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CHROMATOGRAPHIC BEHAVIOUR OF PHENOLS ON THIN LAYERS OF CATION AND ANION EXCHANGERS

II. DOWEX 50-X4 AND REXYN 102

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SUMMARY

The chromatographic characteristics of 58 phenols on Rexyn 102 and Dowex 50-X4 thin layers in both the acidic and sodium salt forms have been studied, using elution with water, water–alcohol mixtures and aqueous salt solutions at different pH values. The influence of the percentage of alcohol, the pH and the ionic strength on the chromatographic behaviour of these phenols was investigated.

The validity of the relationships among the R_F values, the pH of the eluent and the pK_a of the phenol has been verified on Dowex 50-X4 (Na^+) thin layers. It has been shown that from the $R_{F_{ac}}$, $R_{F_{alk}}$ and pK_a values of the different phenols, it is possible to predict their behaviour over the whole pH range and therefore to select the best conditions for their chromatographic separation.

INTRODUCTION

In Part I (ref. 1), 58 phenols were studied on Bio-Rad AG 3-X4A, PEI-cellulose and DEAE-cellulose thin layers. This second part concerns a study of the same phenols on Dowex 50-X4 and Rexyn 102 so that a complete picture of their chromatographic behaviour on cation exchangers with different supports can be seen.

EXPERIMENTAL

Preparation of the layers and of the solutions

The solutions of the phenols were prepared as described previously[1]. The layers of Dowex 50-X4 and Rexyn 102 (Fisher Scientific, Fair Lawn, N.J., U.S.A.) were prepared by mixing 3 g of the resin with 9 g of microcrystalline cellulose in 50 ml of water. Before use, the exchangers were rinsed with water and methanol and dried at room temperature. Water–alcohol mixtures in different ratios, neutral and alkaline salt solutions and equimolecular sodium acetate and acetic acid solutions were employed as eluents.

The chromatographic measurements were made at 25 ± 0.5°. The migration distance was 11 cm unless otherwise stated.

Detection

The detection of the phenols was effected as previously described[1]. The layers, however, must be sprayed with 1 *M* sodium hydroxide solution before their exposure to nitrogen dioxide vapour. Some phenols cannot be detected under such experimental conditions, as is shown in the results in the different tables.

When eluting with alkaline solutions, the detection of hydroquinone is not possible as it is probably oxidized. The same effect occurs, although to a lesser extent, by catechol, pyrogallol and gallic acid. These latter compounds, however, can be detected doubling their amounts on the layer.

RESULTS AND DISCUSSION

Dowex 50-X4 (H^+)

Table I shows the results for Dowex 50-X4 in the acid form when eluting with water and water–ethanol mixtures. On eluting with water, most phenols with a primary or a secondary amine group in the aromatic ring remain at the starting point, with the exception of 2-aminophenol-4-sulphonic acid and 2,4-dinitro-4'-hydroxydiphenylamine-3'-sulphonic acid, which have high R_F values.

As regards the other phenols, the results in Table I show that they move from the starting point to different extents. The chromatographic behaviour of the phenols, when eluting with water, is determined by the presence of one or more substituent groups in the ring and overall by the nature of such substituents. With respect to phenol, the introduction into the ring of a chlorine atom or a methyl group causes a remarkable decrease in the R_F value and this decrease becomes larger as the number of substituents increases.

Also, mono- and dinitrophenols exhibit a higher affinity than phenol towards the exchanger. With dinitrophenols, however, it should be noted that their affinity towards the exchanger decreases as their acidity increases. Such characteristics are exemplified by picric acid, which, owing to its high acidity, almost runs with the solvent front.

The introduction of one or more hydroxyl groups into the ring, on the contrary, does not result in any essential change with respect to phenol.

With water–alcohol eluents, for most phenols an increase in R_F value is observed compared with water alone. An exception is shown by some phenols with an amine group, which remain at the starting point independently of the percentage of alcohol in the eluent. Anomalous behaviour is that shown by 2-aminophenol-4-sulphonic acid, whose R_F value decreases as the percentage of ethanol in the eluent is increased owing to its low solubility in this solvent.

With water–ethanol in a 1:2 ratio, a levelling of the R_F values of many phenols is observed. For this reason, we considered it useful to examine the chromatographic behaviour of these compounds with water–alcohol mixtures owing to the importance of such eluents in "solubilization chromatography".

