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STUDIES ON THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

XVI. THE BEHAVIOUR OF SOME SUBSTITUTED INDANOLS ON LAYERS OF CELLULOSE IMPREGNATED WITH SIMPLE AMIDES

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SUMMARY

Indanols have been chromatographed on thin layers of cellulose impregnated with simple amides as stationary phases and hexane as the mobile phase. The R_F values have been shown to vary with the impregnation coefficient of the mobile phase. Differences in the behaviour of the amides have been correlated with differences in their molecular dimensions. The effects of molecular structure on the chromatographic behaviour are reflected in the successful separation of 4-indanol and its homologues from the 5-indanol series.

INTRODUCTION

A recent investigation[1] has shown that the highest degree of reproducibility of R_F values in reversed-phase thin-layer chromatography is obtained when the impregnant is dissolved in the solvent used to slurry the impregnant support and the resultant sample-loaded chromatograms are eluted in a double saturation chamber (*i.e.* a glass sandwich made of the chromatoplate, a glass former and a cover plate enclosed in a disposable polythene bag which served as the chromatographic chamber[2]). In this investigation indanols were used as model compounds, the mobile phase being aqueous ethanol (37.5 % v/v) with ethyl oleate supported on cellulose as the stationary phase.

The R_F values for the compounds were shown to be less reproducible if the stationary phase was chromatographed into the support medium and/or when the chromatograms were eluted in conventional tanks.

Where a high degree of reproducibility of R_F values was obtained it was attributed to constancy of the A_M/A_S ratio in the equation[3]

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

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In cases where the A_M/A_S term is constant an alternative chromatographic parameter, the R_M value, has been derived[4], *viz.*:

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

This term, which is more directly related to the partition coefficient, α, than is the simple R_F term, has been used to substantiate the validity of the MARTIN additivity principle[5]. The R_M theory, as applied to paper chromatography has been exhaustively reviewed[6]. It has also been used successfully in relating the molecular structure of phenols to their thin-layer chromatographic behaviour[7–13].

Eqns. (1) and (2) may be combined, *viz.*:

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

Provided that A_M remains constant, α being constant by definition, then linear plots of R_M *vs.* $\log A_S$ (or more specifically the log of the concentration of the stationary phase in the solvent used for slurrying the support mechanism) should indicate the constancy of the A_S term. For methylated phenols chromatographed on layers of cellulose impregnated with formamide[14,15] or with N-methylated formamides[15], using hexane as the mobile phase, this has been shown to be approximately so.

In the present investigation, the chromatographic system of cellulose impregnated with amides as the stationary phase and hexane as the mobile phase has been re-investigated using a different type of solute in an attempt to confirm our previous findings[15]. Indanols, phenols in which one side of the molecule bears a five-membered ring, were chosen for the investigation because we wished to observes if any stereo-specific interaction in the alignment of the phenolic molecule with the amide surface resulted from the presence of the fused ring.

EXPERIMENTAL

Chromatography

Full details of the experimental procedure are given in previous papers[14,15]. Cellulose (15 g MN 300 HR) was slurried with solutions (65 cm^3) of amides (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 *M*) in acetone. The mixture was used to coat glass plates (5 × 20 cm × 20 cm) at an applied layer thickness of 0.3 mm.

The chromatoplates, spotted with indanols (1 μl of 0.25 % solutions in cyclohexane), were eluted with hexane (40 ml) in our double saturation chamber[2].

The visualisation of the indanols with alkaline potassium permanganate was carried out as previously reported[1].

2,6-Dimethylphenol was used as an internal standard on all plates from which R_F values of indanols were obtained. The high degree of reproducibility ($\pm$0.01 R_F unit) for this internal standard was as reported in earlier papers[14,15]. The R_F values of the indanols were also reproducible to $\pm$0.01 R_F units. These R_F values (the mean of at least 5 determinations) and their R_M values are quoted in Tables I–III.

The determination of the amounts of amide above and below the solvent front on eluted chromatograms

In order to determine the onset of double fronting[14,15] the amounts of amide above and below the solvent front were determined.

