CHROM. 16,464

RELATIONSHIP BETWEEN THE CHROMATOGRAPHIC BEHAVIOUR OF DERMORPHIN-RELATED OLIGOPEPTIDES AND THE COMPOSITION OF THE MOBILE PHASE IN REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY: COMPARISON OF EXTRAPOLATED R_M VALUES

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(Received November 21st, 1983)

SUMMARY

The R_M values of a series of dermorphin-related oligopeptides were determined in two reversed-phase thin-layer chromatographic systems, the mobile phase being an aqueous buffer alone or mixed with various amounts of methanol or acetone. The linear relationship between the chromatographic behaviour and the composition of the mobile phase yielded very similar extrapolated R_M values at 0% of organic solvent in both systems. This shows that the extrapolated R_M values are independent of the nature of the organic solvent. In other words, the extrapolated R_M values should be related to the partitioning of the compounds between water and silicone oil in a standard system where all the compounds can be compared.

INTRODUCTION

The lipophilic character of drugs is important in determining their biological activity. The R_M values in reversed-phase thin-layer chromatography (TLC) can be determined very easily and are suitable as a measure of the hydrophobicity of molecules. The stationary phase is usually non-polar, such as silica gel impregnated with silicone oil, and the mobile phase is polar, *e.g.*, water–acetone or methanol mixtures. The linear relationship between the chromatographic behaviour and the composition of the mobile phase yields extrapolated R_M values at 0% of organic solvent in the

mobile phase, which should be related to the partitioning of the compounds between water and silicone oil[1]. However, there are very few papers dealing with a more detailed analysis of the relationship between R_M values and the composition of the mobile phase. Soczewinski and Matysik[2] described the relationship between R_M values and the composition of the mobile phase in liquid–liquid chromatography. Karger et al.[3] more recently discussed some aspects of the influence of the nature of the organic solvent on chromatographic behaviour. Draffehn et al.[4] gave an interpretation of R_M versus acetone concentration curves.

In previous work we determined the R_M values of a series of dermorphin-related oligopeptides in a reversed-phase TLC system where the mobile phase was an aqueous buffer alone or mixed with various amounts of methanol[5]. The purpose of this work was to compare the extrapolated R_M values of the same series of compounds obtained with the addition of acetone or methanol to the mobile phase.

EXPERIMENTAL

The TLC technique has been described previously[1,5]. Glass plates measuring 20×20 cm were coated with silica gel G; in order to obtain better control of the pH of the stationary phase a slurry of silica gel G was prepared with 0.09 N sodiumhydroxide solution[6]. A non-polar stationary phase was obtained by impregnating the silica gel G layer with silica DC 200 (350 cS) (Applied Sciences Labs.). The impregnation was carried out by developing the plates in a 5% silicone solution in diethyl ether. Eight plates could be impregnated in a single chromatographic chamber, containing 200 ml of the silicone solution. The plates were left in the chamber for 12 h, i.e., for several hours after the silicone solution had reached the top of the plates. This method of impregnating silica gel G layers has also been used by several other investigators, as reported by Seydel and Schaper[7]. The chromatographic chamber was saturated with the mobile phase vapour according to Stahl[8].

A migration of 10 cm was obtained on all the plates by cutting the layer at 12 cm and spotting the compounds on a line 2 cm from the lower edge of the plate. The mobile phase saturated with silicone was an aqueous buffer (sodium acetate–Veronal buffer, 1/7 M at pH 7.0), alone or mixed with various amounts of acetone. In the previous work the organic solvent was methanol[5]. Two plates were developed simultaneously in a chromatographic chamber containing 200 ml of mobile phase. The dermorphin-related derivatives were dissolved in methanol (1–2 mg/ml) and 1 μl of solution was spotted on the plates in random positions in order to avoid any systematic error. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. After a few minutes at 120°C, yellow spots appeared on an intense pink background. The R_M values were calculated by means of the equation

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

RESULTS

Spraying of the developed plates with potassium permanganate resulted in the

appearance of round spots at different distances from the starting line. A few compounds, *viz.*, the five most hydrophilic ones, allowed reliable R_F values to be determined when the mobile phase was the aqueous buffer alone. The R_M values obtained for these five compounds are very similar to those previously determined under the same conditions[5]. Those R_M values for compounds 1, 10, 15 and 21 at 0% are reported in Table I. Only for compound 18 is a slightly different R_M value reported. In order to obtain suitable R_M values for the more lipophilic compounds, it was necessary to add an organic solvent to the mobile phase. Table I gives the R_M values obtained with the addition of acetone to the mobile phase and those previously determined in the methanol system. Higher and/or positive R_M values indicate compounds more lipophilic than those represented by lower and/or negative R_M values. The R_M values obtained with the acetone system are lower than those obtained with the same methanol concentrations, owing to the higher eluting power of acetone.