Fig. 1 shows some peculiar trends of R_F values with increasing percentages of alcohol in the eluent. Such trends refer to phenols whose affinity towards the ex-

TABLE I

R_F VALUES OF PHENOLS ON DOWEX 50-X4 (H^+) AND REXYN 102 (H^+) THIN LAYERS

Phenol	*Dowex 50-X4 (H^+)*			*Rexyn 102 (H^+)*
	H_2O	*H_2O–C_2H_5OH (4:1)*	*H_2O–C_2H_5OH (1:2)*	*0.5 M HCl in H_2O–C_2H_5OH (4:1)*
Phenol	0.54	0.57	0.92	0.50
Guaiacol	0.41	0.45	0.92	0.45
Hydroquinone	0.56	0.61	0.94	0.68
Catechol	0.57	0.57	0.93	0.63
Resorcinol	0.54	0.60	0.94	0.65
Orcinol	0.40	0.48	0.94	0.56
Pyrogallol	0.61	0.65	0.89	0.71
Phloroglucinol	0.52	0.70	0.90	0.70
Pyrocatechic acid	0.37	0.49	0.90	0.50
Gallic acid	0.38	0.50	0.90	0.52
o-Cresol	0.35	0.43	0.92	0.41
m-Cresol	0.35	0.43	0.92	0.41
2,6-Dimethylphenol	0.01	0.02	n.d.	0.02
2,3-Dimethylphenol	0.25	0.32	0.90	0.27
3,4-Dimethylphenol	0.26	0.34	0.90	0.27
3,5-Dimethylphenol	0.26	0.34	0.90	0.27
m-Nitrophenol	0.28	0.30	0.89	0.31
o-Nitrophenol	n.d.*	n.d.	n.d.	n.d.
p-Nitrophenol	0.23	0.30	0.90	0.29
2,5-Dinitrophenol	0.22	0.28	0.88	0.21
2,4-Dinitrophenol	0.25	0.29	0.88	0.23
2,6-Dinitrophenol	0.35	0.38	0.90	0.23
Picric acid	0.90	0.90	0.96	0.28
m-Aminophenol	0.00	0.00	0.00	0.71
o-Aminophenol	0.00	0.00	0.00	0.68
p-Aminophenol	0.00	0.00	0.00	0.72
5-Aminosalicylic acid	0.00	0.00	0.00	0.55
4-Aminosalicylic acid	0.00	0.00	0.00	0.49
3-Hydroxyanthranilic acid	0.00	0.00	0.00	0.57
2-Aminophenol-4-sulphonic acid	0.91	0.85	0.76	0.87
4-Amino-2-nitrophenol	0.00	0.00	0.00	0.58
2-Amino-5-nitrophenol	0.00	0.02	0.13	0.53
2-Amino-4-nitrophenol	0.00	0.00	0.00	0.58
2-Amino-4,6-dinitrophenol	0.00	0.04	0.32	0.22
2-Amino-3,4,6-trichlorophenol	0.00	0.01	0.44	0.18
p-Chlorophenol	0.25	0.30	0.92	0.26
m-Chlorophenol	0.25	0.30	0.92	0.25
o-Chlorophenol	n.d.	n.d.	n.d.	n.d.
p-Bromophenol	0.20	0.23	0.91	0.19
o-Bromophenol	n.d.	n.d.	n.d.	n.d.
3,4-Dichlorophenol	0.11	0.14	0.91	0.10
3,5-Dichlorophenol	0.12	0.16	0.91	0.10
2,4-Dichlorophenol	n.d.	0.14	0.91	0.14
2,3-Dichlorophenol	n.d.	0.14	0.91	0.14
2,5-Dichlorophenol	n.d.	n.d.	n.d.	n.d.
2,6-Dichlorophenol	n.d.	n.d.	n.d.	0.18
β-Naphthol	0.08	0.14	0.86	0.10

(Continued on p. 368)

TABLE I *(continued)*

Phenol	*Dowex 50-X4 (H^+)*			*Rexyn 102 (H^+)*
	H_2O	*H_2O–C_2H_5OH (4:1)*	*H_2O–C_2H_5OH (1:2)*	*0.5 M HCl in H_2O–C_2H_5OH (4:1)*
α-Naphthol	0.07	0.13	0.86	0.10
1,5-Naphthalenediol	0.08	0.14	0.86	0.20
2-Hydroxy-1-naphthaldehyde	0.04	0.07	0.78	0.03
7-Amino-2-naphthol	0.00	0.00	0.00	0.34
1-Amino-7-naphthol	0.00	0.00	0.00	0.34
5-Amino-1-naphthol	0.00	0.00	0.00	0.36
4-Hydroxydiphenylamine	0.00	0.01	0.03	0.42
3-Hydroxydiphenylamine	0.00	0.01	0.43	0.21
2,4-Dinitro-4′-hydroxydiphenylamine	0.01	0.07	0.80	0.03
2,4-Dinitro-4′-hydroxydiphenylamine-3′-sulphonic acid	0.90	0.91	0.96	0.20
4-Hydroxyazobenzene	0.01	0.03	0.86	0.01

