STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

VIII. THE REVERSED PHASE THIN-LAYER CHROMATOGRAPHY OF SOME HALOGENATED PHENOLS AND SOME HALOGENO-; ALKYL-SUBSTITUTED PHENOLS*

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INTRODUCTION

Normal paper chromatography[1–6] has been used for the separation of halogenated phenols by a number of workers, but relatively few workers[7–10] have attempted the separation of these phenols by reversed phase paper chromatography.

GREBENOVSKY[7] separated six chlorophenols by chromatographing them on papers impregnated with formamide, sandwiched between glass plates to reduce the loss of the volatile phenols, with xylene as the eluent.

GESSNER AND SMITH[8] reported the separation of 3- and 4-chlorophenols using formamide as the impregnant and hexane as the mobile phase.

MARCINKIEWICZ AND GREEN[9], using the system ethyl oleate–25 % aqueous ethanol, found that the monohalogenophenols, fluoro-, chloro-, bromo- and iodo-, separated according to the molar volumes of the halogen atoms, and that the negative inductive effects of the halogen atoms played no part in influencing the separation. For any given halogen atom, they failed to separate the isomeric monohalogenophenols. Altering the system to Trigol–diisopropyl ether did not resolve the isomeric monochlorophenols.

More recently, VACEK, STOTA AND STANEK[10] have carried out reversed phase separation of the 19 members of the homologous series of chlorinated phenols, using papers impregnated with olive oil as the stationary phase, and Britton–Robinson buffers of different pH as the mobile phases. They observed that the R_F values decreased with an increase in the number of chlorine substituents. An *ortho* effect was observed when one or both *ortho* positions were chlorinated. This effect was attributed to steric hindrance of the attachment of the phenolate ion to the stationary phase, or, in particular, to the carbonyl groups therein.

In earlier papers[11–13], we have considered the behaviour of substituted phenols on alumina surfaces. We have also considered the behaviour of nitrophenols[14], and alkylated phenols[15] chromatographed with a nonpolar stationary phase (ethyl oleate) and a polar mobile phase (aqueous ethanol). We have stated that hydrogen

* For Parts I, II, III, IV, V, VI and VII of this series, see refs. 11, 17, 16, 14, 12, 15 and 13.

bonding between the mobile phase and the phenolic group plays a very important part in the separation process. In nitrophenols, the nitro group also plays an important role by two mechanisms:

(1) Altering the polarity of the phenolic group;

(2) Being solvated on the oxygen atoms.

When the nitro group is present in the 2-position, intramolecular hydrogen bonding occurs, thus reducing the availability of both groups for solvation. It is also possible that there is some steric hindrance of solvation of the phenolic group by the bulky nitro group, but it is not possible to assess this because its contribution is probably masked by the intramolecular hydrogen bonding between the phenolic group and the adjacent nitro group, and by the solvation of the oxygen atom, of the nitro group, not involved in intramolecular hydrogen bonding. In the alkyl phenols[15], the steric effects were observed to be more pronounced since the polarity effects were small.

In halogenated phenols, it should be possible to see the combination of both these effects simultaneously. We have, therefore, chromatographed a series of halogenated phenols, and some halogeno-; alkyl-substituted phenols in the system ethyl oleate–aqueous ethanol. The reversed phase thin-layer separation of these compounds has not previously been reported.

EXPERIMENTAL

The method of preparing the layers, cellulose impregnated with ethyl oleate, and the solvent systems (25 % and 37.5 % v/v aqueous ethanol) have been reported previously[14–16].

The phenols were applied to the layers by means of our multiple spotting device[17].

The chromatograms were eluted by an ascending technique, at a constant temperature of 25° ± 0.5°, using our double saturation chamber[17].

RESULTS

The results shown in Tables I and II are the mean of at least 4 runs on plates carrying an internal standard. The R_F values of the standard were reproducible to within ± 0.01 R_F units of a pre-determined mean. The R_F values of the individual phenols are also reproducible to ± 0.01 R_F units.

DISCUSSION

The two mobile phases used, 25 % aqueous ethyl alcohol, and 37.5 % aqueous ethyl alcohol are designated systems 1 and system 2, respectively.

It can be seen from the Tables I and II that an increase in the ethanolic content of the eluent system results in higher R_F values for all compounds. This may be attributed to an increased solubility of the hydrophobic part of the molecule in the organic part of the mobile phase. However, it is considered that the main cause of removal of the phenols from the interface between the stationary and mobile phases will be solvation of the phenol group.

