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Short Communication

Determination of lipophilicity by means of reversed-phase thin-layer chromatography
II. Influence of the organic modifier on the slope of the thin-layer chromatographic equation

G.L. Biagi*[,a], A.M. Barbaro[a], A. Sapone[a], M. Recanatini[b]

[a]*Dipartimento di Farmacologia, Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy*

[b]*Dipartimento di Scienze Farmaceutiche, Università di Bologna, Via Belmeloro 6, 40126 Bologna, Italy*

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Abstract

The R_M values measured for a series of steroids were very close to those determined more than 15 years ago using the same chemicals. This finding supports the reliability of R_M values as a lipophilicity parameter. However, the point at issue in this work was the influence of the organic solvent in the mobile phase on the slopes of the TLC equations. In fact, the slopes of the TLC equations were shown to be related to the reciprocal of the solvent strength ($1/E_0$). As a consequence, the ratio between the slopes of the TLC equations in different solvent systems are close to the ratio between the $1/E_0$ values for the corresponding solvent pairs. A further interesting aspect seems to arise from the analysis of the equations correlating slopes and intercepts of the TLC equations. In particular, the ratios between the b values of such equations in different solvent systems are close to the ratios between the corresponding E_0 values.

1. Introduction

The partition coefficient, P, between water and n-octanol is currently used as an expression of the lipophilic character of a given compound. The chromatographic R_M value measured by means of reversed-phase TLC has been proposed as a reliable alternative to the classical log P [1–3]. In a previous paper, the analysis of more than 700 TLC equations allowed to consolidate the main aspects of our chromatographic work [4], which can be summarized as follows.

(a) The relationship between R_M values and concentration of the organic modifier in the mobile phase can be described by a linear equation. The intercepts of the TLC equations represent the extrapolated R_M values, *i.e.*, the theoretical R_M values at 0% organic solvent. In this way a theoretical R_M value can be calculated even for those compounds which do not migrate with an aqueous buffer alone. In other words the intercepts of the TLC equations can be considered as a measure of the partitioning of compounds between silicone oil and an aqueous buffer, *i.e.*, in a standard system, where all the compounds can be compared on the basis of their lipophilicity.

* Corresponding author.

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(b) As the more hydrophilic compounds can migrate in a reliable way even at 0% organic solvent, their experimental and extrapolated R_M values can be compared. The very good correlation, with intercept and slope close to 0 and 1, respectively, can support the validity of the extrapolation procedure.

(c) The very close overlap of the extrapolated R_M values obtained with different organic solvents in the mobile phase shows that the intercepts of the TLC equations are not dependent on the nature of the organic modifier. Again, it comes out that the extrapolated R_M values can be considered as a measure of the partitioning in a standard system represented only by water and silicone oil.

(d) A linear relationship can be shown between slopes and intercepts of the TLC equations. This phenomenon was observed earlier in HPLC by several workers and in particular was studied by Valkó and co-workers for a series of related compounds (see ref. 4 and references cited therein). The negative slope of the equation describing such a relationship means that lipophilic compounds are more sensitive to the variation of polarity of the mobile phase. Because of the relationship between slopes and intercepts, the use of the slopes of the TLC equations as an alternative lipophilic parameter to be used in QSAR studies has been proposed [5]. However an interesting point is that such a relationship can be found only when dealing with strictly congeneric compounds. Therefore, as it is also very difficult to define congenerity in terms of chromatographic behaviour and the variability of the slopes is much smaller than that of the intercepts [4], at the moment the extrapolated R_M values seem to be best suited as a measure of lipophilicity.

Another aspect of the chromatographic determination of lipophilicity that deserves attention is the influence of different organic modifiers on the slope of the TLC equation. We have already suggested that the intercept is not dependent on the nature of the organic modifier. The aim of this work was to investigate the influence of the organic solvent on the slope of the TLC equation. Some preliminary results have already been published [2,6]. This work is based on data previously described for triazines, prostaglandins, dermorphins, naphthalenes and quinolines [4]. New data for a series of steroids were also taken into consideration.

2. Experimental

Details of the reversed-phase TLC technique have been described previously [4,7]. For the present series of steroids the mobile phase was sodium acetate–Veronal buffer (pH 7.0) alone or mixed with various amounts of acetone (4–70%), acetonitrile (4–70%) or methanol (4–70%). The non-polar stationary phase was obtained in the usual way by impregnating the silica gel GF_{254} layer with silicone oil. The general formulae for the chemical series under investigation are shown in Fig. 1.

