmalol (compounds 12 and 13 in Fig. 4a) is characterized by lower

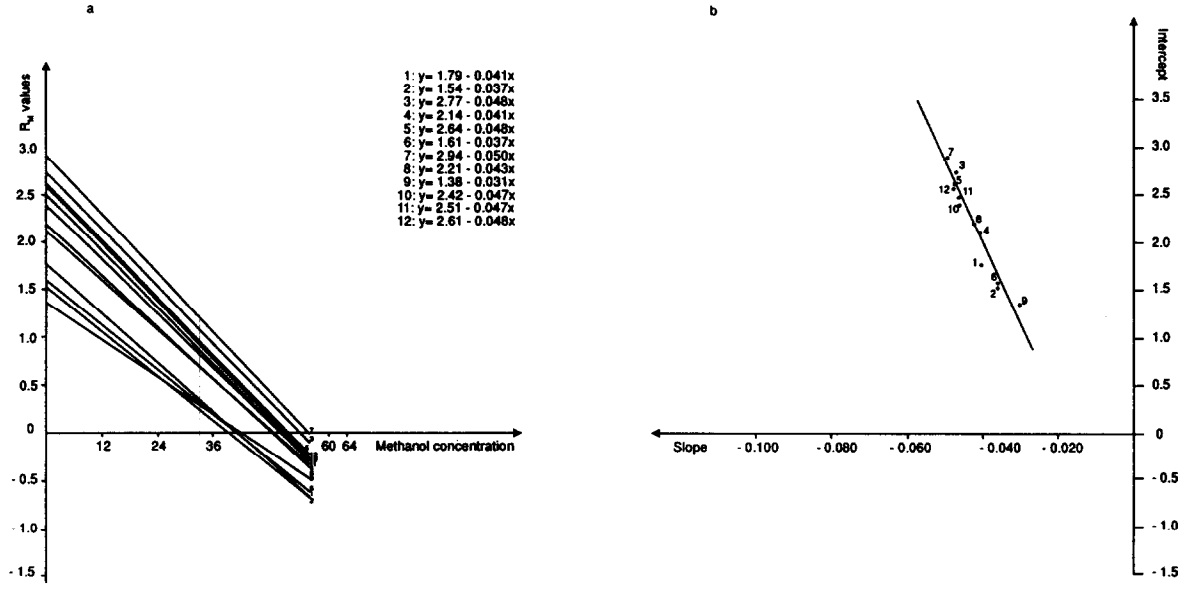


Fig. 3. (a) Relationship between R_M values and methanol concentration in the mobile phase, as described by the TLC equations for twelve prostaglandins. (b) Relationship between slope and intercept of the TLC equations for the prostaglandins in (a) as described by eqn. 37 in Table IV.