Table I shows the R_F values obtained in the two systems for phenol containing halogen groups only.

TABLE I

REVERSED PHASE THIN-LAYER CHROMATOGRAPHY OF HALOGENOPHENOLS

Support: 15 g cellulose impregnated with a solution of ethyl oleate in diethyl ether (70 ml of a 0.75 % v/v solution).
System 1: 25 % aqueous ethanol; system 2: 37.5 % aqueous ethanol.

Key	*Phenol*	R_F *in system 1*	R_F *in system 2*
1	Phenol	0.795	0.900
2	2-Chloro	0.535	0.790
3	3-Chloro	0.430	0.670
4	4-Chloro	0.460	0.680
5	2,3-Dichloro	0.280	0.530
6	2,4-Dichloro	0.220	0.480
7	2,5-Dichloro	0.200	0.460
8	2,6-Dichloro	0.310	0.550
9	3,4-Dichloro	0.215	0.460
10	3,5-Dichloro	0.140	0.355
11	2,4,5-Trichloro	0.075	0.235
12	2,4,6-Trichloro	0.150	0.275
13	2,3,4,6-Tetrachloro	0.045	0.135
14	2,3,4,5,6-Pentachloro	0.020	0.020
15	2-Fluoro	0.755	0.860
16	3-Fluoro	0.690	0.795
17	4-Fluoro	0.700	0.830
18	2-Bromo	0.480	0.730
19	3-Bromo	0.370	0.595
20	4-Bromo	0.390	0.600
21	2-Iodo	0.340	0.560
22	3-Iodo	0.280	0.490
23	4-Iodo	0.290	0.510
24	2,4-Dibromo	0.130	0.350
25	3,5-Dibromo	0.100	0.220
26	2,4,6-Tribromo	0.070	0.210
27	2,4,6-Triiodo	0.070	0.070
28	2,4-Diiodo-6-chloro	0.000	0.090
29	2,6-Diiodo-4-chloro	0.000	0.080
30	2,6-Diiodo-4-bromo	0.000	0.070

MARCINKIEWICZ AND GREEN[9] failed to separate the 2-, 3- and 4-substituted isomers of the four monohalogenophenols. The results reported above (Table I) indicate that it is possible to separate the 2-isomer from the 3- and 4-compounds. The R_F values of the 3- and 4-compounds also show marginal differences, the former having the lower value for all four halogeno substituents.

In an earlier paper[14], it was shown that the 2-nitrophenols had lower R_F values in the system ethyl oleate–aqueous ethanol than had either the 3- or 4-nitrophenols, and it was stated that in the 2-isomer, the formation of a strong internal hydrogen bond reduced the ability of the phenolic group to be solvated and hence lowered the R_F values.

Also in the system ethyl oleate–aqueous ethanol was it shown that 2-alkylated phenols[15] had lower R_F values than had either the corresponding 3- or 4-isomers. The reduction in R_F values here was attributed to steric hindrance of solvation of the phenolic group by the *ortho*-alkyl groups.

The R_F values of the 2-halogenophenols are higher than the R_F values of the corresponding 3- and/or 4-isomers. MARCINKIEWICZ AND GREEN[9] in order to account