3. Results

The data obtained with the present series of steroids show the usual linear relationship between R_M values and organic modifier concentration in the mobile phase. The intercepts (a) and slopes (b) of the TLC equations are reported in Table 1. It is interesting that the extrapolated R_M values in Table 1 are very close to those obtained more than 15 years ago in our laboratory for the same compounds in an acetone system [8]. Although two compounds, corticosterone and prednisone, show some deviation, this certainly supports the reliability of the R_M values as a lipophilic parameter. The intercepts in Table 1 also show that the extrapolated R_M values from TLC systems with different organic solvents are very similar. The correlations between the extrapolated R_M values are described by the following equations:

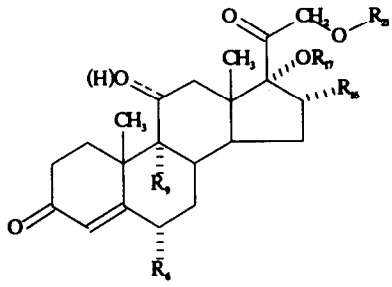
$$R_{M\ \text{methanol}} = 0.116\ (\pm 0.061) + 0.947\ (\pm 0.040) R_{M\ \text{acetone}} \quad (1)$$

$(n = 15;\ r = 0.989;\ s = 0.077;\ P < 0.005)$

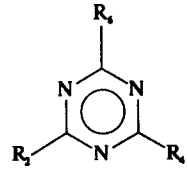
$$R_{M\ \text{acetonitrile}} = -0.014\ (\pm 0.048) + 0.966\ (\pm 0.032) R_{M\ \text{acetone}} \quad (2)$$

$(n = 15;\ r = 0.993;\ s = 0.061;\ P < 0.005)$

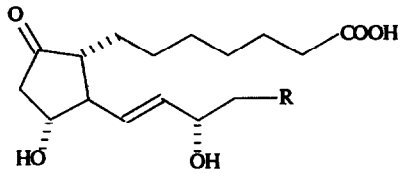
Steroids:



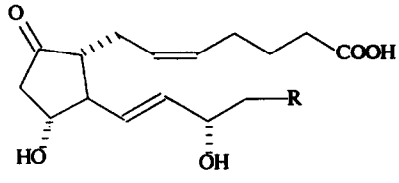
Triazines:



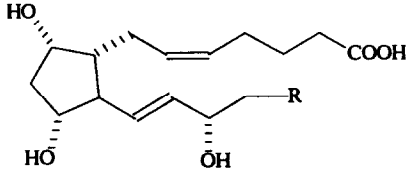
Prostaglandins:



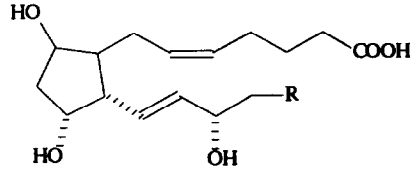
PGE_1 derivatives



PGE_2 derivatives



$PGF_{2\alpha}$ derivatives



$PGF_{2\beta}$ derivatives

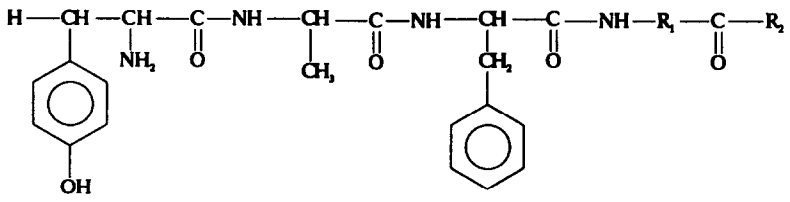
Fig. 1.

$$R_{M\ \text{acetonitrile}} = -0.060\ (\pm 0.098) + 0.989\ (\pm 0.063) R_{M\ \text{methanol}} \quad (3)$$

$$(n = 15;\ r = 0.975;\ s = 0.117;\ P < 0.005)$$

The slopes and intercepts very close to 1 and 0, respectively, support our hypothesis that the nature of the organic modifier does not affect the intercept of the TLC equations [4]. Further,

Dermorphins:



Naphthalenes & Quinolines:

naphthalenes quinolines

Fig. 1. General formulae for the chemical series in Table 2.

from the data in Table 1 it can be shown that there is a linear relationship between slopes and intercepts of the TLC equations, as already pointed out previously for many series of compounds [4]. The equations describing such a linear relationship for the present series of ster-