TABLE IV

RELATIONSHIP BETWEEN INTERCEPTS AND SLOPES OF TLC EQUATIONS

Chemical class	TLC mobile phase		$R_{M\,extrap} = a + b$ slope					Eqn. No.	Ref.
	Solvent	pH	a	b	n	r	s		
Steroids	Acetone	7.0	−2.294 (±0.076)	−81.625 (±1.203)	88	0.991	0.203	29	24
Nitroimidazo-thiazoles	Acetone	9.0	−1.095 (±0.117)	−78.201 (±3.125)	47	0.966	0.136	30	26
Phenols	Acetone	7.0	−1.166 (±0.137)	−75.383 (±4.357)	28	0.959	0.201	31	30
Triazines	Acetone	7.0	−1.258 (±0.308)	−69.484 (±8.208)	20	0.894	0.173	32	32
	Acetonitrile	7.0	−1.287 (±0.597)	−74.194 (±16.475)	20	0.728	0.265	33	32
	Methanol	7.0	−1.602 (±0.707)	−109.73 (±26.023)	20	0.705	0.279	34	32
Prostaglandins	Acetone	7.0	−2.289 (±0.762)	−61.014 (±10.555)	12	0.877	0.261	35	33
	Acetonitrile	7.0	−2.970 (±1.226)	−73.829 (±18.141)	12	0.790	0.284	36	25
	Methanol	7.0	−1.499 (±0.313)	−86.005 (±7.187)	12	0.967	0.140	37	33
5-Nitroimidazoles	Methanol	7.0	−0.848 (±0.062)	−66.689 (±2.625)	22	0.985	0.120	38	37
Dermorphins	Acetone	7.0	−1.710 (±0.228)	−56.775 (±3.466)	23	0.963	0.263	39	38
	Methanol	7.0	−1.054 (±0.197)	−69.317 (±4.055)	23	0.966	0.249	40	38
4-Nitropyrazoles	Acetone	1.2	−1.593 (±0.137)	−84.834 (±4.397)	32	0.962	0.237	41	25
	Acetone	7.0	−1.645 (±0.122)	−79.483 (±3.494)	32	0.972	0.205	42	25
1,4-Dihydropy-ridines	Acetone	7.0	−1.167 (±0.199)	−69.429 (±3.850)	53	0.930	0.176	43	25
Naphthalenes and quinolines	Acetone	9.0	−1.356 (±0.193)	−62.704 (±4.065)	44	0.922	0.265	44	25
	Methanol	9.0	−1.051 (±0.186)	−87.810 (±6.060)	44	0.913	0.277	45	25
	Acetone	9.0	−1.430 (±0.166)	−64.502 (±3.511)	57	0.928	0.244	46	25

slopes. In fact, these two compounds do not fit the straight line in Fig. 4b and were omitted from the calculation of eqn. 48 (Table V).

The equations in Table V describe the relationship between the slopes and intercepts for two other series of compounds, *i.e.*, cardiac glycosides and benzodiazepines. Ouabain was omitted from eqn. 47 because of its large deviation. In the benzodiazepine series six compounds markedly deviated from the linear relationship: four of them are characterized by very low slopes of their TLC equations and by the presence of a basic chain $CH_2CH_2N(C_2H_5)_2$ in position 1 of the 1,4-diazepine nucleus; two other compounds show higher slopes and the presence of a COOH group. These compounds were omitted from the calculation of eqn. 49.

The analysis of the TLC equations for penicillins and xanthones revealed the presence of a number of subclasses within each series (Table

