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Influence of mobile phase composition on evaluation of lipophilicity by partition chromatography

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ABSTRACT

    The problems of the concentration dependence of retention indices and the applicability of extrapolated values in the evaluation of lipophilicity were studied. The reversed-phase high-performance liquid chromatography of arylalkanoic acids were carried out with experimental data for substituted estra-1,3,5 (10)-trienes, benzodiazepines, dermorphine derivatives and dansylamides selected from the literature for this purpose. Fair linear relationships between slopes of concentration dependences and extrapolated and non-extrapolated values of R_M and log k' were found. Equivalence of these indices in the evaluation of lipophilicity can be inferred. Statistically significant dependences of log P ($\Sigma\pi$) values on concentration slopes make it possible to use them as new parameters of lipophilicity. The goodness of fit of these relationships increases when the values of $E_T(30)$, as a measure of the solvatochromic solvent polarity of mobile phases, are used instead of the change in modifier concentration.

INTRODUCTION

    Lipophilicity is one of the inherent properties of chemical compounds, affecting their biological activity. It plays a determinant role in the transport of compounds through a biological system and it may also influence the formation of a complex between a compound and a receptor or a biomacromolecule at the site of action. Partition chromatography is widely used in the evaluation of lipophilicity [1–4]. Application of partition chromatography for such a purpose follows from the relationship between suitable retention indices and partition coefficient, P_s, determined in the chromatographic system. A suitable index in thin-layer chromatography (TLC) is R_M, which is related to the experimental R_F value and depends linearly on log P_s [5]. The logarithm of the capacity factor, log k' [6,7], is equivalent to R_M in high-performance liquid chromatography (HPLC). R_M or log k' values can be used directly as lipophilicity parameters. Another possibility is the application of linear relationships between retention indices and log P, determined in the reference system octanol–water, for the calculation of log P from experimental values of R_M or log k'. In such a case, it is necessary that Collander's linear relationship [8] holds for the chromatographic and the reference system octanol–water:

$$\log P = a \log P_s + b \tag{1}$$

It follows that

$$\log P = a\, R_M\, (\log k') + b \tag{2}$$

The statistical significance of these relationships depend on the type of chromatographic system and a suitable choice of the series of compounds tested.

The use of retention indices extrapolated to pure water as a mobile phase is a commonly discussed problem accompanying the utilization of chromatographic parameters for the evaluation of lipophilicity. Methanol, acetone or acetonitrile is usually used as a modifier and their concentration has a significant effect on the mobility of separated compounds. Soczewinski and Wachtmeister [9] found that R_M values are frequently linearly dependent on the composition of the mobile phase. For a binary mixture, this function can be expressed as

$$R_M = \varphi_1\, R_M(1) + \varphi_2\, R_M(2) \tag{3}$$

where φ_1 and φ_2 are the volume functions of the two components in the binary mobile phase and $R_M(1)$ and $R_M(2)$ are the R_M values in pure components of the mobile phase. It follows from eqn. 3 that

$$R_M = R_M^0 - a\, \varphi \tag{4a}$$

where φ is the fraction of the modifier and R_M^0 is the value of R_M when $\varphi = 0$. For reversed-phase HPLC, Schoenmakers et al. [10] suggested that the relationship between solute retention ($\log k'$) and the concentration φ of the modifier can be expressed by the equation

$$\log k' = \log k_0' + A\, \varphi^2 - B\varphi \tag{4b}$$

where A and B are constants. Snyder et al. [11] showed that a linear relationship expressed by the equation

$$\log k' = \log k_0' - a\, \varphi \tag{4c}$$

is valid over a limited range of concentration φ of the modifier.

Such expressions can be used for calculation of theoretical values of retention indices, R_M^0 and $\log k_0'$, extrapolated to pure water. A number of workers [12–15] consider that these values are more suitable as lipophilicity parameters than experimental values determined at a particular concentration of the modifier in the mobile phase. There are two reasons for this consideration: (a) R_M or $\log k'$ scales are extended, which allows better resolution in the characterization of lipophilicity; (b) lipophilicity is better expressed by R_M^0 or $\log k_0'$ calculated in the extrapolated system, as such a system is closer to the reference system octanol–water. Whereas the first reason is clear-cut, the second is less so. Chromatographic indices determined at different φ were often found to be equivalent for the evaluation of lipophilicity.

