Effect of TLC Support Characteristics and Coating on the Lipophilicity Determination of Phenols and Aniline Derivatives

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Abstract

The lipophilicities of 18 ring-substituted phenol and aniline derivatives are determined on alumina and silica TLC supports impregnated with 2.5 and 10% (v/v) paraffin oil in *n*-hexane using a mixture of water–methanol as the mobile phases. The effect of calculated physicochemical parameters and chromatographic conditions on the retention is elucidated by stepwise regression analyis. It is established that the degree of impregnation exerts a greater impact on the retention than the character of the support. Not only the calculated lipophilicity but also various sterical and polarity parameters significantly influence the retention, indicating the involvement of factors other than hydrophobic forces in the retention mechanism.

Introduction

The application of quantitative structure activity relationship (QSAR) studies has considerably facilitated the rational design of new bioactive molecules such as pharmaceuticals (1,2), pesticides (3), and others. A wide variety of molecular parameters have been calculated and tentatively applied for the assessment of the relationship between biological activity and physicohemical characteristics (4). Lipophilicity is one of the molecular parameters most frequently used in QSAR studies (5). Because of their advantageous application parameters, various reversed-phase (RP) liquid chromatographic methods have been extensively used for the determination of lipophilicity (6,7). These techniques require only a small amount of compounds, and they do not need to be very pure because the impurities are readily separated during the chromatographic process. It has been established that both planar [thin-layer chromatography (TLC)] and high-performance liquid chromatography (HPLC) are equally adequate for the determination of lipophilicity (8), and the lipophilicity values measured by HPLC and TLC are generally well correlated (9).

The determination of lipophilicity (R_M) by reversed-phase (RP)-TLC is rapid, needs no complicated instrumentation, and is easy to carry out. In recent years the lipophilicity of a considerable number of molecules has been measured by RP-TLC and the relationship between biological activity and lipophilicity has been assessed. Thus, the lipophilicity values of semisynthetic cephalosporins (10), antiarrhythmic and antihypertensive 1-[2-hydroxy-3-(4-aryl-1-piperazinyl)propyl]-pyrrolidin-2-1 or 1-[2-acetoxy-3-(4-aryl-1-piperazinyl)propyl]-pyrrolidin-2-1 derivatives (11), benzimidazoles (12), 2,4-dihydroxythiobenzanilides (13), and carbonyl derivatives of 2-aminoimidazolines have been determined (14,15). Good correlations have been found between the biological activity and R_M values of benzimidazole (16), 1,2,4-triazole derivatives (17), and potential fungicides (18).

Because the R_M value generally depends linearly on the concentration of the organic modifier in the mobile phase, the value has been frequently extrapolated to a zero concentration of organic component, which increases in this manner the reliability of the determination of lipophilicity (19).

However, chromatographic methods have some drawbacks. The adsorption characteristics of the original supports more or less remain even after coating the surface of the support with the apolar ligand (20). This side effect influences retention and subsequently modifies lipophilicity. It has also been observed many times that not only the adsorption characteristics of the support but also the concentration of the hydrophobic ligand on the surface exert a marked influence on the R_M value (21).

The objectives of the investigations were the determination of the lipophilicity of some ring-substituted phenol and aniline derivatives, the elucidation of the influence of the character of the support and the concentration of hydrophobic ligand on the retention of solutes, and the assessment of the relationship between the RP-TLC retention of solutes and their calculated physicochemical parameters.