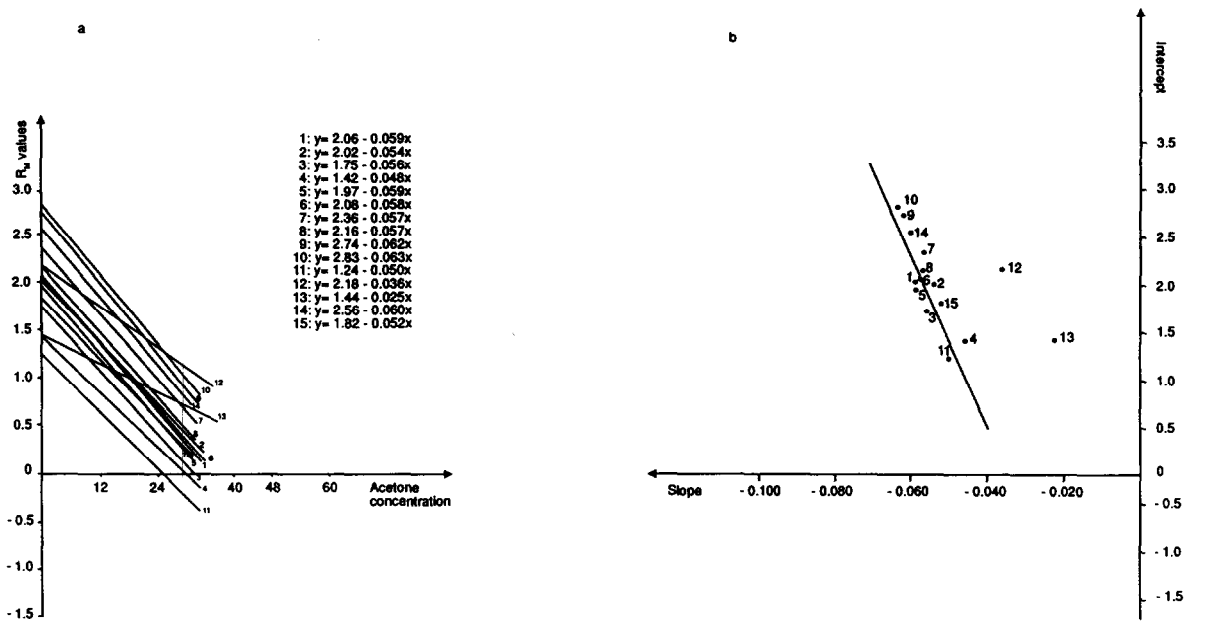


Fig. 4. (a) Relationship between R_M values and organic solvent concentrations, as described by the TLC equations for fifteen β-carbolines. The slopes of the TLC equations of compounds 12 and 13 are clearly lower. (b) Relationship between slope and intercept of the TLC equations for the β-carbolines in (a) as described by eqn. 48 in Table V. Both harmaline (compound 12) and harmalol (compound 13) were omitted from the calculation of the equation.

VI). As regards the penicillins at pH 1.2, eqn. 50 was calculated with ten derivatives bearing a COOH group as the only ionizable one and eqn. 51 with six compounds having both an amino and a carboxyl group. Carbenecillin, because of its large deviation, was omitted from both equations (Fig. 5a). At pH 7.0, eqn. 52 was calculated with all the penicillins except the prodrugs bacampicillin, talampicillin and lenampicillin, where the carboxyl group is esterified (Fig. 5b). Despite its large deviation in Fig. 5b there was no apparent reason for excluding compound 1 from the calculation of eqn. 52.

In the case of xanthone compounds the original series of 41 derivatives had to be split into four subclasses characterized by different substituents in positions 3 and 4 (Table VII). Eqn. 53 was calculated for the 3-alkoxy or 3-NH_2-

TABLE V

RELATIONSHIP BETWEEN INTERCEPTS AND SLOPES OF TLC EQUATIONS

Chemical class	TLC mobile phase		$R_{M\,extrap} = a + b$ slope					Eqn. No.	Ref.
	Solvent	pH	a	b	n	r	s		
Cardiac glycosides	Acetone	7.2	−1.550 (±0.219)	−63.492 (±3.646)	40	0.943	0.303	47	34
β-Carbolines	Acetone	9.0	−3.218 (±0.802)	−93.760 (±14.140)	13	0.894	0.220	48	27
Benzodiazepines	Acetone	7.0	−2.557 (±0.454)	−89.642 (±9.240)	33	0.867	0.231	49	29

TABLE VI

RELATIONSHIPS BETWEEN INTERCEPTS AND SLOPES OF TLC EQUATIONS

Chemical class	TLC mobile phase		$R_{M\,extrap} = a + b$ slope					Eqn.	Ref.
	Solvent	pH	a	b	n	r	s		
Penicillins	Acetone	1.2	−2.143 (±0.299)	−81.782 (±6.380)	10	0.976	0.132	50	28
			−1.949 (±0.274)	−48.528 (±5.249)	6	0.977	0.190	51	28
	Acetone	7.0	−2.540 (±0.422)	−53.744 (±7.092)	14	0.909	0.335	52	28
Xanthones	Methanol	9.0	0.507 (±0.181)	−91.006 (±6.678)	19	0.957	0.129	53	35
			0.594 (±0.026)	−69.490 (±0.810)	4	0.999	0.006	54	35
			−0.185 (±0.565)	−79.518 (±14.861)	7	0.923	0.140	55	35
			−0.831 (±0.139)	−83.796 (±3.938)	11	0.990	0.060	56	35

