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# REVERSED-PHASE AND SOAP THIN-LAYER CHROMATOGRAPHY OF PHENOLS

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## SUMMARY

The chromatographic characteristics of 60 phenols on layers of silanized silica gel alone and impregnated with anionic and cationic detergents have been investigated using elution with water–alcohol mixtures at different pH. The validity of the relationships between the $R_F$ values, the pH of the eluent and the $pK_a$ of the phenols has been verified on thin layers of silanized silica gel alone and impregnated with 4% DBS. Many interesting separations of polyhydroxybenzenes and dichloro-, trichloro-, dinitro- and alkylphenols have been carried out.

## INTRODUCTION

Reversed-phase chromatography on silanized silica gel and soap thin-layer chromatography (TLC) have been used with good results in recent years in the study of basic organic compounds[1–6]. In this study we have applied such techniques to the separation of acidic organic compounds. The compounds considered included alkylphenols, halogenated phenols, nitrophenols and polyhydroxybenzenes, many of which we have already studied on anion and cation exchangers with cellulose, paraffinic and polystyrene matrices[7–9]. It is possible, therefore, to compare the results achieved on layers of these exchangers and those of silanized silica gel alone and impregnated with detergents.

## EXPERIMENTAL

The test compounds (Supelco, Bellefonte, PA, U.S.A.) were dissolved in 95% ethanol. The concentration of the solutions were 1–2 mg/ml and 1-$\mu$l volumes were deposited on the layer. With halogenated phenols, more concentrated solutions (6–10 mg/ml) were employed. Fresh solutions were used for those phenols which easily decompose (pyrogallol, gallic acid and pyrocatechic acid).

The phenols were detected by the Boute reaction[10], exposing the wet layers successively to nitrogen dioxide and ammonia vapours. The solution of 2,6-dibromophenol is violet coloured. A violet spot, due to impurities in the commercial product,

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is visible at the application point before the exposure of the layer to nitrogen dioxide vapour.

The layers (thickness 300 $\mu$m) were prepared with a Chemetron automatic apparatus by mixing 20 g of silanized silica gel 60 HF ($C_2$) (Merck, Darmstadt, G.F.R.) in 50 ml of 95% ethanol with a known concentration of detergent. The detergents used were triethanolamine dodecylbenzenesulphonate (DBS) and N-dodecylpyridinium chloride (N-DPC).

All the work was carried out at 25°C. The migration distance was 11 cm unless otherwise stated.

## RESULTS AND DISCUSSION

Table I gives the $R_F$ values of 60 phenols on layers of silanized silica gel alone (columns A) and impregnated with 4% DBS (columns B), eluting with aqueous–organic solutions containing the same percentage of methanol (30%) but with different pH values (apparent pH between 5 and 11.3).

The DBS concentration refers to the alcoholic solution in which the silanized silica gel was suspended when the layers were prepared.

*Layers of silanized silica gel alone*

On these layers, the chromatographic behaviour of the phenols depends on their acid–base characteristics, as the $R_F$ values increase with the increase in the apparent pH of the eluent owing to the progressive deprotonation of the phenolic OH group.

On the basis of the p$K_a$ values reported in Table I, the $R_F$ values obtained with the solution at pH 5 refer to the non-dissociated form of most phenols, and those with the solution at pH 11.3 refer to their deprotonated form.

The influence of the substituent groups on the chromatographic behaviour of the compounds can be seen from the behaviour of the phenol and of the eluent at the lowest pH value, so that the deprotonation effect can be excluded in the retention mechanism.

The introduction into the ring of $CH_3$, $CH_2CH_3$, $NO_2$, Br and Cl groups involves an increase in the retention by the layer, whereas the opposite behavior is observed on introduction of an OH group. This last occurrence is similar to that observed on the same layer with the introduction of an OH group into the aromatic ring of catecholamines[1] and of an $NH_2$ group in the case of primary aromatic amines[3]. The behaviour of polyhydroxybenzenes, in contrast, is completely different from that observed on Dowex 50-X4 ($Na^+$) thin layers, where such compounds are less retained than phenol[8].

The influence of the ionic strength on the chromatographic behaviour of phenols is shown from the data in columns 4A and 4B in Table I; on changing the ionic strength of the eluent from 0.1 to 0.01 a considerable increase in the $R_F$ values is observed. Such behaviour is similar to that obtained on Dowex 50-X4 ($Na^+$) layers[8].