R_M values extrapolated from a wide range of organic solvent concentrations

In the methanol system we had considered the R_M values to bear a linear relationship to the composition of the mobile phase up to a 50% methanol concentration[5]. In a similar way, the equations describing the linear relationship between the R_M values and the acetone concentrations up to 40% were calculated and are reported in Table II. The R_M values obtained with concentrations of acetone or methanol higher than 40 and 50%, respectively, were not used because they were considered to be out of the range of linearity. The correlation coefficients provided by the equations using the methanol system are higher than those obtained with the data using the acetone system. This means that the linear relationship between R_M values and the composition of the mobile phase up to 50% methanol fits the data better than that describing the relationship between R_M values and acetone concentrations in the mobile phase up to 40%. The theoretical R_M values at 0% methanol or acetone in the mobile phase, *i.e.*, in a standard system where all the compounds might be compared, are represented by the intercepts of the equations reported in Table II.

A highly significant relationship between the extrapolated R_M values in the methanol and acetone systems is shown by the equation

$$R_{M_{CH_3OH}} = 1.768 - 0.482\, R_{M_{(CH_3)_2CO}} \qquad \begin{array}{ccc} n & r & s \\ 23 & 0.987 & 0.163 \end{array} \qquad (1)$$

$$(F = 817.07;\ P < 0.005)$$

Eqns. 2 and 3 show a very high correlation coefficient between the R_M values in both systems and the $\Sigma\pi$ values calculated from the data of Hansch and Leo[9] and Fauchère *et al.*[10] and are reported in Table III.

$$R_{M_{CH_3OH}} = 1.601 + 0.859\, \Sigma\pi \qquad \begin{array}{ccc} n & r & s \\ 23 & 0.943 & 0.343 \end{array} \qquad (2)$$

$$(F = 168.35;\ P < 0.005)$$

$$R_{M_{(CH_3)_2CO}} = 1.195 + 0.466\, \Sigma\pi \qquad \begin{array}{ccc} & & \\ 23 & 0.915 & 0.232 \end{array} \qquad (3)$$

$$(F = 108.61;\ P < 0.005)$$

TABLE I

R_M VALUES OF DERMORPHIN-RELATED OLIGOPEPTIDES AT INCREASING METHANOL OR ACE-TONE CONCENTRATIONS IN THE MOBILE PHASE

Compound	Aqueous buffer (0%)	Acetone concentration (%)								
		4	8	12	16	20	24	28	32	36
1	1.40	0.83	0.48	0.22	−0.10	−0.14	−0.22	−0.30	−0.40	−0.66
2	−	1.04	0.77	0.42	−0.04	0.00	−0.14	−0.21	−0.35	−0.58
3	−	−	1.08	0.82	0.27	0.16	0.16	0.05	−0.13	−0.51
4	−	−	−	1.13	0.56	0.39	0.43	0.25	0.02	−0.27
5	−	−	−	−	0.81	0.61	0.60	0.39	0.15	−0.14
6	−	−	−	−	0.75	0.70	0.56	0.43	0.05	−0.16
7	−	−	−	−	0.72	0.65	0.52	0.36	0.10	−0.17
8	−	−	−	−	0.87	0.65	0.71	0.46	0.23	−0.10
9	−	−	1.34	1.02	0.48	0.41	0.29	0.18	−0.03	−0.27
10	1.44	0.94	0.50	0.24	−0.16	−0.14	−0.19	−0.31	−0.45	−0.71
11	−	1.08	0.69	0.45	0.03	−0.02	−0.10	−0.17	−0.34	−0.58
12	−	−	1.24	0.81	0.32	0.18	0.17	0.02	−0.26	−0.48
13	−	−	−	−	1.29	1.07	1.12	0.92	0.62	0.27
14	−	−	−	−	1.52	1.32	1.34	0.96	0.83	0.54
15	0.98	0.45	0.23	−0.13	−0.51	−0.46	−0.60	−0.71	−0.75	−1.04
16	−	1.14	0.80	0.47	0.08	0.04	0.01	−0.11	−0.28	−0.49
17	−	−	−	−	0.91	0.86	0.73	0.52	0.32	0.03
18	1.38	0.87	0.60	0.29	−0.09	−0.11	−0.26	−0.28	−0.43	−0.66
19	−	−	−	−	0.81	0.72	0.59	0.43	0.16	−0.12
20	−	−	−	−	1.36	1.23	1.15	0.94	0.68	0.30
21	1.06	0.51	0.19	−0.17	−0.41	−0.45	−0.63	−0.69	−0.70	−0.97
22	−	−	1.12	0.83	0.39	0.26	0.18	0.08	−0.12	−0.34
23	−	−	−	−	1.07	1.02	0.84	0.68	0.38	0.03

