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

I. BIO-RAD AG 3-X4A, PEI-CELLULOSE AND DEAE-CELLULOSE

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SUMMARY

The chromatographic behaviour of 58 phenols on thin layers of Bio-Rad AG 3-X4A, PEI-cellulose and DEAE-cellulose has been studied with acidic and alkaline solutions, neutral and alkaline alcohol solutions and organic solvent mixtures as eluents. The selectivity of AG 3-X4A layers is ascribed to interactions between the functional group of the resin and the phenolic hydroxyl group.

Many separations among the 58 phenols by two-dimensional chromatography and with successive developments with the same eluent have been effected. The acid–base and the chromatographic characteristics have been compared for most of the phenols.

INTRODUCTION

The chromatographic behaviour of phenols on columns of ion exchangers[1–7] and of polystyrene-based resins without functional groups[8] has been widely investigated. In thin-layer chromatography, silica gel[9–13], alumina[13,14] and polyamide[13,15–25] layers have been employed. Only one paper concerns the use of ion exchangers on thin layers and particularly the behaviour of six phenols on Dowex 50-X4 (Na^+ and H^+) with water–methanol mixtures as eluents[26]. The behaviour of these compounds on ion-exchange papers has not been widely investigated[27,28]. It was considered useful, therefore, to extend the investigation of the behaviour of this important class of compounds on thin layers of several ion exchangers in order to find the best conditions for their separation.

This paper concerns Bio-Rad AG 3-X4A (a polystyrene-based anion exchanger), polyethyleneimine (PEI)-cellulose and O-(diethylaminoethyl) (DEAE)-cellulose layers. Bio-Rad AG 3-X4A was used in the free base form in order to study the interactions between the functional group of the resin and the phenolic hydroxyl group.

EXPERIMENTAL

Most phenols were dissolved in ethanol, with the exception of those that contain a carboxylic or a sulphonic group which were dissolved in water. The concentrations of the solutions were 1–2 mg/ml and 1-μl volumes were deposited on the layers. With chloro-, bromo- and dichloro-phenols, more concentrated solutions (6–12 mg/ml) were employed. Fresh solutions were used for those phenols which easily decompose (pyrogallol and *o*- and *p*-aminophenol).

Detection

The phenols were detected by the Boute reaction[29], exposing the layers successively to nitrogen dioxide and to ammonia vapours. Other developers tried were diazotized benzidine and *p*-nitroaniline, sodium molybdate, potassium permanganate in acidic solution and phosphotungstomolybdic acid (Folin–Ciocalteu reagent); these detection reagents, however, were less satisfactory.

Preparation of the layers

Layers with a thickness of 300 μm were obtained with a Chemetron automatic apparatus. In order to obtain Bio-Rad AG 3-X4A in the free base form (C_6H_5–NR_2), the commercial product (Bio-Rad Labs., Richmond, Calif., U.S.A.) was treated with sodium hydroxide solution, rinsed with water and methanol and dried at room temperature. The Bio-Rad AG 3-X4A (200–400 mesh) layers were prepared by mixing 3 g of the resin with 9 g of microcrystalline cellulose (E. Merck, Darmstadt, G.F.R.) in 50 ml of water. The Bio-Rad AG 3-4XA (Cl^-) layers were prepared in the same way after rinsing the commercial product with water, methanol and drying at room temperature. The PEI-cellulose (E. Merck) and DEAE-cellulose (Serva, Heidelberg, G.F.R.) layers were prepared from 10 and 6 g of the exchanger, respectively, in 40 ml of water.

The chromatographic development was carried out at 25 ± 0.5° and the migration distance was 11 cm unless otherwise stated.

RESULTS AND DISCUSSION

Bio-Rad AG 3-X4A

On layers of this exchanger in the free base form, the elution of 58 phenols with water, aqueous solutions of polar solvents (methanol, ethanol, isopropanol, acetonitrile and dimethylformamide) and organic solvents alone and in mixtures (ethyl acetate, benzene, chloroform, ethyl acetate–dimethylformamide and ethanol–dimethylformamide) was studied.

The best results from an analytical standpoint were achieved by eluting with water–alcohol solutions, because with organic solvents or water alone it was not possible to obtain a migration of the phenols from the starting point. The organic solvent mixtures proved unsatisfactory as they gave rise to elongated spots. Of the aqueous–organic solutions, methanol–water gave the best results in the separation of the phenols, with a short elution time. With this eluent, furthermore, compact spots were obtained. In Table I are reported the R_F values of the phenols on Bio-Rad AG 3-X4A layers on elution with 50 and 95% methanol (columns 1 and 2), 0.5 *M*

TABLE I

R_F VALUES OF PHENOLS ON LAYERS OF BIO-RAD AG 3-X4A IN THE FREE BASE AND CHLORIDE FORMS

Eluents: (1) 50% methanol; (2) 95% methanol; (3) 0.5 *M* NH_3 in 50% ethanol; (4) 0.5 *M* NH_3 in 95% ethanol; (5) acetic acid–cyclohexane (7:93).

