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Determination of the hydrophobicity parameter R_{Mw} by reversed-phase thin-layer chromatography

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Abstract

Experimental conditions were established that permit the determination of chromatographic lipophilicity parameters on the basis of thermodynamically true R_M values. The superiority of methanol as compared with other modifiers (*e.g.*, acetonitrile) is substantiated; physico-chemical reasons are discussed. Solvent pH influences only the silanophilic effect; hence, with highly ionized structures, determination at neutral pH is to be preferred. Extrapolation to modifier-free conditions (R_{Mw} value) is essential to diminish the contribution of polar interactions. Advantages and disadvantages of non-linear extrapolation procedures are compared with linear regression. Correctly estimated R_{Mw} values coincide numerically not only with log k_w values, as theoretically expected, but also with partitioning data from the octanol–water system.

1. Introduction

The prime role of lipophilicity among quantitative structure–activity relationship (QSAR) parameters is undisputed. The classical approach to quantifying lipophilicity, by octanol–water partitioning, is being supplanted by chromatographic procedures, in particular high-performance liquid chromatography on reversed-phase RP-18 phases (RP-HPLC). Using methanol as a modifier, water-extrapolated log k values (log k_w) derived in this system show an excellent correlation with octanol–water partition coefficients, as summarized by Braumann in a valuable review [1]. As thin-layer chromatographic (TLC) procedures and HPLC exhibit the same dependence on the stationary and mobile phases, this correlation should also apply to TLC.

R_M values, obtained by RP-TLC, have a long tradition as lipophilicity parameters (see, *e.g.*, ref. 2), but they are often viewed as "quick and dirty" parameters. In a previous paper [3], we showed that this view is due to incorrect measurement of R_M. First, it is essential to determine R'_F values with the aid of front markers, which are the only means of calculating thermodynamically true R_M values. Because R_M values depend significantly on modifier content, Biagi *et al.* [4] preferred R_{Mw} values, *i.e.*, R_M extrapolated to 100% water, as lipophilicity parameters in QSAR studies. The theoretical and experimental correctness of such an extrapolation were demonstrated by Soczewinski and Wachtmeister [5]. However, a linear dependence of R_M

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holds only at low modifier content φ; at high φ, the R_M values deviate from linearity [4,6].

Despite pronounced differences in experimental procedures, the basic partitioning conditions are similar in RP-TLC and RP-HPLC. The stationary phase, *i.e.*, silica gel etherified with octadecanol, is identical with that used on RP-TLC plates. Hence the partitioning process is governed by identical physico-chemical parameters when the same solvent is used [1,7]. Correspondingly, also in RP-HPLC it is common to apply water-extrapolated values ($\log k_w$) as lipophilicity parameters. Also for RP-HPLC it has been shown by several workers [8–11] that in case of high modifier contents the $\log k'$ values can deviate from linearity and linear extrapolations therefore lead to erroneous $\log k_w$.

In this work, we investigated the questions of linearity and extrapolation to R_{Mw} on the basis of thermodynamically true R_M values. In addition, a comparison of such R_{Mw} data with other lipophilicity data is given; experimental lipophilicity parameters such as $\log P_{Oct}$ and $\log k_w$ and also calculation parameters such as Σf [12] are included Finally, we report on the influence of acetonitrile as modifier and of the solvent pH on R_{Mw}.

2. Experimental

Precoated TLC plates (RP-18 F_{254S}, 20×10 cm) purchased from Merck (Darmstadt, Germany) were used. Compared with differently coated silica gel plates [4,6], these plates have the considerable advantage of high stability, permitting their use for large ranges of varying modifier/buffer contents; they are also similar to the material used in RP-HPLC. As solvent we used methanol–buffer mixtures with methanol contents between 20 and 100% (v/v) or acetonitrile–buffer mixtures with modifier contents between 40 and 75% (v/v) in 5% increments. Tris buffer [pH 7.4 (ionic strength 0.1 mol/l)] was used, prepared with water obtained from a Milli-Q Plus water system (Millipore, Bedford, MA, USA). In some instances, commercially available buffer (pH 12) from Riedel-de Haën (Seelze, Germany) was used. TLC was performed in twin-trough chambers (Camag, Muttenz, Switzerland), which were placed in an incubator adjusted to 30°C.

Detailed experimental conditions for the determination and calculation of thermodynamically true R'_F and R_M values have already been published [3]. For determining the thermodynamically true position of the front, KI was used. A 0.5-μl volume of an ethanolic solution of the test compounds was applied to the plates with the aid of a Nanomat II (Camag). Positioning of the starting points (Z_0) and the positions after the runs (Z_X) were exactly evaluated with the aid of a CD 50 densitomer (Desaga, Heidelberg, Germany). The R'_F value of a test compound X is calculated according to

$$R'_{FX} = 0.99(Z_X - Z_0)/(Z_{KI} - Z_0) \quad (1)$$

$Z_X - Z_0$ and $Z_{KI} - Z_0$ characterize the migration distances of the test compound X and of the front marker KI; the correction factor 0.99 corresponds to the front gradient [3,7,13].

From the R'_F values, the thermodynamically true R_M values were calculated according to the well known procedure of Bate-Smith and Westall [14]:

$$R_{MX} = \log(1/R'_{FX} - 1) \quad (2)$$

2.1. Test compounds

The compounds tested are listed in Table 1. Compounds **19–21** were kindly provided by Professor Weber, Department of Pharmaceutical Chemistry, University of Düsseldorf. The remaining compounds were obtained from Aldrich (Milwaukee, WI, USA).

2.2. Statistics

All statistical procedures were run with Graph Pad InPlot, version 4.04 (Graph Pad Software, San Diego, CA, USA). Deviations are given as 95% confidence intervals.