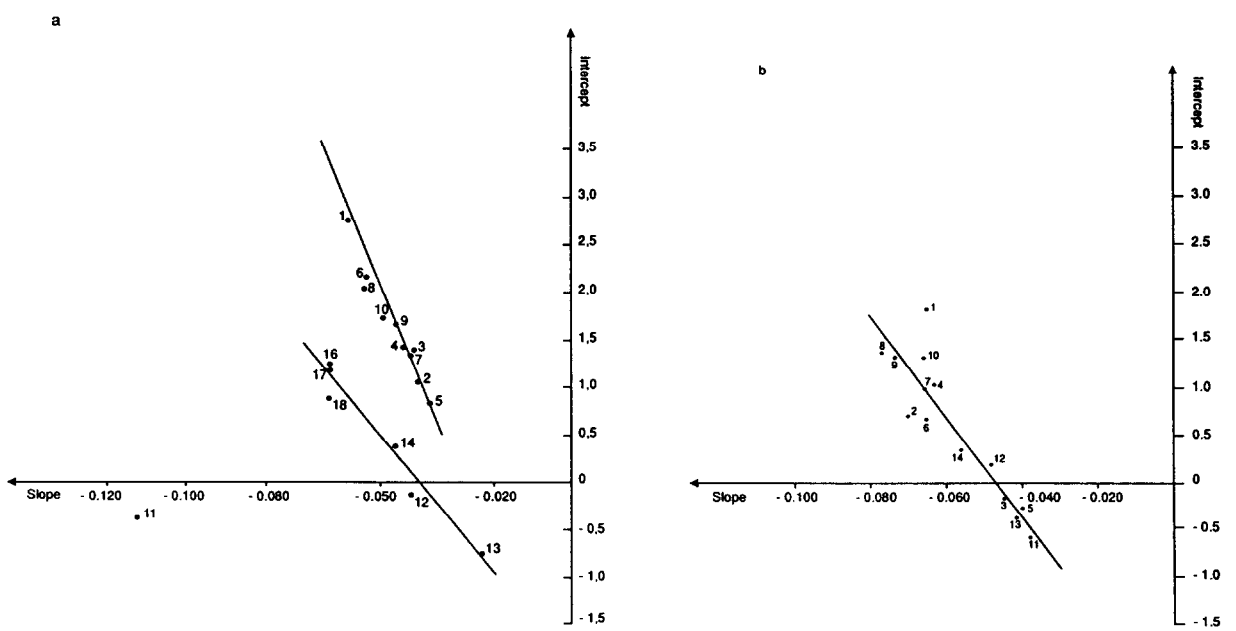
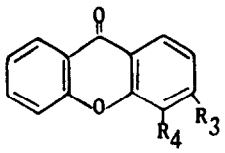


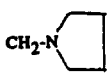
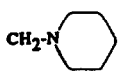
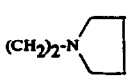
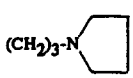
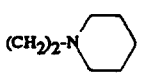
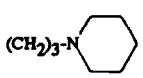
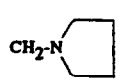
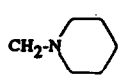
Fig. 5. (a) Relationship between slope and intercept of the TLC equations for penicillins at pH 1.2, as described by eqns. 50 (compounds 1–10) and 51 (compounds 12–14, 16–18) in Table VI. Carbenecillin (compound 11) deviated from both equations. (b) Relationship between slope and intercept of the TLC equations for penicillins at pH 7.0, as described by eqn. 52 in Table VI.

TABLE VII

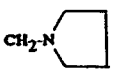
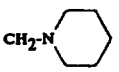
STRUCTURES OF THE XANTHONE DERIVATIVES FITTING EQNS. 53–56



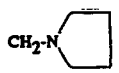
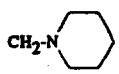
Eqn. 53

No.	R_3	R_4
11	OCH_3	
17	OCH_3	
23	OCH_3	CH_2-N(CH_3)$_2$
29	OCH_3	CH_2-N(C_2H_5)$_2$
36	OCH_3	
37	OCH_3	
38	OCH_3	
39	OCH_3	
40	OCH_3	$(CH_2)_2$-N(CH_3)$_2$
41	OCH_3	$(CH_2)_3$-N(CH_3)$_2$
42	OCH_3	$(CH_2)_2$-N(C_2H_5)$_2$
43	OCH_3	$(CH_2)_3$-N(C_2H_5)$_2$
15	$OCH(CH_3)_2$	
21	$OCH(CH_3)_2$	
27	$OCH(CH_3)_2$	CH_2-N(CH_3)$_2$