In alkaline media, gallic and pyrocatechic acids, phloroglucinol, pyrogallol and the three esters of gallic acid are oxidized in air and become visible as brown spots (elongated in some instances) before the exposure of the layers to nitrogen

TABLE I

$R_F$ VALUES OF PHENOLS ON THIN LAYERS OF SILANIZED SILICA GEL (A) ALONE AND (B) IMPREGNATED WITH 4% DBS SOLUTION

Eluents: (1) 0.1 *M* $CH_3COOH$ + 0.1 *M* $CH_3COONa$ in 30% $CH_3OH$ (pH 5.00); (2) 0.1 *M* $KH_2PO_4$ in 30% $CH_3OH$ (pH 7.00); (3) 0.1 *M* $NH_3$ + 0.1 *M* $NH_4Cl$ in 30% $CH_3OH$ (pH 9.02); (4) 1 *M* $NH_3$ + 0.1 *M* NaCl in 30% $CH_3OH$ (pH 11.30); (5) 1 *M* $NH_3$ in 30% $CH_3OH$ (pH 11.30).

| *Phenol* | *1* | | *2* | | *3* | | *4* | | *5* | | $pK_a$(25°C)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | |
| Phenol | 0.36 | 0.35 | 0.36 | 0.35 | 0.37 | 0.35 | 0.51 | 0.47 | 0.73 | 0.62 | 10.02 |
| *m*-Cresol | 0.21 | 0.19 | 0.22 | 0.19 | 0.24 | 0.20 | 0.38 | 0.31 | 0.53 | 0.40 | 10.09 |
| *p*-Cresol | 0.21 | 0.19 | 0.22 | 0.19 | 0.23 | 0.19 | 0.35 | 0.29 | 0.48 | 0.36 | 10.27 |
| *o*-Cresol | 0.22 | 0.20 | 0.22 | 0.20 | 0.23 | 0.20 | 0.35 | 0.29 | 0.48 | 0.35 | 10.32 |
| 3,5-Dimethylphenol | 0.13 | 0.12 | 0.13 | 0.12 | 0.13 | 0.12 | 0.24 | 0.21 | 0.33 | 0.25 | 10.19 |
| 3,4-Dimethylphenol | 0.14 | 0.12 | 0.14 | 0.12 | 0.14 | 0.12 | 0.22 | 0.19 | 0.29 | 0.24 | 10.36 |
| 2,5-Dimethylphenol | 0.13 | 0.11 | 0.13 | 0.11 | 0.13 | 0.11 | 0.22 | 0.19 | 0.26 | 0.21 | 10.41 |
| 2,3-Dimethylphenol | 0.13 | 0.11 | 0.13 | 0.11 | 0.13 | 0.11 | 0.22 | 0.19 | 0.26 | 0.20 | 10.54 |
| 2,4-Dimethylphenol | 0.12 | 0.11 | 0.12 | 0.11 | 0.13 | 0.11 | 0.20 | 0.18 | 0.26 | 0.19 | 10.60 |
| 2,6-Dimethylphenol | 0.13 | 0.12 | 0.13 | 0.12 | 0.13 | 0.12 | 0.20 | 0.18 | 0.25 | 0.20 | 10.63 |
| 2,3,5-Trimethylphenol | 0.07 | 0.06 | 0.07 | 0.06 | 0.07 | 0.06 | 0.13 | 0.10 | 0.14 | 0.12 | — |
| 2,4,6-Trimethylphenol | 0.07 | 0.06 | 0.07 | 0.06 | 0.07 | 0.06 | 0.13 | 0.10 | 0.13 | 0.10 | 10.88 |
| 2,3,6-Trimethylphenol | 0.07 | 0.06 | 0.07 | 0.06 | 0.07 | 0.06 | 0.13 | 0.10 | 0.14 | 0.11 | — |
| *m*-Ethylphenol | 0.11 | 0.11 | 0.11 | 0.11 | 0.12 | 0.11 | 0.25 | 0.21 | 0.32 | 0.28 | 9.9** |
| *p*-Ethylphenol | 0.12 | 0.11 | 0.12 | 0.11 | 0.12 | 0.11 | 0.23 | 0.20 | 0.28 | 0.24 | 10.0** |
| *o*-Ethylphenol | 0.11 | 0.10 | 0.11 | 0.10 | 0.11 | 0.10 | 0.20 | 0.18 | 0.25 | 0.21 | 10.2** |
| *o*-Chlorophenol | 0.20 | 0.19 | 0.21 | 0.19 | 0.32 | 0.24 | 0.69 | 0.58 | 0.93 | 0.89 | 8.48 |
| *m*-Chlorophenol | 0.16 | 0.12 | 0.16 | 0.12 | 0.20 | 0.14 | 0.50 | 0.42 | 0.82 | 0.64 | 9.02 |