* n.d. = not determined.

changer changes from high values (curves 6, 7, 8 and 9) to small values (curve 1). With the exception of the phenols with an amine group (curves 8 and 9), the greatest change in the R_F values occurs at alcohol contents between 20 and 50%. With alcohol contents less than 20%, a series of straight lines is obtained, in accordance with the results observed by Sherma and Hood[2] for some phenols on layers of the same exchanger with water–methanol mixtures as eluents.

The trends of these curves can be explained by assuming that the addition of alcohol to the eluent causes, other than an increase in the solubility in the mobile phase, a decrease in the interactions between the phenols and the exchanger. Such interactions seem to be completely eliminated for alcohol contents in the eluent above 50%.

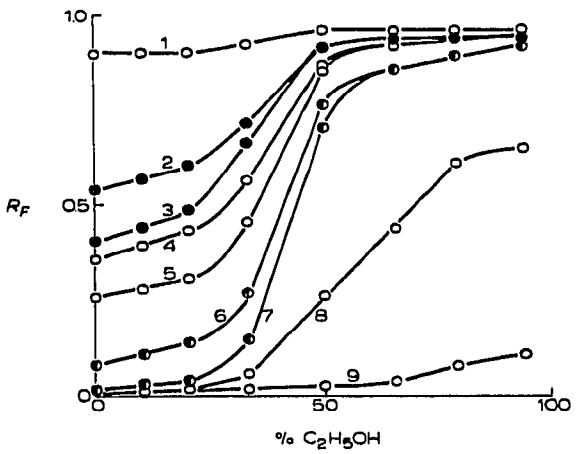

Fig. 1. R_F values of phenols on Dowex 50-X4 (H^+) thin layers *versus* ethanol content in the eluent. 1, Picric acid; 2, resorcinol; 3, orcinol; 4, *m*-cresol; 5, *m*-chlorophenol; 6, α-naphthol; 7, 4-hydroxyazobenzene; 8, 3-hydroxydiphenylamine; 9, 4-hydroxydiphenylamine.

The behaviour of the aminophenols (see curves 8 and 9) seems to contradict this assumption; it must be pointed out, however, that in this case the interactions between the amine group of these two compounds and the sulphonic group of the exchanger[3] play an important role. Such interactions are less affected by the increase in the alcohol content in the eluent than those between the phenols and the matrix of the exchanger.

Among the separations possible on the basis of the R_F values, we effected the following: picric acid, 2,6-dinitrophenol and 2,5-dinitrophenol (water); phenol and α-naphthol (4:1 water–ethanol); and 4-hydroxydiphenylamine, 3-hydroxydiphenylamine, 2,4-dinitro-4'-hydroxydiphenylamine-3'-sulphonic acid and 2,4-dinitro-4'-hydroxydiphenylamine (1:2 water–ethanol). It should be noted that the phenols with an amine group can be separated from all the others and, further, that some of these same phenols may be separated from each other under suitable elution conditions.

Rexyn 102 (H^+)

On this exchanger, there are no appreciable differences in the chromatographic behaviour of the phenols on elution with water and water–ethanol mixtures compared with that observed on Dowex 50-X4 (H^+) under the same conditions.

The use of acidic water–alcohol solutions as eluents does not affect the R_F values of the phenols without an amine group, whereas it causes a noticeable increase in the R_F values of the aminophenols, as the results in Table I show.

On the basis of the same behaviour of the phenols on Rexyn 102 (H^+) and Dowex 50-X4 (H^+), we can assume that the influence of the interactions between the two exchangers and the phenols is similar. As the interactions of the paraffinic matrix with the aromatic compounds are weaker than those of the polystyrene ones[4], the similar behaviour of the phenols must be ascribed to the different ionic environments in these two exchangers.

The following separations have been effected on Rexyn 102 (H^+) layers with 0.5 *M* hydrochloric acid in 4:1 water–ethanol as eluent: 4-hydroxydiphenylamine and 3-hydroxydiphenylamine; and α-naphthol, 2-hydroxy-1-naphthaldehyde and 1,5-naphthalenediol. In both instances the migration distance was 13 cm.

Dowex 50-X4 (Na^+)

On these layers, eluting with water and with water–alcohol mixtures, there are no appreciable differences in the chromatographic behaviour of the phenols compared with that observed on the same exchanger in the acidic form (even if the R_F values are generally lower), with the exception of the aminophenols, which are less retained on Dowex 50-X4 (Na^+) layers.