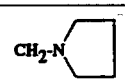
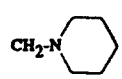
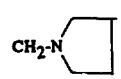
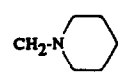
Eqn. 54

No.	R_3	R_4
33	$OCH(CH_3)_2$	CH_2-N(C_2H_5)$_2$
13	NH_2	
19	NH_2	
31	NH_2	$CH_2N(C_2H_5)_2$

Eqn. 54

No.	R_3	R_4
10	H	
16	H	
22	H	CH_2-N(CH_3)$_2$
28	H	CH_2-N(C_2H_5)$_2$

Eqn. 55

No.	R_3	R_4
12	Cl	
18	Cl	
30	Cl	CH_2-N(C_2H_5)$_2$
14	NO_2	
20	NO_2	

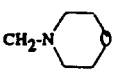
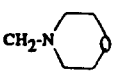
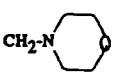
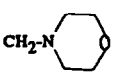
(Continued on p. 356)

TABLE VII (*continued*)

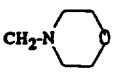
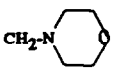
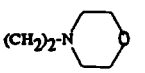
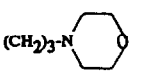
Eqn. 53

No.	R_3	R_4
26	NO_2	CH_2-N(CH_3)$_2$
32	NO_2	CH_2-N(C_2H_5)$_2$

Eqn. 56

No.	R_3	R_4
4	H	
5	OCH_3	
6	Cl	
7	NH_2	

Eqn. 54

No.	R_3	R_4
8	NO_2	
9	$OCH(CH_3)_2$	
34	OCH_3	
35	OCH_3	
1	H	H
2	OCH_3	H
3	Cl	H

substituted derivatives, eqn. 54 for the 3-H-substituted derivatives and eqn. 55 for the 3-Cl- or 3-NO_2-substituted derivatives. Compounds lacking the 4-aminoalkyl moiety or bearing a 4-morpholinoalkyl moiety were excluded from eqns. 53, 54 and 55. They fitted eqn. 56 (Fig. 6).

Interestingly, the analysis of the chromatographic data on cephalosporins [28] and xanthines and adenosines [36] revealed that these chemical series do not follow the general behaviour so far described. In fact, in the case of cephalosporins there was no correlation between the slopes and intercepts at either pH 1.2 or 7.0 (Fig. 7). For xanthines and adenosines it was not possible to find any structural meaning for some groupings of data (Fig. 8).

Finally, it is important to note that the low correlation coefficient of some of the equations in Tables IV, V and VI might also be due to the fact that the variability of the slopes is lower than that of the intercepts. The lower variability of the slopes yields lower values of the sum of the products of deviations of x and y values (Σxy). Therefore, since $r = \Sigma xy/\sqrt{\Sigma x^2 \Sigma y^2}$, the consequence is a low correlation coefficient.

DISCUSSION AND CONCLUSIONS

The present analysis of our chromatographic work, including some recent unpublished data, points to the reliability of two basic features of the R_M values, which we have been showing in many papers. They can be illustrated by eqns. 19 and 28. The correlation between experimental and extrapolated R_M values strongly supports the validity of the extrapolation technique. The correlation between extrapolated R_M values obtained using different organic solvents is another important support for the reliability of the extrapolated R_M values as an expression of the partitioning between an aqueous mobile phase and the silicone oil of the stationary phase. In fact, the nature of the organic modifier does not affect the extrapolated R_M values. However, this conclusion is based on the eight equations reported in Table III. It might be questionable