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Experimental

Paraffin oil (paraffinum liquidum, pharmaceutical grade) (22), *n*-hexane (HPLC grade), and methanol (HPLC grade) were acquired from LABORCHEM Kft (Budapest, Hungary). Ring-substituted phenol and aniline derivatives of analytical purity were the products of REANAL Fine Chemicals (Budapest, Hungary). DC-Aluminium-oxide F_{254} (Merck, Darmstadt, Germany) and Polygram UV_{254} silica gel plates (Macherey-Nagel, Düren, Germany) were impregnated in *n*-hexane paraffin oil mixtures (97.5–2.5 and 90–10, v/v) as previously described (23). The application of paraffin-oil-coated plates instead of ready made RP-TLC was motivated by the fact that the objective of the study was the assessment of the effect of support characteristics and degree of coating on the lipophilicity of solutes that can't be elucidated using ready made RP-TLC plates. The IUPAC names of the analytes have been compiled in Table I. They were separately dissolved in methanol at a concentration of 3 mg/mL and the solutions (2 µL) were spotted on the plates. Water–methanol mixtures were used as mobile phases; the methanol concentration ranged from 0 to 25% (v/v) in increments of 2.5%. In order to evaluate the effect of support and the degree of coating on the RP-TLC retention of these solutes, the mobile phases have not been buffered. Developments were carried out in 22- x 22- x 3-cm sandwich chambers at room temperature; the development distance was approximately 16 cm. After the development, the solute spots were revealed by their UV absorbance. Each experiment was run in quadruplicate.

The R_M values characterizing molecular lipophilicity in RP-TLC were calculated for each analyte in each RP-TLC system:

$$R_M = \log(1/R_F - 1) \qquad \text{Eq. 1}$$

where the coefficient of variation of parallel determinations was > 6% and the R_M values were omitted from the following calculations.

Table I. IUPAC Names of Ring Substituted Phenol and Aniline Derivatives

No. of solute	IUPAC name
1	Phenol
2	4-Hydroxyphenol
3	2-Hydroxyphenol
4	2-Methoxyphenol
5	2-Methylphenol
6	3-Methylphenol
7	4-Methylphenol
8	4-Cyanophenol
9	4-Bromophenol
10	4-Chlorophenol
11	3-Fluorophenol
12	3,4-Dihydroxybenzaldehide
13	2-Aminophenol
14	3-Aminophenol
15	4-Aminophenol
16	2-Nitroaniline
17	2-Methoxyaniline
18	4-Methoxyaniline

In order to increase the reliability of the determination of lipophilicity, the R_M were extrapolated to zero methanol concentration (C):

$$R_M = R_{M0} + b.C \qquad \text{Eq. 2}$$

where R_M is the R_M value of an analyte measured at a given methanol concentration; R_{M0} is the theoretical (calculated) R_M value extrapolated to zero methanol concentration (best estimate of lipophilicity); b is the decrease in R_M value caused by the 1% (v/v) increase in the concentration of methanol in the mobile phase. The calculations were performed separately for alumina supports impregnated with 2.5% ($R_{M0(alu2.5)}$) and 10% paraffin oil ($R_{M0(alu10)}$), and for silica supports also impregnated with 2.5% ($R_{M0(sil2.5)}$) and 10% paraffin oil ($R_{M0(sil10)}$). Because the relationships calculated by equation 2 were highly significant, the application of quadratic equations that take into consideration the possible curvature of the correlation were not necessary.

In order to determine the relationships between the R_{M0} values of solutes developed in four different RP-TLC systems and their calculated physicochemical parameters, stepwise regression analysis was employed (24). In the common multivariate regression analysis, the presence of independent variables exerting no significant influence on the change of dependent variable considerably decreases the significance level of the equation. Stepwise regression analysis automatically eliminates from the selected equation the dependent variables that have no significant impact on the dependent variable, which increases in this manner the reliability of calculation. The physicochemical parameters of the calculation included: = Hansch-Fujita's substituent constants characterizing hydrophobicity; H – Ac and H – Do = indicator variables for proton acceptor and proton donor properties, respectively; M – RE = molar refractivity; F and R = Swain and Luton's electronic parameters characterizing the inductive and resonance effects; q = Hammett's constant characterization of the electron-withdrawing power of the substituent; Es = Taft's contant characterizing the steric effects of substituents; and B_1 and B_4 = Sterimol width parameters that were determined by the distance of substituents at their maximum point perpendicular to attachment. The parameters of the solutes were calculated according to the additivity rule from the fragmental constants. The four R_{M0} values were separate from the dependent variables and the physicochemical parameters were the independent variables in each instance. In order to detect the physicochemical parameters that exerted a secondary but significant influence on the R_{M0} values, the same calculations have also been performed and the physicochemical parameters showed a significant effect according to the first stepwise regression analysis that was omitted.