Table 1
R_{Mw} values, obtained by linear regression or according to Eq. 10, as compared with log k_w, log P_{Oct} [27] and Σf values, calculated according to ref. 12

No.	Compound	$R_{Mw} \pm 95\%$ c.i.[a]		Log k_w	Log P_{Oct}	$\Sigma f_{rev.}$
		Linear	Non-linear (Eq. 10)			
1	Benzoic acid	1.649 ± 0.018	1.661 ± 0.136	1.92	1.87	1.84
2	2-Methylbenzoic acid	1.967 ± 0.029	1.974 ± 0.267		2.18	2.14
3	3-Methylbenzoic acid	2.208 ± 0.020	2.218 ± 0.100		2.37	2.36
4	4-Methylbenzoic acid	2.218 ± 0.043	2.270 ± 0.115	2.48	2.27	2.36
5	3,4-Dimethylbenzoic acid	2.668 ± 0.058	2.701 ± 0.300			2.87
6	3-Methoxybenzoic acid	1.804 ± 0.024	1.801 ± 0.169		2.02	1.91
7	4-Methoxybenzoic acid	1.954 ± 0.037	2.002 ± 0.126		1.96	1.91
8	3-Fluorobenzoic acid	1.763 ± 0.030	1.853 ± 0.400		2.15	2.08
9	4-Fluorobenzoic acid	1.797 ± 0.028	1.798 ± 0.191		2.07	2.08
10	3-Chlorobenzoic acid	2.106 ± 0.032	2.096 ± 0.111		2.68	2.57
11	4-Chlorobenzoic acid	2.190 ± 0.027	2.179 ± 0.099	2.70	2.65	2.57
12	3-Bromobenzoic acid	2.265 ± 0.031	2.260 ± 0.150		2.87	2.77
13	4-Bromobenzoic acid	2.368 ± 0.032	2.383 ± 0.145		2.86	2.77
14	3-Iodobenzoic acid	2.536 ± 0.059	2.529 ± 0.185		3.13	3.08
15	4-Iodobenzoic acid	2.628 ± 0.054	2.603 ± 0.154		3.02	3.08
16	4-Butylbenzoic acid	3.940 ± 0.155	4.002 ± 0.594			3.91
17	4-Pentylbenzoic acid	4.429 ± 0.131	4.430 ± 0.332			4.43
18	4-Heptylbenzoic acid	5.440 ± 0.103	5.410 ± 0.380			5.47
19	2-Hydroxybenzoic acid	1.165 ± 0.044	1.169 ± 0.626		2.21	2.16
20	4-Hydroxybenzoic acid	1.068 ± 0.022	1.033 ± 0.910	1.20	1.57	1.50
21	2,4-Dihydroxybenzoic acid	0.872 ± 0.056	0.737 ± 1.275		1.44	1.60
22	1-Naphthalenecarboxylic acid	3.047 ± 0.063	3.016 ± 0.215		3.10	3.12
23	3-Methylphenylacetic acid	2.049 ± 0.029	2.039 ± 0.120		1.86	2.00
24	3-Fluorophenylacetic acid	1.644 ± 0.055	1.624 ± 0.192		1.65	1.72
25	4-Fluorophenylacetic acid	1.649 ± 0.047	1.628 ± 0.161		1.55	1.72
26	4-Chlorophenylacetic acid	2.166 ± 0.036	2.146 ± 0.175		2.12	2.21
27	4-Bromophenylacetic acid	2.306 ± 0.017	2.302 ± 0.087		2.31	2.41
28	3-Phenylpropionic acid	2.095 ± 0.027	2.106 ± 0.133		1.84	2.00
29	4-Phenylbutyric acid	2.527 ± 0.018	2.524 ± 0.083		2.42	2.52
30	Benzophenone	3.361 ± 0.082	3.385 ± 0.239	3.15	3.38	3.05
31	2,6-Dimethylbenzophenone	4.038 ± 0.104	4.029 ± 0.189			3.65
32	2,2′-Dimethylbenzophenone	4.121 ± 0.155	4.116 ± 0.416			3.87
33	2,6,2′,6′-Tetramethylbenzophenone	4.463 ± 0.132	4.418 ± 0.179			4.69
34	2,6,2′,6′-Tetraethylbenzophenone	6.131 ± 0.097	6.169 ± 0.408			6.54
35	4-Bromoacetophenone	2.938 ± 0.052	2.950 ± 0.195		2.43	2.58
36	2-Hydroxybenzamide	1.380 ± 0.013	1.385 ± 0.123	1.24	1.28	1.09
37	4-Hydroxybenzamide	0.460 ± 0.027	0.470 ± 0.166		0.33	0.43
38	Phenol	1.278 ± 0.056	1.250 ± 0.556	1.30	1.46	1.55
39	4-Chlorophenol	2.031 ± 0.026	2.039 ± 0.163	2.24	2.35	2.28
40	4-Bromophenol	2.223 ± 0.023	2.209 ± 0.138		2.43	2.48
41	1-Naphthol	2.572 ± 0.035	2.592 ± 0.133	1.71	2.65	2.84
42	2-Naphthol	2.577 ± 0.041	2.609 ± 0.124	2.15	2.70	2.84
43	4-Methylbenzyl alcohol	1.916 ± 0.026	1.952 ± 0.113		1.59	1.71
44	4-Chlorobenzyl alcohol	2.120 ± 0.012	2.124 ± 0.074		1.96	1.92
45	Imidazole	−0.130 ± 0.022	0.033 ± 1.020		−0.08	0.16
46	2-Methylimidazole	0.014 ± 0.009	0.088 ± 0.638			0.68
47	2-Ethylimidazole	0.155 ± 0.016	0.262 ± 1.116			1.20
48	2-Propylimidazole	0.309 ± 0.009	0.335 ± 0.117			1.72
49	1-Butylimidazole	0.614 ± 0.014	0.602 ± 0.262			2.02
50	2-Phenylimidazole	1.170 ± 0.041	1.094 ± 1.129		1.87	2.08
51	Benzimidazole	0.821 ± 0.032	0.866 ± 0.365		1.20	1.45
52	2-Methylbenzimidazole	0.917 ± 0.018	0.960 ± 0.592		1.43	1.96
53	5,6-Dimethylbenzimidazole	1.685 ± 0.074	1.726 ± 0.515		2.35	2.48