| *p*-Chlorophenol | 0.16 | 0.12 | 0.16 | 0.12 | 0.20 | 0.14 | 0.42 | 0.33 | 0.67 | 0.50 | 9.38 |
| 2,6-Dichlorophenol | 0.12 | 0.10 | 0.16 | 0.13 | 0.56 | 0.55 | 0.72 | 0.73 | 0.95 | 0.88 | 6.79, 6.79*** |
| 2,5-Dichlorophenol | 0.09 | 0.06 | 0.11 | 0.06 | 0.34 | 0.28 | 0.62 | 0.62 | 0.93 | 0.85 | 7.50, 7.35*** |
| 2,3-Dichlorophenol | 0.08 | 0.06 | 0.09 | 0.06 | 0.28 | 0.22 | 0.59 | 0.58 | 0.93 | 0.85 | — , 7.45*** |
| 2,4-Dichlorophenol | 0.08 | 0.05 | 0.08 | 0.05 | 0.22 | 0.16 | 0.55 | 0.52 | 0.81 | 0.78 | 7.89, 7.75*** |
| 3,5-Dichlorophenol | 0.07 | 0.04 | 0.07 | 0.04 | 0.14 | 0.09 | 0.43 | 0.42 | 0.79 | 0.64 | 8.18, 7.93*** |
| 3,4-Dichlorophenol | 0.07 | 0.04 | 0.07 | 0.04 | 0.12 | 0.07 | 0.39 | 0.35 | 0.76 | 0.57 | — , 8.39*** |
| 2,3,6-Trichlorophenol | 0.05 | 0.05 | 0.13 | 0.12 | e.s. | 0.45 | e.s. | 0.49 | 0.83 | 0.82 | 6.12 |
| 2,4,6-Trichlorophenol | 0.04 | 0.04 | 0.09 | 0.08 | 0.37 | 0.37 | 0.49 | 0.47 | 0.81 | 0.73 | 6.42 |
| 2,3,5-Trichlorophenol | 0.03 | 0.03 | 0.05 | 0.04 | 0.26 | 0.25 | 0.43 | 0.43 | 0.76 | 0.69 | 7.23 |
| 2,4,5-Trichlorophenol | 0.03 | 0.03 | 0.05 | 0.04 | 0.25 | 0.22 | 0.43 | 0.43 | 0.78 | 0.70 | 7.33 |
| 2,3,4-Trichlorophenol | 0.03 | 0.03 | 0.05 | 0.03 | 0.23 | 0.18 | 0.43 | 0.43 | 0.77 | 0.68 | 7.59 |
| 3,4,5-Trichlorophenol | 0.02 | 0.02 | 0.03 | 0.02 | 0.12 | 0.09 | 0.33 | 0.34 | 0.64 | 0.52 | 7.74 |
| 2,3,5,6-Tetrachlorophenol | 0.02 | 0.02 | 0.12 | 0.12 | 0.24 | 0.25 | 0.33 | 0.33 | 0.64 | 0.50 | 5.44 |
| 2,3,4,5-Tetrachlorophenol | 0.01 | 0.01 | 0.02 | 0.02 | 0.14 | 0.14 | 0.29 | 0.30 | 0.61 | 0.46 | 6.96 |
| Pentachlorophenol | 0.01 | 0.01 | 0.09 | 0.09 | 0.14 | 0.13 | 0.24 | 0.21 | 0.46 | 0.33 | 5.26 |
| *o*-Bromophenol | 0.18 | 0.15 | 0.18 | 0.15 | 0.26 | 0.20 | 0.68 | 0.59 | 0.93 | 0.88 | 8.44 |
| *m*-Bromophenol | 0.13 | 0.10 | 0.13 | 0.10 | 0.19 | 0.12 | 0.49 | 0.38 | 0.78 | 0.62 | 9.03 |
| *p*-Bromophenol | 0.13 | 0.10 | 0.13 | 0.10 | 0.18 | 0.11 | 0.42 | 0.30 | 0.65 | 0.50 | 9.36 |
| 2,6-Dibromophenol | 0.08 | 0.07 | 0.11 | 0.09 | 0.49 | 0.46 | 0.64 | 0.64 | 0.93 | 0.89 | 6.6 |
| 2,4-Dibromophenol | 0.05 | 0.03 | 0.06 | 0.04 | 0.11 | 0.10 | 0.51 | 0.43 | 0.85 | 0.75 | 7.8 |
| *p*-Nitrophenol | 0.26 | 0.23 | 0.31 | 0.26 | 0.67 | 0.66 | 0.83 | 0.89 | 0.96 | 0.92 | 7.15 |
| *o*-Nitrophenol | 0.20 | 0.17 | 0.23 | 0.19 | 0.62 | 0.61 | 0.79 | 0.83 | 0.96 | 0.92 | 7.23 |
| *m*-Nitrophenol | 0.25 | 0.22 | 0.25 | 0.22 | 0.36 | 0.29 | 0.69 | 0.68 | 0.96 | 0.92 | 8.40 |
| 2,6-Dinitrophenol | 0.53 | 0.68 | 0.54 | 0.76 | 0.66 | 0.77 | 0.71 | 0.78 | 0.96 | 0.92 | 3.71 |
| 2,4-Dinitrophenol | 0.48 | 0.58 | 0.51 | 0.74 | 0.63 | 0.77 | 0.71 | 0.78 | 0.96 | 0.92 | 4.09 |