TABLE I

R_F AND R_M VALUES OF SUBSTITUTED INDANOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH FORMAMIDE

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

Key	*Indanol*	*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	4-Indanol	0.64	−0.250	0.60	−0.176	0.39	+0.195	0.23	+0.525	0.17	+0.689	0.13	+0.826	0.11	+0.908
2	1-Methyl-	0.79	−0.545	0.74	−0.455	0.55	+0.087	0.40	+0.176	0.28	+0.410	0.21	+0.586	0.16	+0.716
3	2-Methyl-	0.79	−0.545	0.74	−0.455	0.55	−0.087	0.40	+0.176	0.28	+0.410	0.21	+0.580	0.16	+0.716
4	5-Methyl-	0.90	−0.955	0.86	−0.788	0.73	−0.432	0.63	−0.231	0.54	−0.070	0.44	+0.105	0.35	+0.269
5	6-Methyl-	0.78	−0.550	0.75	−0.478	0.57	−0.123	0.43	+0.140	0.32	+0.327	0.25	+0.535	0.19	+0.630
6	7-Methyl-	0.72	−0.410	0.68	−0.327	0.50	0.000	0.35	+0.269	0.24	+0.501	0.18	+0.659	0.14	+0.789
7	7-*tert.*-Butyl-	0.94	−1.194	0.91	−1.004	0.83	−0.699	0.76	−0.500	0.68	−0.327	0.60	−0.176	0.55	−0.087
8	5,7-Di-*tert.*-butyl-	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
9	5,7-Di-*tert.*-butyl-3-methyl-	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
10	5-Indanol	0.48	+0.025	0.47	+0.051	0.28	+0.410	0.17	+0.689	0.12	+0.865	0.09	+1.005	0.07	+1.124
11	1-Methyl-	0.68	−0.327	0.64	−0.250	0.47	+0.051	0.33	+0.301	0.23	+0.525	0.17	+0.689	0.14	+0.789
12	3-Methyl-	0.68	−0.327	0.64	−0.250	0.47	+0.051	0.33	+0.301	0.23	+0.525	0.17	+0.689	0.14	+0.789
13	4-Methyl-	0.75	−0.478	0.73	−0.432	0.56	−0.105	0.42	+0.140	0.32	+0.327	0.25	+0.479	0.19	+0.630
14	6-Methyl-	0.79	−0.575	0.78	−0.550	0.60	−0.176	0.46	+0.070	0.36	+0.258	0.28	+0.410	0.21	+0.580
15	7-Methyl-	0.65	−0.269	0.60	−0.176	0.43	+0.123	0.29	+0.389	0.20	+0.602	0.14	+0.789	0.12	+0.865
16	6-*tert.*-Butyl-	1.00	—	1.00	—	1.00	—	1.00	—	0.97	−1.509	0.94	−1.194	0.90	−0.955

TABLE II

R_F AND R_M VALUES OF SUBSTITUTED INDANOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH N-METHYL FORMAMIDE

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

Key	*Indanol*	*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	4-Indanol	0.30	+0.368	0.16	+0.716	0.10	+0.954	0.08	+1.040	0.07	+1.024	0.00	—
2	1-Methyl-	0.43	+0.123	0.23	+0.525	0.14	+0.789	0.11	+0.908	0.08	+1.040	0.06	+1.195
3	2-Methyl-	0.43	+0.123	0.23	+0.525	0.14	+0.789	0.11	+0.908	0.08	+1.040	0.06	+1.195
4	5-Methyl-	0.65	−0.269	0.39	+0.195	0.21	+0.580	0.14	+0.789	0.12	+0.865	0.10	+0.954
5	6-Methyl-	0.45	+0.087	0.23	+0.525	0.13	+0.826	0.10	+0.954	0.11	+0.820	0.10	+0.954
6	7-Methyl-	0.42	+0.140	0.22	+0.550	0.10	+0.954	0.07	+1.124	0.06	+1.195	0.00	—
7	7-*tert.*-Butyl-	0.75	−0.478	0.50	0.000	0.30	+0.368	0.21	+0.580	0.17	+0.689	0.14	+0.789
8	5,7-Di-*tert.*-butyl-	1.00	—	1.00	—	0.95	−1.276	0.92	−1.061	0.87	−0.827	0.82	−0.659
9	5,7-Di-*tert.*-butyl-3-methyl	1.00	—	1.00	—	1.00	—	0.95	−1.276	0.92	−1.061	0.89	−0.907
10	5-Indanol	0.25	+0.478	0.14	+0.789	0.08	+1.061	0.06	+1.195	0.04	+1.380	0.00	—
11	1-Methyl-	0.40	+0.176	0.20	+0.602	0.12	+0.865	0.08	+1.091	0.06	+1.195	0.00	—
12	3-Methyl-	0.40	+0.176	0.20	+0.602	0.12	+0.865	0.08	+1.091	0.06	+1.195	0.00	—
13	4-Methyl-	0.49	+0.017	0.28	+0.410	0.15	+0.750	0.12	+0.865	0.09	+1.005	0.06	+1.195
14	6-Methyl-	0.52	−0.035	0.31	+0.348	0.17	+0.689	0.13	+0.826	0.10	+0.959	0.08	+1.040
15	7-Methyl-	0.38	+0.213	0.18	+0.659	0.10	+0.954	0.07	+1.124	0.06	+1.195	0.00	—
16	6-*tert.*-Butyl-	0.75	−0.478	0.73	−0.432	0.54	−0.070	0.38	+0.213	0.30	+0.368	0.24	+0.500