The behaviour of the phenols at different pH values and ionic strengths is particularly interesting. The R_F of the protonated form of the phenols is only slightly affected by a change in the ionic strength from 0.01 to 0.1, while that of the deprotonated form, depending on the substituent groups in the aromatic ring, may be considerably decreased, for instance for β-naphthol it decreases from 0.86 to 0.66 (see Table II).

On changing the ionic strength from 0.1 to 1 (see Table II), the decrease in the R_F of the deprotonated form is generally greater than that observed in the 0.01–0.1 range. The dependence of the R_F of the phenols on the ionic strength of the eluent

TABLE II

R_F VALUES OF PHENOLS ON DOWEX 50-X4 (Na^+) THIN LAYERS

Phenol	*Eluent*					
	0.1 M Acetate buffer	*0.1 M $NaHCO_3$*	*0.05 M Na_2CO_3*	*1 M NH_3*	*1 M NH_3 + 0.1 M CH_3COONa*	*1 M NH_3 + 1 M CH_3COONa*
Phenol	0.49	0.53	0.65	0.92	0.90	0.73
Guaiacol	0.38	0.41	0.58	0.92	0.89	0.56
Hydroquinone	0.43	n.d.*	n.d.	n.d.	n.d.	n.d.
Catechol	0.44	0.50	0.70	0.94	0.93	e.s.**
Resorcinol	0.41	0.49	0.72	0.94	0.94	0.83
Orcinol	0.33	0.38	0.63	0.94	0.92	0.73
Pyrogallol	0.46	0.71	0.88	0.95	0.95	n.d.
Phloroglucinol	0.39	0.80	0.93	0.95	0.95	0.88
Pyrocathechic acid	0.43	0.95	0.95	0.95	0.95	n.d.
Gallic acid	0.44	0.95	0.95	0.95	0.95	n.d.
o-Cresol	0.35	0.35	0.50	0.92	0.82	0.49
m-Cresol	0.35	0.36	0.53	0.92	0.88	0.56
2,6-Dimethylphenol	0.01	0.01	0.05	n.d.	n.d.	n.d.
2,3-Dimethylphenol	0.24	0.24	0.31	0.90	0.72	0.34
3,4-Dimethylphenol	0.25	0.25	0.41	0.91	0.78	0.39
3,5-Dimethylphenol	0.25	0.25	0.42	0.91	0.78	0.39
m-Nitrophenol	0.21	0.58	0.80	0.94	0.90	0.46
o-Nitrophenol	n.d.	0.91	0.92	0.96	0.92	0.65
p-Nitrophenol	0.18	0.81	0.84	0.94	0.84	0.44
2,5-Dinitrophenol	0.28	0.80	0.80	0.92	0.80	0.45
2,4-Dinitrophenol	0.50	0.72	0.72	0.88	0.72	0.26
2,6-Dinitrophenol	0.79	0.88	0.88	0.94	0.88	0.53
Picric acid	0.65	0.67	0.67	0.82	0.66	0.19
m-Aminophenol	0.06	0.53	0.68	0.94	0.88	0.71
o-Aminophenol	0.03	0.53	e.s.	e.s.	e.s.	e.s.
p-Aminophenol	0.00	e.s.	e.s.	e.s.	e.s.	e.s.
5-Aminosalicylic acid	0.47	0.91	0.91	0.94	0.90	0.65
4-Aminosalicylic acid	0.53	0.91	0.91	0.94	0.90	0.64
3-Hydroxyanthranilic acid	0.54	0.92	0.92	0.94	0.92	0.65
2-Aminophenol-4-sulphonic acid	0.96	0.96	0.96	0.96	0.96	0.96
4-Amino-2-nitrophenol	0.07	0.74	0.87	0.94	0.88	0.66
2-Amino-5-nitrophenol	0.09	0.24	0.65	0.87	0.76	0.49
2-Amino-4-nitrophenol	0.10	0.71	0.78	0.89	0.78	0.51
2-Amino-4,6-dinitrophenol	0.25	0.55	0.56	0.77	0.56	0.18
2-Amino-3,4,6-trichlorophenol	0.02	0.54	0.63	0.84	0.63	0.20
p-Chlorophenol	0.19	0.24	0.65	0.94	0.89	0.53
m-Chlorophenol	0.20	0.31	0.72	0.94	0.90	0.66
o-Chlorophenol	n.d.	n.d.	0.86	0.96	0.95	0.78
p-Bromophenol	0.13	0.21	0.60	0.94	0.88	0.53
o-Bromophenol	0.21	0.51	0.84	0.96	0.94	0.74
3,4-Dichlorophenol	0.08	0.24	0.66	0.94	0.82	0.45
3,5-Dichlorophenol	0.08	0.36	0.75	0.94	0.84	0.50
2,4-Dichlorophenol	0.09	0.58	0.82	0.95	0.90	0.50
2,3-Dichlorophenol	0.09	0.66	0.84	0.95	0.91	0.56
2,5-Dichlorophenol	0.11	0.78	0.88	0.96	0.91	0.59