Phenol	*Bio-Rad AG 3-X4A*					*Bio-Rad AG 3-X4A(Cl^-)*	*pK_a (25°)**
	1	*2*	*3*	*4*	*5*	*2*	
Phenol	0.15	0.58	0.22	0.59	0.09	0.71	9.99
Guaiacol	0.11	0.57	0.20	0.57	0.26	0.67	9.98
Hydroquinone	0.16	0.47	0.07	0.48	0.00	0.61	10.35***
Catechol	0.09	0.36	0.14	0.29	0.00	0.58	9.85***
Resorcinol	0.10	0.38	0.18	0.36	0.00	0.58	9.81***
Orcinol	0.05	0.39	0.15	0.40	0.00	0.57	–
Pyrogallol	0.07	0.16	e.s.**	0.28	0.00	0.45	9.01
Phloroglucinol	0.08	0.20	e.s.	0.16	0.00	0.46	8.45
Pyrocatechic acid	0.00	0.00	0.44	0.06	0.00	0.14	–
Gallic acid	0.00	0.00	0.43	0.03	0.00	0.07	4.41
o-Cresol	0.07	0.49	0.14	0.53	0.15	0.60	10.32
m-Cresol	0.07	0.49	0.14	0.55	0.11	0.60	10.09
2,6-Dimethylphenol	0.05	0.51	0.15	0.56	0.27	0.59	10.63
2,3-Dimethylphenol	0.01	0.44	0.09	0.49	0.15	0.53	10.54
3,4-Dimethylphenol	0.03	0.46	0.13	0.52	0.12	0.56	10.36
3,5-Dimethylphenol	0.02	0.45	0.13	0.52	0.13	0.57	10.19
m-Nitrophenol	0.01	0.23	0.20	0.41	0.02	0.45	8.40
o-Nitrophenol	0.00	0.03	0.22	0.16	e.s.	0.52	7.23
p-Nitrophenol	0.00	0.03	0.23	0.26	0.00	0.39	7.16
2,5-Dinitrophenol	0.00	0.00	0.07	0.13	0.10	0.02	5.22
2,4-Dinitrophenol	0.00	0.00	0.05	0.10	0.01	0.00	4.09
2,6-Dinitrophenol	0.00	0.00	0.04	0.07	0.00	0.00	3.71
Picric acid	0.00	0.00	0.00	0.03	0.00	0.00	0.60
m-Aminophenol	0.26	0.52	0.39	0.53	0.00	0.53	9.99§
o-Aminophenol	0.19	0.50	0.31	0.50	0.00	0.56	9.44§
p-Aminophenol	0.38	0.62	0.53	0.66	0.00	0.67	8.46§
5-Aminosalicylic acid	0.00	0.00	0.57	0.20	0.00	0.02	2.74
4-Aminosalicylic acid	0.00	0.00	0.50	0.18	0.00	0.02	1.7
3-Hydroxyanthranilic acid	0.00	0.00	0.40	0.16	0.00	0.02	–

(Continued on p. 172)