TABLE III

R_F AND R_M VALUES OF SUBSTITUTED INDANOLS ON THIN LAYERS OF CELLULOSE IMPREGNATED WITH N,N'-DIMETHYL FORMAMIDE

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

Key	*Indanol*	*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	4-Indanol	0.28	+0.410	0.13	+0.826	0.09	+1.005	0.06	+1.195	0.00	—
2	1-Methyl-	0.43	+0.123	0.21	+0.580	0.13	+0.826	0.08	+1.061	0.05	+1.279
3	2Methyl-	0.43	+0.123	0.21	+0.580	0.13	+0.826	0.08	+1.061	0.05	+1.279
4	5-Methyl-	0.50	0.000	0.28	+0.410	0.18	+0.659	0.12	+0.865	0.08	+1.061
5	6-Methyl-	0.42	+0.140	0.20	+0.602	0.13	+0.826	0.07	+1.124	0.06	+1.195
6	7-Methyl-	0.40	+0.176	0.19	+0.630	0.09	+1.005	0.06	+1.195	0.00	—
7	7-*tert.*-Butyl-	0.65	−0.269	0.44	+0.105	0.28	+0.410	0.19	+0.630	0.13	+0.826
8	5-7-Di-*tert.*-butyl-	1.00	—	1.00	—	0.82	−0.658	0.74	−0.455	0.64	−0.250
9	5,7-Di-*tert.*-butyl-3-methyl	1.00	—	1.00	—	0.90	−0.955	0.84	−0.721	0.76	−0.500
10	5-Indanol	0.18	+0.630	0.11	+0.908	0.07	+1.124	0.04	+1.380	0.00	—
11	1-Methyl-	0.32	+0.327	0.19	+0.630	0.11	+0.908	0.07	+1.124	0.04	+1.380
12	3-Methyl-	0.32	+0.327	0.19	+0.630	0.11	+0.908	0.07	+1.124	0.04	+1.380
13	4-Methyl-	0.41	+0.149	0.23	+0.518	0.14	+0.789	0.10	+0.954	0.06	+1.195
14	6-Methyl-	0.52	−0.035	0.28	+0.410	0.17	+0.689	0.11	+0.908	0.07	+1.124
15	7-Methyl-	0.30	+0.368	0.17	+0.689	0.10	+0.954	0.06	+1.195	0.00	—
16	6-*tert.*-Butyl-	0.81	−0.609	0.64	−0.250	0.40	+0.070	0.34	+0.288	0.28	+0.410

The chromatoplates, lacking the solute spots, were prepared and eluted as described above. The mobile phase was allowed to rise 12.5 cm (±0.5 cm) above the normal point of application of the solutes. They were then removed from the sandwich chamber and the mobile phase was allowed to evaporate. After this, bands (2 cm in width) were removed from the layers and transferred into flasks containing sodium hydroxide (25 cm^3 of 1.0 N). Ethylene glycol was added and the flask and its contents were heated under conditions of total reflux for 2 h. This resulted in the hydrolysis of the amide. After this time the reaction products together with flask and condenser washings were transferred to erlenmeyer flasks and the excess sodium hydroxide was determined with standard hydrochloric acid. From the results obtained, the amount of sodium hydroxide used in the reaction and hence the amount of amide present in each band was determined.