Some years ago, we compared [16] retention indices R_M and $\log k'$ obtained from TLC and HPLC, respectively, using mobile phase with a modifier or extrapolated to pure water. Eqns. 5 and 7 were obtained for non-extrapolated retention indices, and eqns. 6 and 8 for indices extrapolated to pure water. An increase in selectivity of the evaluation of lipophilicity is indicated by a decrease in the slopes in eqns. 6 and 8. At the same time, however, the statistical significance of both equations is slightly diminished.

$$
\begin{array}{llllll}
 & & n^a & r^a & s^a & F^a \\
\log P = 1.990\, R_M^{50} + 2.399 & & 11 & 0.998 & 0.048 & 2048.8 & (5) \\
\log P = 0.797\, R_M^{0} + 1.763 & & 11 & 0.991 & 0.097 & 494.7 & (6) \\
\log P = 1.790\, \log k'_{60} - 1.971 & & 12 & 0.999 & 0.024 & 8353.2 & (7) \\
\log P = 1.103\, \log k'_{0} - 0.926 & & 12 & 0.991 & 0.097 & 527.0 & (8)
\end{array}
$$

This paper considers more deeply the problem of concentration dependences of retention indices in the evaluation of lipophilicity.
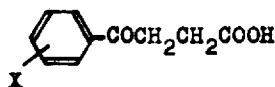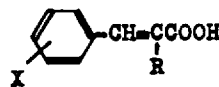
EXPERIMENTAL

TLC of compounds I–III

To prepare the stationary phase, silanized Kieselgel 60 F_{254} (E. Merck, Darmstadt, Germany) was impregnated by washing glass plates (20 × 10 cm) with a 5%

I: X—⟨benzene⟩—CH₂COOH

II: X—⟨benzene⟩—COCH₂CH₂COOH

III: X—⟨benzene⟩—CH=CCOOH (R)
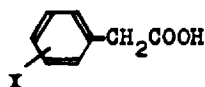
ethereal solution of silicone oil (Lukoil 100; VCHZ, Kolin, Czechoslovakia); the volatile components were evaporated within 16 h at 20°C. A citrate buffer (pH 3.4) containing various percentages of acetone was used as the mobile phase. Solutions (1%) of acids I–III in methanol were prepared and 5-μl samples were applied to the plates 3 cm from the lower edge. After evaporating off methanol at 20°C, ascending one-dimensional TLC was carried out. A chromatographic chamber was equilibrated with the mobile phase for 16 h at 20°C. After migration for 15 cm, the plates were removed and, after the remaining mobile phase had been evaporated off, the acids were detected under UV light (254 nm). Each chromatogram contained six compounds, two acids serving as reference samples. In the individual chromatogram, the R_F values of standards did not differ by more than 0.02.

TLC of compounds IV–VI

Experimental data were taken from the literature [17–20]. TLC of 16α-substituted estra-1,3,5(10)-trienes (IV) was performed [17] on silanized silica gel (PF_{254}) and the R_F values of androstane-type steroids were measured [18] on a silica gel G layer impregnated with silicone oil (DC 200). Acetone–water mixtures were used as the mobile phase in both instances. The R_M values of benzodiazepine derivatives V were obtained [19] on Silcoplat F_{254} impregnated with paraffin oil with acetonitrile–0.06 M KH_2PO_4 (pH 4.6) mixtures as mobile phases. The chromatography of dermorphine-related derivatives VI was performed [20] on silica gel G impregnated with silicone oil (DC 200) with methanol–aqueous buffer (pH 7.0) as mobile phase.

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^{a} The symbols r, s and F are explained under Experimental; n is the number of compounds.

IV

V

L-Tyr-D-Ala-L-Phe-NH-R^1-CONHR^2
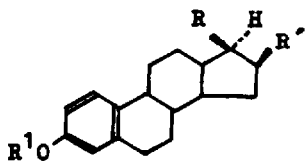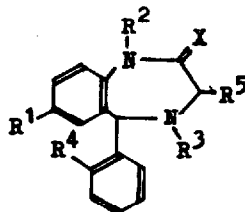
VI

HPLC

Determination of acids **I** was carried out using a liquid chromatograph assembled from a Model 6000 A pump, U6K injector, a Model 440 fixed-wavelength detector and an N730 data module (Waters Assoc., Milford, MA, USA). A μBondapak C$_{18}$ column (30 cm × 3.9 mm I.D.) (Waters Assoc.) and a custom-made column (25 cm × 3.9 mm I.D.), slurry-packed with 5-μm Spherisorb ODS (Phase Separations, Hauppage, NY, USA) were used as stationary phases. A phosphate buffer (pH 3.0) containing various percentages of methanol was used as the mobile phase. The eluent flow-rate was 1 ml/min. UV detection at 280 μm was used, range 0–0.01 a.u. The retention time of sodium nitrite (0.2% solution) was taken as t_o and the capacity factor, k', was evaluated from the retention time, t_r, of the solute, using the relationship $k' = (t_r - t_o)/t_o$.