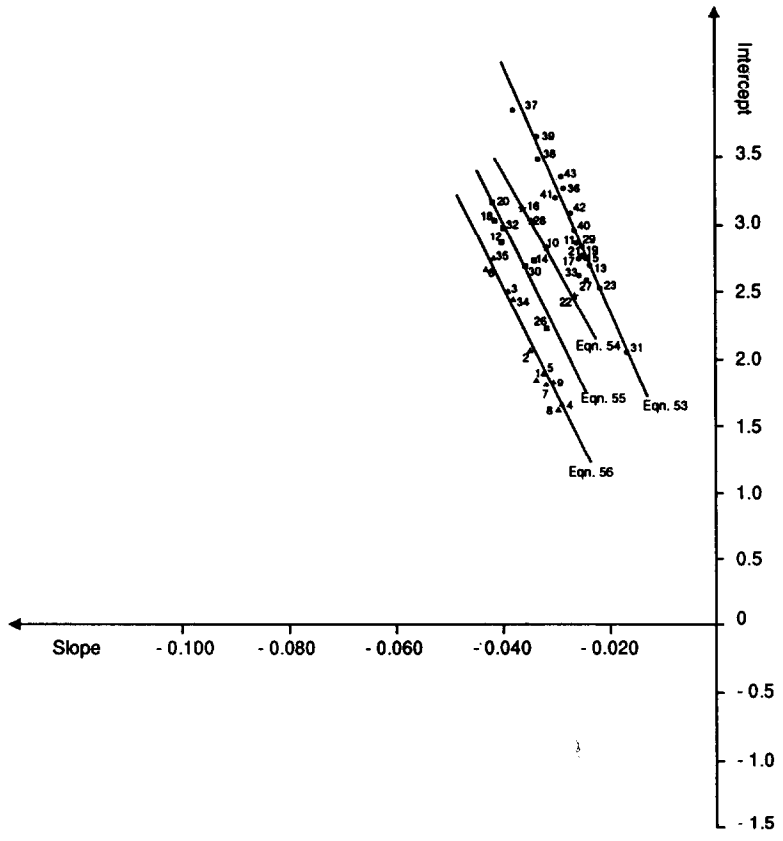


Fig. 6. Relationship between slope and intercept of the TLC equations for xanthone derivatives, as described by eqns. 53, 54, 55 and 56 in Table VII.

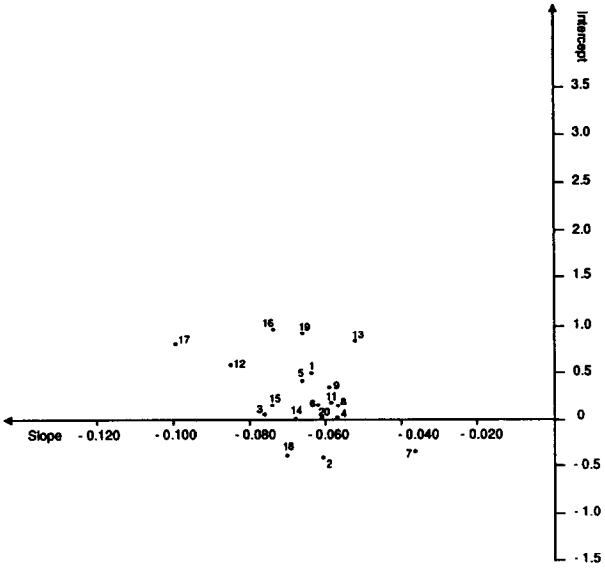


Fig. 7. Plot of slope *vs.* intercept of the TLC equations for cephalosporins at pH 7.0.

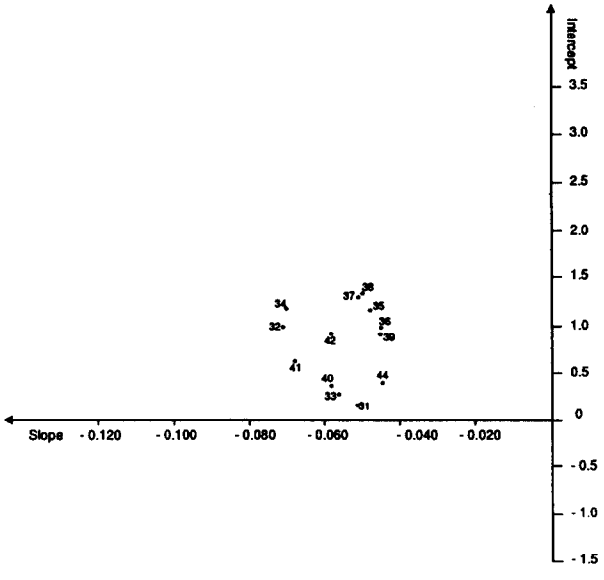


Fig. 8. Plot of slope vs. intercept of the TLC equations for adenosines at pH 7.0.

whether this aspect has general relevance for any chromatographic process. Some HPLC data seem to suggest a different influence of methanol and acetonitrile on the retention of simple organic compounds [45,46].