(Continued on p. 116)

Table 1 (*Continued*)

No.	Compound	$R_{Mw} \pm 95\%$ c.i.[a]		Log k_w	Log P_{Oct}	$\Sigma f_{rev.}$
		Linear	Non-linear (Eq. 10)			
54	4-Nitroaniline	1.415 ± 0.032	1.421 ± 0.171	1.46	1.39	0.76
55	4-Chloroaniline	1.692 ± 0.040	1.695 ± 0.227	1.80	1.83	1.73
56	4-Bromoaniline	1.989 ± 0.031	2.014 ± 0.186	2.01	2.05	1.93
57	2,5-Di-*tert.*-butylaniline	4.383 ± 0.043	4.388 ± 0.188			5.15
58	2-Naphthylamine	2.201 ± 0.043	2.207 ± 0.178		2.28	2.29
59	4-Bromo-1-naphthylamine	3.341 ± 0.033	3.358 ± 0.267			3.22
60	2-Aminobiphenyl	2.988 ± 0.027	2.977 ± 0.112		2.84	2.92
61	2-Aminofluorene	3.421 ± 0.045	3.381 ± 0.387			3.02
62	2-Amino-7-bromofluorene	4.110 ± 0.070	4.134 ± 0.497			3.95
63	2-Amino-1,3-dibromofluorene	4.867 ± 0.026	4.888 ± 0.240			4.88
64	1-Aminoanthracene	3.562 ± 0.037	3.563 ± 0.255			3.58
65	3-Aminofluoranthene	4.588 ± 0.079	4.581 ± 0.444			4.01
66	1-Aminopyrene	4.661 ± 0.089	4.666 ± 0.294 .			4.01
67	Acridine	3.400 ± 0.060	3.386 ± 0.399	2.87	3.40	3.31
68	4-Nitrotoluene	2.605 ± 0.022	2.607 ± 0.115	2.40	2.42	2.38
69	4-Chloronitrobenzene	2.701 ± 0.025	2.734 ± 0.049	2.35	2.41	2.59
70	4-Bromonitrobenzene	2.760 ± 0.037	2.757 ± 0.160		2.55	2.79
71	1-Nitronaphthalene	3.248 ± 0.016	3.260 ± 0.094		3.19	3.15
72	Pentamethylbenzene	4.352 ± 0.182	4.369 ± 0.583	4.70	4.56	4.70
73	Biphenyl	3.920 ± 0.084	3.937 ± 0.342	4.09	4.08	4.02
74	Bibenzyl	4.676 ± 0.072	4.682 ± 0.349	4.92	4.79	4.84
75	Naphthalene	3.168 ± 0.053	3.171 ± 0.179	3.31	3.35	3.39
76	2-Methylnaphthalene	3.747 ± 0.083	3.751 ± 0.253		3.86	3.91
77	2,6-Dimethylnaphthalene	4.290 ± 0.090	4.331 ± 0.196		4.31	4.43
78	1-Phenylnaphthalene	4.529 ± 0.036	4.542 ± 0.105			5.31
79	2,6-Di-*tert.*-butylnaphthalene	6.416 ± 0.137	6.319 ± 0.776			7.55
80	Anthracene	4.228 ± 0.039	4.243 ± 0.151	4.58	4.45	4.68
81	2-Methylanthracene	4.623 ± 0.069	4.632 ± 0.154		5.07	5.20
82	2-Ethylanthracene	5.085 ± 0.092	5.075 ± 0.202			5.72
83	2-Chloroanthracene	4.750 ± 0.170	4.725 ± 0.417			5.41
84	9-Bromoanthracene	4.959 ± 0.163	5.023 ± 0.501			5.61
85	9-Phenylanthracene	5.158 ± 0.037	5.174 ± 0.135			6.60
86	9,10-Diphenylanthracene	6.938 ± 0.197	6.963 ± 0.540			8.52
87	2-Methylphenanthrene	5.158 ± 0.023	5.205 ± 0.332		4.86	5.20
88	1,3,5-Trichlorobenzene	4.047 ± 0.035	4.064 ± 0.221	4.26	4.02	4.29
89	1,2,4,5-Tetrachlorobenzene	4.524 ± 0.103	4.635 ± 0.638	4.65	4.52	5.02
90	Pentachlorobenzene	4.896 ± 0.079	4.901 ± 0.205	5.25	5.03	5.75
91	Hexachlorobenzene	5.360 ± 0.131	5.375 ± 0.354	5.46	5.37	6.48
92	1,4-Dibromobenzene	3.877 ± 0.065	3.873 ± 0.285		3.64	3.97

[a] c.i. = Confidence interval.