HPLC of dansylamides **VII** was performed [21] on Spherisorb S5 ODS-2 with methanol–water as mobile phase.
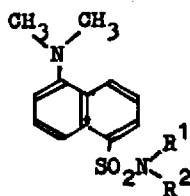
VII

Determination of partition coefficients

Experimental partition coefficients of acids **I–III** were determined by the shake-flask method [22] in octanol–acetate buffer (pH 3.4) at 20°C, with each phase being presaturated with the other. The concentration of acids in both phases was determined spectrophotometrically and the partition coefficients P were calculated as the ratio of concentrations in octanol and aqueous phases ($P = C_o/C_w$).

Determination of eluent polarity

The elution properties of the mobile phase were characterized by the change in its polarity. The values of $E_T(30)$, taken from paper of Johnson *et al.* [23], were used for this purpose. These values are based on the charge-transfer absorption (E_T-30) of 2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridinio) phenolate (Reichardt's dye). This compound exhibits a very large solvatochromic effect on going from a very polar solvent (water, 453 nm) to a non-polar solvent (diphenyl ether, 810 nm). The values of $E_T(30)$ were calculated from corresponding wavelength of maximum absorption. The slopes $a(E_T)$ of the dependences $\log k' = a(E_T)E_T(30) + b$ were taken from ref. 23.

Sample preparation

Synthesis of arylacetic (I) [24,25], aryloxoalkanoic (II) (26) and cinnamic acids (III)[27] are described elsewhere. The origins of the compounds IV–VII are described in refs. 17–20.

Calculations

Log P values of the remaining acids I, II and III were calculated using parameters π according to the relationship $\log P = \log P_H + \Sigma\pi$, where P and P_H are the partition coefficients of a substituted and unsubstituted acid, respectively, and π is the substituent parameter of lipophilicity. Parameters π derived [28] for arylacetic acids were used for acids I whereas parameters π derived [28] for substituted benzoic acids were used for acids II and III. The sums of parameters π for 3-chloro-4-alkoxy derivatives were reduced by 0.23, in accordance with the results [16] of partition chromatography of these derivatives.

The coefficients in regression equations were calculated from the experimental results by multiple regression analysis. The statistical significance of the regression equations was tested by the standard deviation s, the coefficient of multiple correlation r and the Fischer–Snedecor criterion F.

RESULTS AND DISCUSSION

Eqns. 5–8 derived from chromatographic indices of arylacetic acids I [16] show statistical equivalence of both extrapolated and non-extrapolated values used as parameters of lipophilicity. Similar results were obtained also when using μBondapak as the stationary phase, as is evident from eqns. 9 and 10; the corresponding chromatographic indices are summarized in Table I.

	n	r	s	F	
$\log P = 1.548 \log k'_{50} + 1.562$	11	0.995	0.086	808.5	(9)
$\log P = 0.800 \log k'_0 + 0.326$	11	0.996	0.077	1014.8	(10)

The evaluation of the concentration dependences of R_M values (Table II) led to eqns. 11 and 12 for arylacetic (I), eqns. 13 and 14 for aryloxobutanoic (II) and eqns. 15 and 16 for cinnamic acids (III), respectively. The similarity of extrapolated and non-extrapolated R_M values as lipophilicity parameters is obvious.

	n	r	s	F	
$\log P = 1.362\,R_M^{40} + 2.004$	6	0.992	0.108	233.1	(11)
$\log P = 0.599\,R_M^{0} + 1.630$	6	0.995	0.081	415.6	(12)
$\log P = 1.940\,R_M^{45} + 1.931$	5	0.987	0.137	109.8	(13)
$\log P = 0.696\,R_M^{0} + 1.386$	5	0.968	0.211	44.9	(14)
$\log P = 1.792\,R_M^{45} + 2.306$	5	0.993	0.122	224.8	(15)
$\log P = 0.713\,R_M^{0} + 1.737$	5	0.989	0.157	134.6	(16)

Very different values of the slopes of the concentration dependences (cf. Table I) conflict with Snyder et al.'s [11] suggestion that the slopes depend only on the solvent strength of the organic modifier. Apparently, these slopes depend on the nature of the solutes. A decisive property is probably their lipophilicity, because the slopes correlate significantly with $\log P$ according to eqns. 17–19. Eqn. 17 was derived for the slopes of the concentration dependences of R_M values, eqn. 18 for $\log k'$ (Spherisorb) and eqn. 19 for $\log k'$ (μBondapak) in the series of acids I (values of slopes a are summarized in Table I).

	n	r	s	F	
$\log P = 0.557\,a + 1.431$	11	0.984	0.131	267.9	(17)
$\log P = 1.659\,a + 5.424$	12	0.929	0.261	63.4	(18)
$\log P = 0.908\,a - 1.420$	11	0.971	0.195	149.0	(19)