*(Continued on p. 342)*

TABLE I *(continued)*

| *Phenol* | *1* | | *2* | | *3* | | *4* | | *5* | | *pK*$_a$*(25°C)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* | |
| 2,5-Dinitrophenol | 0.32 | 0.31 | 0.49 | 0.65 | 0.63 | 0.73 | 0.70 | 0.77 | 0.96 | 0.92 | 5.22 |
| 3,4-Dinitrophenol | 0.21 | 0.18 | 0.40 | 0.51 | 0.58 | 0.68 | 0.70 | 0.78 | 0.96 | 0.92 | 5.43 |
| 2-Chloro-5-methylphenol | 0.11 | 0.09 | 0.11 | 0.09 | 0.13 | 0.12 | 0.55 | 0.45 | 0.89 | 0.77 | — |
| 4-Chloro-3-methylphenol | 0.08 | 0.06 | 0.08 | 0.06 | 0.09 | 0.06 | 0.28 | 0.20 | 0.51 | 0.30 | — |
| 4-Chloro-2-methylphenol | 0.08 | 0.06 | 0.08 | 0.06 | 0.09 | 0.06 | 0.25 | 0.16 | 0.44 | 0.24 | — |
| Gallic acid | 0.92 | 0.94 | 0.91 | 0.94 | e.s. | e.s. | e.s. | e.s. | e.s. | e.s. | 4.41 |
| Pyrocatechic acid | e.s. | 0.93 | 0.85 | 0.95 | e.s. | e.s. | e.s. | 0.95 | e.s. | 0.95 | — |
| Phloroglucinol | 0.76 | 0.73 | 0.74 | 0.72 | e.s. | e.s. | 0.95 | 0.95 | 0.95 | 0.95 | 8.45 |
| Pyrogallol | 0.74 | 0.68 | 0.74 | 0.67 | e.s. | e.s. | e.s. | e.s. | e.s. | e.s. | 9.01 |
| Resorcinol | 0.58 | 0.58 | 0.56 | 0.55 | 0.56 | 0.55 | 0.74 | 0.70 | 0.95 | 0.87 | 9.81 |
| Hydroquinone | 0.67 | 0.66 | 0.66 | 0.66 | 0.67 | 0.68 | e.s. | e.s. | e.s. | e.s. | 10.35 |
| Orcinol | 0.47 | 0.48 | 0.46 | 0.46 | 0.47 | 0.46 | 0.62 | 0.57 | 0.89 | 0.80 | — |
| BHA[§] | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.04 | 0.03 | 0.05 | 0.03 | — |
| *n*-Propyl gallate | 0.19 | 0.24 | 0.21 | 0.23 | e.s. | e.s. | e.s. | e.s. | e.s. | e.s. | — |
| Octyl gallate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | — |
| Dodecyl gallate | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | — |

* Refs. 11, 12 and 13.
** p$K_a$ values at 25°C.
*** p$K_a$ values at 29°C (ref. 14).
§ 2, (3)-*tert.*-Butyl-4-hydroxyanisole (mixed isomers).

dioxide vapour. Hydroquinone is oxidized only in strongly alkaline media because, on eluting with the solution at pH 9.02, it does not give rise to the brown spot.

*Layers of silanized silica gel impregnated with 4% DBS*

In the presence of DBS, as shown in Table I, a decrease in the $R_F$ values for most compounds is observed, with the exception of the phenols with marked acidic characteristics, such as dinitrophenols and gallic and pyrocatechic acids, for which higher $R_F$ values than those on the layers without detergent are observed.

The affinity sequence of the phenols on the two layers is, however, similar over the whole pH range explored, except for the above-mentioned compounds and some polyhydroxybenzenes. Such behaviour can be used from an analytical standpoint on layers impregnated with detergent for the separation of pyrogallol from phloroglucinol and of dinitrophenols.

A characteristic of these layers is the extraordinary compactness of the spots, so that the separation of compounds that differ only by 0.05 in their $R_F$ values can be carried out. This is important in view of the shorter elution time on the impregnated layer (about 45 min) than on silanized silica gel alone (about 55 min).

On changing the ionic strength of the eluent from 0.1 to 0.01, an increase in the $R_F$ values is observed on layers impregnated with 4% DBS (see columns 4B and 5B in Table I), even if such an increase is generally less marked than on layers without detergent.

Another peculiar characteristic of the impregnated layer is the possibility of using eluents with containing less than 30% of methanol, so that both the retention and the resolving power of the layers can be further increased[5].