3. Results and discussion

3.1. Determination of R_{Mw} values by linear extrapolation

To test the linearity between R_M and methanol content φ, in general 16 data points were available for each test compound. For compounds with $R_{Mw} > 3$, R_M values cannot be calculated with sufficient accuracy at low φ, owing to inaccurate measurement of migration distances around 0.01 mm. For only 4 out of 92 investigated compounds, the R_M values decline linearly up to φ values of 0.85 (Fig. 1A); these four compounds (tri-, tetra-, penta- and hexachlorobenzene, **88–91**) are extremely non-polar. Kamlet *et al.* [15] specified their α and β values, which quantify the proton-accepting and proton-

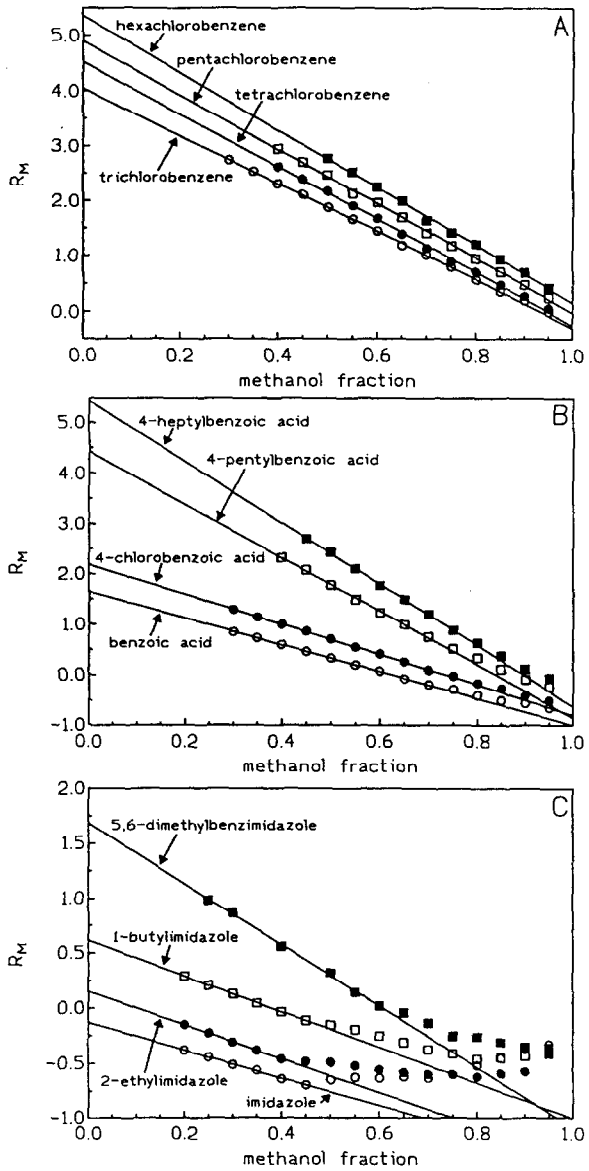

Fig. 1. Dependence of R_M values on the methanol fraction (φ). (A) Non-polar compounds; (B) acids; (C) bases.

donating behaviour, as zero. For all other test compounds, deviations from linearity are found at methanol contents of 45–75% (Fig. 1B and C), presumably owing to the so-called silanophilic effect, which has been described by Nahum and Horvath [10] for RP-HPLC. This effect is based on polar interactions between free silanol moieties of the RP material and polar moieties of the test molecules. In the RP material the silanol groups are only partially etherified with octadecanol for stereochemical reasons; for the Merck plates used in this investigation, the amount of etherification is given as 22% [16]. In solvents with a high buffer content, silanol groups are quantitatively protected by water molecules and the chromatographic process is based almost exclusively on partitioning (reversed-phase behaviour). With increasing modifier content the possibility of polar interactions of the silanol groups increases (normal-phase behaviour).

One way to reduce the silanophilic effect and achieve an improved determination of R_M would be to use Merck RP-18 HPTLC plates, which are coated with a silica gel material with higher etherification with octadecyl groups. However, these plates can only be moistened by solvents containing less than 40% of water. This makes extrapolation to modifier-free conditions less accurate. Similar experience has been reported by Butte *et al.* [17].

The linear part of the relationship between R_M and methanol content was determined by the aid of a computer program [18]. Because the silanophilic effect always initiates an increase in the measured R_M values, the necessary procedure was unequivocally defined. Correspondingly calculated R_{Mw} values are summarized in Table 1.

3.2. Determination of R_{Mw} by non-linear regression

Schoenmakers and co-workers [19,20] described the correlation between log k' and φ with a Scatchard–Hildebrand extended solubility parameter model [21,22] by means of the following equation:

$$\log k' = \log k_w + A\varphi^2 - S\varphi \quad (3)$$

The application of this approach to the determination of R_{Mw} necessitates, for an accurate calculation, the availability of a large number of data points, particularly at low modifier contents; mainly for technical reasons these data were not always available. Log k_w values calcu-

lated according to Eq. 3 have been correlated with log P_{Oct} data by Braumann [1] and El Tayar *et al.* [23]. From these correlations, it is concluded that in the case of lipophilic compounds the above approach yields overestimated parameters.

The shape of the plots of R_M *versus* φ resembles a decreasing exponential in the first part followed by an increasing one. We therefore attempted to describe this pattern empirically by the following equation:

$$R_M = \log(Ae^{-B\varphi} + Ce^{D\varphi}) \quad (4)$$

The decreasing exponential in Eq. 4 expresses the contribution of hydrophobic interactions between the test compound, the stationary hydrophobic phase and the aqueous mobile phase to R_M, while the increasing exponential corresponds to the contribution of polar adsorption. Parameters A, B, C and D were calculated by non-linear regression. If the parameters A, B, C and D are given, R_{Mw} is calculated by setting $\varphi = 0$:

$$R_{Mw} = \log(A + C) \quad (5)$$

Table 2 summarizes some R_{Mw} values, calculated according to this approach for six test compounds representing the chemical classes included in this study. These results agree well with those obtained by linear extrapolation. However, in some instances the iteration program calculated negative values for D, which means that the program fits the plot of R_{Mw} *versus* φ to two decreasing exponentials. This is obviously misleading, as the adsorptive, polar contribution to R_M increases with increasing modifier content. Hence Eq. 4 is not generally applicable.