TABLE I

LIPOPHILICITY AND CHROMATOGRAPHIC BEHAVIOUR OF ACIDS I

Compound	X	Log P	a^a	a^b	μBondapak				
					Log $k'_{60}{}^c$	Log k'_{50}	Log k'_{40}	Log k'_{0}	a
Ia	H	1.45	—	—	−0.293	−0.008	0.135	1.417	3.65
Ib	4-Cl	2.15	1.12	4.66	−0.008	0.245	0.570	2.110	3.73
Ic	4-C_2H_5	2.43	1.71	4.50	0.170	0.566	0.782	2.546	3.96
Id	3-Cl-4-i-C_3H_7O	2.71	2.16	4.86	0.269	0.717	0.954	2.957	4.48
Ie	4-i-C_3H_7	2.85	2.17	4.79	0.334	0.825	1.064	3.130	4.61
If	4-t-C_4H_9	3.13	2.73	5.08	0.536	1.024	1.276	3.464	4.88
Ig	4-n-$C_5H_{11}O$	3.46	3.56	5.01	—	—	—	—	—
Ih	3-Cl-4-CH_3O	1.91	1.12	4.66	−0.093	0.281	0.468	2.151	3.74
Ii	4-i-C_3H_7O	2.26	1.72	4.58	0.070	0.472	0.675	2.482	4.02
Ik	3-Cl-4-CH_2=$CHCH_2O$	2.61	2.11	4.89	0.222	0.675	0.900	2.940	4.53
Il	4-i-C_4H_9	3.35	—	5.08	—	—	—	—	—
Im	4-c-$C_6H_{11}O$	3.91	4.57	5.61	0.912	1.502	1.795	4.452	5.90
In	4-n-$C_6H_{13}O$	3.96	4.74	5.55	0.972	1.575	1.872	4.590	6.03

[a] Slopes of concentration dependences: $R_M = R_M^0 - a\varphi$; R_M values were measured [16] on silanized silica gel impregnated by silicone oil.
[b] Slopes of dependences: $\log k' = \log k'_0 - a\varphi$; $\log k'$ values were obtained [16] on Spherisorb ODS.
[c] Subscripts denote the concentration of modifier in mobile phase (in %, v/v).

Highly significant linear relationships between log P and slopes a (summarized in Table II) were also obtained from TLC data in the series of acids I (eqn. 20), II (eqn. 21) and III (eqn. 22):

		n	r	s	F	
$\log P = 0.425\ a + 1.314$		6	0.996	0.076	472.7	(20)
$\log P = 0.485\ a + 1.102$		5	0.951	0.259	28.6	(21)
$\log P = 0.530\ a + 1.358$		5	0.983	0.196	85.7	(22)

If the linear relationships of slopes a and parameters of lipophilicity, log P, are valid, it can be inferred that similar linear relationships exist also between the slopes and the respective chromatographic values. These dependences have been mentioned [29–31], especially for the extrapolated retention indices measured in methanol–water. The relationships expressed by eqns. 23–28 were calculated from the chromatographic indices of acids I (Table I). Letters A and B in parentheses denote Spherisorb ODS and μBondapak respectively, as stationary phases.

		n	r	s	F	
R_M^0	$= 0.770\ a - 0.474$	11	0.999	0.058	4310.2	(23)
R_M^{50}	$= 0.281\ a - 0.489$	11	0.990	0.059	455.1	(24)
$\log k_0'\ (A)$	$= 1.584\ a - 4.333$	12	0.970	0.154	160.7	(25)
$\log k_{60}'\ (A)$	$= 0.944\ a - 4.129$	12	0.930	0.146	63.6	(26)
$\log k_0'\ (B)$	$= 1.139\ a - 2.197$	11	0.978	0.211	199.7	(27)
$\log k_{60}'\ (B)$	$= 0.589\ a - 1.937$	11	0.980	0.103	223.7	(28)

TABLE II

R_M VALUES OF ACIDS I, II AND III (R = H, CH_3) AT VARIOUS CONCENTRATIONS OF ACETONE IN MOBILE PHASE[a]