TABLE II *(continued)*

Phenol	*Eluent*					
	0.1 M Acetate buffer	*0.1 M $NaHCO_3$*	*0.05 M Na_2CO_3*	*1 M NH_3*	*1 M NH_3 + 0.1 M CH_3COONa*	*1 M NH_3 + 1 M CH_3COONa*
2,6-Dichlorophenol	0.12	0.94	0.95	0.96	0.95	0.65
β-Naphthol	0.04	0.05	0.29	0.86	0.66	0.31
α-Naphthol	0.04	0.06	0.35	0.92	0.75	0.34
1,5-Naphthalenediol	0.03	0.07	e.s.	e.s.	e.s.	e.s.
2-Hydroxy-1-naphthaldehyde	0.04	0.38	0.52	n.d.	n.d.	n.d.
7-Amino-2-naphthol	0.00	0.03	0.26	0.86	0.49	0.28
1-Amino-7-naphthol	0.00	0.05	0.35	0.86	0.61	0.34
5-Amino-1-naphthol	0.00	0.05	0.36	0.86	0.61	0.36
4-Hydroxydiphenylamine	0.00	0.00	0.12	0.78	0.51	0.20
3-Hydroxydiphenylamine	0.01	0.01	0.15	n.d.	0.30	0.10
2,4-Dinitro-4′-hydroxydiphenylamine	0.00	0.02	0.16	0.68	0.38	0.10
2,4-Dinitro-4′-hydroxydiphenylamine-3′-sulphonic acid	0.42	0.74	0.86	0.90	0.85	0.30
4-Hydroxyazobenzene	0.01	0.06	e.s.	0.78	0.45	0.13

* n.d. = not determined.
** e.s. = elongated spot.

can be used, in some instances, in order to obtain or to improve separations among phenols with similar pK_a values and must be taken into account in the determination of the R_F values on this exchanger.

As regards the influence of pH, it must be noted that the protonated form of the phenols exhibits a higher affinity towards the exchanger than the deprotonated form. Such behaviour is similar to that observed by Grieser and Pietrzyk[5] for some phenols on Amberlite XAD-2 columns with water–alcohol eluents.

In that paper, the following relationship was used:

$$K_D = \frac{[HA]_R + [A^-]_R}{[HA]_S + [A^-]_S} \cdot \frac{v}{w} \qquad (1)$$

where K_D is the distribution coefficient, $[HA]_R$, $[A^-]_R$, $[HA]_S$ and $[A^-]_S$ are the concentrations of the protonated and deprotonated form of the phenol in the resin and in the solution, v is the volume of the solution and w is the weight of the resin. Introducing the K_a value into eqn. 1 and rearranging it in order to obtain the distribution coefficient as a function of the hydrogen ion concentration and of K_a, Grieser and Pietrzyk obtained log K_D *versus* pH curves with a shape similar to that of an acid–base titration curve. In these curves, the greatest change in log K_D is observed at a pH value about one unit higher than the pK_a value of the corresponding phenols.

In our case, using the equation

$$K_D = \left(\frac{1}{R_F} - 1\right)\frac{A_1}{A_s} = \frac{[HA]_R + [A^-]_R}{[HA]_S + [A^-]_S} \qquad (2)$$

where A_1 and A_s are the cross-sectional areas of the mobile and stationary phases, respectively, we obtained the relationship

$$\left(\frac{1}{R_F} - 1\right) = \left(\frac{1}{R_{F_{ac}}} - 1\right)\frac{[H^+]}{K_a + [H^+]} + \left(\frac{1}{R_{F_{alk}}} - 1\right)\frac{K_a}{[H^+] + K_a} \qquad (3)$$

where $R_{F_{ac}}$ and $R_{F_{alk}}$ are the R_F values of the protonated and deprotonated form of the phenol obtained by eluting, with a low pH solution (*e.g.*, 1 *M* acetic acid or 0.1 *M* equimolecular acetate buffer) and a high pH solution (*e.g.*, 1 *M* ammonia + 0.1 *M* sodium acetate or 1 *M* ammonia + 0.1 *M* sodium hydroxide), respectively. Applying eqn. 3 to some phenols, the $[(1/R_F) - 1]$ *versus* pH curves reported in Fig. 2 were obtained. The good agreement between the theoretical curves and the experimental values in Table III supports the validity of such an equation in thin-layer chromatography.