DISCUSSION

The effect of the amide loading

The results quoted in Tables I–III show that the R_F values of the indanols

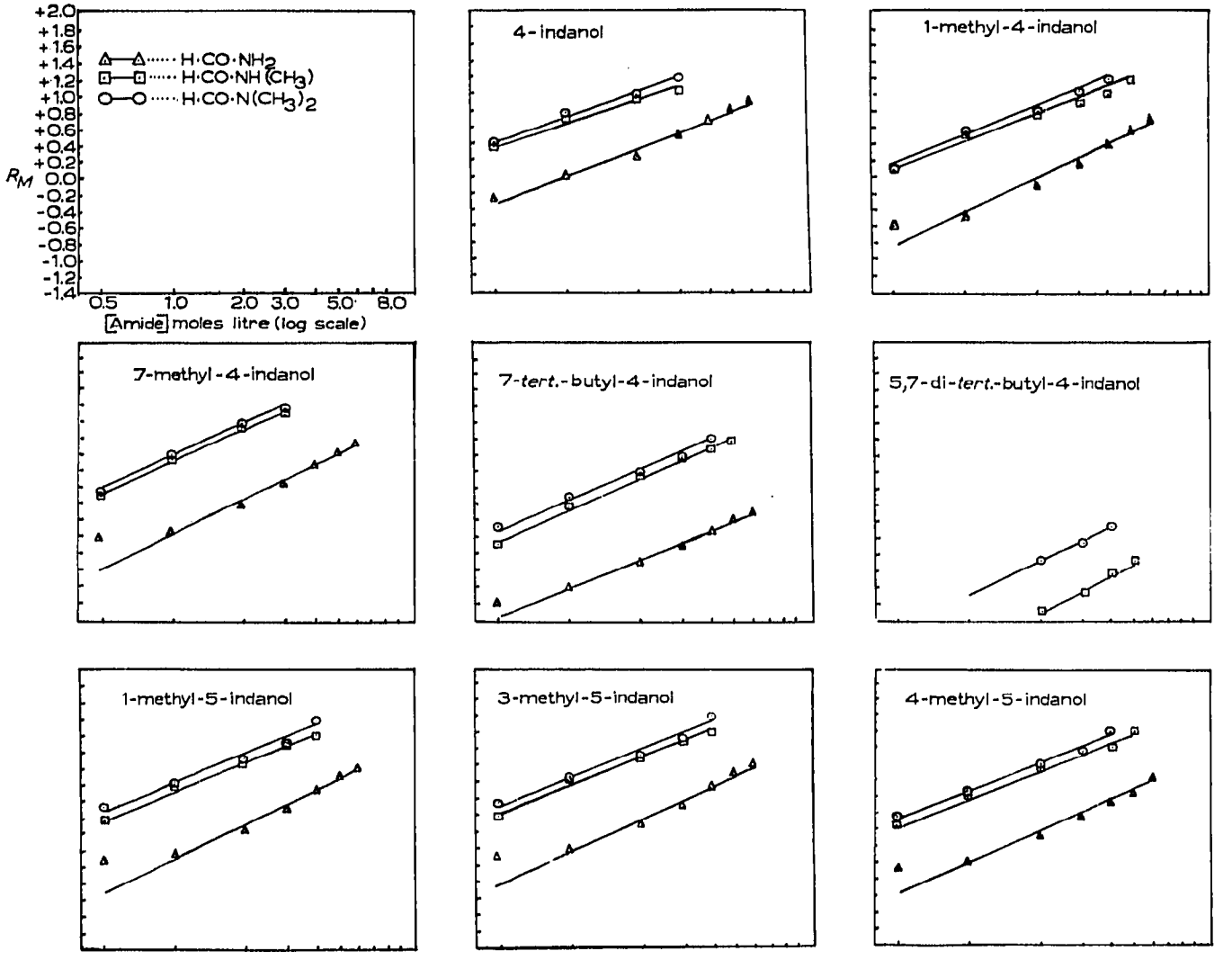
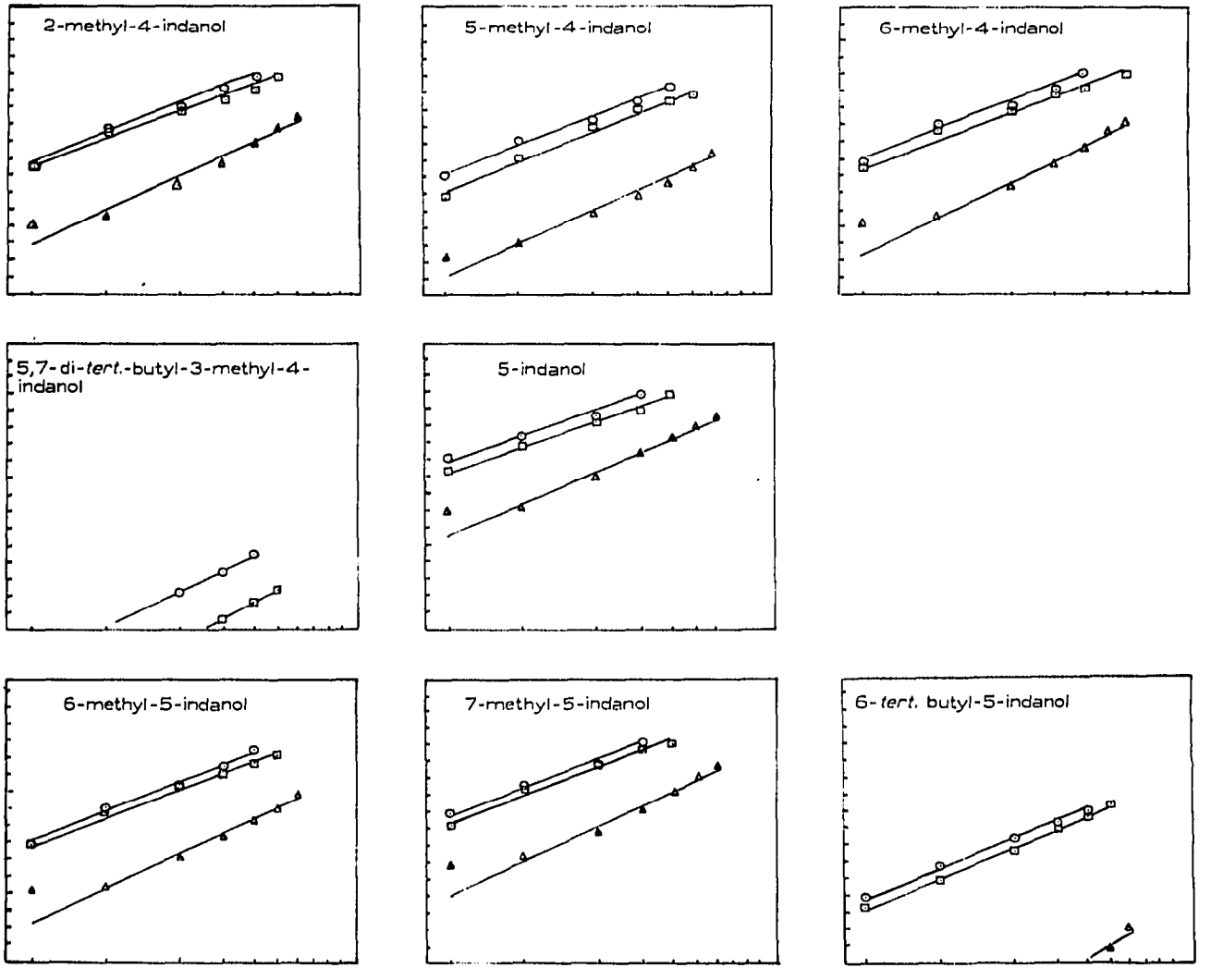


