STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

XV. THE BEHAVIOUR OF METHYLATED PHENOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH FORMAMIDE AND WITH N-METHYLATED FORMAMIDES

R. J. T. GRAHAM, L. S. BARK AND J. DALY

Department of Chemistry and Applied Chemistry, University of Salford, Salford 5, Lancs. (Great Britain)

SUMMARY

The R_M values of phenol and 19 methylated phenols on thin layers of cellulose impregnated with formamide, N-methylformamide or N,N-dimethylformamide are shown to be linearly related to the log of the concentration of the amide in the slurrying medium used in the preparation of the layers. The deviations from the R_M theory occurring at low and high amide concentrations are explained.

INTRODUCTION

The basic theories used in studying the relationship between molecular structure and chromatographic behaviour in open column techniques are those based on the earlier works of CONSDEN, GORDON AND MARTIN[1], MARTIN[2] and BATE-SMITH AND WESTALL[3].

CONSDEN, GORDON AND MARTIN[1], related the R_F value of a compound obtained from paper partition chromatography to the partition coefficient, α, of the compound between the phases used in the system by the equation:

$$\alpha = \frac{A_M}{A_S}\left(\frac{1}{R_F} - 1\right) \qquad (1)$$

where A_M/A_S is the ratio of the thicknesses of the mobile phase and stationary phase.

Assuming the term A_M/A_S to be a constant for a given chromatogram, BATE-SMITH AND WESTALL[3] proposed a new function, R_M, whence

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \qquad (2)$$

This latter expression was used by them to test the additivity principle of chromatographic behaviour proposed by MARTIN[2]. They found the theory to be reasonably confirmed.

Since their work, several workers[4–8] have tested the MARTIN relationship. Some[4–6] have proved its validity, others[7,8] have not. The apparent breakdown of the MARTIN theory can be traced to the failure by the investigators[7,8] to accept the limitations to the theory put forward by MARTIN[2] and BATE-SMITH AND WESTALL[3]. The present situation concerning the validity of the R_M theory has been reviewed by BUSH[9], who found it "to be in accord with subsequent more sophisticated theories of the physical chemistry of solubility properties and of partition coefficients".

However, one of the major reasons for questioning the validity of eqns. (1) and (2), and hence their usefulness in studying structural problems by chromatographic methods, lies in the possible variation of the A_M/A_S ratio on the chromatogram[10–13]. Only if this ratio can be maintained constant is a study of the accuracy of eqns. (1) and (2) meaningful.

In reversed phase systems on paper, GREEN AND MARCINKIEWICZ[14], have shown that the A_S value can be maintained constant by care in the method of impregnating the layers. In the reversed phase thin-layer chromatography of metal ions on layers of cellulose impregnated with tri-*n*-butyl phosphate (TBP) DUNCAN[15] has shown that by incorporating the TBP into the cellulose during the slurrying process, there is little concentration gradient of the stationary phase over the chromatoplate.

Here we report an attempt to prove the validity of eqns. (1) and (2) using the reversed phase thin-layer chromatography of methylated phenols on layers of cellulose impregnated with different amides using hexane as the mobile phase.

We have previously shown the system formamide/hexane to be of use in the thin-layer chromatography of phenols[16].

EXPERIMENTAL

The eluent system

Hexane (BDH Reagent Grade) was purified as previously described[16]. Before its use as the eluent, the hexane was saturated with the appropriate amide.

Impregnants

Reagent grade formamide, N-methylformamide (MMF) and N,N-dimethylformamide (DMF) were further purified by passing them through a short column of activated alumina.

Solutions of these amides (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 *M*) in acetone were prepared.

Cellulose (15 g of MN 300 HR grade) was slurried with the amide solution (65 ml) to give a homogeneous slurry. This slurry was used to coat glass plates to give applied layers of 0.3 mm. The plates were allowed to dry for 15 min to allow the acetone to evaporate. They were then stored in an atmosphere of the impregnant until required.

The rapid and simultaneous application of the phenols (1 μl of 0.25% solutions in cyclohexane), the elution of the chromatograms and the identification of the phenols were carried out as previously described[16].

RESULTS

The results, quoted in Tables I–III, are the mean of at least 5 runs on layers

carrying 2,6-dimethylphenol as an internal standard. In each system over 500 R_F values for this standard were determined. The actual R_F values of the standard did not differ from the mean value by more than $\pm$0.01 R_F unit in any of the systems described.