However, the extrapolated R_M values reported in Table II are very different in the two systems, which is reflected in the intercept and the slope of eqn. 1. Moreover, the extrapolated R_M values in Table II range from 0.829 to 4.065 in the methanol system and from 0.636 to 2.587 in the acetone system, which means a difference on a logarithmic scale of 3.24 and 1.95, respectively. On the other hand, the $\Sigma\pi$ values in Table III range from −1.23 to 2.70 with a much larger difference of 3.93 between the most hydrophilic and the most lipophilic compounds.

As a consequence, eqns. 2 and 3 are completely different. The wide range of the $\Sigma\pi$ values is mainly due to the $\Sigma\pi$ values for compounds 1 and 18, which are among the most hydrophilic compounds and are characterized by an amino group. The use of the π values for such a substituent seems to predict, for the present series of compounds, a degree of hydrophilicity much higher than that measured by the R_M values. This is in agreement with previous findings for a series of xanthone deriva-tives[11]. Without compounds 1 and 18 the $\Sigma\pi$ values range from −0.67 to 2.70 with a difference on a logarithmic scale of 3.37. The corresponding R_M values in the meth-anol and acetone systems range from 0.83 to 4.06 and from 0.64 to 2.59 with differ-ences of 3.23 and 1.95, respectively. Hence, while the spread of the R_M values ob-tained with the methanol system is very similar to that of the $\Sigma\pi$ values, that of the R_M values obtained with the acetone system is narrower. In fact, eqn. 4, which was

		Methanol concentration (%)								
40	48	10	20	30	40	45	50	60	70	80
−0.86	–	0.76	0.33	0.17	−0.03	−0.43	−0.48	−0.51	−0.66	–
−0.78	–	1.02	0.63	0.36	−0.03	−0.28	−0.41	−0.53	−0.72	–
−0.72	–	–	1.03	0.70	0.32	−0.05	−0.25	−0.38	−0.56	−0.50
−0.60	–	–	1.23	0.89	0.40	0.02	−0.14	−0.45	−0.76	–
−0.53	–	–	–	1.20	0.57	0.17	−0.03	−0.36	−0.74	–
−0.61	–	–	–	1.16	0.51	0.15	−0.10	−0.36	−0.69	–
−0.64	–	–	–	1.08	0.58	0.12	−0.10	−0.29	−0.64	–
−0.49	–	–	–	1.20	0.71	0.27	0.05	−0.24	−0.61	–
−0.60	–	–	1.25	0.89	0.36	0.02	−0.20	−0.43	−0.68	–
–	–	0.85	0.40	0.21	−0.06	−0.42	−0.54	−0.57	−0.71	–
−0.86	–	1.12	0.68	0.46	0.15	−0.30	−0.45	−0.48	−0.65	–
−0.86	–	–	0.92	0.58	0.09	−0.29	−0.45	−0.64	–	–
−0.23	−0.32	–	–	–	1.07	0.68	0.40	−0.06	−0.46	−0.55
0.00	−0.24	–	–	–	1.22	0.86	0.52	−0.04	−0.34	−0.37
–	–	0.39	0.05	−0.13	−0.39	−0.66	−0.78	−0.76	–	–
−0.72	–	1.03	0.61	0.38	0.10	−0.30	−0.50	−0.58	–	–
−0.35	−0.52	–	–	1.20	0.57	0.17	−0.06	−0.35	−0.67	–
–	–	0.84	0.43	0.23	0.09	−0.39	−0.46	−0.56	−0.63	–
−0.51	−0.54	–	–	1.22	0.62	0.26	−0.05	−0.33	−0.75	–
−0.15	−0.38	–	–	–	1.15	0.77	0.42	−0.01	−0.46	−0.48
–	–	0.44	0.01	−0.12	−0.39	−0.63	−0.66	−0.74	–	–
−0.62	–	–	1.01	0.65	0.22	−0.09	−0.26	−0.49	–	–
−0.19	−0.54	–	–	1.24	0.72	0.34	0.03	−0.28	−0.68	–