TABLE I *(continued)*

Phenol	*Bio-Rad AG 3-X4A*					*Bio-Rad AG 3-X4A(Cl⁻)*	*pK_a (25°)**
	1	*2*	*3*	*4*	*5*	*2*	
2-Aminophenol-4-sulphonic acid	0.00	0.00	0.56	0.24	0.00	0.01	–
4-Amino-2-nitrophenol	0.06	0.28	0.45	0.23	0.01	0.43	–
2-Amino-5-nitrophenol	0.03	0.20	0.19	0.23	0.00	0.27	–
2-Amino-4-nitrophenol	0.01	0.05	0.27	0.18	0.00	0.30	–
2-Amino-4,6-dinitrophenol ..	0.00	0.00	0.03	0.04	0.00	0.00	–
2-Amino-3,4,6-trichlorophenol	0.00	0.00	0.03	0.07	0.10	0.32	–
p-Chlorophenol	0.06	0.39	0.11	0.41	0.05	0.52	9.38
m-Chlorophenol	0.03	0.37	0.11	0.41	0.06	0.52	9.02
o-Chlorophenol	0.04	0.35	0.18	0.38	0.15	0.55	8.48
p-Bromophenol	0.02	0.34	0.09	0.40	0.06	0.48	8.87
o-Bromophenol	0.03	0.30	0.15	0.35	0.19	0.50	8.42
3,4-Dichlorophenol	0.00	0.26	0.11	0.33	0.05	0.46	– 8.39§§
3,5-Dichlorophenol	0.01	0.20	0.04	0.32	0.11	0.46	8.18 7.93§§
2,4-Dichlorophenol	0.00	0.14	0.10	0.25	0.14	0.45	7.89 7.75§§
2,3-Dichlorophenol	0.00	0.12	0.11	0.25	0.14	0.49	– 7.45§§
2,5-Dichlorophenol	0.00	0.10	0.11	0.25	0.14	0.47	7.50 7.35§§
2,6-Dichlorophenol	0.00	0.01	0.08	0.14	0.25	0.45	6.79 6.79§§
β-Naphthol	0.00	0.27	0.02	0.31	0.07	0.37	9.51
α-Naphthol	0.00	0.27	0.02	0.30	0.07	0.38	9.34
1,5-Naphthalenediol	0.00	0.14	0.02	0.16	0.00	0.26	–
2-Hydroxy-1-naphthaldehyde	0.00	0.00	0.00	0.00	0.00	0.08	–
7-Amino-2-naphthol	0.05	0.31	0.12	0.30	0.00	0.33	–
1-Amino-7-naphthol	0.02	0.28	0.10	0.25	0.00	0.31	–
5-Amino-1-naphthol	0.02	0.26	0.07	0.20	0.00	0.27	–
4-Hydroxydiphenylamine	0.00	0.25	0.03	0.29	0.10	0.35	–
3-Hydroxydiphenylamine	0.00	0.23	0.02	0.25	0.08	0.32	–
2,4-Dinitro-4'-hydroxydiphenylamine	0.00	0.12	0.01	0.16	0.00	0.24	–
2,4-Dinitro-4'-hydroxydiphenylamine-3'-sulphonic acid	0.00	0.00	0.00	0.03	0.00	0.01	–
4-Hydroxyazobenzene	0.00	0.13	0.02	0.22	0.27	0.35	–

* Refs. 8, 30 and 31.
** e.s. = elongated spot.
*** pK_a values at 20°.
§ pK_a values at 21°.
§§ pK_a values at 29° (ref. 32).

ammonia in 50 and 95% ethanol (columns 3 and 4) and acetic acid–cyclohexane (column 5), and on Bio-Rad AG 3-X4A (Cl^-) layers on elution with 95% methanol (column 6). The phenols were chosen, as far as possible, on the basis of the substituent group and arranged in each class according to the decreasing sequence of their pK_a values.

On elution with 50% methanol (column 1), most phenols and particularly those which contain two aromatic nuclei remain at the starting point. As the concentration of methanol in the eluent is increased (column 2), a remarkable increase in the R_F values of most phenols is observed.

The results obtained with such eluents show that the chromatographic behaviour of these compounds is noticeably affected by the type and number of substituent groups in the ring. In particular, the introduction into the ring of a group with marked acidic characteristics ($-SO_3H$, $-COOH$) or that remarkably increases the acid strength of phenol ($-NO_2$), causes a sharp increase in the affinity of the resin towards such compounds. In fact, *o*- and *p*-nitrophenol, dinitrophenols, picric acid and hydroxybenzoic, aminohydroxybenzoic and aminohydroxybenzenesulphonic acids remain virtually at the starting point, independent of the concentration of methanol in the eluent.

It should be noted that such compounds have pK_a values $\leqslant 7.23$. For phenols with pK_a values > 7.23, there is generally good agreement, in each class, between their chromatographic behaviour and acid–base characteristics. For most isomers, in fact, the R_F sequence is the same as that of the pK_a values (the greater the pK_a, the higher is the R_F value). Exceptions to this rule are *p*-aminophenol with respect to the *o*- and *m*-isomers and phloroglucinol with respect to pyrogallol. There is no correlation between the pK_a and R_F values for phenols of different classes.

On the basis of the behaviour of the phenols on this resin when eluting with methanol–water solutions, and particularly the stronger retention of the phenols with more acidic characteristics, the selectivity of the resin towards these compounds can be assumed to be determined by interactions between the nitrogen of the functional group of the resin and the phenolic hydroxyl group. The chromatographic behaviour of the phenols, under the same elution conditions, on the resin in the chloride form (column 6) supports such an assumption. In fact, the following occurrences should be taken into account:

(a) a remarkable increase in the R_F values on this resin with respect to that in the free base form;

(b) a general levelling of the R_F values, particularly in the case of the isomers of a given class.