Compound	X (R[b])	Log P	R_M^{60}	R_M^{55}	R_M^{50}	R_M^{45}	R_M^{40}	R_M^0	a^c
Ia	H	1.45[d]	–	−0.48	–	−0.44	−0.43	−0.297	0.33
Ie	4-i-C_3H_7	2.85	−0.05	−0.03	0.13	0.33	0.64	1.944	3.48
Io	4-$C_6H_5CH_2O$	2.85[d]	−0.03	0.04	0.345	0.48	0.76	2.299	3.98
If	4-t-C_4H_9	3.13	0.02	0.12	0.25	0.56	0.84	2.438	4.16
Ip	3-Cl-4-$C_6H_5CH_2O$	3.43[d]	0.02	0.21	0.45	0.77	0.95	2.900	4.84
Ig	4-n-$C_5H_{11}O$	3.46	0.05	0.25	0.50	0.85	1.04	3.118	5.16
IIa	H	1.30[d]	−0.38	–	−0.35	−0.315	−0.27	−0.068	0.53
IIb	4-i-C_3H_7O	2.18	0.31	−0.09	0.01	0.18	0.31	1.404	2.74
IIc	4-i-C_3H_7	2.70	−0.05	0.02	0.18	0.29	0.45	1.448	2.54
IId	3-Cl-4-i-C_3H_7O	2.78	0	0.14	0.35	0.46	0.72	2.094	3.52
IIe	4-i-C_4H_9	3.20	0.02	0.23	0.45	0.675	0.89	2.638	4.37
IIIa	H (H)	2.13[d]	−0.32	−0.30	−0.26	−0.16	−0.09	0.374	1.20
IIIb	4-i-C_3H_7 (H)	3.53	0.05	0.21	0.41	0.73	0.91	2.702	4.48
IIIc	4-i-C_4H_9 (H)	4.03	0.155	0.33	0.74	0.93	1.07	3.075	4.86
IIId	H (CH_3)	2.58[d]	−0.15	−0.04	0.075	0.23	0.37	1.407	2.62
IIIe	4-t-C_4H_9 (CH_3)	4.26	0.315	0.58	0.84	1.06	1.38	3.445	5.22

[a] Superscripts on R_M denote the concentration of acetone in mobile phase (in vol. %).
[b] R belongs to acids III.
[c] Slopes of concentration dependences $R_M = R_M^0 - a\varphi$.
[d] Experimental values.

A similar result was obtained in the TLC of acids I–III (Table II). The linear relationships between slopes and chromatographic values are highly significant, as is evident from eqns. 29 and 30 derived for acids I, 31 and 32 for acids II and 33 and 34 for acids III.

		n	r	s	F	
R_M^0	$= 0.710\,a - 0.525$	6	1.000	0.019	$> 10^4$	(29)
R_M^{40}	$= 0.309\,a - 0.496$	6	0.995	0.061	385.3	(30)
R_M^0	$= 0.707\,a - 0.438$	5	0.998	0.080	638.9	(31)
R_M^{45}	$= 0.256\,a - 0.445$	5	0.988	0.066	123.2	(32)
R_M^0	$= 0.707\,a - 0.544$	5	0.999	0.078	1040.5	(33)
R_M^{45}	$= 0.298\,a - 0.537$	5	0.997	0.049	427.9	(34)

These relationships are of great importance for solving the question related to the equivalence of extrapolated and non-extrapolated retention indices, used as criteria of lipophilicity. If the linear relationship given by eqn. 4a holds and if, at the same time, the slope a is a linear function of extrapolated index according to eqn. 35:

$$a = m\,R_M^0 + n \tag{35}$$

then:

$$R_M = \varphi\,(mR_M^0 + n) + R_M^0 \tag{36}$$
$$R_M = (\varphi\,m + 1)\,R_M^0 + \varphi\,n \tag{37}$$

This result shows that there is a linear relationship between both indices:

$$R_M = a'R_M^0 + b' \tag{38}$$

Both parameters can be mutually substituted as criteria of lipophilicity in given range of concentrations of the modifier.

We have found that the validity of these relationships can be extended to other series of compounds, the retention characteristics of which were taken from the literature. Thirty structurally related 16α-substituted estra-1,3,5(10)-trienes (IV) [17] were used to derive eqns. 39 and 40. These equations characterize linear relationships between extrapolated and non-extrapolated R_M values and the slopes of the concentration dependences. Another series of compounds were selected from the paper by Biagi et al. [18]. Eqns. 41 and 42 were derived for androstane-type steriods (compounds 57–96 in Table I in ref. 18), whose R_M^{45} values were obtained experimentally and not by extrapolation. Also in this instance, a linear relationship exists between R_M and the slopes a of the concentration dependences.

		n	r	s	F	
R_M^0	$= 0.866\,a - 0.545$	30	0.988	0.124	1124.7	(39)
R_M^{45}	$= 0.415\,a - 0.540$	30	0.950	0.124	261.8	(40)
R_M^0	$= 0.801\,a - 2.099$	36	0.995	0.151	3182.3	(41)
R_M^{45}	$= 0.364\,a - 2.083$	36	0.971	0.155	566.7	(42)

Another example was taken from the series of benzodiazepine derivatives V. From the experimental results (Table III in ref. 19), the relationship between extrapolated R_M^0 values and slopes a of the concentration dependences is expressed by the equation

TABLE III

CHROMATOGRAPHIC INDICES OF DANSYLAMIDES VII[a]