For the systems formamide and N-methylformamide, no difficulty was experienced in obtaining reproducible R_F values. In the case of N,N-dimethylformamide, however, it was noticed that if the prepared plates were exposed to the laboratory atmosphere, the R_F values increased with the length of time of standing exposure. It may be seen from the tables that higher R_F values are associated with lower degrees of impregnation of the layers. It was therefore concluded that under such conditions, evaporation caused substantial loss of N,N-dimethylformamide, which boils approximately 30° below the other two amides. However, when the plates were stored in a vessel containing a little DMF, reproducible R_F values were obtained. Subsequently, in order to make direct comparison between the results obtained from the different layers, the prepared plates were stored in the appropriate amide environment.

The values for individual phenols were also reproducible to within $\pm$0.01 R_F units of the mean value quoted.

DISCUSSION

The composition of the phases

Amides were chosen as the stationary phases because evidence is available from infra-red spectroscopy[17,18] to show the proton acceptor property of the carbonyl group towards the phenolic hydrogen atom.

Hexane was chosen as the mobile phase because we have previously shown that this removes the phenols from the stationary phase by means of solvation of the hydrophobic part of the molecule[16]. Thus interactions between the mobile phase and the solute functional group, and between the mobile phase and the substrate are at a minimum. These last two limitations are necessary in a system where the validity of the R_M theory is being studied as a result of variations in the A_S value.

The effect of the amide loading

Since all experimental parameters other than the concentration of the amide in the slurrying solvent are kept constant, the decrease in the R_F values of the phenols with the increase in the amide concentrations must be regarded as caused by the alteration (*i.e.* increase) in the degree of impregnation of the cellulose support This is to be expected from eqn. (1).

Suitable rearrangement of eqns. (1) and (2) gives:

$$R_M = \log \alpha - \log A_M + \log A_S \qquad (3)$$

From this equation, it may be seen that a linear relationship between R_M and the logarithm of the amide concentration should obtain, provided the cross-sectional area of the mobile phase does not alter (the partition coefficient is by definition independent of the volumes of the phases used).

Fig. 1 shows plots of R_M values *vs.* concentration of the amide (log scale) in the slurrying solution. It can be seen that while these are linear over the bulk of the range of concentration studied, deviations occur at low loadings and at high loadings.

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TABLE I

R_F AND R_M VALUES OF METHYLATED PHENOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH FORMAMIDE

Concentration of formamide in slurrying solvent (moles litre^{-1}).

	Key Phenol	*0.5*		*1.0*		*2.0*		*3.0*		*4.0*		*5.0*		*6.0*	
		R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1	Phenol	0.18	+0.659	0.12	+0.865	0.06	+1.195	0.04	+1.38	0.03	+1.52	0.02	+1.600	0.00	
2	2-Methyl	0.40	+0.176	0.32	+0.329	0.20	+0.602	0.13	+0.79	0.105	+0.92	0.08	+1.061	0.08	+1.061
3	3-Methyl	0.28	+0.410	0.18	+0.659	0.10	+0.954	0.06	+1.195	0.05	+1.279	0.04	+1.380	0.04	+1.380
4	4-Methyl	0.28	+0.410	0.18	+0.659	0.10	+0.954	0.06	+1.195	0.05	+1.279	0.04	+1.380	0.04	+1.380
5	2,3-Dimethyl	0.56	−0.105	0.45	+0.087	0.31	+0.348	0.23	+0.550	0.16	+0.716	0.12	+0.865	0.13	+0.826
6	2,4-Dimethyl	0.59	−0.158	0.48	+0.025	0.36	+0.250	0.27	+0.432	0.20	+0.602	0.14	+0.789	0.15	+0.750
7	2,5-Dimethyl	0.63	−0.231	0.48	+0.025	0.35	+0.269	0.265	+0.433	0.195	+0.616	0.14	+0.789	0.14	+0.789
8	2,6-Dimethyl	0.78	−0.550	0.75	−0.478	0.64	−0.250	0.53	−0.052	0.45	+0.087	0.36	+0.250	0.36	+0.250
9	3,4-Dimethyl	0.38	+0.213	0.27	+0.432	0.18	+0.659	0.125	+0.845	0.09	+1.005	0.07	+1.124	0.06	+1.195
10	3,5-Dimethyl	0.46	+0.070	0.33	+0.301	0.225	+0.537	0.16	+0.716	0.12	+0.865	0.10	+0.959	0.09	+1.005
11	2,3,4-Trimethyl	0.66	−0.301	0.57	−0.125	0.44	+0.105	0.33	+0.301	0.25	+0.470	0.20	+0.602	0.20	+0.602
12	2,3,5-Trimethyl	0.68	−0.327	0.60	−0.176	0.50	0.00	0.39	+0.195	0.31	+0.348	0.26	+0.450	0.25	+0.477
13	2,3,6-Trimethyl	0.87	−0.827	0.84	−0.727	0.78	−0.50	0.70	−0.368	0.61	−0.195	0.53	−0.061	0.53	−0.061
14	2,4,5-Trimethyl	0.68	−0.327	0.60	−0.176	0.50	0.00	0.39	+0.195	0.31	+0.348	0.26	+0.450	0.25	+0.477
15	2,4,6-Trimethyl	0.89	−0.907	0.86	−0.788	0.80	−0.602	0.74	−0.455	0.64	−0.250	0.56	−0.105	0.55	−0.087
16	3,4,5-Trimethyl	0.48	+0.025	0.38	+0.213	0.24	+0.501	0.17	+0.689	0.135	+0.806	0.11	+0.820	0.10	+0.954
17	2,3,4,5-Tetramethyl	0.73	−0.432	0.68	−0.327	0.60	−0.176	0.46	+0.06	0.38	+0.213	0.32	+0.327	0.32	+0.327
18	2,3,4,6-Tetramethyl	0.92	−1.061	0.91	−1.004	0.87	−0.827	0.80	−0.602	0.76	−0.500	0.72	−0.410	0.72	−0.410
19	2,3,5,6-Tetramethyl	0.95	−1.272	0.94	−1.194	0.90	−0.955	0.845	−0.738	0.79	−0.575	0.74	−0.455	0.73	−0.432
20	2,3,4,5,6-Pentamethyl	1.00		1.00		0.94	−1.194	0.90	−0.955	0.85	−0.780	0.82	−0.658	0.80	−0.602