calculated without compounds 1 and 18, with a slope very close to 1 is much better than eqn. 2. Eqn. 5, which shows a much lower slope, still indicates the difference between the R_M values from the two TLC systems.

$$R_{M_{CH_3OH}} = 1.411 + 0.991\ \Sigma\pi \qquad \begin{array}{ccc} n & r & s \\ 21 & 0.964 & 0.272 \end{array} \qquad (4)$$

$$(F = 248.32;\ P < 0.005)$$

$$R_{M_{(CH_3)_2CO}} = 1.044 + 0.571\ \Sigma\pi \qquad \begin{array}{ccc} 21 & 0.968 & 0.147 \end{array} \qquad (5)$$

$$(F = 281.44;\ P < 0.005)$$

R_M values extrapolated from a narrow range of organic solvent concentrations

If the extrapolated R_M values represented the partitioning of the compounds between the silicone oil of the stationary phase and a mobile phase constituted only by water, we would expect that both systems would give the same extrapolated R_M values. With this in mind we re-examined the relationship between R_M values and the composition of the mobile phase. Fig. 1 gives some experimental data. For com-

TABLE II

EQUATIONS DESCRIBING THE LINEAR RELATIONSHIP BETWEEN THE R_M VALUES OF DERMOR-PHIN-RELATED DERIVATIVES AND THE COMPOSITION OF THE MOBILE PHASE
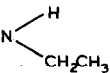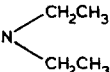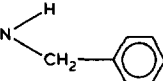
$$\text{H—CH—CH—C—NH—CH—C—NH—CH—C—NH—R}_1\text{—C—R}_2$$

with substituents: OH (Tyr), NH$_2$, O, CH$_3$, O, CH$_2$ (Phe), O, O

Tyr   D-Ala   Phe   Gly or β-Ala
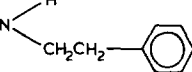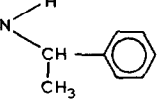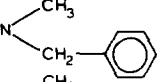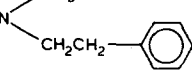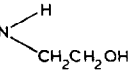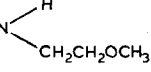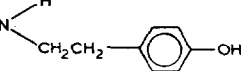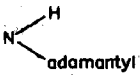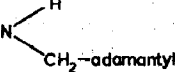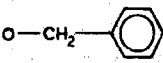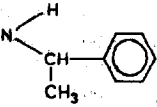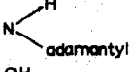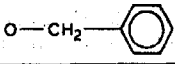
Com-pound	Structure		TLC equation					
	R_1	R_2	Acetone system			Methanol system		
			$R_M = a^\star$	$b^\star$	$r^\star$	$R_M = a^\star$	$b^\star$	$r^\star$
1	CH$_2$	N(H)(H)	1.242	−0.056	0.909	1.219	−0.035	0.979
2	CH$_2$	N(H)(CH$_2$CH$_3$)	1.020	−0.046	0.966	1.382	−0.036	0.998
3	CH$_2$	N(CH$_2$CH$_3$)(CH$_2$CH$_3$)	1.341	−0.050	0.968	1.951	−0.043	0.991
4	CH$_2$	N(H)(CH$_2$—C$_6$H$_5$)	1.597	−0.052	0.965	2.241	−0.048	0.993
5	CH$_2$	N(H)(CH$_2$CH$_2$—C$_6$H$_5$)	1.901	−0.058	0.977	3.087	−0.063	0.996
6	CH$_2$	N(H)(CH(CH$_3$)—C$_6$H$_5$)	1.945	−0.060	0.975	3.070	−0.064	0.999
7	CH$_2$	N(CH$_3$)(CH$_2$—C$_6$H$_5$)	1.890	−0.059	0.974	2.928	−0.061	0.992
8	CH$_2$	N(CH$_3$)(CH$_2$CH$_2$—C$_6$H$_5$)	2.015	−0.059	0.970	2.997	−0.059	0.993
9	CH$_2$	N(H)(cyclopentyl)	1.601	−0.054	0.976	2.304	−0.050	0.995
10	CH$_2$	N(H)(CH$_2$CH$_2$OH)	1.054	−0.052	0.945	1.305	−0.037	0.988
11	CH$_2$	N(H)(CH$_2$CH$_2$OCH$_3$)	1.051	−0.047	0.973	1.518	−0.038	0.985
12	CH$_2$	N(H)(CH$_2$CH$_2$—C$_6$H$_4$—OH)	1.486	−0.057	0.973	1.931	−0.047	0.993