Nahum and Horváth [10] developed an equation that separates two contributions to log k' depending on the buffer content of the eluent. Owing to the above-mentioned free silanol groups, they assumed the simultaneous existence of both reversed-phase (solvophobicity) and normal-phase (silanophilicity) behaviour. Solvophobic behaviour is expressed as follows:

$$k_1 = Ae^{B\psi} \quad (6)$$

where ψ defines the water content. In normal-phase chromatography with polar adsorbents such as silica gel, the interdependence between the retention factor and the composition of a binary solvent is expressed as follows [24,25]:

$$k_2 = 1/(C + D\psi) \quad (7)$$

The entire retention factor k' is then the sum of k_1 and k_2:

$$k' = Ae^{B\psi} + 1/(C + D\psi) \quad (8)$$

or, in logarithmic form,

$$\log k' = \log[Ae^{B\psi} + 1/(C + D\psi)] \quad (9)$$

As already outlined above, this calculation can also be applied to the determination of R_{Mw}.

Table 2
Comparison of some R_{Mw} values, obtained by non-linear regression (Eq. 4), with linearly extrapolated data

No.	Compound	$R_{Mw} \pm 95\%$ c.i.[a]		Δ
		Nonlinear regression by Eq. 4	Linear regression	
1	Benzoic acid	1.682 ± 0.061	1.649 ± 0.018	0.033
26	Benzophenone	3.457 ± 0.121	3.361 ± 0.082	0.096
40	Imidazole	−0.075 ± 0.173	−0.130 ± 0.022	0.055
50	4-Chloroaniline	1.691 ± 0.054	1.692 ± 0.040	−0.001
72	Anthracene	4.197 ± 0.065	4.228 ± 0.039	−0.031
82	Pentachlorobenzene	4.896 ± 0.148	4.896 ± 0.079	0.000

[a] c.i. = Confidence interval.

Calculation of the parameters A, B, C and D and setting $\psi = 1$ (modifier free) then gives

$$R_{Mw} = \log[Ae^B + 1/(C + D)] \quad (10)$$

Correspondingly calculated R_{Mw} data are summarized in column 2 in Table 1. Linear extrapolation and non-linear regression according to Horváth (Eq. 10) results in almost identical R_{Mw} values ranging between −0.13 and 6.98, as shown by the regression equation

$$R_{Mw,Hor} = 0.997(\pm 0.005)R_{Mw,lin} + 0.016(\pm 0.017) \quad (11)$$

$n = 92$; $s = 0.039$; $r = 0.9997$; $F = 144\,000$

The mean difference between the two calculation procedures is 0.01 ± 0.04; only in three cases it is greater than 0.1.

Although non-linear regression according to Nahum and Horváth [10] is the better approach for the determination of R_{Mw}, our results demonstrate that the far more convenient linear extrapolation yields almost identical results, if carefully applied. In addition, confidence intervals are significantly smaller in the latter instance, as expected from the calculation procedure.

3.3. Influence of solvent pH on R_M

Concerning the influence of solvent pH on the R_M values of strong bases, we have already reported [3] that the silanophilic effect, but not the lipophilic distribution, depends on solvent pH. For a validation of this hypothesis, we selected some imidazole derivatives exhibiting pK values around 7 [26]. R_{Mw} values were measured at pH 7.4 (50% dissociation) and 12 (no protonation). As shown in Table 3, the measured data are well correlated, with the greatest deviation being 0.067. This is shown by the regression equation

$$R_{Mw,pH7.4} = 0.969(\pm 0.042)R_{Mw,pH12} + 0.010(\pm 0.036) \quad (12)$$

$n = 9$; $s = 0.031$; $r = 0.9988$; $F = 2973$

The influence of solvent pH is shown in detail in Fig. 2. At higher pH, polar interactions are

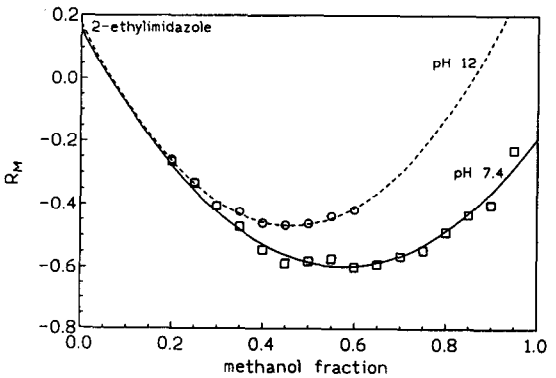

Fig. 2. Influence of pH on R_M values. Curves were calculated according to Horváth (see also Eq. 10).

Table 3
R_{Mw} values of imidazoles

No.	Compound	R_{Mw} (pH 7)	n	R_{Mw} (pH 12)	n	Δ
45	Imidazole	−0.130	6	−0.130	4	0.000
46	2-Methylimidazole	0.014	5	0.037	3	−0.023
47	2-Ethylimidazole	0.155	5	0.173	3	−0.018
48	2-Propylimidazole	0.309	7	0.253	4	0.056
49	1-Butylimidazole	0.614	6	0.611	5	0.003
50	2-Phenylimidazole	1.170	4	1.188	3	−0.018
51	Benzimidazole	0.821	9	0.803	8	0.018
52	2-Methylbenzimidazole	0.917	5	0.949	4	−0.032
53	5,6-Dimethylbenzimidazole	1.685	6	1.752	5	−0.067

Data measured at pH 7.4 are compared with values derived at pH 12.0 and their differences (Δ) are given. n = Number of data points included for linear extrapolation.

strengthened. Accordingly, the curve shape is linear between 20 and 40% methanol at pH 7.4, but only up to 30% methanol at pH 12. Hence, uncertainties in the extrapolation to R_{Mw} arise, which underlie the deviations in Eq. 12. At low methanol contents, where R_M depends solely on the lipophilic distribution, the data coincide almost exactly.