TABLE II

R_F AND R_M VALUES OF METHYLATED PHENOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH N-METHYLFORMAMIDE

Concentration of N-methylformamide in slurrying solvent (moles litre^{-1}).

Key Phenol	*0.5*		*1.0*		*2.0*		*3.0*		*4.0*		*5.0*	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1 Phenol	0.16	+0.716	0.09	+1.005	0.05	+1.279	0.035	+1.510	0.02	+1.69	0.00	
2 2-Methyl	0.38	+0.213	0.23	+0.525	0.12	+0.865	0.095	+0.978	0.075	+1.09	0.05	+1.278
3 3-Methyl	0.26	+0.454	0.16	+0.716	0.085	+1.032	0.05	+1.27	0.04	+1.38	0.03	+1.51
4 4-Methyl	0.26	+0.454	0.16	+0.716	0.085	+1.032	0.05	+1.27	0.04	+1.38	0.03	+1.51
5 2,3-Dimethyl	0.52	−0.035	0.30	+0.368	0.16	+0.716	0.12	+0.865	0.09	+1.005	0.07	+1.124
6 2,4-Dimethyl	0.54	−0.087	0.32	+0.327	0.18	+0.659	0.13	+0.826	0.105	+0.952	0.08	+1.061
7 2,5-Dimethyl	0.54	−0.087	0.32	+0.327	0.18	+0.659	0.13	+0.826	0.105	+0.952	0.08	+1.061
8 2,6-Dimethyl	0.67	−0.307	0.43	+0.123	0.24	+0.501	0.18	+0.659	0.14	+0.789	0.115	+0.886
9 3,4-Dimethyl	0.36	+0.250	0.21	+0.580	0.12	+0.865	0.08	+1 091	0.06	+1.195	0.05	+1.279
10 3,5-Dimethyl	0.44	+0.105	0.24	+0.501	0.135	+0.806	0.095	+0.978	0.075	+1.091	0.06	+1.195
11 2,3,4-Trimethyl	0.60	−0.176	0.33	+0.301	0.20	+0.602	0.135	+0.806	0.10	+0.954	0.08	+1.06
12 2,3,5-Trimethyl	0.62	−0.213	0.37	+0.231	0.23	+0.525	0.155	+0.736	0.125	+0.845	0.10	+0.954
13 2,3,6-Trimethyl	0.78	−0.550	0.56	−0.105	0.36	+0.250	0.26	+0.454	0.21	+0.580	0.17	+0.689
14 2,4,5-Trimethyl	0.62	−0.213	0.37	+0.231	0.23	+0.525	0.155	+0.736	0.125	+0.845	0.10	+0.954
15 2,4,6-Trimethyl	0.78	−0.550	0.56	−0.105	0.36	+0.250	0.26	+0.454	0.21	+0.580	0.17	+0.689
16 3,4,5-Trimethyl	0.44	+0.105	0.28	+0.410	0.17	+0.689	0.11	+0.908	0.08	+1.04	0.07	+1.124
17 2,3,4,5-Tetramethyl	0.64	−0.250	0.41	+0.158	0.25	+0.477	0.19	+0.630	0.135	+0.806	0.11	+0.908
18 2,3,4,6-Tetramethyl	0.82	−0.658	0.70	−0.368	0.46	+0.07	0.33	+0.301	0.24	+0.501	0.20	+0.602
19 2,3,5,6-Tetramethyl	0.84	−0.721	0.72	−0.410	0.48	+0.025	0.35	+0.269	0.25	+0.477	0.21	+0.58
20 2,3,4,5,6-Pentamethyl	0.87	−0.827	0.76	−0.500	0.52	−0.035	0.38	+0.213	0.28	+0.410	0.23	+0.325