TABLE II

REVERSE PHASE CHROMATOGRAPHY OF PHENOLS CONTAINING ALKYL AND HALOGENO GROUPS

Key	*Phenol*	R_F *in system 1*	R_F *in system 2*
31	4-Chloro-3-methyl	0.310	0.560
32	4-Chloro-2,3-dimethyl	0.115	0.430
33	4-Chloro-2,5-dimethyl	0.115	0.450
34	4-Chloro-2,6-dimethyl	0.100	0.410
35	4-Chloro-3,5-dimethyl	0.125	0.500
36	4-Chloro-2,3,5-trimethyl	0.050	0.290
37	4-Chloro-3-methyl-5-ethyl	0.090	0.340
38	2-Chloro-4,5-dimethyl	0.210	0.570
39	2,4-Dichloro-6-methyl	0.050	0.235
40	2,4-Dichloro-3,5-dimethyl	0.055	0.240
41	2,4-Dichloro-3,6-dimethyl	0.035	0.150
42	2,6-Dichloro-4-methyl	0.160	0.370
43	2,6-Dichloro-3,4-dimethyl	0.090	0.270
44	2,4,6-Trichloro-3-methyl	0.070	0.160
45	2,4,6-Trichloro-3,5-dimethyl	0.020	0.090
46	2,4,6-Trichloro-3-methyl-5-ethyl	0.000	0.050
47	2-Bromo-4-methyl	0.300	0.660
48	2-Bromo-4-cyclohexyl	0.000	0.100
49	2-Bromo-4-phenyl	0.000	0.190
50	2-Bromo-3,4,6-trimethyl	0.050	0.195
51	2-Bromo-3-methyl-4,6-di-*tert.*-butyl	0.000	0.030
52	2,4-Dibromo-5-methyl	0.040	0.190
53	2,4-Dibromo-6-methyl	0.050	0.155
54	2,4-Dibromo-6-*tert.*-butyl	0.000	0.060
55	2,4-Dibromo-6-cyclohexyl	0.000	0.030
56	2,4-Dibromo-6-phenyl	0.000	0.060
57	2,4-Dibromo-3,6-dimethyl	0.030	0.120
58	2,4-Dibromo-5,6-dimethyl	0.040	0.115
59	2,4-Dibromo-3,5,6-trimethyl	0.000	0.060
60	2,6-Dibromo-4-methyl	0.070	0.255
61	2,6-Dibromo-4-*tert.*-butyl	0.000	0.120

for the lack of differences in the R_F values of the isomeric monohalogenophenols, stated that their results could be ascribed to the absence of hydrogen bonding between the phenolic group and the 2-halogeno-group. WULF and co-workers[8], from an analysis of the infrared spectra of 2-chlorophenol in the overtone region, observed the existence of *cis* and *trans* isomers of the compound, with the *cis* form predominating. This they attributed to stabilisation of the *cis* form by intramolecular hydrogen bonding. GOODE AND IBBITSON[19] calculated the dipole moments of the *cis* form (2.99D) and the *trans* form (0.75D) of 2-chlorophenol. The measured value for the dipole moment of this compound (1.33D) lies between the two calculated values. They therefore concluded intramolecular hydrogen bonding was possible in this compound, and also presented evidence, from dipole moment studies for its existence in 2,4,6-trichloro- and 2,4,6-tribromophenols. The moments of these last two being determined in the nonpolar solvent benzene.

To explain the R_F values of the 2-halogenophenols observed in this study, it is necessary to postulate that any internal hydrogen bond formed between the halogen atom and the phenolic group is one which is easily broken under the influence of a

highly polar solvent, and that once it is broken there are available at the same end of the molecule two solvation points, *viz.* the phenolic group and the halogen atom.

Some evidence for this instability of the intramolecular hydrogen bond, formed between the halogen atom and the phenolic group, has been given by BAKER AND KAEDING[20] from their studies of unsymmetrical 2,6-dihalogenophenols by infrared spectroscopy. They were of the opinion that there was an orbital–orbital repulsive energy arising from the overlap of the mutually exclusive O–H bonding orbital and the donated lone pair orbital; the amount of overlap, and hence the degree of repulsion, being related to the geometry of the lone pair orbitals. The net result of this repulsive force being to bend the O–H... halogen bond at an angle in excess of 85° from the optimum co-linear position, thus weakening the bond. In the case of the 3- and 4-isomers, however, solvation of the phenolic group tilts the rest of the molecule into the stationary phase and so reduces the availability of the 3- and/or 4-halogen atoms for solvation by the mobile phase. The R_F values for the 2-halogenophenols are lower than those of phenol, suggesting that even if the above mechanism does operate, the presence of the halogen atom in the 2-position still exerts some steric effect.

For the series of monohalogenated phenols, fluoro-, chloro-, bromo-, iodo-, MARCINKIEWICZ AND GREEN[9] found separations to occur according to the molar volumes of the halogen atoms, and that their —*I* effects played little or no part in their separation. We also find the order of R_F values to be: fluoro < chloro < bromo < iodo, *i.e.* in order of the molar volumes of the halogen atoms.