3.4. Influence of the modifier on R_M

The pronounced influence of the modifier on the quality of chromatographic data has been comprehensively described by Braumann [1]. The distribution of the test compounds into the octadecyl phase of the RP-18 phase depends significantly on physico-chemical properties of the modifier such as dipole moment or proton-donating properties. In this respect, the properties of acetonitrile are distinctly different from those of water, whereas methanol is very similar. Accordingly, methanol has to be viewed as the modifier of choice for the chromatographic determination of lipophilicity. These considerations are impressively substantiated by our present investigations. R_{Mw} values determined with acetonitrile as modifier (Table 4) are significantly lower than the data measured in the methanol system:

$$R_{Mw,ACN} = 0.679(\pm 0.070)R_{Mw,MeOH} + 0.232(\pm 0.217) \quad (13)$$

$$n = 22;\ s = 0.179;\ r = 0.9766;\ F = 412$$

The mean difference is 0.69 ± 0.41. Also in comparison with log P_{Oct} the acetonitrile-related data show a significant negative deviation with a mean value of 0.87 ± 0.30. Correspondingly, the correlations of the chromatographic data with log P_{Oct} are less significant in the case of the

Table 4
R_{Mw} values of some selected test compounds measured in the acetonitrile system, and the differences from the values obtained in the methanol system

No.	Compound	$R_{Mw,ACN} \pm 95\%$ c.i.[a]	$\Delta R_{Mw,MeOH}$
1	Benzoic acid	1.360 ± 0.112	0.289
2	2-Methylbenzoic acid	1.548 ± 0.065	0.419
4	4-Methylbenzoic acid	1.561 ± 0.049	0.657
5	3,4-Dimethylbenzoic acid	1.756 ± 0.050	0.912
6	3-Methoxybenzoic acid	1.476 ± 0.098	0.328
7	4-Methoxybenzoic acid	1.441 ± 0.109	0.513
8	3-Fluorobenzoic acid	1.530 ± 0.092	0.233
9	4-Fluorobenzoic acid	1.507 ± 0.095	0.290
10	3-Chlorobenzoic acid	1.696 ± 0.071	0.410
11	4-Chlorobenzoic acid	1.711 ± 0.059	0.479
12	3-Bromobenzoic acid	1.779 ± 0.054	0.486
13	4-Bromobenzoic acid	1.773 ± 0.056	0.595
14	3-Iodobenzoic acid	1.867 ± 0.072	0.669
15	4-Iodobenzoic acid	3.998 ± 0.072	0.748
30	Benzophenone	2.757 ± 0.097	0.604
31	2,6-Dimethylbenzophenone	3.239 ± 0.286	0.799
32	2,2′-Dimethylbenzophenone	3.277 ± 0.356	0.844
33	2,6,2′,6′-Tetramethylbenzophenone	3.582 ± 0.226	0.881
34	2,6,2′,6′-Tetraethylbenzophenone	3.998 ± 0.060	2.133
72	Pentamethylbenzene	3.119 ± 0.092	1.233
73	Biphenyl	2.843 ± 0.115	1.077
75	Naphthalene	2.489 ± 0.074	0.679

[a] c.i. = Confidence interval.

acetonitrile system (Eq. 14) as compared with the methanol system (Eq. 15):

$$R_{Mw,ACN} = 0.677(\pm 0.126) \log P_{Oct} + 0.028(\pm 0.360) \quad (14)$$

$n = 17$; $s = 0.180$; $r = 0.9476$; $F = 132$

3.5. Comparison of R_{Mw} with log P_{Oct}

For 65 of 92 test compounds (see Table 1), log P_{Oct} values have been published [27]. For the latter a measuring accuracy of 0.3 log units is generally accepted [28]; 52 pairs are located within this range.

Correlations between log P_{Oct} and R_{Mw} data, obtained either by linear extrapolation (Eq. 15) or by non-linear regression (Eq. 16), yield the following results:

$$R_{Mw,lin} = 1.008(\pm 0.064) \log P_{Oct} - 0.151(\pm 0.183) \quad (15)$$

$n = 65$; $s = 0.294$; $r = 0.9699$; $F = 1000$

$$R_{Mw,Hor} = 1.009(\pm 0.066) \log P_{Oct} - 0.147(\pm 0.190) \quad (16)$$

$n = 65$; $s = 0.304$; $r = 0.9679$; $F = 935$

The average R_{Mw} values are 0.13 ± 0.29 lower than the corresponding log P_{Oct} values. In five cases (three benzoic acids and two imidazoles), the values were more than two standard deviations lower. The R_{Mw} values of all acids exhibit lower values than log P_{Oct} with a mean difference of 0.24 ± 0.31. Omission of these acids results in correlation equations with regression coefficients approximating 1 and with intercepts only marginally differing from zero:

$$R_{Mw,lin} = 1.000(\pm 0.062) \log P_{Oct} - 0.059(\pm 0.197) \quad (17)$$

$n = 40$; $s = 0.260$; $r = 0.9827$; $F = 1070$

$$R_{Mw,Hor} = 0.998(\pm 0.063) \log P_{Oct} - 0.036(\pm 0.202) \quad (18)$$

$n = 40$; $s = 0.267$; $r = 0.9818$; $F = 1013$

Not surprisingly, the mean differences for this set of 40 compounds are as low as 0.06 ± 0.26, ranging within the measurement accuracy of 0.3 units, as accepted for log P.