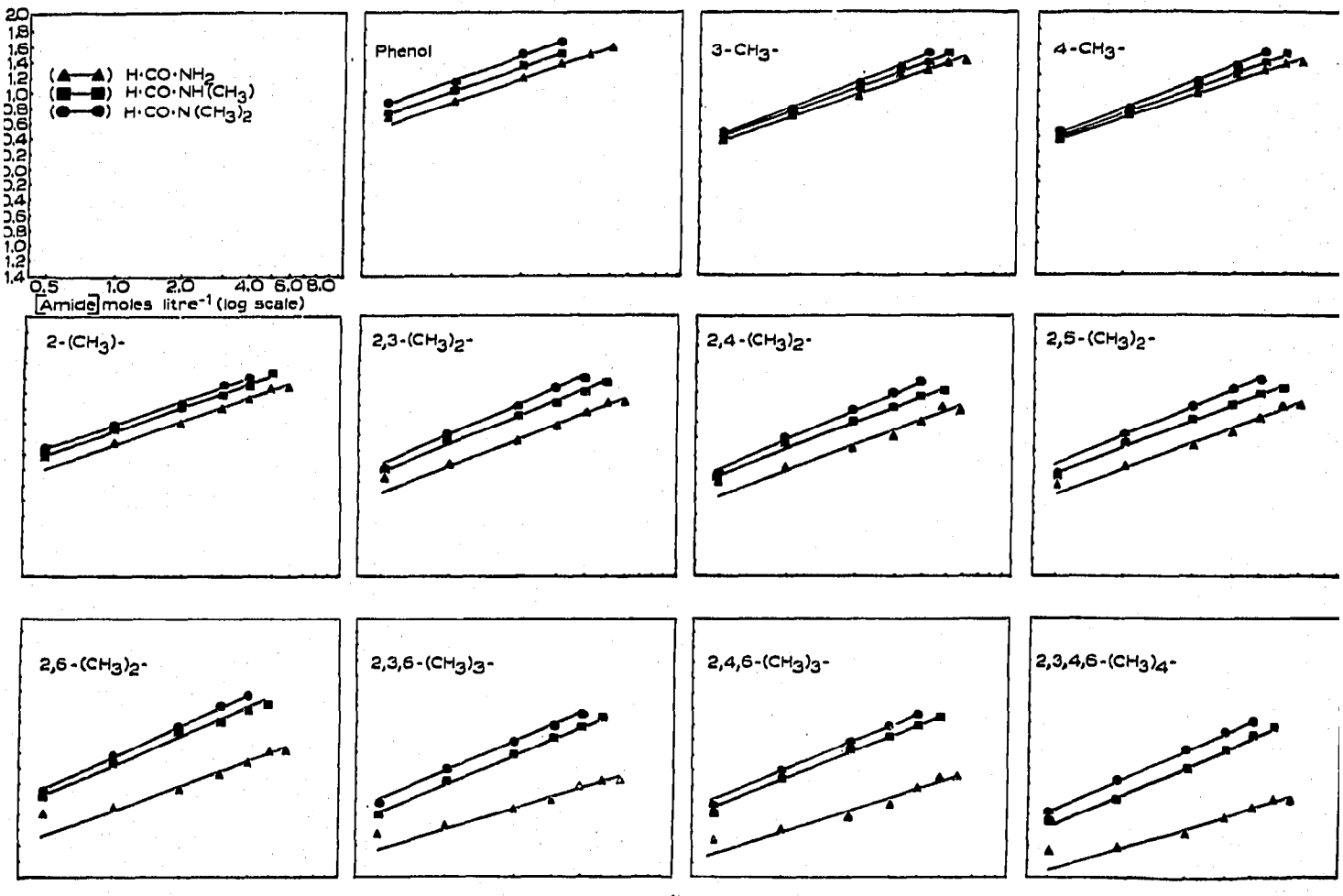
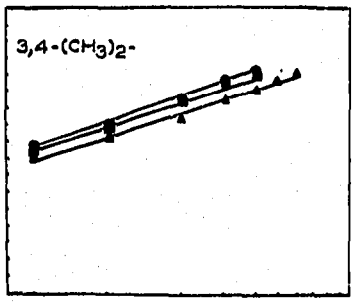