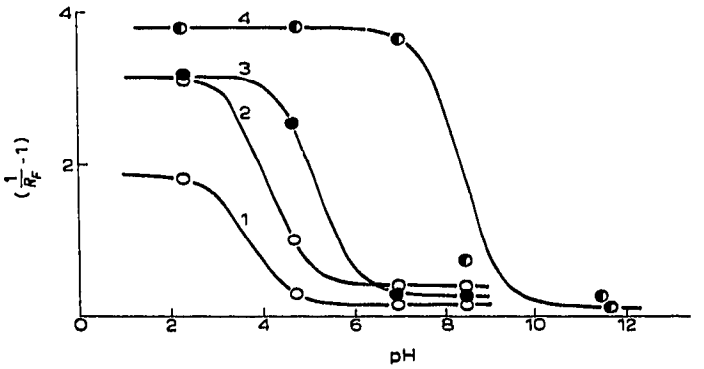

Fig. 2. $(1/R_F - 1)$ *versus* pH plots for phenols on Dowex 50-X4 (Na^+) thin layers. 1, 2,6-Dinitrophenol ($pK_a = 3.71$); 2, 2,4-dinitrophenol ($pK_a = 4.09$); 3, 2,5-dinitrophenol ($pK_a = 5.22$); 4, *m*-nitrophenol ($pK_a = 8.40$).

In these curves, the major change in $[(1/R_F) - 1]$ with pH occurs at pH = pK_a. In fact, differentiating eqn. 3 twice with respect to log $[H^+]$ and equating to zero the relationship obtained, it can be seen that the mean value of $[(1/R_{F_{ac}}) - 1]$ and $[(1/R_{F_{alk}}) - 1]$ is achieved at pH = pK_a.

As regards the application of eqn. 3, it should be noted that the $[(1/R_F) - 1]$ quantity, contrary to the case with R_F, is rarely used in thin-layer chromatography. We tried, therefore, to explain the R_F in eqn. 3. Fig. 3 shows the R_F *versus* pH curves of some phenols and, as in the case of the curves in Fig. 2, there is good agreement in most instances between the theoretical curves and the experimental values (see Table III). It should be noted in these curves that the mean R_F value (R_{F_m}) of $R_{F_{ac}}$ and $R_{F_{alk}}$ occurs at a pH value higher than that corresponding to the pK_a value. Differentiating R_F twice with respect to log $[H^+]$ and equating to zero, the following relationship is obtained:

$$[H^+] = K_a \cdot \frac{R_{F_{ac}}}{R_{F_{alk}}} \qquad (4)$$

TABLE III

R_F AND $(1/R_F - 1)$ VALUES OF PHENOLS ON DOWEX 50-X4 (Na^+) THIN LAYERS OBTAINED WITH ELUENTS AT DIFFERENT pH VALUES

1 *M* acetic acid (pH = 2.35); 0.1 *M* acetic acid + 0.1 *M* sodium acetate (pH = 4.75); 0.05 *M* disodium phosphate (pH = 7.00); 0.1 *M* sodium hydrogen carbonate (pH = 8.50); 0.05 *M* sodium carbonate (pH = 11.50); 1 *M* ammonia + 0.1 *M* sodium acetate (pH = 11.70); 1 *M* ammonia + 0.1 *M* sodium hydroxide (pH = 13.00).

2,5-Dinitrophenol			*2,6-Dinitrophenol*			*2,4-Dinitrophenol*		
pH	R_F	$(1/R_F - 1)$	*pH*	R_F	$(1/R_F - 1)$	*pH*	R_F	$(1/R_F - 1)$
2.35	0.24	3.17	2.35	0.36	1.80	2.35	0.24	3.17
4.75	0.50	1.00	4.75	0.79	0.26	4.75	0.28	2.57
7.00	0.72	0.39	7.00	0.88	0.14	7.00	0.77	0.30
8.50	0.72	0.39	8.50	0.88	0.14	8.50	0.80	0.25
11.70	0.72	0.39	11.70	0.88	0.14	11.70	0.80	0.25

m-Nitrophenol			*Phloroglucinol*		*3,5-Dichlorophenol*		*α-Naphthol*	
pH	R_F	$(1/R_F - 1)$	*pH*	R_F	*pH*	R_F	*pH*	R_F
2.35	0.21	3.76	4.75	0.39	4.75	0.08	4.75	0.04
4.75	0.21	3.76	7.00	0.39	7.00	0.09	7.00	0.04
7.00	0.22	3.55	8.50	0.80	8.50	0.36	8.50	0.06
8.50	0.58	0.72	11.50	0.93	11.50	0.75	11.50	0.35
11.50	0.80	0.25	11.70	0.95	11.70	0.84	11.70	0.75
11.70	0.90	0.11					13.00	0.80