On changing the resin from the free base form to the chloride form, the nitrogen atom of the functional group becomes quaternary; for this reason, the interactions between the functional group of the resin and the phenolic hydroxyl group, which cause the selectivity of the resin in the free base form towards the phenols, disappear. On the resin in the chloride form, the chromatographic behaviour of the phenols is therefore determined mainly by the liquid–liquid partition process, with the exception of those phenols which are, at least partially, in the phenate form and can therefore replace the chloride ion in the resin by an anion-exchange process. The interactions between the tertiary nitrogen atom of the resin in the free base form and the phenolic hydroxyl group seem to be correlated with the formation of a hydrogen bond, as

Bio-Rad AG 3-X4A is a weak base and may act as an anion exchanger only at pH < 7.

On changing from methanol to ethanol, a general increase in the R_F values is observed, particularly for halogenated and methyl- and dimethyl-phenols.

The behaviour of the three dihydroxybenzene isomers is peculiar with ethanol as eluent. Hydroquinone, in fact, has a higher R_F value (0.48) than that observed with 95% methanol, while resorcinol and catechol have smaller R_F values (0.36 and 0.29, respectively) in ethanol.

Influence of the pH of the eluent. When eluting with acetic acid solutions in water and in methanol, the formation of a double front, the first of which is due to acetic acid, is observed. The formation of the double front can be avoided by using acetic acid solutions in cyclohexane.

The R_F values obtained with this eluent are reported in Table I (column 5). The phenols remain at the starting point, with the exception of chloro-, bromo- and dichloro-phenols, methyl- and dimethyl-phenols and a few other compounds. The chromatographic behaviour of the phenols on this exchanger is similar to that observed under the same elution conditions on polyamide layers[25], apart from the stronger retention on Bio-Rad AG 3-X4A.

With 0.5 *M* aqueous ammonia as eluent, most phenols remain at the starting point and there are no appreciable differences with respect to their behaviour with water. The only exceptions are shown by those compounds with a carboxylic group in the molecule. Therefore, the deprotonation of the phenolic hydroxyl group does not involve any difference in the chromatographic characteristics of these compounds.

By adding alcohol to the aqueous ammonia solution, as is observed with water–alcohol mixtures, the R_F values of most phenols increase. On the basis of the R_F values reported in Table I (columns 3 and 4), the phenols can be divided into three groups: the first includes those phenols whose chromatographic behaviour is not appreciably affected by the presence of ammonia; the second those phenols with marked acidic characteristics whose R_F values are noticeably affected by the presence of ammonia in the eluent; and the third those phenols with a carboxylic or a sulphonic group in the ring whose retention increases as the concentration of alcohol in the eluent is increased. The behaviour of the phenols of the last group can be ascribed to a decrease in their solubility in the mobile phase as the concentration of ethanol in the eluent is increased.

For the first two groups, it should be noted that the first includes phenols with pK_a values $>$ *ca.* 8.4 and the second those with $pK_a <$ *ca.* 8.4. Although the pK_a values in the case of aqueous alcoholic solutions are generally different and higher than those in water[32], the higher mobility of the phenols with $pK_a <$ *ca.* 8.4 must be correlated with the deprotonated form in which such compounds mainly occur in aqueous alcoholic ammonia solutions. As interactions due to a hydrogen bond cannot be assumed between the phenate ion and the functional group of the resin in the free base form, it is necessary to identify the parameters that determine the retention of the deprotonated phenols with alcoholic ammonia solutions as eluents. The retention is not due only to interactions between the phenate ion and the microcrystalline cellulose and/or the polystyrene matrix of the exchanger, as the deprotonated phenols show a negligible affinity towards both Amberlite XAD-2 (ref. 8) and Dowex 50-X4 (Na^+) under the same elution conditions on layers prepared in the same way as those

of Bio-Rad AG 3-X4A. A possible explanation is that the tertiary nitrogen atom of the resin, bonded to the aromatic ring, may give rise to resonance forms and therefore to interactions with the phenate ion or with other groups in the molecule.