Fig. 1. R_M values (subst. indanols) *vs.* concentration of amide in the slurrying solvent (log scale).

decrease with an increase in the concentration of the amide in the solvent used to slurry the support medium, *i.e.* with an increase in the impregnation coefficient of the stationary phase. This is to be expected because an increase in the impregnation coefficient is reflected in an increase in the A_S term of eqn. (1). This behaviour parallels the behaviour of the methylated phenols in the same systems[15].

Plots of R_M values of the indanols *vs.* concentration of the amide (log scale) in the slurrying solvent are shown in Fig. 1 from which it can be seen that they are linear over the bulk of the range of concentrations studied. This can be taken as proof of the validity of eqn. (3) and evidence for the constancy of the A_S term (or at least the A_M/A_S ratio) in the system studied.

Deviations from linearity, however, occur at very low or very high amide loadings, the points of deviation for each amide studied being identical with those obtained when the methylated phenols were investigated in the same systems[15], *i.e.* they are independent of the nature of the solutes investigated. This confirms that these deviations are a consequence of the chromatographic phases or more specifically the stationary phase, *i.e.* the deviations at low concentration being attributed to incomplete coverage of the cellulose by the stationary phase while those at high con-



centrations were considered to be a consequence of excess stationary phase being sloughed off the cellulose and pushed ahead of the mobile phase so resulting in the phenomenon of double fronting[15].