Table II gives the $R_F$ values of phenol and alkylphenols, eluting with solutions

TABLE II

$R_F$ VALUES OF PHENOLS ON LAYERS OF SILANIZED SILICA GEL IMPREGNATED WITH 4% DBS SOLUTION

Eluents: ammonia solutions in 20% methanol.

| *Phenol* | *Ammonia concentration (M)* | | | | |
|---|---|---|---|---|---|
| | *1* | *4* | *6* | *8* | *10* |
| Phenol | 0.60 | 0.73 | 0.78 | 0.79 | 0.80 |
| *m*-Cresol | 0.37 | 0.57 | 0.63 | 0.63 | 0.63 |
| *p*-Cresol | 0.31 | 0.48 | 0.54 | 0.57 | 0.57 |
| *o*-Cresol | 0.32 | 0.48 | 0.53 | 0.55 | 0.56 |
| 3,5-Dimethylphenol | 0.21 | 0.37 | 0.42 | 0.44 | 0.46 |
| 3,4-Dimethylphenol | 0.19 | 0.32 | 0.38 | 0.41 | 0.42 |
| 2,5-Dimethylphenol | 0.17 | 0.29 | 0.35 | 0.37 | 0.38 |
| 2,3-Dimethylphenol | 0.15 | 0.26 | 0.32 | 0.34 | 0.34 |
| 2,4-Dimethylphenol | 0.14 | 0.24 | 0.30 | 0.31 | 0.32 |
| 2,6-Dimethylphenol | 0.16 | 0.27 | 0.32 | 0.35 | 0.36 |
| 2,3,5-Trimethylphenol | 0.07 | 0.14 | 0.17 | 0.20 | 0.22 |
| 2,3,6-Trimethylphenol | 0.06 | 0.10 | 0.16 | 0.19 | 0.21 |
| 2,4,6-Trimethylphenol | 0.06 | 0.09 | 0.14 | 0.17 | 0.19 |
| 2,3,5,6-Tetramethylphenol | 0.03 | 0.05 | 0.11 | 0.14 | 0.15 |
| *m*-Ethylphenol | 0.21 | 0.38 | 0.46 | 0.49 | 0.51 |
| *p*-Ethylphenol | 0.19 | 0.34 | 0.38 | 0.43 | 0.44 |
| *o*-Ethylphenol | 0.16 | 0.27 | 0.31 | 0.35 | 0.36 |

with a constant methanol concentration (20%) and increasing ammonia concentrations (from 1 to 10 *M*). Under these elution conditions the behaviour of the alkylphenols in the deprotonated form is pointed out; 2,3,5,6-tetramethylphenol, whose detection is possible only in strongly alkaline medium, has also been studied.

From the data in Table II it should be noted that, as the ammonia concentration in the eluent is increased, an increase in the $R_F$ values is observed for all phenols. This increase is sharp at ammonia concentrations up to 6 *M* and becomes negligible at higher ammonia concentrations. The differences in the $R_F$ values of the isomers, which are not very marked at ammonia concentrations below 4 *M*, reach their highest values at concentrations above 6 *M*.

It should be noted that, relative to phenol, the $R_F$ values of the alkylphenols gradually decrease as the number of methyl groups in the ring increases. Therefore, from the $R_F$ values of an alkylphenol, the number of methyl groups in the molecule can be obtained.

The affinity sequence of the isomers of cresols, ethylphenols and dimethylphenols is opposite to that of their $pK_a$ values.

*Layers of silanized silica gel impregnated with N-DPC*

On these layers the retention of phenols is higher than that on silanized silica gel alone, and is similar to that observed on layers impregnated with DBS, at least for those phenols which are prevalently in the non-dissociated form.

The phenols in the deprotonated form are strongly retained owing to an anion-exchange process with the functional group of N-DPC in addition to the liquid–liquid partition process. As the apparent pH of the eluent is increased, a

decrease in the $R_F$ values is observed, which is different to that found on layers of silanized silica gel alone and impregnated with DBS. Such behaviour causes a levelling of the $R_F$ values of the phenols and therefore the different acid–base characteristics of the compounds cannot be used for their separation.

The best results can be achieved with an increase in the methanol concentration in the eluent (from 30 to 50%) and a decrease in the amount of N-DPC on the layer (from 4 to 1%). For example, on layers impregnated with 1% N-DPC and with 0.1 *M* ammonia solution in 50% methanol as eluent, a great difference between the $R_F$ values of phenol (0.49) and cresols (0.34–0.38) with respect to those of polyhalogenated phenols (0.09–0.13) is observed.

As the concentration of methanol in the eluent is further increased (to 80%) a general increase in the $R_F$ values is obtained, but this does not result in a better resolving power because many compounds give rise to elongated spots.

*Retention mechanism*

The parameters that determine the retention of phenols on layers of silanized silica gel alone and impregnated with DBS are the same that affect retention on cation exchangers[8]. We considered it useful, therefore, to verify also on silanized silica gel layers the validity of the relationship

$$\frac{1}{R_F} - 1 = \left(\frac{1}{R_{F_{ac}}} - 1\right)\frac{[H^+]}{K_a + [H^+]} + \left(\frac{1}{R_{F_{alk}}} - 1\right)\frac{K_a}{K_a + [H^+]} \qquad (1)$$

where $R_{F_{ac}}$ and $R_{F_{alk}}$ are the $R_F$ values of the protonated and deprotonated form of the phenols obtained on eluting with acidic and alkaline solutions, respectively. Applying eqn. 1 to some phenols chosen on the basis of their p$K_a$ values, the curves in Fig. 1, which refer to layers of silanized silica gel, were obtained.