TABLE II (continued)

Com- pound	Structure		TLC equation					
	R_1	R_2	Acetone system			Methanol system		
			$R_M = a^\star$ $b^\star$		$r^\star$	$R_M = a^\star$ $b^\star$		$r^\star$
13	CH_2	N—H, adamantyl	2.388	−0.060	0.948	3.732	−0.067	0.996
14	CH_2	N—H, CH_2—adamantyl	2.587	−0.059	0.962	4.017	−0.070	1.000
15	CH_2	OH	0.636	−0.049	0.951	0.829	−0.032	0.984
16	CH_2	O—CH_2CH_3	1.098	−0.046	0.969	1.411	−0.037	0.987
17	CH_2	O—CH_2—⬡	1.948	−0.055	0.979	3.129	−0.064	0.997
18	$(CH_2)_2$	N—H, H	1.045	−0.051	0.957	1.263	−0.034	0.982
19	$(CH_2)_2$	N—H, CH(—⬡)CH_3	1.998	−0.060	0.976	3.145	−0.064	0.999
20	$(CH_2)_2$	N—H, adamantyl	2.502	−0.061	0.965	4.065	−0.073	1.000
21	$(CH_2)_2$	OH	0.662	−0.049	0.943	0.862	−0.032	0.977
22	$(CH_2)_2$	O—CH_2CH_3	1.365	−0.049	0.980	1.911	−0.043	0.997
23	$(CH_2)_2$	O—CH_2—⬡	2.102	−0.055	0.980	3.093	−0.061	0.997

$\star$ a, b and r are respectively the intercept, slope and correlation coefficient of the equations describing the
lationship between the R_M values and the composition of the mobile phase.

pounds 1, 2, 3, 4, 9, 10, 11, 12, 15, 16, 18, 21 and 22, by taking into consideration
only the lower concentrations of methanol or acetone, the equations reported in
Table III were calculated. Table III also reports the ranges of the organic solvent
concentrations used in calculating such equations. The R_M values obtained with
higher acetone or methanol concentrations were not used because they were out of
the range of a close linear relationship. In fact, at higher acetone or methanol con-
centrations the compounds tend to migrate with the solvent front. The extrapolated
R_M values reported in Table III are very similar in the two systems. It can be also
pointed out that the extrapolated R_M values are very close to the experimental R_M
values at 0% which were available for five compounds (1, 10, 15, 18 and 21). This
could be further evidence of the validity of the extrapolation approach.
        For the more lipophilic compounds 5, 6, 7, 8, 13, 14, 17, 19, 20 and 23, lower
methanol or acetone concentrations did not yield reliable R_M values as the com-
pounds migrated only very little or not at all. In the methanol system an extrapolated
R_M value was calculated by considering the range of concentrations between 30 and
40 and 50% of organic solvent in the mobile phase. In the acetone system the equa-

TABLE III

EQUATIONS DESCRIBING THE RELATIONSHIP BETWEEN R_M VALUES AND COMPOSITION OF THE MOBILE PHASE IN THE REPORTED CONCENTRATION RANGES

a, *b* and *r* defined as in Table II.