The former of these views is difficult to prove but evidence for the latter was forthcoming from two sources,

(a) the R_F values of the solutes (indanols and methylated phenols) tending towards a minimum,

(b) visual inspection of the plates after evaporation of the mobile phase showed that the region beyond the solvent front remained damp at high impregnation coefficients, the extent of the damp zone increasing with an increase in the impregnation coefficient.

From the results in Tables I–III and from Fig. 1, it is apparent that, for the three amides studied, the points at which the linear part of each curve begins and ends are not the same but that they occur at progressively higher impregnation coefficients *viz.* N,N'-dimethylformamide < N-methylformamide < formamide. This confirms our findings for the same systems when methylated phenols were used as the solutes[15].

Because fixed volumes of molar concentrations of the amides in acetone were used to slurry fixed amounts of cellulose, it is to be expected that, for a given concentration of amide in the slurrying solvent, the same number of molecules of each amide would be present on the cellulose and hence that the points of deviation from linearity should coincide for each amide. That this is not so suggests that either our visual appraisal of the onset of double fronting is at fault, or that, while the number of molecules of the stationary phase will have some importance in determining the R_F values, some other effect is superimposed on this primary factor. Here, we suggest that this addition effect will be related to the molecular dimensions of the molecules of the stationary phases.

In order to assess the creditability of these suppositions it was necessary, in the first instance, to determine chemically the amount of amide present above and below the stationary phase. This was done by the hydrolytic method described above.

Layers bearing a high concentration of amide were prepared. For each amide and for each impregnation coefficient investigated (0.5 *M* increments) they were divided into two groups. The first of these were not eluted but the layers were divided into bands, each band was removed from the plate and the total amount of amide in each band was determined. A uniform distribution of the amide over the layer was observed for each concentration.

The second group of plates was eluted in the usual way, the mobile phase was allowed to evaporate and the layers were again divided into bands and the amide content of each band was determined hydrolytically. The results here fell into two groups.

(a) Where the impregnation coefficient was lower than the values shown in Table IV there was little or no difference between the amide concentration in the bands below or above the solvent front, *i.e.* the distribution of the amide over the layer is constant.

(b) Where the impregnation coefficient for the amide was greater than the values shown in Table IV then the amide concentration below the solvent front was fairly uniform whereas the amide concentration in the bands above the solvent front were

variable. In the band immediately above the solvent front this concentration was at its highest for the layer, decreasing with an increase in the distance of the band above the solvent front. Furthermore, the higher the initial impregnation coefficient of the layers of this category the greater the amount of amide found in the zones beyond the solvent front. These results clearly confirm the existence of the phenomena of double fronting.

The results quoted in Table IV, however, show that in the case of N-methylformamide and N,N'-dimethylformamide the onset of double fronting, as determined by chemical means occurred at a slightly lower impregnation coefficient than that expected from the R_F values though there is generally good agreement between the two sets of values.

It is now necessary to correlate these impregnation coefficients with the molecular dimensions of the amides. Two possible dimensions could be used: (a) the molar volume of the amide; (b) the parachor of the amide.

The former, because it is normally determined at the boiling point of the liquid to be investigated, was considered to be inappropriate for consideration in a chromatographic system in which the chromatograms were run at temperatures well below the boiling points of the liquid stationary phases used.

The parachor was therefore chosen as being the more appropriate molecular dimension. Its use for chromatographic systems consisting of liquid stationary phases which are members of the same homologous series is particularly significant because it represents the relative molar volume for each stationary phase when it is measured under conditions of unit surface tension, *i.e.* the molecular interactions of the stationary phases will be approximately equal and the spreadability of the phases over the support will be the same.

From values quoted in a standard text[16] we have computed the parachors of the three amides. From the ratios of these we have calculated, relative to formamide, the impregnation coefficients (*i.e.* their molar concentrations in the slurrying solvent) at which double fronting should begin (Table IV). These values show excellent agreement with (a) those obtained by the constancy of R_F values and (b) those obtained chemically for the onset of this phenomenon.

From these results we can rationalise the observed differences in the points of deviation from linearity for the three amides. Thus, for the same impregnation coefficient, formamide because it has the smallest molecular dimensions will cover a smaller

TABLE IV

MOLARITY OF AMIDE IN THE SLURRYING SOLVENT AT WHICH THE PHENOMENON OF DOUBLE FRONTING OCCURS

(a) = based on point at which R_F values become constant; (b) = determined by the hydrolytic methods; (c) = determined from ratios of parachor values for the amides.