The influence of support characteristic and the degree of impregnation on the lipophilicity values has also been elucidated by stepwise regression analysis. The dependent variables were the R_{M0} values determined in each RP-TLC system. The physicochemical parameters, the presence of alumina or silica supports (characterized by the dummy variable 1 or 0), and the degree of impregnation (2.5 and 10) were the independent variables.

The number of accepted independent variables was not limited and the acceptance limit was set to a 95% significance level for each stepwise regression analysis.

Software for stepwise regression analysis was purchased from CompuDrug (Budapest, Hungary).

Results and Discussion

The solutes showed regular retention behavior in each RP-TLC system. Their retention decreased uniformly with increasing methanol concentration in the mobile phase regardless of the type of support and the degree of impregnation. This result indi-

Table II. Parameters of Linear Relationship Between the RM Value of Solutes and the Concentration of Methanol in the Mobile Phase*

No. of solute	R_{M0}	$-b.10^2$	$s_b \cdot 10^3$	$r_{calc.}$
1	0.16	1.83	3.60	0.9150
2	0.65	1.57	2.29	0.9420
3	–0.01	1.04	2.09	0.8967
4	0.47	1.75	2.03	0.9620
5	0.63	1.67	1.49	0.9770
6	0.63	1.65	1.78	0.9668
7	0.64	1.64	1.65	0.9713
8	near to the solvent front			
9	0.81	0.85	1.11	0.9520
10	0.64	0.74	0.78	0.9687
11	0.20	0.38	0.84	0.8807
12	remained on the start			
13	0.65	1.03	1.40	0.9484
14	–0.20	1.09	0.89	0.9804
15	0.38	1.86	1.58	0.9790
16	0.72	2.29	1.59	0.9859
17	0.36	2.20	1.91	0.9782
18	0.10	2.41	1.80	0.9837

* The support was alumina impregnated with paraffin oil–n-hexane (2.5:97.5, v/v).

Table III. Parameters of Linear Relationship Between the RM Value of Solutes and the Concentration of Methanol in the Mobile Phase*

No. of solute	R_{M0}	$-b.10^2$	$s_b \cdot 10^3$	$r_{calc.}$
1	0.67	1.86	1.45	0.9823
2	0.67	1.07	2.18	0.8951
3	0.35	1.35	1.31	0.9732
4	0.99	2.42	2.09	0.9786
5	1.13	2.13	1.68	0.9818
6	1.13	2.11	1.89	0.9769
7	1.12	2.04	2.06	0.9706
8	near to the solvent front			
9	1.32	1.37	2.49	0.9136
10	1.13	1.24	1.78	0.9433
11	0.69	0.94	2.17	0.8701
12	elongated spot shape			
13	0.98	1.31	2.18	0.9259
14	0.16	1.63	1.63	0.9712
15	0.48	1.97	1.73	0.9777
16	1.16	2.66	0.90	0.9966
17	0.80	2.53	1.27	0.9926
18	0.53	2.76	1.33	0.9931

* The support was alumina impregnated with paraffin oil–n-hexane (10:90, v/v).

Table IV. Parameters of Linear Relationship Between the RM Value of Solutes and the Concentration of Methanol in the Mobile Phase*

No. of solute	R_{M0}	$-b.10^2$	$s_b \cdot 10^3$	$r_{calc.}$
1	near to the solvent front			
2	–0.62	1.86	2.90	0.9345
3	–0.37	1.86	1.95	0.9686
4	0.45	2.20	1.99	0.9765
5	0.57	1.18	1.37	0.9834
6	0.56	1.76	1.19	0.9867
7	0.57	1.76	1.32	0.9838
8	0.35	1.88	0.92	0.9928
9	1.03	1.77	1.06	0.9894
10	0.83	1.51	0.88	0.9900
11	elongated spot shape			
12	0.01	2.43	2.06	0.9793
13	–0.20	1.28	2.35	0.9114
14	–0.34	1.59	2.00	0.9560
15	elongated spot shape			
16	0.95	2.14	1.72	0.9811
17	0.55	2.26	3.60	0.9316
18	0.14	1.79	4.02	0.8756

* The support was silica impregnated with paraffin oil–n-hexane (2.5:97.5, v/v).