Compound	Acetone				Methanol				$\Sigma\pi$
	$R_M = a$	*b*	*r*	Concentration range (%)	$R_M = a$	*b*	*r*	Concentration range (%)	
1	1.288	−0.090	0.988	0–16	1.365	−0.054	0.994	0–20	−1.23
2	1.445	−0.090	0.993	4–16	1.410	−0.039	1.000	10–20	0.08
3	1.938	−0.101	0.979	8–16	1.951	−0.043	0.991	20–50	1.18
4	2.173	−0.093	0.955	12–20	2.241	−0.048	0.993	20–50	0.78
5	2.560	−0.076	0.994	28–40	3.087	−0.063	0.996	30–50	1.34
6	2.758	−0.083	0.993	28–40	3.070	−0.064	0.999	30–50	1.34
7	2.692	−0.082	0.988	28–40	2.928	−0.061	0.992	30–50	1.34
8	2.728	−0.080	0.994	28–40	2.997	−0.059	0.993	30–50	1.90
9	2.237	−0.107	0.989	8–16	2.304	−0.050	0.995	20–50	0.91
10	1.348	−0.095	0.996	0–16	1.417	−0.052	0.997	0–20	−0.59
11	1.410	−0.085	0.995	4–16	1.560	−0.044	1.000	10–20	0.06
12	2.170	−0.115	0.999	8–16	1.931	−0.047	0.993	20–50	0.67
13	3.625	−0.095	0.993	28–40	3.732	−0.067	0.996	40–50	2.14
14	4.192	−0.104	0.985	28–40	4.017	−0.070	1.000	40–50	2.70
15	0.916	−0.089	0.993	0–16	0.938	−0.047	0.988	0–20	−0.67
16	1.500	−0.088	0.999	4–16	1.450	−0.042	1.000	10–20	0.38
17	2.595	−0.073	0.991	28–40	3.129	−0.064	0.997	30–50	1.66
18	1.314	−0.088	0.994	0–16	1.358	−0.048	0.997	0–20	−0.95
19	2.625	−0.078	0.996	28–40	3.145	−0.064	0.999	30–50	1.62
20	3.545	−0.091	0.993	28–40	4.065	−0.073	1.000	40–50	2.42
21	0.960	−0.090	0.989	0–16	1.028	−0.053	0.995	0–20	−0.39
22	1.875	−0.091	0.993	8–16	1.911	−0.043	0.997	20–50	0.66
23	2.741	−0.074	0.996	28–40	3.093	−0.061	0.997	30–50	1.94

tions were calculated with R_M values in the range between 28 and 40% of acetone in the mobile phase. The R_M values obtained with higher acetone or methanol concentrations were not used because they were out of the range of a close linear relationship. The equations are reported in Table III and show very similar extrapolated R_M values in the two TLC systems. A very good correlation between the extrapolated R_M values from both systems, as reported in Table III, is shown by eqn. 6.

$$R_{M_{CH_3OH}} = -0.031 + 1.083\, R_{M_{(CH_3)_2CO}} \qquad \begin{array}{ccc} n & r & s \\ 23 & 0.976 & 0.214 \end{array} \qquad (6)$$

$$(F = 430.83;\ P < 0.005)$$

The intercept and a slope very close to 0 and 1, respectively, indicate the overlapping of the R_M values extrapolated from the two solvent systems.

A highly significant correlation with the $\Sigma\pi$ values is shown by eqns. 7 and 8.

$$R_{M_{CH_3OH}} = 1.666 + 0.819\, \Sigma\pi \qquad \begin{array}{ccc} n & r & s \\ 23 & 0.935 & 0.351 \end{array} \qquad (7)$$