Analytical applications. The data in Table I can be used for many interesting separations of phenols both within a given class and among different classes. With 95% methanol as the eluent, the separation of 4-amino-2-nitrophenol, 2-amino-5-nitrophenol, 2-amino-4-nitrophenol and 2-amino-4,6-dinitrophenol has been effected with a migration distance of 14 cm. With three successive developments in the same eluent and with a migration distance of 12 cm, the separation shown in Fig. 1 was obtained; this separation includes five of the six dichlorophenols and their separation from the chlorophenols. Fig. 1 also shows the separation of *m*-nitrophenol from *p*-nitrophenol. With two successive developments in 95% ethanol, the different behaviour of the three dihydroxybenzenes (hydroquinone, resorcinol and catechol) can be used from an analytical standpoint. The different behaviour of some phenols with alcoholic ammonia solutions as eluents can be used in two-dimensional chromatography. Fig. 2 shows the separation of twelve phenols with 95% methanol and 0.5 *M* ammonia in 50% ethanol as eluents. The behaviour of phenols with acetic acid in cyclohexane as eluent is very interesting from an analytical point of view. In fact, the *ortho*-isomers can be separated from the other isomers, *e.g.*, *o*-chlorophenol from the other two isomers, *o*-bromophenol from *p*-bromophenol, and 2,6-dichlorophenol and 2,6-dimethylphenol from their isomers. Also, the separation of phenol from guaiacol and of these two from dihydroxy- and trihydroxy-benzene is very interesting.

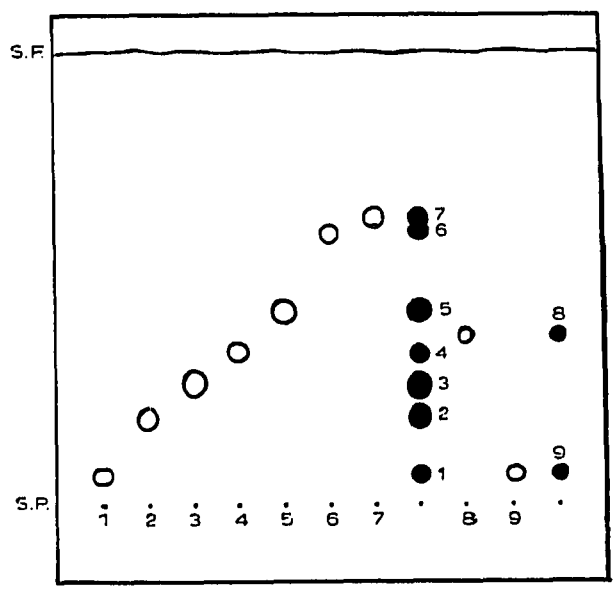

Fig. 1. Thin-layer chromatogram of chloro- and nitro-phenols on Bio-Rad AG 3-X4A in the free base form. Three successive developments in 95% methanol. Migration distance 12 cm. Spots: (1) 2,6-dichlorophenol; (2) 2,5-dichlorophenol; (3) 2,4-dichlorophenol; (4) 3,5-dichlorophenol; (5) 3,4-dichlorophenol; (6) *o*-chlorophenol; (7) *p*-chlorophenol; (8) *m*-nitrophenol; (9) *p*-nitrophenol. S.F. = Solvent front; S.P. = starting point. Black spots: mixture.