The most interesting aspect arising from this paper is the finding that the linear relationship between slopes and intercepts of the TLC equations seems to be another basic feature of the chromatographic determination of lipophilicity. In order to understand the relationship between slopes and intercepts of the TLC equations, the physico-chemical meaning of these two parameters must be discussed. As mentioned above, the intercept of the TLC equation can be considered as a measure of the partitioning of the compounds between a polar mobile phase and a non-polar stationary phase, *i.e.*, as the result of the balance between the interactions with the non-polar phase and the interactions with the polar phase.

On the other hand, less attention has been devoted to the physical meaning of the slope of the HPLC or TLC equations. As already pointed out, the slope of the TLC equation indicates the rate at which the solubility of the compound increases in the mobile phase. The increased solubility is due to the decreased polarity of the mobile phase, altering the balance of polar and non-polar interactions between each phase and the solute. According to Horváth *et al.* [40], the increased migration can be related to a decrease in the surface tension of the mobile phase, thus resulting in a better solubility. From a molecular point of view, Murakami [47] has interpreted the slope in terms of the so-called "displacement model" as the number of mobile phase solvent molecules in the solvation sphere of the solute. This is the number of solvent molecules that are released after the formation of the stationary phase–solute complex, and it depends on the area of the apolar surface characterizing any organic compound and on the type and number of polar substituents present in the solute structure [17]. Therefore, Murakami [47] pointed out again the importance of polar and non-polar interactions in determining the relationship between retention and the composition of the mobile phase.

As a consequence of the above considerations both the intercept and slope of the TLC equation seem to be related to the same physico-chemical factors, and therefore they should be interrelated. However, the data in Tables IV, V and VI and those of Valkó [16] and Kuchar *et al.* [20] show that the correlation holds only within series of congeneric compounds. An attempt to explain this aspect could be based on the concept of "hydrophobic surface availability", introduced by Kaibara *et al.* [48]. The retention should depend not just on the hydrophobic surface area but also on that part of it actually available for the interactions with the non-polar stationary phase. The shape of this surface might be the critical factor for differentiating series of congeneric compounds. This interpretation could be in agreement with the definition of a congeneric series proposed by Ariëns [49], i.e. "a three-dimensional homology of the various molecular fragments in the chemical framework of the series". From this point of view all the members of the series listed in Table IV seem to be congeneric. On the other hand, the series reported in Tables V and VI are characterized by the presence of compounds which do not fit the same linear equation. Whereas for the series in Table V this concerns only a few compounds as shown in Fig. 4, for the penicillins and xanthones (Table VI) a number of subclasses can be identified (Figs. 5 and 6). As the number of outliers in the series of penicillins and xanthones is sufficiently high, an equation for each subclass could be calculated.

In particular, congenerity might be broken down by the presence of ionizable groups which could modify the interactions of the compounds with the polar and/or non-polar phase. In fact, this is the case for the benzodiazepine, penicillin and β-carboline series. Benzodiazepines bearing the basic chain $CH_2CH_2N(C_2H_5)_2$ or a COOH group deviated from eqn. 49. At pH 1.2 penicillins bearing only one COOH group and those bearing both one NH_2 and one COOH group fitted eqns. 50 and 51 respectively. Carbenecillin with two COOH groups deviated from both equations. The presence of the second (non-ionized) carboxyl group can probably alter the shape of the hydrophobic surface of carbenecillin

with respect to those characterizing the compounds fitting eqns. 50 and 51. At pH 7.0 a similar mechanism could induce the deviation of ester prodrugs from eqn. 52. Finally, it must be noted that the two β-carbolines excluded from the calculation of eqn. 48 are characterized by the non-aromatic C ring, which implies the presence of a dihydropyridine ring instead of a pyridine ring. This again might be the cause of different polar and/or non-polar interactions.