The theoretical curves were constructed according to the p$K_a$ values relative to solutions containing 30% methanol and drawn by adding 0.58 to the corresponding

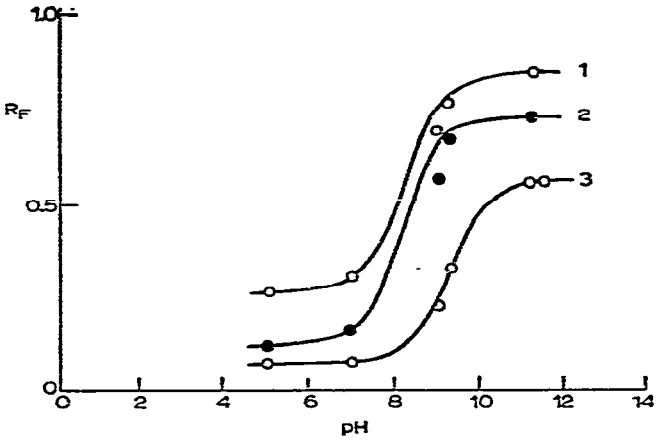


Fig. 1. $R_F$ values *versus* the apparent pH of the eluent for phenols on silanized silica gel thin layers. (1) *p*-Nitrophenol (p$K_a$ = 7.73); (2) 2,6-dichlorophenol (p$K_a$ = 7.37); (3) 2,4-dichlorophenol (p$K_a$ = 8.47). The p$K_a$ values refer to aqueous—organic solutions containing 30% methanol.

$pK_a$ in aqueous solution[15]. The experimental points are those reported at the different apparent pH values of the eluent in Table III; they are in good agreement with the theoretical curves and support the validity of eqn. 1 even on these layers. The experimental points at pH 9.02 are lower than the theoretical values on curves 1 and 2, owing to an apparent pH value on the layer that is smaller than that of the eluent for the formation of a pH gradient on the layer similar to that observed on cation exchangers[8].

TABLE III

$R_F$ VALUES OF PHENOLS ON SILANIZED SILICA GEL THIN LAYERS OBTAINED WITH ELUENTS AT DIFFERENT pH VALUES

Eluents: 0.1 *M* $CH_3COOH$ + 1 *M* $CH_3COONa$ in 30% $CH_3OH$ (pH 5.00); 0.1 *M* $KH_2PO_4$ in 30% $CH_3OH$ (pH 7.00); 0.1 *M* $NH_3$ + 0.1 *M* $NH_4Cl$ in 30% $CH_3OH$ (pH 9.02); 0.2 *M* $NH_3$ + 0.1 *M* $NH_4Cl$ in 30% $CH_3OH$ (pH 9.30); 1 *M* $NH_3$ + 0.1 *M* NaCl in 30% $CH_3OH$ (pH 11.30); 2 *M* $NH_3$ + 0.1 *M* NaCl in 30% $CH_3OH$ (pH 11.60).

| *p-Nitrophenol* | | *2,6-Dichlorophenol* | | *2,4-Dichlorophenol* | |
|---|---|---|---|---|---|
| $R_F$ | *pH* | $R_F$ | *pH* | $R_F$ | *pH* |
| 0.26 | 5.00 | 0.12 | 5.00 | 0.08 | 5.00 |
| 0.31 | 7.00 | 0.16 | 7.00 | 0.08 | 7.00 |
| 0.67 | 9.02 | 0.56 | 9.02 | 0.22 | 9.02 |
| 0.75 | 9.30 | 0.66 | 9.30 | 0.32 | 9.30 |
| 0.83 | 11.30 | 0.72 | 11.30 | 0.55 | 11.30 |
| | | | | 0.56 | 11.60 |

*Analytical applications*

Among the separations that can be effected on the basis of the $R_F$ values obtained with the different eluents, we carried out separations of the three chloro- and bromophenols and of the two tetrachlorophenols, on layers of silanized silica gel alone and impregnated with 4% DBS. In comparison with reversed-phase chromatography, however, soap TLC permits the separation of a larger number of phenols, as smaller differences in their $R_F$ values are necessary owing to the compactness of the spots.

For example, the separation of the three methylchlorophenols cannot be performed on layers of silanized silica gel alone, although the difference between the $R_F$ values of 4-chloro-2-methylphenol and 4-chloro-3-methylphenol is greater than that observed on impregnated layers (see columns 5A and 5B in Table I), where such a separation can be effected by eluting with ammonia solution at pH 11.30, both alone and in the presence of 0.1 *M* sodium chloride. It should be noted that the difference in the $R_F$ values of the two above-mentioned isomers is only 0.04 with the eluent of higher ionic strength.