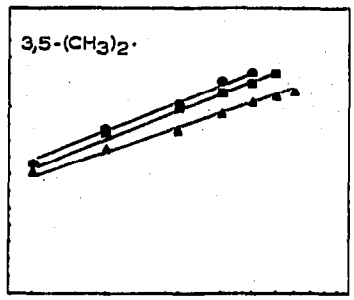
Fig. 1. R_M values (methylated phenols) *vs.* concentration of amide in the slurrying solvent (log scale).

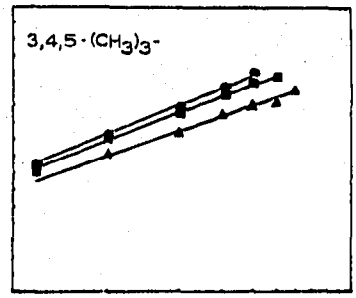
In the former case, we suggest that the interface between the mobile phase and stationary phase is not a simple hexane–amide interface but that the cellulose support is playing a significant part in the chromatographic mechanism[16]. At low concentrations there is competition between the cellulose hydroxyl groups and the phenolic group for the oxygen atom of the carbonyl group, *viz.* hydrogen bonds formed between the cellulose and the amide have to be broken before the phenol can be taken up by the surface. Some evidence for the possibility of such an equilibrium is obtained from the appearance of the spots on the developed chromatograms. At low concentrations of amide, the spots are diffuse and at very low concentrations they become streaked. (The 0.5 M loading represents the lowest loading at which the spots are sufficiently discrete for reliable R_F values to be obtained.)

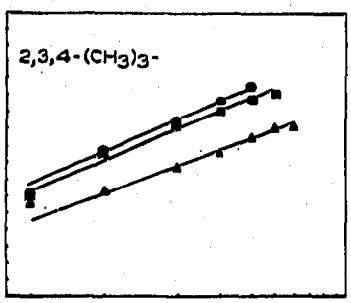
At higher concentrations of amide, the part played by the cellulose becomes progressively less significant, until it eventually becomes zero. This point is located where the linear parts of the curves begin. Once this point is reached, we regard the cellulose as being totally covered by the amide.

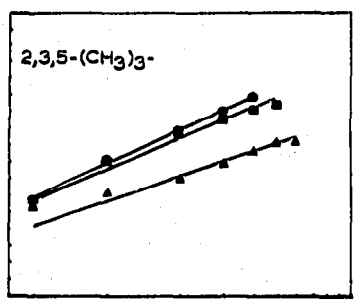
In the region below this point, where the cellulose is significant, the chromatographic mechanism is not solely one of partition, but is also one of adsorption. The

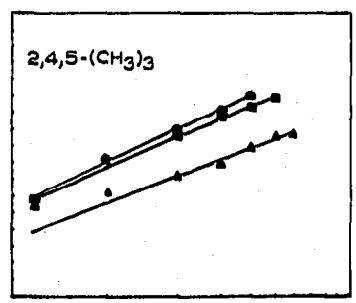


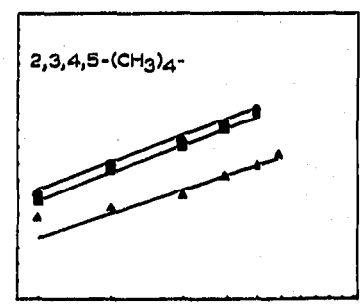


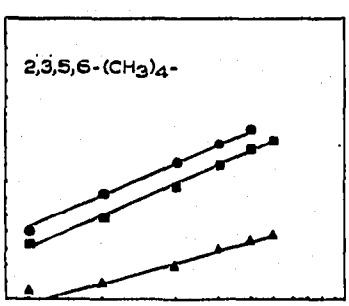


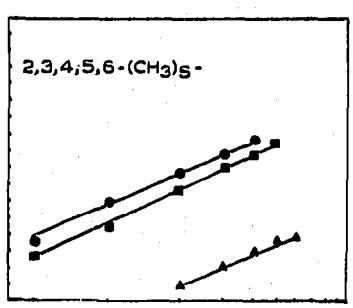












formation of hydrogen bonds between the support, *i.e.* the cellulose, is regarded as a major factor contributing to the deviation from the R_M theory.