In order to explain the deviation of ouabain from eqn. 47, the presence of a higher number of OH groups on its genin (ouabagenin) when compared with the other genins of the series (digoxigenin, digitoxigenin, gitoxigenin, k-strophanthidin, cannogenin and scillarenin) (Fig. 1) can be pointed out. In this case a different frame of intra- and intermolecular interactions could change the shape of the hydrophobic surface. The most striking example of the influence of structural differences on the slopes of TLC equations is provided by the xanthone derivatives. The series is characterized in position 3 of the xanthone nucleus by the presence of a number of substituents with different electronic properties and in position 4 by the presence of different aminoalkyl groups or by the lack of any substituent at all (Table VII).

The straight lines in Fig. 6 referring to the subclasses of Table VII show that when the amine in position 4 is dimethylamine, diethylamine, pyrrolidine or piperidine the properties of the substituents in position 3 make a difference among the compounds bearing the same amine (eqns. 53, 54 and 55). On the other hand, if the amine in position 4 is morpholine or if there is no aminoalkyl group in that position, all the compounds are congeneric despite the different substitution in position 3 (eqn. 56). The reason for the grouping of the xanthones into four subclasses could be found in different ionization patterns altering the availability of the hydrophobic surface. In fact, when there is no aminoalkyl group in position 4, the molecules are non-ionizable, whereas the presence of the aminoalkyl moiety in position 4 introduces an ionizable group. The experimental pK_a values of these compounds are not available. However, they should not be far from the pK_a values of the corresponding methylamines [50]: trimethylamine (9.76), methyldiethylamine (10.29), N-methylpyrrolidine (10.46), N-methylpiperidine (10.08) and N-methylmorpholine (7.41). At the pH of our TLC system (9.0), the compounds with a morpholino group should be mostly non-ionized; in fact, they fit the same equation as the 4-unsubstituted compounds. On the other hand, the presence of dimethylamino-, diethylamino-, pyrrolidino- or piperidinoalkyl groups renders the molecules more ionized at pH 9.0, which is not far from their pK_a values.

The extent to which these molecules are ionized can be influenced by the electronic character of the substituents in position 3, and the subclasses fitting eqns. 53, 54 and 55 can be identified on the basis of the electronic properties of these substituents. Eqn. 53 groups the compounds bearing alkoxy and NH_2 substituents, which might increase the pK_a of the amino group via an electron-donating effect, stabilizing the cation. The H substituent does not exert any electronic effect (eqn. 54). The Cl and NO_2 substituents are electron-withdrawing groups, able to lower the basic ionization constant. Consistently, the curve representing eqn. 55 lies close to that representing eqn. 56, that was calculated for the non-ionized compounds. As regards cephalosporins and also xanthines and adenosines, it was not possible to establish any meaningful relationships between slopes and intercepts. One must conclude that the members of these series of compounds are not congeneric from the chromatographic point of view. This does not imply that any series of cephalosporins or xanthines and adenosines would behave in the same way. At this stage of our work we are not able to interpret in structural terms the reasons for this chromatographic non-congenerity.

In conclusion, the relationship between slopes and intercepts of the TLC equations can be considered as a basic aspect of the chromatographic determination of lipophilicity, provided that one is dealing with strictly congeneric compounds. Recently, the slope of TLC or HPLC equations has been proposed as an alternative lipophilic parameter to be used in QSAR studies [20]. However this suggestion deserves some comment. The relationship holds only for a

series of strictly congeneric compounds and it is very difficult to define *a priori* congenerity in terms of chromatographic behaviour. The variability of the slopes is much smaller than that of the corresponding R_M values. In the case of a linear relationship with another variable, this implies a lower correlation and therefore a lower predictive value of the model.

Work is in progress aimed at collecting experimental and/or calculated log P values for all the compounds studied in our laboratory, with a view to an analysis of their relationship with the intercepts or slopes. This study will allow us to establish which of the two chromatographic parameters is best suited as an alternative measure of lipophilicity. A general equation correlating R_M and log P values could also be used as a calibration graph for the prediction of log P values from R_M measurements. Preliminary results have already pointed out the significant correlation existing between R_M and experimental and/or calculated log P [4,5].

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