Table V. Parameters of Linear Relationship Between the RM Value of Solutes and the Concentration of Methanol in the Mobile Phase*

No. of solute	R_{M0}	$-b.10^2$	$s_b \cdot 10^3$	$r_{calc.}$
1	near to the solvent front			
2	–0.35	1.56	2.82	0.9145
3	–0.09	1.63	1.77	0.9664
4	0.77	2.17	1.90	0.9778
5	0.86	1.60	1.27	0.9817
6	0.85	1.59	0.61	0.9956
7	0.86	1.61	0.82	0.9923
8	0.65	1.80	0.79	0.9943
9	1.30	1.16	2.55	0.9345
10	1.04	0.89	1.61	0.9144
11	elongated spot shape			
12	0.28	1.51	2.75	0.9134
13	0.09	1.57	0.96	0.9890
14	–0.07	1.74	1.21	0.9858
15	elongated spot shape			
16	1.21	2.30	1.80	0.9819
17	0.97	2.66	2.46	0.9752
18	0.54	2.39	2.18	0.9760

* The support was silica impregnated with paraffin oil–n-hexane (10:90, v/v).

cates that the R_{M0} value of analytes can be safely calculated using equation 2.

The parameters of equation 2 have been compiled in Tables II, III, IV, and V. The equation fit the experimental data well, and the significance levels in each instance were > 95% (see calculated *r* values). The ratio of variance that was explained varied between 75.71% and 99.32%, which proved the reliability of the method. The parameters of equation 2 showed marked variations among the solutes, indicating that they can be separated by RP-TLC under appropriate conditions. It can be further established that not only the type of solute but also the character of the RP-TLC system (support and degree of impregnation) exert a marked influence on the retention parameters. Because TLC can be employed as a pilot method for HPLC (25,26), the data can be used for the prediction of the behavior of the analytes even on octadecyl-coated alumina stationary phase in RP-HPLC.

The parameters of the equations decribing the relationships between the R_{M0} values determined in various RP-TLC systems and their calculated physicochemical parameters have been listed in Tables VI and VII. Stepwise regression analysis showed significant correlations in each instance, the significance level being always over 95% (compare $F_{calc.}$ values with tabulated values of $F_{5\%}$) suggesting that these calculated parameters exert a significant impact on the retention and they can be used for the prediction of the retention of similar analytes. However, the ratio of variance explained on the alumina support is relatively low, indicating that other physicochemical parameters not included in the calculations may have also influenced the retention. The retention of solutes mainly depends on the polarity parameters, which suggests the importance of hydrophilic binding forces (probably electronic interactions) between the surface of stationary phase and the polar substructures of solutes. This finding can be tentatively explained by the assumption that paraffin oil does not entirely cover the adsorption centers of the polar supports and some remain available for the small solute molecules even after impregnation.

Interestingly, the calculated lipophilicity values ( ) are significantly related to the R_{M0} values only in the case of alumina support at the higher degree of impregnation (equation III in Table VI), and the same relationship is of secondary importance in the case of lower degree of impregnation (equation II in Table VI).