On closer inspection (see also Fig. 3), the halogenated and hydroxylated benzoic acids are seen to be underestimated in comparison with log P_{Oct}. An extraordinarily strong deviation is found for salicylic acid (**19**) and some substituted imidazoles (**50–53**). It remains to be clarified whether the chromatographic or the partitioning approach supplies more precise data. Nevertheless, it is striking that all these compounds are polar. Octanol–water distribution coefficients of ionizable compounds depend strictly on the compound pK values and the pH of the buffer phase. Hence an exact determination of log P necessitates precise measurements of both log D and pK. Mannhold *et al.* [29] demonstrated the profound influence of questionable pK values on transforming log D into log P. On the other hand, chromatography-based lipophilicity determinations are independent of pK/pH, are only influenced by one parameter and therefore represent from our point of view the more accurate data.

A number of workers [1,17,30–34] have concluded that R_{Mw} or log k_w values better correlate

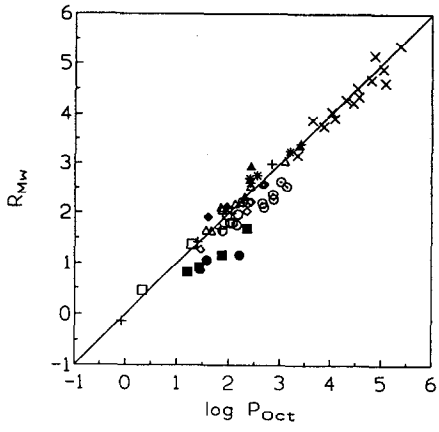

Fig. 3. Correlation of R_{Mw} with log P_{Oct} values. × = Non-polar compounds (**72–92**); ● = hydroxybenzoic acids (**19–21**); ⊙ = halogenated benzoic acids (**8–15**); ○ = remaining benzoic acids (**1–7**, **16–18**); △ = phenones (**30**, **35**); □ = benzamides (**36**, **37**); ◇ = phenols (**38–42**); ■ = substituted imidazoles (**50–53**); + = amines (**45**, **54–56**, **58**, **60**, **67**); * = nitro compounds (**68–71**).

with log P_{Oct} than R_M or log k' values measured at one given modifier content. Regression equations with respect to the varying modifier contents have been calculated according to

$$R_{M,\varphi} = a \log P_{Oct} + b \quad (19)$$

Fig. 4 shows the slopes a and the intercepts b for the individual regression equations as a function of the modifier content φ. In the range 20–45% methanol content, R_M determinations were not possible for some test compounds owing to their high lipophilicity. Correspondingly, the number of test compounds (n) included differs for the various methanol–buffer mixtures.

Our investigations substantiate the findings of

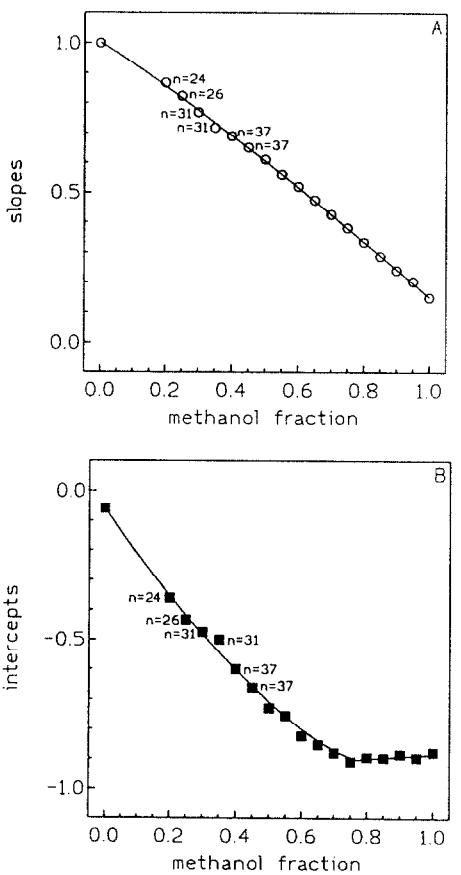

Fig. 4. (A) Slopes and (B) intercepts of the function $R_M = A \log P_{Oct} + B$, calculated for various methanol fractions φ. For $\varphi > 0.45$ up to 1.0 the number of included compounds is 65 (for further explanations, see text).

the above-mentioned workers that correlations between R_M and log P_{Oct} are optimum when using the extrapolated data (see Eqs. 15–18). With increasing methanol content the correlation coefficient decreases from 0.97 to 0.80. This decreasing interrelation is due to additional polar effects, which significantly emerge with increasing modifier content.

Fig. 4 clearly demonstrates that with decreasing modifier content the slope of Eq. 19 approximates to 1 and the intercept approximates to zero. Correspondingly, R_{Mw} and log P_{Oct} can be considered as interchangeable lipophilicity parameters. These results coincide completely with the investigations of Braumann [1] and several other workers [17,31,33] concerned with RP-HPLC. The conclusion of Braumann that log k_w has to be accepted as an *a priori* parameter for lipophilicity is identically applicable to R_{Mw} from our investigations.

3.6. Comparison between R_{Mw} and log k_w

According to our introductory remarks and the considerations detailed above, R_{Mw} and log k_w should be correlated, provided they have been measured under comparable conditions. For 25 of the test compounds studied here, log k_w values were available from the literature [1,31,34–39]. Their correlation with our R_{Mw} data gives the following equation:

$$R_{Mw} = 0.905(\pm 0.091) \log k_w + 0.246(\pm 0.298) \quad (20)$$

$$n = 25;\ s = 0.296;\ r = 0.9737;\ F = 421$$

The most discrepant compounds are 1-naphthol (**41**) and acridine (**67**), the R_{Mw} data for which are almost identical with log P_{Oct}. Omitting these two yields

$$R_{Mw} = 0.931(\pm 0.069) \log k_w + 0.111(\pm 0.229) \quad (21)$$

$$n = 23;\ s = 0.219;\ r = 0.9869;\ F = 787$$

Also in this correlation a certain trend to somewhat lower R_M as compared with log k_w emerges; nevertheless, the theoretically expected

coincidence between these parameters seems to be proved.