Table 1
TLC equations of steroids when the mobile phase was a mixture of acetone, acetonitrile or methanol and aqueous buffer (pH 7.0)

Compound	$R_M = a + b$ (% organic modifier)						Previous R_M [a]
	Acetone		Acetonitrile		Methanol		
	a	*b*	*a*	*b*	*a*	*b*	
Hydrocortisone	1.04	−0.041	1.03	−0.037	1.10	−0.023	0.96
Hydrocortisone 21-acetate	1.46	−0.046	1.36	−0.042	1.54	−0.027	1.58
Corticosterone	1.47	−0.046	1.49	−0.040	1.51	−0.027	1.27
Corticosterone 21-acetate	1.82	−0.048	1.64	−0.041	1.90	−0.029	1.92
Deoxycorticosterone	1.86	−0.051	1.79	−0.047	1.79	−0.028	1.78
Deoxycorticosterone 21-acetate	2.48	−0.060	2.43	−0.052	2.48	−0.037	2.49
Prednisolone	1.02	−0.043	0.98	−0.037	1.10	−0.027	0.90
Prednisolone 21-acetate	1.52	−0.046	1.53	−0.039	1.52	−0.027	1.54
6α-Methylprednisolone	1.27	−0.043	1.19	−0.039	1.22	−0.028	1.32
6α-Methylprednisolone 21-acetate	1.84	−0.052	1.75	−0.047	1.96	−0.030	1.98
Dexamethasone	1.23	−0.043	1.20	−0.040	1.40	−0.027	1.30
Dexamethasone 21-acetate	1.99	−0.055	2.06	−0.048	1.87	−0.030	1.99
Prednisone	1.01	−0.040	1.02	−0.034	1.15	−0.024	0.84
6α-Fluoroprednisolone 21-acetate	1.40	−0.046	1.38	−0.042	1.43	−0.027	1.57
Triamcinolone	0.30	−0.030	0.32	−0.028	0.33	−0.020	0.24

[a] See ref. 8.

oids are reported in Table 2, where the corresponding equations for triazines, dermorphins, prostaglandins, naphthalenes and quinolines are also listed [4]. All the steroids in Table 1 fit Eqs. 4–6 and therefore can be considered as congeneric from a chromatographic point of view. As already remarked [4], the poor correlation coefficients of some equations in Table 2 might be mainly due to the fact that the variability of the slopes is lower than that of the intercepts.

However, the point at issue here is the influence of the organic modifier on the slope of the TLC equation itself. The absolute values of the negative slopes of the TLC equations of steroids in Table 1 are ranked for each compound as follows: acetone > acetonitrile > methanol. The ranking of the slopes of the TLC equations is also illustrated in Fig. 2 for deoxycorticosterone 21-acetate and triamcinolone. The mean values in Table 3 show that the same ranking holds for all other series of compounds taken into consideration. The above ranking of slopes indicates that the migration of a given compound is more sensitive to the increasing concentration of acetone in mobile phase than to those of acetonitrile or methanol in this order. In previous papers [2,6] we have shown a relationship between the slopes of the TLC equations in different solvent systems and the solvent strength parameter (E_0) of acetone, acetonitrile and

Table 2
Relationship between intercepts and slopes of TLC equations

Chemical class	TLC mobile phase		$R_{M\ extrap} = a + b$ (slope)						Eq. No.
	Solvent	pH	a	b	n	r	s	F	
Steroids[a]	Acetone	7.0	−1.905 (±0.174)	−72.872 (±3.735)	15	0.983	0.098	380.6	4
	Acetonitrile	7.0	−1.873 (±0.283)	−80.365 (±6.864)	15	0.956	0.154	137.1	5
	Methanol	7.0	−1.878 (±0.390)	−122.802 (±14.126)	15	0.924	0.197	75.57	6
Triazines[b]	Acetone	7.0	−1.258 (±0.308)	−69.484 (±8.208)	20	0.894	0.173	71.66	7
	Acetonitrile	7.0	−1.287 (±0.597)	−74.194 (±16.475)	20	0.728	0.265	20.28	8
	Methanol	7.0	−1.602 (±0.707)	−109.73 (±26.023)	20	0.705	0.279	17.78	9
Prostaglandins[b]	Acetone	7.0	−2.289 (±0.762)	−61.014 (±10.555)	12	0.877	0.261	33.41	10
	Acetonitrile	7.0	−2.970 (±1.226)	−73.829 (±18.141)	12	0.790	0.284	16.56	11
	Methanol	7.0	−1.499 (±0.313)	−86.005 (±7.187)	12	0.967	0.140	143.2	12
Dermorphins[b]	Acetone	7.0	−1.710 (±0.228)	−56.775 (±3.466)	23	0.963	0.263	268.3	13
	Methanol	7.0	−1.054 (±0.197)	−69.317 (±4.055)	23	0.966	0.249	292.2	14
Naphthalenes and quinolines[b]	Acetone	9.0	−1.356 (±0.193)	−62.704 (±4.065)	44	0.922	0.265	237.9	15
	Methanol	9.0	−1.051 (±0.186)	−87.810 (±6.060)	44	0.913	0.277	210.0	16