The parameters of the equation describing the dependence of all R_{M0} values on the physicochemical parameters and RP-TLC conditions have been compiled in Table VIII. The equation selected by stepwise regression analysis fit the original data well and the significance level was over 99.9%. The results entirely support our previous quantitative conclusions. The calculated lipophilicity values had the highest impact on the retention (see path coefficient, b' values), which proved that the mode of separation is based on partititon in these RP-TLC systems. The degree of impregnation exerted the second highest impact, which empha-

Table VI. Parameters of Relationships Between the RM0 Values of Solutes and Their Physicochemical Parameters, Alumina Support Impregnated With 2.5 and 10% Paraffin Oil in n-Hexane, and Results of the Stepwise Regression Analysis (n = 16)

	No. of equation			
Parameter	I*	II†	III‡	IV§
a	0.19	0.56	1.02	0.67
b1	0.80	0.18	0.27	-0.31
sb1	0.37	0.07	0.06	0.13
b2	–0.46	–	–	–0.43
sb2	0.13	–	–	0.15
b'1 (%)	37.87	–	–	46.03
b'2 (%)	62.13	–	–	53.97
r2	0.5682	0.3217	0.5544	0.6324
Fcalc	8.55	6.34	17.42	11.18
F5%	3.80	4.60	4.60	3.80

* Equation I: $R_{M0(Alu2.5)} = a + b_1.sigma + b_2.Es$
† Equation II: $R_{M0(Alu2.5)} = a + b_1.$
‡ Equation III: $R_{M0(Alu10)} = a + b_1.$
§ Equation IV: $R_{M0(Alu10)} = a + b_1.H – Do + b_2.Es$

Table VII. Parameters of Relationships Between the RM0 Values of Solutes and Their Physicochemical Parameters, Silica Support Impregnated With 2.5 and 10% Paraffin Oil in n-Hexane, and Results of Stepwise Regression analysis (n = 15)

	No. of equation			
Parameter	I*	II†	III‡	IV§
a	0.94	0.56	0.95	0.67
b1	–0.87	–0.45	–1.01	–0.46
sb1	0.11	0.09	0.10	0.08
b2	–0.35	0.12	0.34	0.13
sb2	0.11	0.02	0.10	0.02
b3	–	0.68	–	0.60
sb3	–	0.18	–	0.17
b'1 (%)	71.47	34.38	75.15	34.60
b'2 (%)	28.53	39.85	24.85	43.05
b'3 (%)	–	25.80	–	22.35
r2	0.8683	0.9159	0.9001	0.9290
Fcalc.	39.57	39.92	54.07	48.01
F5%	3.88	3.59	3.88	3.59

* Equation I: $R_{M0(Si2.5)} = a + b_1.H – Do + b_2.Es.$
† Equation II: $R_{M0(Si2.5)} = a + b_1.H – Ac + b_2.M – RE + b_3.R.$
‡ Equation III: $R_{M0(Si10)} = a + b_1.H – Do + b_2.B1.$
§ Equation IV: $R_{M0(Si10)} = a + b_1.H – Ac + b_{2£4,}.M – RE + b_3.R.$

Table VIII. Parameters of Relationships Between the RM0 Values of Solutes, Their Physicochemical Parameters, the Character of Support and the Degree of Impregnation*

No. of independent variables	b	s_b	b'(%)
	0.21	0.05	23.02
H – Do	–0.21	0.10	11.59
M – RE	0.05	0.01	17.24
o	0.89	0.23	14.82
Effect of support	0.21	0.06	12.47
Degree of impregnation	0.05	0.01	20.86

* n = 62, a = 0.53, r2 = 0.7665, Fcalc = 30.09, and F99.9% = 4.73.

sized the importance of the amount of hydrophobic ligands in the retention.

Hoewever, the data clearly showed that polarity parameters also influenced retention, which suggested a mixed retention mechanism.

Conclusion

It can be concluded from the results of stepwise regression analyses that the retention of ring-substituted phenol and aniline derivatives in various RP-TLC systems mainly depends on their lipophilicity and on the degree of impregnation of the polar support. However, hydrophilic (electronic) interactions also play a considerable role in the retention, indicating the involvement of polar interactive forces. The considerable differences among the R_M values determined under different RP-TLC conditions indicate that the accurate prediction of the log *P* value by RP-TLC methods is questionable and the results have to be treated with caution.

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