Over the region of amide concentrations where the graphs are truly linear, we regard the mechanism as solely one of partition. Over this range the R_M theory is valid except for constitutive effects, *e.g.* in the case of nuclear substituents, positional effects. These are subdivided into the position of the substituent relative to that of the chromatographically effective functional group (the phenolic group) and the positions of the substituents relative to each other. Such effects have been discussed previously[16,19–21].

Attempts to load the cellulose with very high concentrations of amides resulted in the excess amide being displaced from the cellulose as a result of the mobile phase acting as a molecular plough. It is suggested that it is this molecular plough effect which causes deviation from linearity at high amide concentrations. This effect has been previously noted in reversed phase thin-layer chromatographic systems in which tri-*n*-butyl phosphate has been used as the impregnant[22].

The curves for the 20 compounds show a close parallelism in most cases. We propose that this indicates that the effective chromatographic mechanism is the same

TABLE III

R_F AND R_M VALUES OF METHYLATED PHENOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH N,N-DIMETHYLFORMAMIDE

Concentration of N,N-dimethylformamide in slurrying solvent (moles litre^{-1}).

	Key Phenol	*0.5*		*1.0*		*2.0*		*3.0*		*4.0*	
		R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
1	Phenol	0.14	+0.865	0.07	+1.124	0.03	+1.510	0.02	+1.69	0.00	
2	2-Methyl	0.35	+0.269	0.21	+0.580	0.12	+0.865	0.075	+1.09	0.06	+1.95
3	3-Methyl	0.25	+0.477	0.14	+0.789	0.07	+1.124	0.035	+1.33	0.03	+1.510
4	4-Methyl	0.25	+0.477	0.14	+0.789	0.07	+1.124	0.035	+1.33	0.03	+1.510
5	2,3-Dimethyl	0.50	0.00	0.26	+0.454	0.13	+0.826	0.08	+1.04	0.06	+1.195
6	2,4-Dimethyl	0.53	−0.052	0.28	+0.410	0.145	+0.771	0.09	+1.00	0.07	+1.124
7	2,5-Dimethyl	0.53	−0.052	0.27	+0.432	0.135	+0.806	0.085	+1.03	0.065	+1.158
8	2,6-Dimethyl	0.64	−0.250	0.38	+0.213	0.20	+0.602	0.13	+0.826	0 095	+0.978
9	3,4-Dimethyl	0.34	+0.288	0.19	+0.630	0.10	+0.954	0.06	+1.195	0.05	+1.279
10	3,5-Dimethyl	0.43	+0.123	0.22	+0.550	0.12	+0.865	0.07	+1.124	0.055	+1.234
11	2,3,4-Trimethyl	0.59	−0.158	0.31	+0.348	0.17	+0.689	0.11	+0.908	0.075	+1.091
12	2,3,5-Trimethyl	0.62	−0.213	0.36	+0.250	0.20	+0.602	0.13	+0.826	0.09	+1.005
13	2,3,6-Trimethyl	0.72	−0.410	0.48	+0.025	0.29	+0.388	0.20	+0.602	0.15	+0.750
14	2,4,5-Trimethyl	0.62	−0.213	0.36	+0.250	0.20	+0.602	0.13	+0.826	0.09	+1.005
15	2,4,6-Trimethyl	0.74	−0.455	0.50	0.00	0.30	+0.368	0.21	+0.580	0.155	+0.73
16	3,4,5-Trimethyl	0.42	+0.140	0.26	+0.454	0.14	+0.789	0.085	+1.032	0.06	+1.195
17	2,3,4,5-Tetramethyl	0.60	−0.176	0.38	+0.213	0.23	+0.550	0.17	+0.689	0.12	+0.865
18	2,3,4,6-Tetramethyl	0.78	−0.550	0.565	−0.114	0.34	+0.288	0.24	+0.501	0.18	+0.659
19	2,3,5,6-Tetramethyl	0.79	−0.575	0.57	−0.123	0.36	+0.250	0.25	+0.477	0.185	+0.644
20	2,3,4,5,6-Pentamethyl	0.82	−0.658	0.60	−0.176	0.40	+0.176	0.285	+0.399	0.21	+0.580