Compound	R^1	R^2	a[b]	Log k_0'	Log k_{60}'
VIIa	CH_3	H	3.64	2.78	0.535
VIIb	C_2H_5	H	3.78	3.02	0.70
VIIc	C_3H_7	H	4.03	3.34	0.905
VIId	C_4H_9	H	4.24	3.66	1.13
VIIe	C_5H_{11}	H	4.53	4.01	1.38
VIIf	C_6H_{13}	H	4.85	4.43	1.60
VIIg		$(CH_2)_4$	3.83	3.36	1.04
VIIh		$(CH_2)_5$	4.05	3.71	1.32
VIIi		$CH_2CH_2OCH_2CH_2$	3.63	3.06	0.82
VIIk	CH_3	CH_3	3.70	3.11	0.875
VIIl	C_2H_5	C_2H_5	3.99	3.55	1.18
VIIm	CH_3	$t\text{-}C_4H_9$	4.12	3.74	1.32
VIIn	CH_3	C_4H_9	4.33	3.98	1.46
VIIo	C_2H_5	$i\text{-}C_3H_7$	4.13	3.75	1.34
VIIp	C_3H_7	C_3H_7	4.54	4.23	1.62
VIIr	C_4H_9	C_4H_9	5.15	4.96	2.08
VIIs	CH_3	$C_6H_5CH_2$	4.47	4.12	1.54

[a] Experimental data were taken from ref. 21.
[b] Slopes of concentration dependences log $k' = \log k_0' - a\varphi$.

$$R_M^0 = 0.524\ a - 1.040 \qquad \begin{array}{cccc} n & r & s & F \\ 18 & 0.951 & 0.248 & 150.1 \end{array} \qquad (43)$$

The statistical significance of the relationships between log P $(\Sigma\pi)$ and lipophilic retention quantities generally increases with increasing structural similarity of the compounds studied. The same observation holds for the relationships between the slopes of concentrations dependences and corresponding retention indices. These concentration slopes of log k' values measured for dansylamides VII (Table III) [21] were correlated with extrapolated log k_0' values. Eqn. 44 was derived for the whole series of amides[a], eqn. 45 for N-alkylamides and eqn. 46 for N,N-dialkylamides.

$$\begin{array}{lcccc} & & n & r & s & F \\ \log k_0' = 1.282\ a - 1.662 & & 17 & 0.975 & 0.131 & 283.6 & (44) \\ \log k_0' = 1.350\ a - 2.101 & & 6 & 0.999 & 0.028 & 2488.1 & (45) \\ \log k_0' = 1.246\ a - 1.424 & & 11 & 0.997 & 0.046 & 1413.2 & (46) \end{array}$$

The increase in statistical significance with increasing structural similarity in eqns. 45 and 46 is obvious. Even more significant results were obtained with eqns. 47–49 derived for retention indices evaluated in 60% methanol as mobile phase.

$$\begin{array}{lcccc} & & n & r & s & F \\ \log k_{60}' = 0.839\ a - 2.280 & & 17 & 0.925 & 0.153 & 88.9 & (47) \\ \log k_{60}' = 0.884\ a - 2.653 & & 6 & 0.997 & 0.033 & 765.9 & (48) \\ \log k_{60}' = 0.816\ a - 2.079 & & 11 & 0.991 & 0.051 & 479.1 & (49) \end{array}$$

[a] Bis-2-hydroxyethylamide (compound 12 in ref. 21) was omitted as an outlier, probably owing to the presence of a hydroxy group in the alkylamide moiety.

Similar results were obtained from the chromatography of dermorphine related derivatives **VI** measured by Barbaro *et al.* [20]. The relationships were studied in structurally closed series of monoalkyl amides (Table IV). The dependences of $\Sigma\pi$ values on extrapolated and non-extrapolated R_M values are expressed by eqns. 50 and 51. The relationships between the slopes a of the concentration dependences and R_M values are also valid, as shown by eqns. 52 and 53. The corresponding relationship between slopes a and lipophilic parameters $\Sigma\pi$ is given by eqn. 54.

$$
\begin{array}{lllll}
 & n & r & s & F \\
\Sigma\pi = 0.976\, R_M^0 - 1.465 & 12 & 0.980 & 0.207 & 247.8 \quad (50)\\
\Sigma\pi = 2.216\, R_M^{45} + 0.820 & 12 & 0.970 & 0.256 & 158.2 \quad (51)\\
R_M^0 = 0.732\, a - 1.357 & 12 & 0.987 & 0.168 & 384.7 \quad (52)\\
R_M^{45} = 0.298\, a - 1.493 & 12 & 0.921 & 0.179 & 56.2 \quad (53)\\
\Sigma\pi = 0.705\, a - 2.739 & 12 & 0.955 & 0.311 & 104.3 \quad (54)
\end{array}
$$

An interesting result was obtained when slopes a taken from different concentration ranges (for compounds **VIa, f** and **g**) were used. In this instance, eqn. 55 with lower significance was obtained:

$$
\begin{array}{lllll}
 & n & r & s & F \\
\Sigma\pi = 0.768\, a - 3.235 & 12 & 0.866 & 0.527 & 29.9 \quad (55)
\end{array}
$$