DIKSTEIN[21], in his thermodynamic derivation of the MARTIN[22] postulate, has shown the partition coefficient of a molecule is partly determined by its molar volume, thus, in a homologous series, it is to be expected that changes in R_F/R_M values, consequent upon the addition of successive numbers of the same substituent, are also partly determined by the molar volume of the substituent. For the homologous series of chlorinated phenols, the effect of increased chlorine substitution in the phenyl ring is to lower the R_F values and raise the R_M values. Since the separation of monohalogenophenols has been explained in terms of changes in the molar volumes of the halogen atoms, it can also be suggested that molar volume is a significant factor in determining the R_F values of the chlorinated phenols, and that the series, therefore,

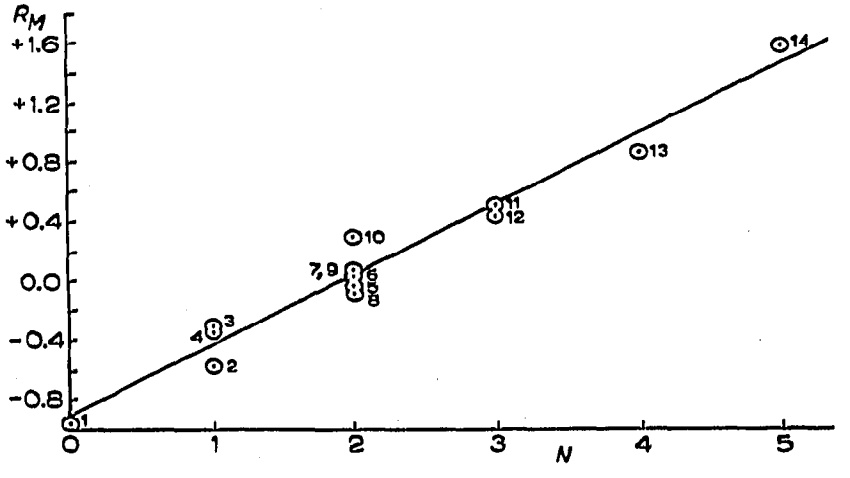

Fig. 1. R_M values of chlorinated phenols *vs.* number of chlorine atoms (N). System 2 (aqueous ethanol, 37.5 %, v/v) as the mobile phase and ethyl oleate as the stationary phase.

obeys the MARTIN relationship approximately. That this is so is shown in Fig. 1. However, the results and the figures indicate that positional effects are also important. The behaviour of the chlorinated phenols is further complicated by the fact that the inductive effect of the chlorine atom, causing a drift of electrons away from the ring, is opposed by release of electrons by mesomerism resulting from the interaction of the lone pairs of electrons in the halogen atom. It is therefore suggested that a combination of the mesomeric effect and the molar volume effect, overrides the inductive effect and results in the order of R_F value change for the members of the homologous series of chlorinated phenols being in the same direction as that observed for the nuclear methylated phenols in the same system. It is also in agreement with the behaviour of the nuclear methylated and nuclear chlorinated phenoxyacetic acids observed by BARK AND GRAHAM[23–25].

The effect of the increase in size, and hence molar volume, of the halogen atoms on the R_F values of the monohalogenated phenols is also seen for the polyhalogenophenols by comparing the R_F values of the dibromo, tribromo-, and triiodophenols with their chloro analogues, and by comparing the values for the three compounds 2,4-diiodo-6-chloro-, 2,6-diiodo-4-chloro-, and 2,6-diiodo-4-bromophenols (compounds 28–30 in Table I).

One of the postulates of the MARTIN relationship[22] is that the effect of the addition of a group to a molecule is independent of the groups already in the molecule. In the field of paper chromatography, we have shown[23–26] that for nuclear substituted compounds this postulate is not strictly correct. The nature of the groups already present and their positions relative to the incoming substituent play a part in determining the effect of the substituent on the chromatographic behaviour of the compound.

It is to be expected that a similar situation obtains in all types of chromatography, and that the MARTIN relationship is only approximate and that the number and nature of the substituents and their relative positions, will have an effect in determining the chromatographic behaviour of a compound. We have shown the effects of this interdependence of nuclear substituents on the behaviour of nitrophenols[12,14] and halogeno-; alkyl-substituted phenols[11] when chromatographed by adsorption chromatography on alumina surfaces. From a consideration of the thin layer chromatographic behaviour of a series of amino acids, PATAKI[27] has also concluded that the change in R_F values, caused by the addition of a given group to a molecule, is dependent on the nature of the groups already present in the molecule.

Since there is an interdependency of group effect in the behaviour of the halogeno-alkylated phenols when chromatographed by adsorption chromatography using a polar stationary phase and a nonpolar mobile phase[11], a similar effect should be evident when these compounds are chromatographed by partition chromatography where the polarities of the phases are reversed. In order to verify this we have compared calculated R_F and R_M values for these compounds, with experimental values (see Table III). The calculated R_M values were obtained using a method similar to that used by GREEN and co-workers[28] to calculate atomic ΔR_M values. (The experimental values necessary to calculate these parameters for alkyl groups are taken from some of our previously reported work[15] and the values for the chloro atoms, from the results reported in this present paper.)