[a] This work.
[b] See ref. 4.

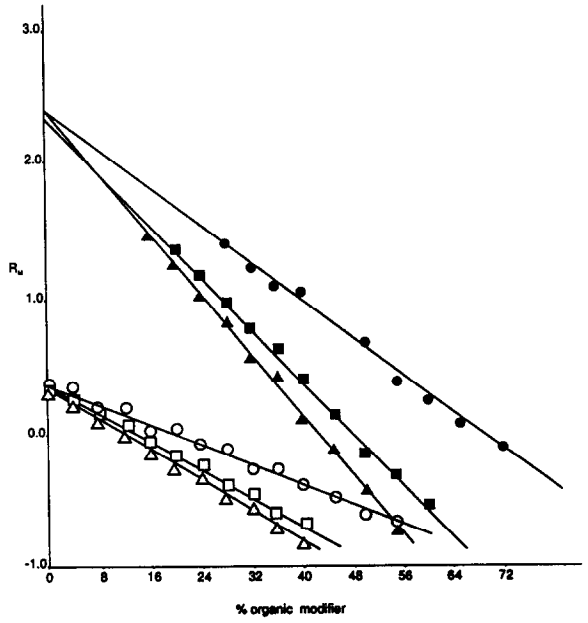


Fig. 2. Influence of the nature of the organic modifier on the slope of the linear relationship between R_M values and organic modifier concentration as described by the TLC equations for deoxycorticosterone 21-acetate (● = methanol; ■ = acetonitrile; ▲ = acetone) and triamcinolone (○ = methanol; □ = acetonitrile; △ = acetone).

methanol, when considered in a reversed-phase TLC system ($1/E_0$) [9,10]. The mean slopes of the TLC equations previously calculated for triazines, dermorphins, prostaglandins, naphthalenes and quinolines and also those newly obtained for steroids are listed in Table 3. The ratios between slopes in different systems and the ratios between the $1/E_0$ values are also reported in Table 3. As already pointed out, some of the original TLC equations had to be recalculated [4]. Therefore, some of the mean slopes reported in Table 3 are different from those reported previously [2,6]. In no way do the new data change the general trend. In particular, the ratios between the mean slopes of the TLC equations in different solvent systems are close to the ratios between the $1/E_0$ values for the corresponding solvent pairs. It can be noted that the slopes in the acetone system seem to be lower than expected on the basis of the $1/E_0$ values. In fact, the acetone/methanol slope ratio (1.53) is lower than the corresponding reciprocal solvent strength ratio (1.70). Despite some deviation of the experimental data from the theoretical values, the whole picture seems to be consistent. In other words, it appears reasonable

Table 3
Ratios between slopes in different TLC systems

Compounds	Mean slopes in solvent systems			Ratios		
	Acetone	Acetonitrile	Methanol	Acetone/ acetonitrile	Acetone/ methanol	Acetonitrile/ methanol
Steroids	−0.046 (±0.002)	−0.041 (±0.001)	−0.027 (±0.001)	1.12	1.70	1.52
Triazines	−0.037 (±0.001)	−0.036 (±0.001)	−0.027 (±0.001)	1.03	1.37	1.33
Prostaglandins	−0.072 (±0.002)	−0.067 (±0.001)	−0.043 (±0.002)	1.07	1.67	1.56
Dermorphins	−0.064 (±0.003)	–	−0.047 (±0.003)		1.36	
Naphthalenes and quinolines	−0.046 (±0.001)	–	−0.030 (±0.001)		1.53	
$\bar{x} \pm$ S.E.[a]				1.07 ± 0.03	1.53 ± 0.07	1.47 ± 0.07
Solvent strength ($1/E_0$)	1.78	1.54	1.05	1.15	1.70	1.47

[a] Mean ± standard error.

to relate the TLC data to the solvent strengths of the organic modifiers.