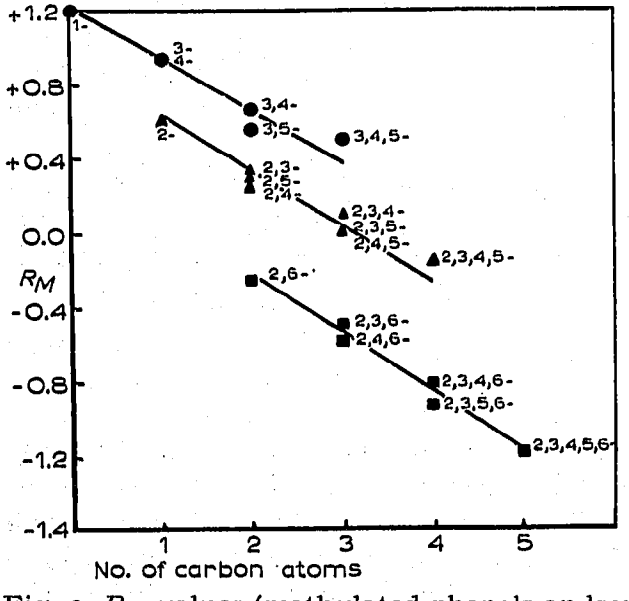
in all cases and is that the phenols are held onto the amide surface by the formation of hydrogen bonds between the hydrogen atom of the phenolic group and the carbonyl oxygen atom. Removal of the phenol from this surface is a result of solvation of the hydrophobic part of the phenolic molecule by the mobile phase. Infra-red studies[17] have shown that when phenols interact with amides to form a hydrogen bonded phenol–amide complex, they do so in a 1:1 ratio. The slopes of the linear regions of the curves in Fig. 1 generally approximate to 1. This correlation suggests that it might be possible to use reversed phase thin-layer chromatography to determine the stoichiometry of the reaction between the chromatographed species and the stationary phase. This has been done for some inorganic species[23].

The nature of the amide

The results in Tables I–III show that at a comparable loading, the R_F values are highest on layers impregnated with formamide and lowest on those impregnated with N,N-dimethylformamide, with the values for layers impregnated with N-methylformamide being between these two. The presence of a methyl group in the substituted amides strengthens the phenol–amide hydrogen bond by the inductive release of electrons to the carbonyl oxygen atom, the effect of the second methyl group is not, however, as great as that of the first. This may in part be attributed to the steric restriction of free rotation about the carbon-nitrogen bond and the resultant loss of the mesomerism which is thought to play some part in the hydrogen bonding of phenols to amides.

Chromatography and molecular structure

Tables I–III show that in all systems the addition of successive methyl groups to the aromatic nucleus increases the R_F values relative to phenol. It is seen however,



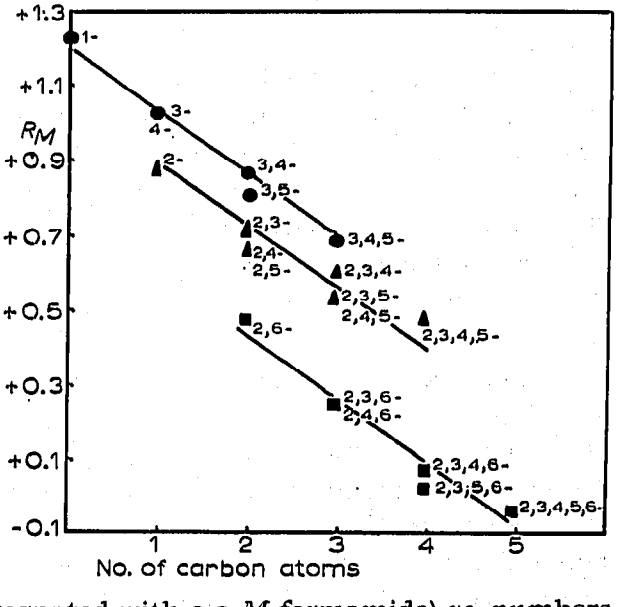


Fig. 2. R_M values (methylated phenols on layers impregnated with 2.0 M formamide) *vs.* numbers of side chain carbon atoms.