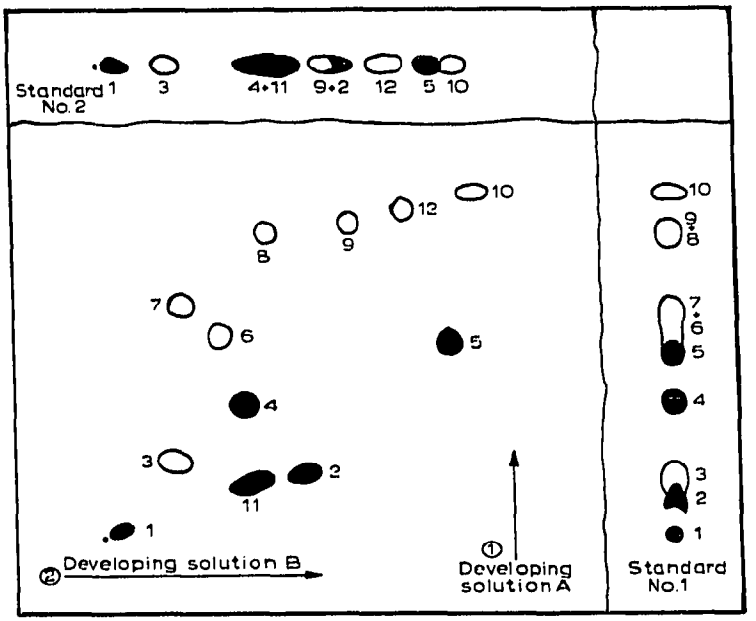

Fig. 2. Two-dimensional separation of phenols on layers of Bio-Rad AG 3-X4A in the free base form. 1, Two successive developments in 95% methanol (solution A); 2, two successive developments with 0.5 *M* NH_3 in 50% ethanol (solution B). Migration distance 12 cm in both directions. Black spots: phenols that are coloured before spraying. Spots: (1) 2-amino-4,6-dinitrophenol; (2) 2-amino-4-nitrophenol; (3) 2,5-dichlorophenol; (4) 2-amino-5-nitrophenol; (5) 4-amino-2-nitrophenol; (6) *o*-chlorophenol; (7) *p*-chlorophenol; (8) phenol; (9) *o*-aminophenol; (10) *p*-aminophenol; (11) *o*-nitrophenol; (12) *m*-aminophenol. Black spots: mixture.

PEI-cellulose and DEAE-cellulose

In Table II are reported the R_F values of the same phenols on PEI-cellulose layers on elution with water, water–methanol, 0.1 *M* sodium hydrogen carbonate solution and acetic acid in cyclohexane.

TABLE II

R_F VALUES OF PHENOLS ON PEI-CELLULOSE THIN LAYERS

Eluents: (1) water; (2) water–methanol (1:4); (3) 0.1 *M* sodium hydrogen carbonate solution; (4) acetic acid–cyclohexane (7:93).

Phenol	*Eluent*			
	1	*2*	*3*	*4*
Phenol	0.85	0.96	0.85	0.24
Guaiacol	0.84	0.96	0.83	0.73
Hydroquinone	0.82	0.95	0.78	0.00
Catechol	0.82	0.95	0.78	0.00
Resorcinol	0.77	0.94	0.75	0.00
Orcinol	0.72	0.94	0.68	0.00
Pyrogallol	0.72	0.87	0.69	0.00
Phloroglucinol	0.58	0.89	0.57	0.00
Pyrocatechic acid	0.05	0.58	0.31	0.00

TABLE II *(continued)*

Phenol	*Eluent*			
	1	*2*	*3*	*4*
Gallic acid	0.03	0.34	0.20	0.00
o-Cresol	0.84	0.95	0.83	0.54
m-Cresol	0.84	0.95	0.83	0.38
2,6-Dimethylphenol	0.07	0.90	0.06	0.91
2,3-Dimethylphenol	0.77	0.95	0.76	0.63
3,4-Dimethylphenol	0.76	0.95	0.75	0.45
3,5-Dimethylphenol	0.76	0.95	0.75	0.52
m-Nitrophenol	0.74	0.92	0.69	0.03
o-Nitrophenol	n.d.*	0.92	n.d.	n.d.
p-Nitrophenol	0.72	0.92	0.66	0.01
2,5-Dinitrophenol	0.22	0.72	0.40	0.59
2,4-Dinitrophenol	0.02	0.12	0.33	0.35
2,6-Dinitrophenol	0.01	0.09	0.35	0.23
Picric acid	0.00	0.02	0.21	0.00
m-Aminophenol	0.85	0.93	0.81	0.00
o-Aminophenol	0.89	0.93	0.83	0.00
p-Aminophenol	0.91	0.93	0.89	0.00
5-Aminosalicylic acid	0.46	0.26	0.55	0.00
4-Aminosalicylic acid	0.02	0.10	0.43	0.00
3-Hydroxyanthranilic acid	0.05	0.44	0.50	0.00
2-Aminophenol-4-sulphonic acid	0.04	0.03	0.48	0.00
4-Amino-2-nitrophenol	0.70	0.82	0.63	0.03
2-Amino-5-nitrophenol	0.47	0.81	0.40	0.00
2-Amino-4-nitrophenol	0.58	0.82	0.51	0.00
2-Amino-4,6-dinitrophenol	0.01	0.09	0.22	0.05
2-Amino-3,4,6-trichlorophenol	0.30	0.92	0.24	0.56
p-Chlorophenol	0.75	0.95	0.75	0.31
m-Chlorophenol	0.75	0.95	0.74	0.32
o-Chlorophenol	n.d.	0.95	n.d.	0.81
p-Bromophenol	0.70	0.95	0.69	0.32
o-Bromophenol	n.d.	0.95	n.d.	0.86
3,4-Dichlorophenol	0.59	0.95	0.58	0.33
3,5-Dichlorophenol	0.58	0.95	c.s.**	0.49
2,4-Dichlorophenol	n.d.	0.95	0.42	0.82
2,3-Dichlorophenol	n.d.	0.95	0.41	0.82
2,5-Dichlorophenol	n.d.	0.95	0.42	0.82
2,6-Dichlorophenol	n.d.	0.95	0.45	0.94
β-Naphthol	0.45	0.88	0.41	0.31
α-Naphthol	0.49	0.89	0.44	0.43
1,5-Naphthalenediol	0.36	0.92	0.27	0.00
2-Hydroxy-1-naphthaldehyde	0.05	0.88	0.03	0.95
7-Amino-2-naphthol	0.57	0.84	0.37	0.00
1-Amino-7-naphthol	0.59	0.85	0.40	0.00
5-Amino-1-naphthol	0.57	0.84	0.40	0.00
4-Hydroxydiphenylamine	0.57	0.95	0.52	0.15
3-Hydroxydiphenylamine	0.47	0.95	0.43	0.14
2,4-Dinitro-4'-hydroxydiphenylamine	0.32	0.88	0.18	0.04
2,4-Dinitro-4'-hydroxydiphenylamine-3'-sulphonic acid	0.00	0.03	0.13	0.00
4-Hydroxyazobenzene	0.19	0.92	0.15	0.36