Amide	*Molarity*		
	(*a*)	(*b*)	(*c*)
Formamide	6.0	6.0	6.0
N-Methylformamide	5.0	4.5	4.5
N,N'-Dimethylformamide	4.0	3.5	3.5

area of the cellulose than will either of the other two amides. Therefore it will require a higher impregnation coefficient of this substrate than either of the other two before either the cellulose can be regarded as being fully covered, (*i.e.* before the lower break point of the linear part of the curve is reached), or before double fronting becomes a problem (*i.e.* the upper break point of the linear part of the curve is reached). Similarly a higher impregnation coefficient of N-methylformamide than of N,N'-dimethylformamide is needed to reach the same points. These views therefore confirm our thesis that the molecular dimensions of a stationary phase are of considerable importance in determining the appropriate amount of stationary phase to be used in order to devise a chromatographic system in which the mechanism can be considered to be a simple partition system beween two liquid phases. The significance of these observations cannot be stressed too highly, particularly if the results of the chromatographic investigations are to be used either for the correlation of chromatographic behaviour of solutes with their molecular structures, or if they are to be used to interpret the various interactions which are likely to occur between the three components of the chromatographic system, *viz.* solute, mobile phase and stationary phase.

The chromatographic behaviour of the indanols

In the first instance the indanols investigated are best considered as being a part of a homologous series.

An important observation is that the R_F values of 4-indanol in all systems are higher than the comparable values for the isomeric 5-indanol. The ΔR_M values for these two (within the limits of experimental error and within the limitations of the R_M theory[6]) are comparable with the ΔR_M values for the pair 2-methylphenol and 4-methylphenol for the same system[15]. Because we have already shown that the ΔR_M values for the latter pair are a result of steric hindrance of the hydrogen bonding between the phenolic proton and the carbonyl oxygen of the amide substrate, it is reasonable to suppose that such a steric effect exists between the *peri*-CH_2 group of the fused ring and the hydroxyl group. Evidence for such an interaction in naphthols, tetralols and anthrols has been given by MARCINKIEWICZ *et al.*[17]. Using the system ethyl oleate–aqueous ethanol, GRAHAM[1] observed slight separation of these two isomeric indanols but the ΔR_M values for the two were much smaller than those reported here. Undoubtedly the reason for the difference in the results obtained in the two investigations is the easier solvation of the phenolic group by the free molecules of the mobile phase in the earlier system[1] compared with the more difficult solvation of the same group by the support stabilised molecules of the stationary phases (*i.e.* the amides) used in the present investigation. In the 5-indanol, of course, the fused ring is remote from the phenolic group and hence does not interfere with the latter's hydrogen bonding with the stationary phase.

The importance of the presence of absence of this effect is seen when we consider the methyl substituted compound of each parent indanol. The general effect of methylation of the parent compound is to increase the R_F value of the substituted compound relative to the parent. This effect is superimposed on the other constitutive effects already existing in the molecule. Thus the methylated 4-indanols all have higher R_F values than the corresponding isomeric methylated 5-isomers and the ΔR_M value approximate to the ΔR_M values attributed to the *ortho* effect of the *peri*-CH_2 group.

This is of significance because in the only previous attempted separation of these compounds by thin-layer chromatography it was observed that the small *peri ortho* effect observed for the parent compound lost its significance in the methylated compounds[1].

Over and above this, the increase in the R_F values of the methylated compounds is dependent on the position of the methyl group relative to the hydroxyl group. Thus the 5-methyl-4-indanol has higher R_F values than its isomers. Similarly the R_F values of the 4-methyl-5-indanol and the 6-methyl-5-indanols are higher than their isomers. These higher values can undoubtedly be attributed to the fact that the methyl group is *ortho* to they hydroxyl group in these compounds and hence they exert a steric effect on the approach of the phenolic group to the amide substrate thereby reducing the hydrogen bond interaction between the two.