$$(F = 146.54;\ P < 0.005)$$
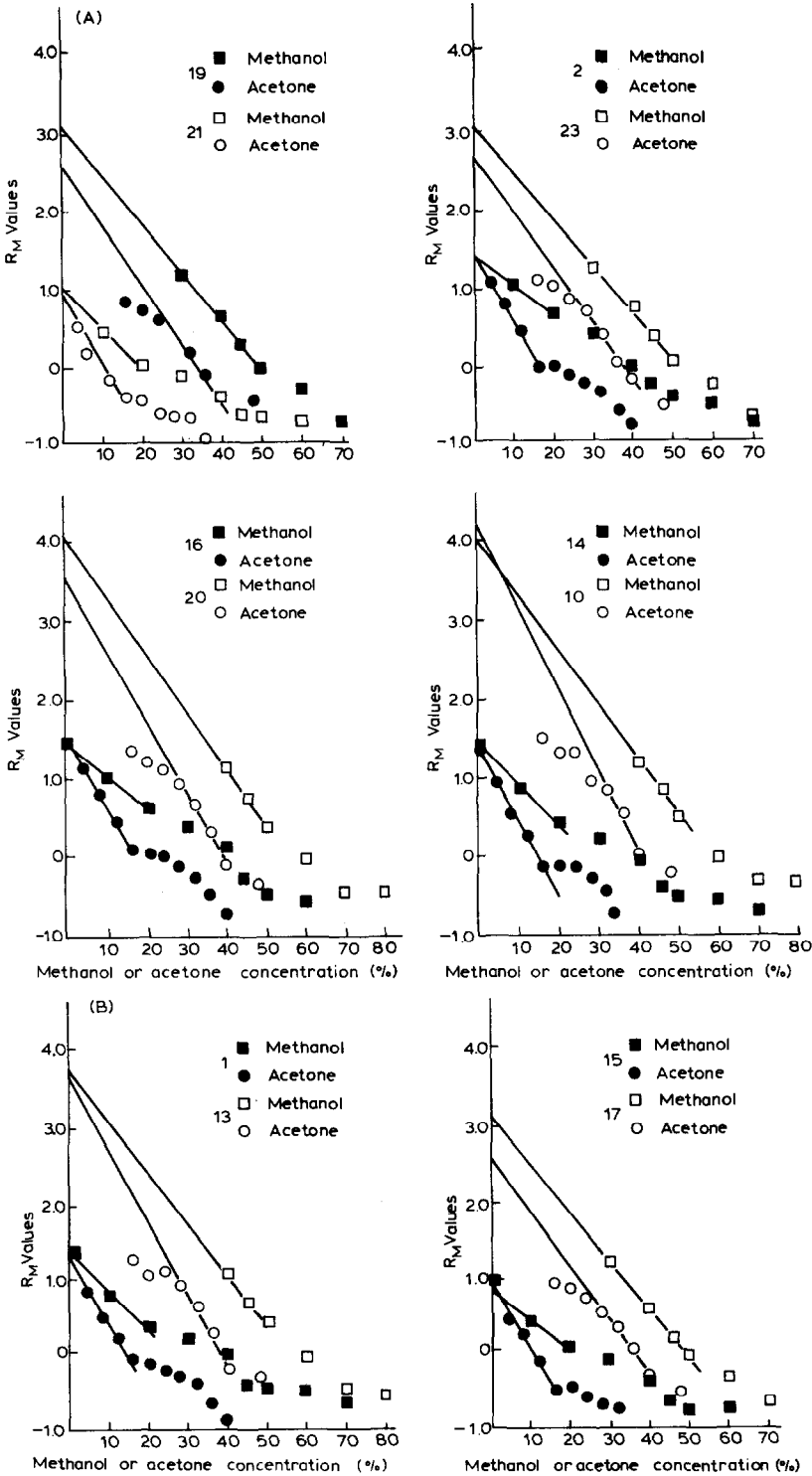
Fig. 1. Relationship between R_M values and the organic solvent concentration in the mobile phase.

$$R_{M_{(CH_3)_2CO}} = 1.583 + 0.737 \, \Sigma\pi \qquad\qquad 23 \quad 0.933 \quad 0.321 \qquad (8)$$

$$(F = 142.23; \, P < 0.005)$$

In particular, eqns. 7 and 8 are much more similar than eqns. 2 and 3, this being due to eqn. 6, which takes into account R_M values extrapolated from a narrow range of organic solvent concentrations (*i.e.* in the range of close linear relationship). However, the slopes of eqns. 7 and 8 are less than 1. In Table III the extrapolated R_M values range from 0.94 to 4.06 in the methanol system and from 0.92 to 4.19 in the acetone system, which means a difference on a logarithmic scale of 3.12 and 3.27, respectively, *i.e.*, a 1320- and 1870-fold difference in lipophilic character. The $\Sigma\pi$ values range from -1.23 to 2.70, which means a difference between the most hydrophilic and the most lipophilic compounds of 3.93, *i.e.*, an 8500-fold difference in lipophilicity.