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

Most phenols are eluted with water (column 1), with the exception of those with marked acidic characteristics, of which the most retained are those with a carboxylic or a sulphonic group in the aromatic ring.

On elution with water–alcohol mixtures (column 2), an increase in the R_F values is observed for all of the phenols with the exception of 5-aminosalicylic and, to a lesser extent, 2-aminophenol-4-sulphonic acid whose R_F values are lower than in water.

Some phenols behave in a peculiar manner on PEI-cellulose when eluted with water; diffuse spots are obtained so that their detection is impossible. This effect also occurs on DEAE-cellulose and on microcrystalline cellulose and may be decreased by the addition of alcohol.

The chromatographic behaviour of most phenols on PEI-cellulose when eluted with water and water–alcohol solutions is similar to that observed on microcrystalline cellulose with the same eluents. The phenols with a carboxylic or a sulphonic group in the aromatic ring and generally those with $pK_a < 7$ are more retained on PEI-cellulose.

Influence of the pH of the eluent. On elution with alkaline 0.1 *M* sodium hydrogen carbonate solution, a decrease in the R_F values compared with the behaviour on elution with water is observed for those phenols with pK_a values > 7. Such behaviour can be ascribed to the increase of the ionic strength of the eluent, as observed in the case of primary aromatic amines[33]. For phenols with marked acidic characteristics ($pK_a < 7$), a remarkable increase in the R_F values is observed, corresponding to a sharp increase in the proportion of the deprotonated form of these compounds and with the presence of hydrogen carbonate ions in the eluent, which replace the phenate ion by means of an ion-exchange process. As an increase in the proportion of the deprotonated form should also be observed for those phenols with pK_a values between 7 and 8, the only explanation for the decrease in the R_F values of these compounds is the lower pH of the layer with respect to that of the eluent[33].

On elution with acetic acid in cyclohexane, many phenols remain at the starting point. Those which are eluted are those having R_F values > 0 on Bio-Rad AG 3-X4A layers under the same elution conditions (see column 5 in Table I).

The chromatographic behaviour of the phenols on DEAE-cellulose layers is similar to that on PEI-cellulose under the same conditions.

Analytical applications. On PEI-cellulose layers, many separations can be effected. With 0.1 *M* sodium hydrogen carbonate solution as eluent, hydroquinone, pyrogallol, phloroglucinol and gallic and pyrocatechic acids can be separated. This interesting separation is reported in Fig. 3. With water–methanol (1:4), 5-aminosalicylic, 4-aminosalicylic and 3-hydroxyanthranilic acids can be separated.

With acetic acid in cyclohexane as eluent the following separations were effected: *o*-chlorophenol from the *m*- and *p*-isomers, *o*-bromophenol from *p*-bromophenol, α-naphthol from β-naphthol and 2,6-dichlorophenol from 2,4-dichlorophenol and 3,4-dichlorophenol.