Fig. 3. R_M values (methylated phenols on layers impregnated with 2.0 M N-methylformamide) *vs.* numbers of side chain carbon atoms.

that the positions of the methyl group within the nucleus are also effective in governing the pattern of chromatographic behaviour. Thus as is shown in the tables and in Figs. 2–4 (for 2.0 *M* amide impregnation) the phenols may be divided into three groups, *viz.*

(a) phenols containing no group *ortho* to the phenolic group,
(b) phenols containing one group *ortho* to the phenolic group,
(c) phenols containing two groups *ortho* to the phenolic group.

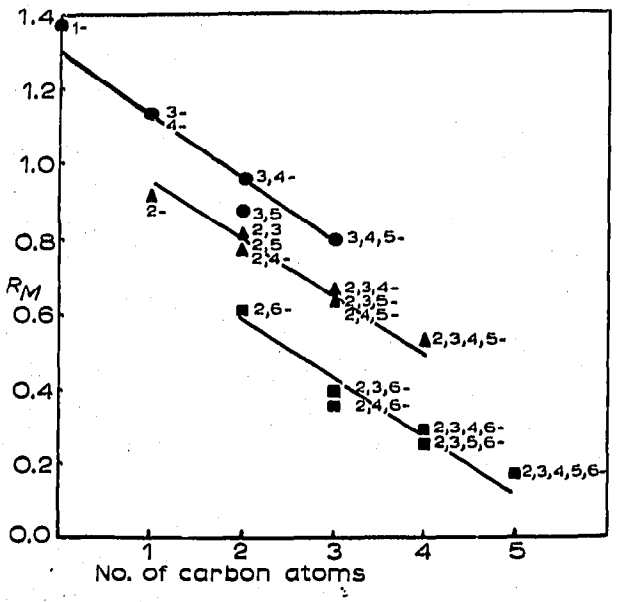


Fig. 4. R_M values (methylated phenols on layers impregnated with 2.0 *M* N,N-dimethylformamide) *vs.* numbers of side chain carbon atoms.

It is suggested that the increase in R_F values consequent upon the *ortho* effect is a result of the steric hindrance of the approach of the phenolic group to the carbonyl group of the surface.

Within each of these groups some separation of isomers occurs as a result of the buttressing effect which is found when two methyl groups occupy adjacent positions in the aromatic nucleus; such behaviour has been previously discussed in detail[16].

CONCLUSION

Over a certain range of amide concentrations a linear relationship exists between R_M values obtained from reversed-phase thin-layer chromatograms and the log of the concentration of the impregnant applied to the substrate, thus substantiating the validity of the relationship between R_F/R_M values and partition coefficient, α. Deviations occur when the amount of impregnant is insufficient to minimise the effect of the impregnant support so that the chromatographic mechanism is both partition and adsorption and also when the loading of impregnant is so high that the impregnant is removed from the layers by the action of the mobile phase.

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DISCUSSION

DE LIGNY: Professor BRENNER remarked this morning that the reproducibility of R_F values in paper and thin-layer chromatography is not good enough to allow their application in physical-chemical investigations. We have now learned that Dr. GRAHAM was able to demonstrate the *ortho* effect of a single methyl group in phenols. These results are the more striking when it is remembered that, until very recently, it appeared impossible to demonstrate the *ortho* effect of a single methyl group in phenols: in all textbooks on physical organic chemistry it is stated that there is no such effect on the ionization constants of methylphenols and even in 1964 HEINEN (*Dissertation*, Utrecht) was still unable to demonstrate an *ortho* effect in an infrared investigation of the complex formation between methylphenols and acetone. It was only by recourse to MC DANIEL's and BROWN's refined method for the determination of *ortho* effects and having at our disposal very accurate data that we were able to demonstrate an *ortho* effect in the above-mentioned cases.

HAIS: I wonder whether Dr. GRAHAM does not speak about *ortho* effect in a broader sense including steric hindrance of the approach of solvent molecules, whereas Dr. DE LIGNY has in mind the traditional more restricted meaning of the term.

GRAHAM: Though the values quoted must include both polar and steric effects, I believe that the *ortho* effect here is caused primarily by the steric hindrance of approach of the phenol to the amide surface and that the contribution of polar effect is probably small.

DE LIGNY: *Ortho* effects can be caused by different mechanisms, *e.g.* intramolecular hydrogen bonding. However, I think that both Dr. GRAHAM and I speak about steric hindrance of solvation (which is about equivalent to steric hindrance of hydrogen bonding) by the presence of *ortho*-methyl groups; or about the *ortho* effect "in a broader sense" in your terminology.