The above results are based on the analysis of the slope of the TLC equations in different organic modifier systems. Interestingly, the same conclusions can be drawn by examining the b values of the equations correlating intercepts and slopes of the TLC equations, *i.e.*, taking into consideration the equations in Table 2. According to our hypothesis, a given compound is more sensitive to the changing concentration of the organic modifier when this is acetone rather than acetonitrile or methanol. As a consequence, for a given series of compounds, the slopes of the TLC equations in an acetone system cover a wider range than those in acetonitrile or methanol systems. This point can be illustrated by the equations in Table 2, correlating slopes and intercepts of the TLC equations. The higher absolute b values of Eqs. 6, 9, 12, 14 and 16 in Table 2 indicate a narrower range of variations of the slopes of the TLC equations in the methanol system. In the acetonitrile (Eqs. 5, 8 and 11) and acetone (Eqs. 4, 7, 10, 13 and 15) systems, wider ranges of variations of the slopes of the TLC equations lead to lower b values. As a consequence, the ranking of the b values in Table 2 for each series of compounds is methanol > acetonitrile > acetone, *i.e.*, opposite to that shown for the mean slopes in Table 3. As the b values in Table 2 seem to depend on the organic modifiers, they should be related to the solvent strengths. In fact, in Table 4 the ratios between the b values in Table 2 are close to the corresponding ratios between the E_0 values. Because of the inverse ranking of the slopes in Table 2 with respect to the ranking of the mean slopes in Table 3, the comparisons in Table 2 were carried out on the basis of the E_0 values instead of the $1/E_0$ values. The deviations of the experimental ratios from the theoretical values in Table 4 show the same trend as observed in Table 3.

4. Discussion and conclusions

The present data on steroids are in agreement with previous findings for many series of chemical agents, *i.e.*, the extrapolated R_M values are not dependent on the nature of the organic modifier, and the intercepts and slopes of the TLC equations of strictly congeneric compounds are linearly related. Further, it is important to point out that the R_M values measured here are very close to those determined more than 15 years ago in our laboratory for the same chemicals. This finding is very important as it certainly supports the reliability of the R_M values as a lipophilicity parameter.

As regards the influence of the organic solvent in the mobile phase on the slopes of the TLC

Table 4
Ratios between slopes of the equations in Table 2

Compounds	Slopes in solvent systems			Ratios		
	Acetone	Acetonitrile	Methanol	Acetone/ acetonitrile	Acetone/ methanol	Acetonitrile/ methanol
Steroids	−72.872	−80.365	−122.802	0.91	0.59	0.65
Triazines	−69.484	−74.194	−109.730	0.94	0.63	0.68
Prostaglandins	−61.014	−73.829	−86.005	0.83	0.71	0.86
Dermorphins	−56.775		−69.317		0.82	
Naphthalenes and quinolines	−62.704		−87.810		0.71	
$\bar{x} \pm$ S.E.[a]				0.89 ± 0.03	0.69 ± 0.04	0.73 ± 0.06
Solvent strength (E_0)	0.56	0.65	0.95	0.86	0.59	0.68

[a] Mean ± standard error.

equations, the present data show that this aspect can be studied from two different points of view, *i.e.*, either considering the slopes of the TLC equations or taking advantage of the equations relating slopes and intercepts of the TLC equations themselves. In fact, the ratios between the slopes of both kinds of equations in different solvent systems are close to the ratios between appropriate expressions of the corresponding solvent strengths of the organic modifiers (Tables 3 and 4). In fact, the slopes of these equations depend on the solvent strength of the organic modifier, *i.e.*, on the higher sensitivity of a given compound to acetone rather than acetonitrile or methanol. Accordingly, the results obtained with a given organic solvent in the mobile phase can be related to those obtained with a different organic solvent, by taking into consideration the solvent strengths of the organic modifiers. Finally, the relationship between TLC data and solvent strengths does not depend on the structure of the chemical series taken into consideration. In fact, despite some deviations from the theoretical values, the results outlined above were obtained by studying six chemical series showing wide structural variety. This again allows the TLC method to be considered as a general procedure for the determination of lipophilicity.

5. References

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