With water, as shown in the chromatogram in Fig. 4, the following separations were effected: (1) α-naphthol, 2-hydroxy-1-naphthaldehyde, 1-amino-7-naphthol and 1,5-naphthalenediol; (2) 3-hydroxydiphenylamine, 4-hydroxydiphenylamine, 4-hydroxyazobenzene, 2,4-dinitro-4′-hydroxydiphenylamine and 2,4-dinitro-4′-hydroxydiphenylamine-3′-sulphonic acid; and (3) 2-amino-3,4,6-trichlorophenol, 2,5-dinitro-

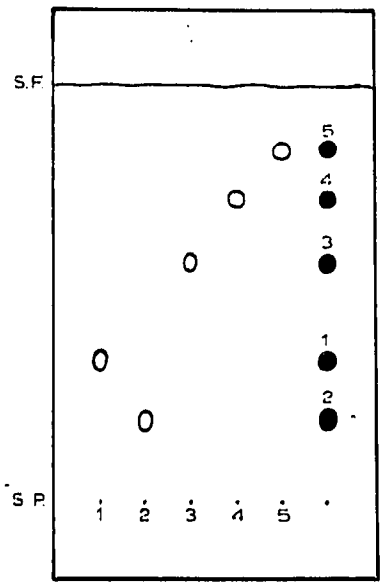

Fig. 3. Thin-layer chromatogram of phenols on PEI-cellulose. Eluent: 0.1 *M* $NaHCO_3$. Spots: (1) pyrocatechic acid; (2) gallic acid; (3) phloroglucinol; (4) pyrogallol; (5) hydroquinone. Black spots: mixture.

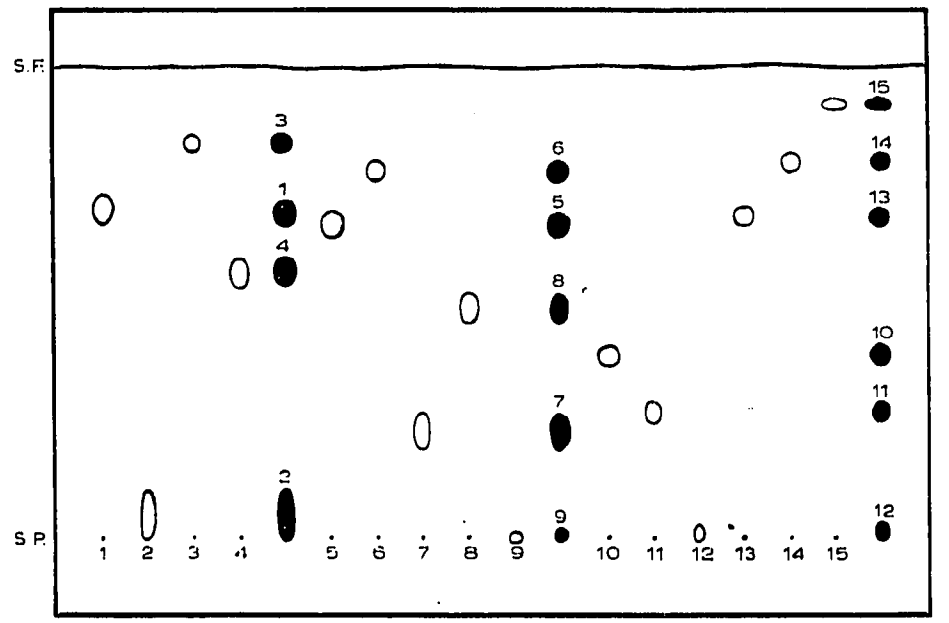

Fig. 4. Thin-layer chromatogram of phenols on PEI-cellulose. Two successive developments in water. Migration distance 12.5 cm. Spots: (1) α-naphthol; (2) 2-hydroxy-1-naphthaldehyde; (3) 1-amino-7-naphthol; (4) 1,5-naphthalenediol; (5) 3-hydroxydiphenylamine; (6) 4-hydroxydiphenylamine; (7) 4-hydroxyazobenzene; (8) 2,4-dinitro-4′-hydroxydiphenylamine; (9) 2,4-dinitro-4′-hydroxydiphenylamine-3′-sulphonic acid; (10) 2-amino-3,4,6-trichlorophenol; (11) 2,5-dinitrophenol; (12) 2-amino-4,6-dinitrophenol; (13) 2-amino-5-nitrophenol; (14) 2-amino-4-nitrophenol; (15) 4-amino-2-nitrophenol. Black spots: mixture.

phenol, 2-amino-4,6-dinitrophenol, 2-amino-5-nitrophenol, 2-amino-4-nitrophenol and 4-amino-2-nitrophenol. It should be noted that the last separation cannot be effected either on microcrystalline cellulose or on DEAE-cellulose layers, because on the former 2-amino-4-nitrophenol and 4-amino-2-nitrophenol exhibit similar R_F values (0.59 and 0.62, respectively), while on the latter 2-amino-4-nitrophenol and 2-amino-5-nitrophenol show the same chromatographic behaviour (R_F = 0.39 for both phenols).

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