From our calculations it is apparent that the calculated R_F values are generally

TABLE III

COMPARISON OF EXPERIMENTAL AND CALCULATED PARAMETERS

Solvent system 2 used.

Phenol	*Experimental*		*Calculated*	
	R_F	R_M	R_F	R_M
4-Chloro-3-methyl	0.560	—0.105	0.535	—0.061
4-Chloro-2,3-dimethyl	0.430	+0.123	0.320	+0.332
4-Chloro-2,5-dimethyl	0.450	+0.096	0.320	+0.332
4-Chloro-2,6-dimethyl	0.410	+0.158	0.260	+0.459
4-Chloro-3,5-dimethyl	0.500	0.000	0.385	+0.205
4-Chloro-2,3,5-trimethyl	0.290	+0.389	0.165	+0.698
4-Chloro-3-methyl-5-ethyl	0.340	+0.288	0.190	+0.639
2-Chloro-4,5-dimethyl	0.570	—0.123	0.525	—0.043
2,4-Dichloro-6-methyl	0.235	+0.513	0.265	+0.446
2,4-Dichloro-3,5-dimethyl	0.240	+0.501	0.210	+0.585
2,4-Dichloro-3,6-dimethyl	0.150	+0.750	0.160	+0.720
2,6-Dichloro-4-methyl	0.370	+0.231	0.460	+0.072
2,6-Dichloro-3,4-dimethyl	0.270	+0.432	0.315	+0.337
2,4,6-Trichloro-3-methyl	0.160	+0.720	0.200	+0.599
2,4,6-Trichloro-3,5-dimethyl	0.090	+1.005	0.135	+0.865
2,4,6-Trichloro-3-methyl-5-ethyl	0.050	+1.274	$>$0.005	+2.399

lower (and the R_M values consequently higher) than those obtained by experiment.

It thus appears though the combined electronic effects of the halogen atoms and the alkyl groups are relatively small, and generally overshadowed by other more gross effects, *e.g.*, molar volume considerations, the effect on the chromatographic behaviour is not negligible. Examples of this effect are to be found in many of the compounds listed.

For example, consideration of the values obtained for 4-chloro-3-methylphenol indicate that solvation of the phenolic group is less than expected. This can be explained by the fact that the inductive effects of the alkyl and chloro groups act in opposition to one another, the net result being a lower electron density on the oxygen of the phenolic group than expected, and hence lower solvation. This is evidenced by the experimental R_F values obtained being considerably lower than those calculated.

The positional effect of the substituents is more pronounced in the mixed substituted phenols than in the simple ones. Although in some cases the R_F values of the halogeno-; alkylphenols are intermediate between those of the alkylphenols and the halogenophenols this is not invariably the case. The positional effects are particularly noticeable when a "2" substituent or "2,6" substituents are involved and it is expected because *ortho*-halogeno groups increase the R_F values relative to those of their 3- or 4-isomers, whilst *ortho*-alkyl groups reduce them. These effects are shown in Table IV and emphasise the interdependence of substituent groups not only on the nature of the groups already in the molecule but also at the relative positions of all groups within a molecule.

In Table V the results obtained by chromatographing the bromo-alkylphenols are given. This enables the effect of substituting the larger bromine atom for the smaller chlorine atom to be evaluated. The results are qualitatively similar to those obtained for the chloro-substituted compounds. However, because of the increased

TABLE IV

THE EFFECTS OF THE RELATIVE POSITIONS OF CHLORO ON METHYL GROUPS

Phenol	R_F *in system 1*	R_F *in system 2*
2,4,5-Trimethyl*	0.300	0.570
4-Chloro-2,5-dimethyl	0.115	0.430
2,4,5-Trichloro	0.075	0.235
2,4,6-Trimethyl*	0.280	0.540
4-Chloro-2,6-dimethyl	0.100	0.410
2,4-Dichloro-6-methyl	0.050	0.235
2,6-Dichloro-4-methyl	0.160	0.370
2,4,6-Trichloro	0.150	0.275
2,3,4,6-Tetramethyl*	0.180	0.370
2,6-Dichloro-3,4-dimethyl	0.090	0.270
2,4,6-Trichloro-3-methyl	0.070	0.160
2,3,4,6-Tetrachloro	0.045	0.135

* The R_F values for the simple methylated phenols have been obtained for the same systems, and are taken from ref. 15.

size and reduced electronegativity of the bromine atom, the R_F values are somewhat lower than those of the corresponding chlorine analogue.