The wide range in the π system is still due to the calculated $\Sigma\pi$ values for compounds 1 and 18. Without these compounds the $\Sigma\pi$ values range from -0.67 to 2.70 with a difference of 3.37, which is much closer to the differences of 3.12 and 3.27, respectively, in the range of the R_M values in the two systems.

$$\begin{array}{cccc} & n & r & s \end{array}$$

$$R_{M_{CH_3OH}} = 1.473 + 0.954 \, \Sigma\pi \qquad 21 \quad 0.960 \quad 0.278 \qquad (9)$$

$$(F = 221.04; \, P < 0,005)$$

$$R_{M_{(CH_3)_2CO}} = 1.412 + 0.856 \, \Sigma\pi \qquad 21 \quad 0.956 \quad 0.260 \qquad (10)$$

$$(F = 203.19; \, P < 0.005)$$

Eqns. 9 and 10, calculated without compounds 1 and 18, show a slope closer to 1 and very similar intercepts. This means that under these conditions the R_M and π systems are very similar in representing the lipophilic character of the compounds.

RESULTS AND CONCLUSION

Extrapolation from the methanol or the acetone system yields very similar R_M values. The results show that the linear relationship between R_M values and the mobile phase composition allows one to calculate extrapolated R_M values that are not dependent on the nature of the organic solvent. In other words, the R_M values at 0% of organic solvent in the mobile phase should be a measure of the partitioning between water and silicone oil, *i.e.*, in a standard system where all the compounds could be compared. All this is also supported by the present finding that for compounds 1, 10, 15, 18 and 21 the experimental R_M values with water as the mobile phase are very similar to the extrapolated R_M values in two different TLC systems. The correlation coefficients reported in Table III, which are higher than those in Table II, show that the linear regression in the ranges of concentrations of organic solvents that were used in order to calculate the extrapolated R_M values fit the data very closely.

The most important point is obviously the choice of the range of acetone or methanol concentrations to be used in calculating the extrapolated R_M values.

We did not use the R_M values obtained with acetone or methanol concentrations higher than 40 and 50%, respectively, because they were out of the range of linearity. According to Karger et al.[3], the deviations from linearity could be explained by structural variations in the solvent system as the concentration of the organic component is increased. The addition of an organic solvent to water should initially result in only a minor perturbation of the water structure. As the concentration of acetone or methanol in water is increased, a point must be reached when at least some of structural aspects of the bulk water begin to change. The concentration at which this takes place will vary with the nature of the organic solvent[3]. The linear relationship between R_M values and methanol concentrations holds over a wider range than that observed in the acetone system. This might depend on the fact that methanol can act as both a proton donor and acceptor[3]. However, the critical concentration seems to change not only with the nature of the organic solvent but also with the lipophilic character of molecules. In fact, in the acetone system the critical concentration is 16–20% for the most hydrophilic compounds and 40% for the most lipophilic compounds. Similarly in the methanol system the corresponding critical concentrations are 20 and 50%, respectively (see the concentration ranges in Table III).

On the other hand, for the more lipophilic compounds lower acetone or methanol concentrations did not yield suitable R_M values as the compounds migrated very little or not at all. This might be due to the poor solubility of the compounds in the water alone or with small amounts of acetone or methanol present and/or to the fact that the mobile phase does not completely wet the stationary phase[3].

An interesting point arises from a comparison of the slopes of the straight lines describing the relationship between R_M values and the composition of the mobile phase in both the methanol and acetone systems. In Table III the slopes in the methanol system have a mean value of 0.055 ± 0.002 and those in the acetone system a mean value of -0.089 ± 0.002. The more negative slopes in the acetone system are certainly related to the higher eluting power of acetone and indicate that the same decrease in R_M value is given by a smaller increase in the acetone concentration. It can be pointed out that the ratio between the above mean values is 1.62, which is very close to the ratio of 1.70 between the solvent strength parameters (E_0) of acetone and methanol when considered in a reversed-phase chromatographic system[12,13].

Karger et al.[3] explained the more negative slopes in acetone by the fact that acetone provides only proton-accepting ability. Therefore, we might expect that acetone might cause changes in the hydrogen-bonded network of water molecules more readily than methanol. In fact methanol, can act as both a proton donor and acceptor. On the other hand, the solvent strength is related to the proton donor and/or acceptor nature of the organic solvent[13].

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