Fig. 2a shows the separation of the six dichlorophenols on layers of silanized silica gel impregnated with 4% DBS, eluting with 0.2 *M* ammonia and 0.1 *M* ammonium chloride in 30% methanol (apparent pH 9.30); such a separation cannot be achieved on layers without detergent (Fig. 2b). With 0.1 *M* ammonia and 0.1 *M* ammonium chloride in 30% methanol as the eluent (apparent pH 9.02) and with

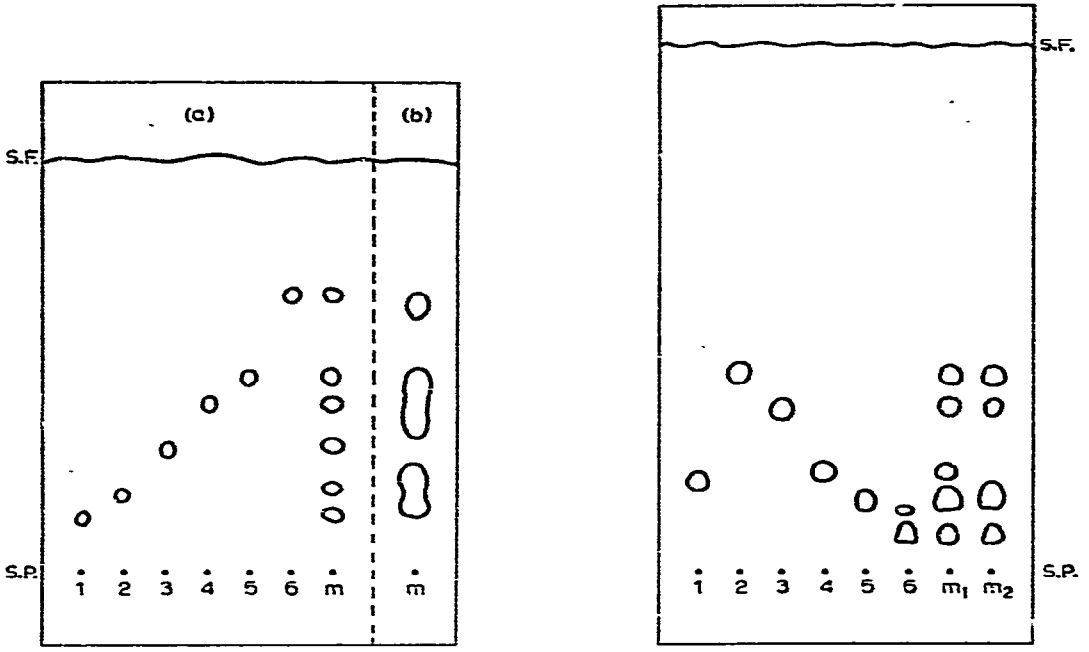


Fig. 2. Thin-layer chromatogram of dichlorophenols on (a) silanized silica gel impregnated with 4% DBS solution and (b) silanized silica gel alone. Eluent: 0.2 *M* ammonia + 0.1 *M* ammonium chloride in 30% methanol (pH = 9.30). (1) 3,4-Dichlorophenol; (2) 3,5-dichlorophenol; (3) 2,4-dichlorophenol; (4) 2,3-dichlorophenol; (5) 2,5-dichlorophenol; (6) 2,6-dichlorophenol; (m) mixture. S.P. = start point; S.F. = solvent front.

Fig. 3. Thin-layer chromatogram of trichlorophenols on silanized silica gel impregnated with 4% DBS solution. Migration distance: 14 cm. Eluent: 0.1 *M* ammonia + 0.1 *M* ammonium chloride in 30% methanol (pH = 9.02). (1) 2,4,5-Trichlorophenol; (2) 2,3,6-trichlorophenol; (3) 2,4,6-trichlorophenol; (4) 2,3,5-trichlorophenol; (5) 2,3,4-trichlorophenol; (6) 3,4,5-trichlorophenol; ($m_1$) mixture of 1, 2, 3, 4, 5 and 6; ($m_2$) mixture of 1, 2, 3, 5 and 6. S.P. = start point; S.F. = solvent front.

a migration distance of 14 cm, the separation shown in Fig. 3 was obtained on layers impregnated with 4% DBS; this separation, however, does not concern all six trichlorophenols which are present in mixture $m_1$ because 2,4,5-trichlorophenol and 2,3,4-trichlorophenol can not be separated, as indicated in the chromatogram relative to mixture $m_2$. With 3,4,5-trichlorophenol, two spots are observed owing to the presence of by-products (probably 2,3,4-trichlorophenol).

Fig. 4 shows the separation of the four dinitrophenols and of a large number of polyhydroxybenzenes on impregnated layers using 0.1 *M* acetate buffer in 30% methanol as eluent and with a long migration distance (15 cm) so that phloroglucinol can be separated from pyrogallol. On layers of silanized silica gel alone the separation of the four dinitrophenols is difficult owing to the small difference in the $R_F$ values of 2,5-dinitrophenol and 2,4-dinitrophenol.