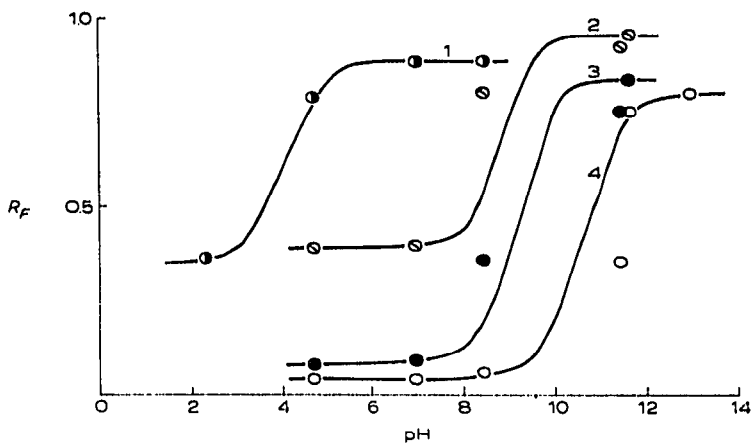

Fig. 3. R_F *versus* pH plots for phenols on Dowex 50-X4 (Na^+) thin layers. 1, 2,6-Dinitrophenol (pK_a = 3.71); 2, phloroglucinol (pK_a = 8.45); 3, 3,5-dichlorophenol (pK_a = 8.18); 4, α-naphthol (pK_a = 9.34).

On the basis of eqn. 4, we can predict that the R_{F_m} value will be shifted more with respect to the pH = pK_a value the lower is the $R_{F_{ac}}/R_{F_{alk}}$ ratio. The experimental points at pH 8.5 and 11.5, which do not fit the theoretical curves in Figs. 2 and 3, were obtained with 0.1 *M* sodium hydrogen carbonate and 0.05 *M* sodium carbonate solutions.

In the first case, R_F values higher than those predicted at pH 8.5 on the basis of the theoretical curves in Figs. 2 and 3 are obtained for *m*-nitrophenol, phloroglucinol and 3,5-dichlorophenol; with sodium carbonate, on the contrary, the above-mentioned phenols and α-naphthol exhibit lower R_F values than those predicted on the basis of the starting pH of the eluent. Such disagreement between theoretical and experimental values may be correlated with the different pH on the layer with respect to that of the eluent. In fact, with the method described in a previous paper[6], we have measured the pH on the layer and found pH values between 9.2 and 9.5 for sodium hydrogen carbonate and between 10.3 and 9.8 for sodium carbonate (see Table IV).

Referring the $[(1/R_F) - 1]$ and R_F values to the pH values reported in Table IV, there is good agreement in the curves in Figs. 2 and 3 between the theoretical and experimental results with these eluents.

TABLE IV

HYDROGEN ION CONCENTRATION GRADIENT ALONG THE LAYER OF DOWEX 50-X4 (Na^+) FOR 0.1 *M* $NaHCO_3$, 0.05 *M* Na_2CO_3 AND 0.05 *M* $NaHCO_3$ + 0.05 *M* Na_2CO_3 SOLUTIONS IN THE ELUENT

*Distance** (cm)	*pH*		
	0.1 M $NaHCO_3$	*0.05 M* Na_2CO_3	*0.05 M* $NaHCO_3$ + *0.05 M* Na_2CO_3
0– 2	9.2	10.3	10.0
2– 4	9.3	10.2	9.9
4– 6	9.3	10.1	9.8
6– 8	9.4	10.0	9.8
8–10	9.5	9.8	9.7

* Distances of the front and rear limits of the band (2 × 10 cm) from the starting point.

The above effects, which might be a serious limitation in the use of eqn. 3, are, however, very important as they show a close correlation between the difference in the experimental R_F value compared with the theoretical value and that of the pH values on the layer and in the eluent. It follows, therefore, that, on the basis of eqn. 3, the pH on the layer can be determined from the R_F value.