The relationships between log P ($\Sigma\pi$) and the concentration slopes show the ability of this quantity to characterize the lipophilicity of the solutes evaluated. The fit of these relationships increases if the elution properties of the mobile phase are characterized by the change in polarity instead of the change in modifier concentration. Johnson *et al.* [23] used the values of $E_T(30)$ as a solvatochromic solvent polarity scale

TABLE IV

LIPOPHILICITY AND CHROMATOGRAPHIC INDICES OF DERMORPHINE-RELATED DERIVATIVES VI[a]

Compound	R^1	R^2	a^b	R_M^0	R_M^{45}	$\Sigma\pi$
VIa	CH_2	C_2H_5	3.6 (3.9^c)	1.382	-0.28	0.08
VIb	CH_2	$C_6H_5CH_2$	4.8	2.241	0.02	0.78
VIc	CH_2	$C_6H_5CH_2CH_2$	6.3	3.087	0.17	1.34
VId	CH_2	$C_6H_5CH(CH_3)$	6.4	3.070	0.15	1.34
VIe	CH_2	$c\text{-}C_5H_9$	5.0	2.304	0.02	0.91
VIf	CH_2	$HOCH_2CH_2$	3.7 (5.2^d)	1.305	-0.42	-0.59
VIg	CH_2	$CH_3OCH_2CH_2$	3.8 (4.4^c)	1.518	-0.30	0.06
VIh	CH_2	$4\text{-}HO\text{-}C_6H_4CH_2$	4.7	1.931	-0.29	0.67
VIi	CH_2	1-Adamantyl	6.7	3.732	0.68	2.14
VIk	CH_2	1-Adamantyl-CH_2	7.0	4.017	0.86	2.70
VIl	CH_2CH_2	$C_6H_5CH(CH_3)$	6.4	3.145	0.26	1.62
VIm	CH_2CH_2	1-Adamantyl	7.3	4.065	0.77	2.42

[a] Experimental data and $\Sigma\pi$ values for chosen compounds were taken from ref. 20.
[b] Slopes of concentration dependences $R_M = R_M^0 - a\varphi$ from a wide concentration range (20–70%) of methanol.
[c] From concentration range 10–20% of methanol.
[d] From concentration range 0–20% of methanol.

TABLE V

LOG P VALUES AND SLOPES OF DEPENDENCES BETWEEN LOG k' AND $E_T(30)$ POLARITY SCALE[a]

Compound	$a(E_T)$	Log P^b	Compound	$a(E_T)$	Log P^b
Phenols			*Anilines*		
2-OH	0.159	0.88	H	0.195	0.90
3-OH	0.140	0.80	N-C$_2$H$_5$	0.330	2.16
4-OH	0.101	0.59	N,N-(CH$_3$)$_2$	0.384	2.31
4-CH$_3$	0.271	1.95	N,N-(C$_2$H$_5$)$_2$	0.541	3.31
4-NO$_2$	0.269	1.91	N-C$_4$H$_9$	0.529	3.58
H	0.209	1.48	2-CH$_3$	0.245	1.32
2,5-(NO$_2$)$_2$	0.318	2.00	3-CH$_3$	0.253	1.40
2,6-(NO$_2$)$_2$	0.294	1.55	4-CH$_3$	0.254	1.39
2-Br	0.311	2.35	2,4-(CH$_3$)$_2$	0.314	2.31
2-Cl	0.293	2.17	4-OCH$_3$	0.213	0.95
2-C$_2$H$_5$	0.342	2.47	3-Cl	0.321	1.90
2-CH$_3$	0.277	1.95	3,4-Cl$_2$	0.430	2.69
2-NO$_2$	0.292	1.79	4-Br	0.335	2.05
3,4-(CH$_3$)$_2$	0.322	2.23	3-NO$_2$	0.262	1.37
3,5-(CH$_3$)$_2$	0.332	2.35	1-Naphthylamine	0.361	2.22
3-Br	0.342	2.63	2-Naphthylamine	0.371	2.28
3-Cl	0.324	2.50	*Heterocyclics*		
3-C$_2$H$_5$	0.339	2.40	Pyridine	0.170	0.64
3-CH$_3$	0.273	1.95	2-NH$_2$-pyridine	0.125	0.20
3-NO$_2$	0.277	2.00	3-NH$_2$-pyridine	0.156	0.49
4-Br	0.340	2.60	4-CH$_3$-pyridine	0.236	1.22
4-Cl	0.320	2.35	4-C$_2$H$_5$-pyridine	0.282	1.69[c]
4-C$_2$H$_5$	0.341	2.58	Pyrazine	0.102	−0.22
1-Naphthol	0.383	2.98	2-CH$_3$-Pyrazine	0.142	0.23
2,4,5-Cl$_3$	0.448	3.72	2,5-(CH$_3$)$_2$-pyrazine	0.182	0.63
2,4,6-Cl$_3$	0.430	3.69	Quinoline	0.298	2.03
4-Cl-3-CH$_3$	0.370	3.10	2-CH$_3$-Quinoline	0.336	2.59
2,3,5,6-Cl$_4$	0.496	4.10	8-CH$_3$-Quinoline	0.347	2.60
4-t-C$_4$H$_9$	0.416	3.31			
2,4-Br$_2$	0.432	3.43			
2,3-(CH$_3$)$_2$	0.338	2.33			
2,6-(CH$_3$)$_2$	0.335	2.36			