The ΔR_M values for the *ortho*-methyl group in the *o*-methyl-5-indanols compared with the non *ortho* isomers are of the order expected for such a group from our previously observed results for simple phenols. In the case of the *o*-methyl-4-indanol, however, the ΔR_M values for the *ortho*-methyl group is much higher than expected for a single *ortho*-methyl group, but it must be remembered that in this compound the phenolic group is sterically hindered on both sides, on the one side by the *peri* CH_2 group and on the other by the methyl group. The greater than expected result is in accord with our previous finding that the steric effect of the second methyl group in 2,6-dimethylphenol (compared with 2-methylphenol) is much greater than that of the first (*i.e.* 2-methylphenol compared with 4-methylphenol)[15].

Ortho effects aside, some other effects are worthy of consideration. Firstly, the chromatographic behaviour of the methyl group is independent of the nature of the ring (*i.e.* aromatic or alicyclic) into which it is substituted. This is an important observation because the major difference between the methyl group in the aromatic ring and the same group in the alicyclic ring lies in differences in their electronic effects consequent upon the group being part of a conjugated system in the former and not in the latter. This suggests that hyperconjugation as a contributory constitutive effect plays little part in the chromatography of these compounds, thus confirming the similar observation for these compounds in a different chromatographic system by GRAHAM[1]. Other workers[17,18] have attempted to use the concept of hyperconjugation as a constitutive effect in explaining observed chromatographic behaviour of phenolics but alternative explanations of their results have been expressed[19]. Overall, therefore, and supported by a rational review of the phenomenon of hyperconjugation[20], the existence of this effect as a constitutive chromatographic parameter must be treated with some caution.

Finally, in connection with the methylated indanols, we observe that the 7-isomer has the lowest R_F values and hence is just separable from the other non *ortho* methylated isomers.

Changing the nature of the substitutent from a methyl group to a *tert.*-butyl group has the result of increasing the size of the non-polar part of the molecule and hence its solvation by the non-polar mobile phase. This causes an increase in R_F values. This is to be expected from our previous studies in the methylated phenols[15] and from the application of the MARTIN additivity principle[5]. The relationship between the size of a substituent and its steric effect is clearly shown in the results for 7-*tert.*-butyl-4-indanol and 6-*tert.*-butyl-5-indanol. In the former the steric effect results

from the smaller *peri*-CH_2 group whilst in the latter it stems from the *tert.*-butyl group and hence this latter compound has the higher R_F value of the two.

Consideration of Tables I–III and of Fig. 1 show that the R_F values of all the compounds studied depend upon the nature of the amide investigated; the values obtained on formamide being far higher than the values for the other two amides. The differences in values for the indanols on N-methylformamide and N,N'-dimethylformamide, however, are much smaller. This behaviour again parallels the behaviour of the methylated phenols[15] in the same systems. Such behaviour is turned to advantage in the case of the two compounds, 5,7-di-*tert.*-butyl-4-indanol and 5,7-di-*tert.*-butyl-3-methyl-4-indanol (compounds 8 and 9 in Tables I–III). Although listed as two compounds, the original sample was supplied as a single compound (No. 8). When this was chromatographed on N-methylformamide and N,N'-dimethylformamide at high impregnation coefficients the sample was resolved into two distinct spots of equal size and the problem was to identify them. The ΔR_M values in the two systems were too small to suggest a tri-*tert.*-butyl compound but suggested the possible presence of a methyl group in the compound with the higher R_F values. The original compound 8 was synthesised by butylation of 4-indanol obtained from coal tar fractions and it is known that the 4-indanol is often contaminated with 3-methyl-4-indanol[21]. Thus butylation of impure 4-indanol would yield a mixture of compounds 8 and 9. For this reason we have tentatively identified compound No. 9 as the 5,7-di-*tert.*-butyl-3-methyl-4-indanol. However, it must be stated that the presence of the methyl group in the 3 position will result in a steric effect on the OH group–substrate hydrogen bond. This was taken into consideration in attempting to identify compound No. 9. The ΔR_M values of compounds 8 and 9 are of the order expected for an *ortho*-methyl group. Hence, though positive identification of the structure of compound 9 on the basis of its R_F values above is not possible, it seems probable that the compound has been correctly identified.

CONCLUSIONS

The results of this investigation show that the chromatographic behaviour of indanols on amides is dependent upon: (a) the impregnation coefficient of the amide used, (b) the molecular dimensions of the amides, (c) hydrogen bonding between the proton of the phenolic group and the carbonyl oxygen atom of the substrate, (d) steric effects inherent in the molecules to be separated.

The above factors are all combined to given successful resolution of the 4-indanol series from the corresponding 5-indanols.

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