Analytical applications

In Table V are reported the separations of some isomers eluted with solutions at different pH values. Such separations can all be predicted on the basis of the R_F *versus* pH curves with the exception of that of the aminonitrophenols, whose pK_a values are unknown. For chloro- and bromophenols, the eluent was a solution of sodium carbonate and hydrogen carbonate in order to have a pH about 9.8 on the layer because, on the basis of the R_F *versus* pH curves, at this pH there is the best resolution of the R_F values of different isomers. As shown by the results in Table IV, the carbonate–hydrogen carbonate mixture involves a pH between 10 and 9.7 on the layer. In this case, the use of the ammonium buffer must be avoided, as the pH on the layer changes owing to the exchange reaction between the ammonium ions and the sodium ions of the exchanger.

TABLE V

SEPARATIONS OBTAINED ON THIN LAYERS OF DOWEX 50-X4 (Na^+)

Migration distance 12.5 cm.

Mixture	*Eluent*	R_F *value*
o-Chlorophenol	0.05 *M* $NaHCO_3$ + 0.05 *M* Na_2CO_3	0.84
m-Chlorophenol		0.61
p-Chlorophenol		0.47
o-Bromophenol	0.05 *M* $NaHCO_3$ + 0.05 *M* Na_2CO_3	0.81
p-Bromophenol		0.40
2,6-Dichlorophenol	0.05 *M* $NaHCO_3$ + 0.05 *M* Na_2CO_3	0.95
2,3-Dichlorophenol		0.80
3,5-Dichlorophenol		0.64
3,4-Dichlorophenol		0.50
2,6-Dichlorophenol	0.1 *M* $NaHCO_3$	0.94
2,3-Dichlorophenol		0.62
3,5-Dichlorophenol		0.33
3,4-Dichlorophenol		0.21
o-Nitrophenol	0.1 *M* $NaHCO_3$	0.86
p-Nitrophenol		0.75
m-Nitrophenol		0.52
2,6-Dinitrophenol	0.1 *M* $NaHCO_3$	0.82
2,5-Dinitrophenol		0.73
2,4-Dinitrophenol		0.65
2,6-Dinitrophenol	0.1 *M* acetate buffer	0.69
2,4-Dinitrophenol		0.42
2,5-Dinitrophenol		0.24
4-Amino-2-nitrophenol	0.1 *M* Na_2CO_3	0.83
2-Amino-4-nitrophenol		0.70
2-Amino-5-nitrophenol		0.61
2-Amino-4,6-dinitrophenol		0.50

The dichlorophenols, on the contrary, may be better separated from each other with 0.1 *M* sodium hydrogen carbonate solution than with carbonate–hydrogen carbonate mixtures, as predicted from the R_F *versus* pH curves.

The nitrophenols were developed in sodium hydrogen carbonate solution, as *o*-nitrophenol cannot be detected with eluents of lower pH (*e.g.*, 0.05 *M* disodium hydrogen orthophosphate solution at pH 7.00).

As regards the dinitrophenols, a good separation of these isomers with both alkaline and acidic eluents can be usefully effected. The separation obtained in alkaline medium, that is, at a pH value at which the three isomers are completely deprotonated, is due to the different influence of the ionic strength of the solution on such isomers.

Rexyn 102 (Na^+)

On this exchanger, when eluting with water, most phenols run with the solvent front. Also with 0.1 *M* sodium hydrogen carbonate solution many phenols exhibit high R_F values (≥ 0.90), with the exception of most phenols with two aromatic nuclei

(R_F between 0.4 and 0.8), 2-amino-5-nitrophenol ($R_F = 0.81$), 2-amino-4,6-dinitrophenol ($R_F = 0.79$), 2-amino-3,4,6-trichlorophenol ($R_F = 0.84$), *p*-bromophenol ($R_F = 0.76$) and 3,4-dichlorophenol ($R_F = 0.85$). The different behaviour of the phenols with water and with sodium hydrogen carbonate can be ascribed to the different ionic strengths of the two solutions. The pH on the layer, in fact, is about 10 with both eluents and is determined by the alkaline reaction of the functional groups of the exchanger. On eluting with 0.1 *M* acetate buffer, a decrease in the R_F values is observed only for those phenols which are the most retained with sodium hydrogen carbonate. Such behaviour is correlated with the pH on the layer (between 8.4 and 10) less in this case than with the two previous eluents. The results achieved with 0.1 *M* sodium hydrogen carbonate are interesting from an analytical standpoint, as the following separations can be effected: 7-amino-2-naphthol and 5-amino-1-naphthol (migration distance 12 cm); and α-naphthol and β-naphthol (migration distance 14 cm).

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