[a] The slopes $a(E_T)$ were taken from ref. 23. HPLC measurements were performed on Unisil Q C$_{18}$ as stationary phase; acetonitrile–water mixtures were used as mobile phase.
[b] Log P values were taken from ref. 32.
[c] Log P for 2-ethylpyridine.

for characterization of the mobile phase composition (see Experimental). The relationships of log k' *vs.* $E_T(30)$ gave statistically more significant linear equations than the corresponding concentration dependences. We have used these slopes, $a(E_T)$, to correlate them with corresponding values of log P^a in the series of phenols (eqn. 56), anilines (eqn. 57) and heterocyclics (eqn. 58). The corresponding data are summarized in Table V. For the common group of the mentioned compounds, the less significant

[a] The log P values were taken from ref. 32; only compounds with experimental log P were used in regression analysis.

eqn. (59) was derived. Taking into account that $\log P$ values were taken from the literature [32], and not measured in one laboratory, the correlations are very good.

	n	r	s	F	
$\log P = 9.330\, a(E_T) - 0.627$	32	0.975	0.184	572.4	(56)
$\log P = 7.350\, a(E_T) - 0.448$	16	0.981	0.157	351.0	(57)
$\log P = 11.262\, a(E_T) - 1.333$	11	0.996	0.099	995.2	(58)
$\log P = 9.088\, a(E_T) - 0.738$	59	0.951	0.295	534.4	(59)

The quantity $a(E_T)$ is the slope of the $\log k'$ vs. $E_T(30)$ relationships.

The experimental values of slopes $a(E_T)$ for the series of aromatic compounds measured on various types of stationary phase (Table VI) were taken from the same source [23]. Eqn. 60 for Sepralyte C_2, eqn. 61 for Sepralyte C_4 and eqn. 62 for Sepralyte C_8 were derived. The greatest deviation was observed for nitrobenzene; it decreases with the length of the silanizing group.

	n	r	s	F	
$\log P = 6.636\, a(E_T) - 0.049$	16	0.974	0.172	254.6	(60)
$\log P = 6.012\, a(E_T) - 0.219$	16	0.959	0.213	160.8	(61)
$\log P = 6.534\, a(E_T) - 0.991$	14	0.988	0.126	478.1	(62)

TABLE VI

LOG P VALUES AND SLOPES OF DEPENDENCES BETWEEN LOG k' AND $E_T(30)$ POLARITY SCALE[a]

Compound	$a(E_T)$[b]	$a(E_T)$[c]	$a(E_T)$[d]	Log P[e]
1,3,4-Trimethylbenzene	0.533	0.606	0.690	3.42
Anthracene	0.707	0.753	0.824	4.45
Benzene	0.331	0.395	0.472	2.13
Biphenyl	0.616	0.687	0.765	4.05
Ethylbenzene	0.461	0.548	0.670	3.15
Bromobenzene	0.451	0.510	0.591	2.99
Chlorobenzene	0.422	0.489	0.571	2.84
Iodobenzene	0.483	0.550	0.625	3.25
Naphthalene	0.519	0.547	0.644	3.37
Nitrobenzene	0.362	0.418	0.472	1.85
Toluene	0.392	0.467	–	2.73
Butylbenzene	0.651	0.778	0.826	4.26
Propylbenzene	0.546	0.684	0.733	3.68
p-Xylene	0.470	0.542	0.628	3.15
o-Xylene	0.454	0.612	–	3.12
Fluorobenzene	0.361	0.430	0.508	2.27

[a] The slopes $a(E_T)$ were taken from ref. 23. HPLC was performed on Sepralyte as stationary phase with methanol–water as mobile phase.
[b] Sepralyte C_2 was used.
[c] Sepralyte C_4 was used.
[d] Sepralyte C_8 was used.
[e] Log P values were taken from ref. 32.

CONCLUSIONS

It can be inferred from the fair linear regression relationships between the slopes of concentration dependences and extrapolated or non-extrapolated values of retention indices that there is an equivalence of these indices in the evaluation of lipophilicity. The statistically significant dependences of log P ($\Sigma\pi$) values on concentration slopes make it possible to use them as new parameters of lipophilicity. The goodness of fit of these relationships increased when a suitable measure of the polarity of mobile phases was used instead of the change in modifier concentration.

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