Finally, numerous separations of mixtures of phenol and alkylphenols can be effected on layers impregnated with 4% DBS. For example, separations of phenol, *m*-cresol, *p*-cresol, 3,5-dimethylphenol, 2,3-dimethylphenol, 2,3,5-trimethylphenol

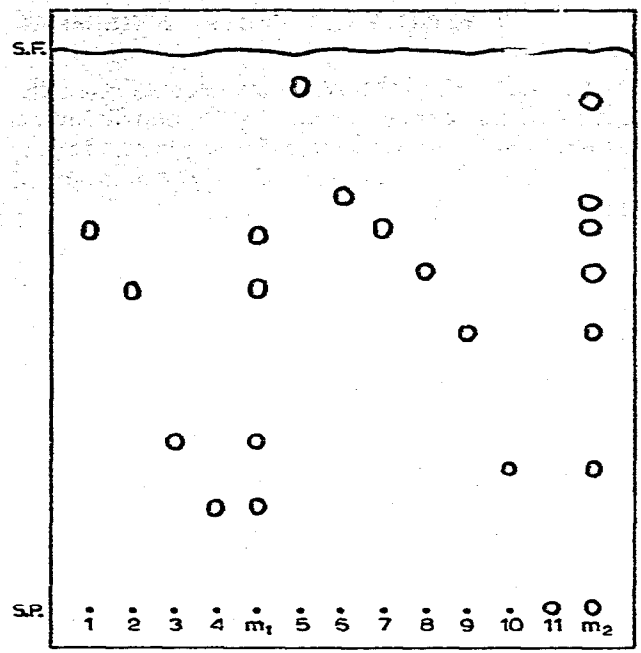


Fig. 4. Thin-layer chromatogram of dinitrophenols and polyhydroxybenzenes on silanized silica gel impregnated with 4% DBS solution. Migration distance: 15 cm. Eluent: 0.1 *M* acetic acid + 0.1 *M* sodium acetate in 30% methanol (pH = 5.00). (1) 2,6-Dinitrophenol; (2) 2,4-dinitrophenol; (3) 2,5-dinitrophenol; (4) 3,4-dinitrophenol; ($m_1$) mixture of dinitrophenols; (5) gallic acid; (6) phloroglucinol; (7) pyrogallol; (8) resorcinol; (9) orcinol; (10) *n*-propyl gallate; (11) octyl gallate; ($m_2$) mixture of polyhydroxybenzenes. S.P. = start point; S.F. = solvent front.

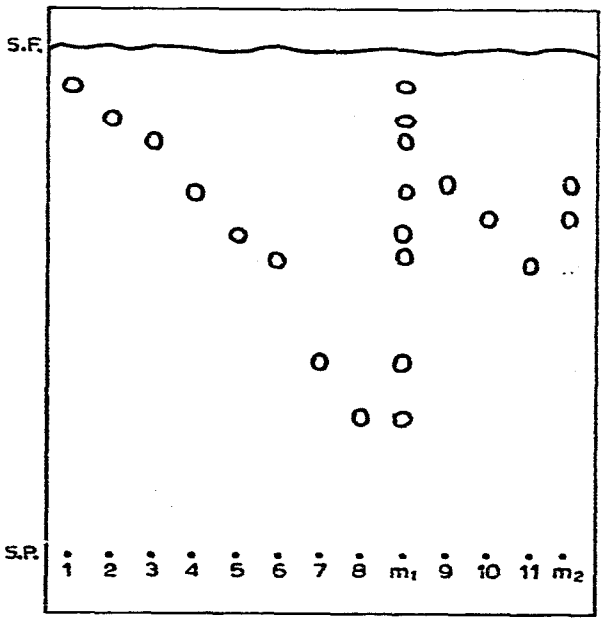


Fig. 5. Thin-layer chromatogram of phenol and of some mono- and polyalkyl derivatives on silanized silica gel impregnated with 4% DBS solution. Migration distance: 13.5 cm. Two successive developments with 10 *M* ammonia in 20% methanol. (1) Phenol; (2) *m*-cresol; (3) *p*-cresol; (4) 3,5-dimethylphenol; (5) 2,5-dimethylphenol; (6) 2,3-dimethylphenol; (7) 2,3,5-trimethylphenol; (8) 2,3,5,6-tetramethylphenol; ($m_1$) mixture; (9) *m*-ethylphenol; (10) *p*-ethylphenol; (11) *o*-ethylphenol; ($m_2$) mixture of ethylphenols. S.P. = start point; S.F. = solvent front.

and 2,3,5,6-tetramethylphenol and of the three ethylphenols have been effected by eluting with solutions with a constant methanol concentration (20%) and different ammonia concentrations (6, 8 and 10 *M*). With two successive developments in the same eluent (10 *M* ammonia in 20% methanol) the separation of a large number of alkylphenols was achieved (see Fig. 5).

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