For a given series of bromo-substituted phenols, the effect of the addition of an alkyl group is the expected one. Thus for the 2,4-dibromo-methyl-substituted phenols, the addition of successive methyl groups progressively lowers the R_F values. The presence of a bulky *tert.*-butyl group in the *ortho* position has the expected result of

TABLE V

THE EFFECTS OF THE ADDITION OF ALKYL GROUPS TO BROMINATED PHENOLS

Phenol	R_F *in system 1*	R_F *in system 2*
2-Bromo-4-methyl	0.300	0.660
2-Bromo-4-cyclohexyl	0.000	0.100
2-Bromo-4-phenyl	0.000	0.190
2-Bromo-3,4,6-trimethyl	0.050	0.195
2-Bromo-3-methyl-4,6-di-*tert.*-butyl	0.000	0.030
2,4-Dibromo-5-methyl	0.040	0.190
2,4-Dibromo-6-methyl	0.050	0.155
2,4-Dibromo-6-*tert.*-butyl	0.000	0.060
2,4-Dibromo-6-cyclohexyl	0.000	0.030
2,4-Dibromo-6-phenyl	0.000	0.060
2,4-Dibromo-3,6-dimethyl	0.030	0.120
2,4-Dibromo-5,6-dimethyl	0.040	0.115
2,4-Dibromo-3,5,6-trimethyl	0.000	0.060
2,6-Dibromo-4-methyl	0.070	0.255
2,6-Dibromo-4-*tert.*-butyl	0.000	0.120

sterically hindering solvation of the phenolic group and so lowering the R_F values. Separation of the isomeric pair 2,4-dibromo-6-methyl- and 2,6-dibromo-4-methylphenol is dependent upon the nature of the group in the 6-position. In the series 2,4-dibromo-6-methylphenol addition of further methyl groups to the molecule expectedly lowers the R_F values.

CONCLUSION

From the chromatographic behaviour of halogenated phenols, the halogeno-; alkyl-substituted phenols, chromatographed in the reversed-phase thin-layer chromatographic system ethyl oleate–aqueous ethanol, it is suggested the mechanism of the chromatographic process is dissolution of the phenol in the hydrophobic stationary phase, followed by the removal of the phenol from the interface as a result of solvation of the phenolic group by the polar mobile phase.

For the simple halogenophenols, the molar volume of the halogen group is considered to be of greater importance in governing the chromatographic process than is the inductive effect of the group. Positional effects are also apparent.

In halogeno-; alkyl-substituted phenols the importance of the effects of the molar volumes of the groups results in the change in R_F values, consequent upon substitution, to be in the same direction for both halogen groups and alkyl group.

This enables approximate R_F values to be calculated on the basis of the MARTIN additivity principle. However, the lack of agreement between calculated and experimental results indicates that the polar effects of the halogen group and alkyl groups, while independently small, are present and affect the overall chromatographic behaviour of the molecule.

It is concluded that in sterically compact molecules such as nuclear substituted compounds, it is not possible to calculate, to better than a first approximation, chromatographic parameters for substituents. As previously concluded from the behaviour of phenols chromatographed by adsorption chromatography the electronic effects of a nuclear substituent are transferred to the whole of the molecule; the ease of delocalisation of the electrons in the nucleus makes it impossible to localise the electronic effects of a substituent.

Thus, the effect of any substituent will depend on the nature of the groups already present in the molecule, and the relative position of the chromatographically functional groups.

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SUMMARY

Sixty-one halogenated and halogeno-; alkyl-substituted phenols have been chromatographed by reversed-phase thin-layer chromatography on cellulose impregnated with ethyl oleate using aqueous ethanol (25 % and 37.5 % v/v) as mobile phases.

The R_F values of the halogenated phenols decrease with an increase in the number of halogen atoms in the molecule suggesting that the effect of molar volumes of the halogen atoms is more important in governing the separation process than are electronic effects. Positional effects are also of importance.

In the halogeno-; alkyl-substituted phenols, the additivity of group R_M parameters enables approximate R_F values to be calculated. The more pronounced positional and electronic effects of the groups in these latter compounds cause deviations between experimental and calculated values.

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