Correspondingly, we view RP-TLC as a feasible alternative to lipophilicity determination by HPLC. One of the major advantages of RP-TLC is its rapidity. As described in detail (see Experimental), 30 compounds can be tested simultaneously. This number can even be increased by the double use of at least some starting positions. One has only to guarantee that the compounds sharing a starting position should differ in lipophilicity by at least one unit. Lipophilicity can easily be determined ahead of time by calculating the Σf values.

3.7. Comparison between R_{Mw} and Σf

We compared our R_{Mw} data with calculated lipophilicity values using the Σf system of Rekker [11,40–42]. The correlation between R_{Mw} and the calculated data is given by

$$R_{Mw,lin} = 0.914(\pm 0.057)\Sigma f_{rev.} + 0.013(\pm 0.204) \quad (22)$$

$$n = 92;\ s = 0.444;\ r = 0.9585;\ F = 1017$$

The high interrelation, also found in this comparison, again substantiates the general applicability of R_{Mw} as a reliable lipophilicity parameter.

References

[1] T. Braumann, *J. Chromatogr.*, 373 (1986) 191.
[2] E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
[3] K. Dross, Ch. Sonntag and R. Mannhold, *J. Chromatogr.*, 639 (1993) 287.
[4] G.L. Biagi, A.M. Barbaro, M.F. Gamba and M.C. Guerra, *J. Chromatogr.* 41 (1969) 371.
[5] E. Soczewinski and C.A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
[6] G.L. Biagi, M.C. Guerra and A.M. Barbaro, *J. Med. Chem.*, 13 (1970) 944.
[7] F. Geiss, *Fundamentals of Thin Layer Chromatography*, Hüthig, Basle, 1987.
[8] N.El Tayar, H. van de Waterbeemd and B. Testa, *J. Chromatogr.* 320 (1985) 293.
[9] N. Tanaka and E.R. Thornton, *J. Am. Chem. Soc.*, 99 (1977) 7300.
[10] A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
[11] K.E. Bij, Cs. Horváth, W.R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
[12] R.F. Rekker and R. Mannhold, *Calculation of Drug Lipophilicity*, VCH, Weinheim, 1992.
[13] S.L. Bolotov, *Kontakte (Darmstadt)*, 2 (1990) 36.
[14] E.C. Bate-Smith and R.G. Westall, *Biochim. Biophys. Acta*, 4 (1950) 427.
[15] M.J. Kamlet, R.M. Doherty, M.H. Abraham, Y. Marcus and R.W. Taft, *J. Phys. Chem.*, 92 (1988) 5244.
[16] Merck, Darmstadt, personal communication.
[17] W. Butte, C. Fooken, R. Kluusmann and D. Schüller, *J. Chromatogr.*, 214 (1981) 59.
[18] S. Lehmkühler, *Statistik-Programm "Finke MediStat", Version 2.2*, 1991.
[19] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
[20] R. Tijssen, H.A.H. Billiet and P.J. Schoemakers, *J., Chromatogr.*, 122 (1976) 185.
[21] J.H. Hildebrand and R.L. Scott, *The Solubility of Non-Electrolytes*, Dover, New York, 1964.
[22] J.H. Hildebrand, J.M. Prausnitz and R.L. Scott, *Regular and Related Solutions*, Van Nostrand-Reinhold, New York, 1970.
[23] N. El Tayar, H. van de Waterbeemd and B. Testa, *J. Chromatogr.*, 320 (1985) 305.
[24] C.F. Simpson, *Practical High-Performance Liquid Chromatography*, Heyden, London, 1976, pp. 81–96.
[25] J. Narkiewicz, M. Jaroniec, M. Borowka and A. Patrykiejew, *J. Chromatogr.*, 157 (1978) 1.
[26] H.J. Roth, K. Eger and R. Troschütz, *Pharmazeutische Chemie II—Arzneistoffanalyse*, Georg Thieme, Stuttgart, 1985.
[27] C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley-Interscience, New York, 1979.
[28] J.C. Dearden and G.M. Bresnen, *Quant. Struct.–Act. Relat.*, 7 (1988) 133.
[29] R. Mannhold, K.P. Dross and R.F. Rekker, *Quant. Struct.–Act. Relat.*, 9 (1990) 21.
[30] N.El Tayar, H. van de Waterbeemd and B. Testa, *Quant. Struct.–Act. Relat.*, 4 (1985) 69.
[31] W.E. Hammers, G.J. Meurs and C.L. de Ligny, *J. Chromatogr.*, 247 (1982) 1.
[32] J.E. Haky and A.M. Young, *J. Liq. Chromatogr.* 7 (1984) 675.
[33] T. Braumann, H.-G. Genieser, C. Lüllmann and B. Jastorff, *Chromatographia*, 24 (1987) 777.
[34] M. Harnisch, H.J. Möckel and G. Schulz, *J. Chromatogr.*, 282 (1983) 315.
[35] D.J. Minick, D.A. Brent and J. Frenz, *J. Chromatogr.*, 461 (1989) 177.
[36] T. Braumann, G. Weber and L.H. Grimme, *J. Chromatogr.*, 261 (1983) 329.
[37] H.A. Cooper and R.J. Hurtubise, *J. Chromatogr.*, 360 (1986) 313.

[38] T.L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 218 (1981) 409.
[39] R.W. Ross and C.A. Lau-Cam, *J. Chromatogr.*, 370 (1986) 403.
[40] R.F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
[41] G.G. Nys and R.F. Rekker, *Eur. J. Med. Chem.*, 9 (1974) 361.
[42] R.F. Rekker and H.M. de Kort, *Eur. J. Med. Chem.*, 14 (1979) 479.