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

III. AG 1-X4 AND BD-CELLULOSE

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SUMMARY

Fifty-eight phenols were chromatographed by elution on thin layers of anion exchangers with aqueous, aqueous–organic and organic solutions. The influence of the matrix and the functional group of the exchanger and of the eluent on the adsorption of the phenols was investigated. Analytical applications of these exchangers are reported.

INTRODUCTION

The chromatographic behaviour of 58 phenols on thin layers of anion exchangers (AG 3-X4A, PEI-cellulose and DEAE-cellulose) and cation exchangers (Rexyn 102 and Dowex 50-X4) was described in previous papers[1,2].

This paper describes studies with AG 1-X4 and BD-cellulose (anion exchangers) in order to have a complete picture of the chromatographic behaviour of this class of compounds on ion exchangers and to investigate the influence on the retention mechanism both of the matrix and of the functional group of the exchanger. The study on benzoylated DEAE-cellulose (BD-cellulose) is very interesting from this point of view, as such an exchanger has the same functional group as DEAE-cellulose and a different matrix owing to the benzoylation of the hydroxyl groups of cellulose[3].

EXPERIMENTAL

The solutions of the phenols were prepared as previously described[1]. The AG 1-X4 (Bio-Rad Labs., Richmond, Calif., U.S.A.) layers in different forms and the BD-cellulose (Serva, Heidelberg, G.F.R.) layers were prepared by mixing 3 g of resin with 9 g of microcrystalline cellulose (E. Merck, Darmstadt, G.F.R.) in 50 ml of water. AG 1-X4 in the acetate and hydroxide forms was obtained from the commercial product (200–400 mesh) in the chloride form by treatment with 0.5 *M* sodium acetate and 0.5 *M* sodium hydroxide solution, respectively, until the chloride ion had completely disappeared.

The polystyrene-based exchangers, as AG 1-X4, were rinsed with water and methanol and then dried at room temperature.

The detection of the phenols was carried out as previously described[1]. The chromatographic measurements were carried out at 25 ± 0.5°. The migration distance was 11 cm unless otherwise stated.

RESULTS AND DISCUSSION

Influence of the functional group of the exchanger

On Dowex 50-X4 in the acid and the sodium salt forms, on eluting with methanol or ethanol, the phenols with no amino groups have $R_F \geqslant 0.95$ (ref. 2). Such behaviour has been found, on eluting with ethanol, on columns of Amberlite XAD-2, a polystyrene-based resin without functional groups[4].

From the data on Dowex 50-X4 and Amberlite XAD-2, it can be concluded that the exchanger matrix (alone or with a sulphonic group) does not give rise to interactions with the phenols such that their detection could be possible with column (K_d) or thin-layer (R_F) chromatographic parameters. On AG 1-X4 (Cl^-), on the contrary, as can be seen in Table I (column 1), the phenols are retained to a greater extent and some of them also in a different way. The presence of the functional group therefore seems to determine the retention of the phenols on this last exchanger.

A behaviour similar to that of the phenols on AG 1-X4 (Cl^-) is also observed, on eluting with methanol, on AG 3-X4A (Cl^-), a polystyrene-based resin with the functional group $-\overset{+}{N}(R)(R)-H\ Cl^-$, which differs from that of AG 1-X4 (Cl^-) in having a hydrogen atom instead of an alkyl group.

The comparison between these two exchangers indicates that the phenol functional group interactions are independent of the number of alkyl groups bound to the quaternary nitrogen atom.

Some workers[5,6] have assumed that the adsorption of the phenol on columns of polystyrene-based anion exchangers, on eluting with organic solvents, may be correlated with the establishment of hydrogen bonds and/or π-bonds between the phenol and the counter ion of the resin. We considered it useful, therefore, to study the behaviour of phenols on AG 1-X4 in the chloride, acetate and hydroxide forms in order to explain the role of the counter ion in the adsorption mechanism. Table I gives the R_F values of the phenols on the exchanger in the three different forms on eluting with methanol (eluent 1). On AG 1-X4 (Cl^-), the R_F values are, in most instances, higher than those relating to the exchanger in the acetate form and overall greater than those on the exchanger in the hydroxide form. The R_F sequence on the exchanger in the three forms is therefore $R_F\,(Cl^-) > R_F\,(CH_3COO^-) \gg R_F\,(OH^-)$.

As regards the influence of the substituents on the retention of the phenols, on changing from the exchanger in the chloride form to that in the hydroxide form, the greatest differences are shown by those compounds with higher acidities than the phenol.

It is therefore evident that the differences in the chromatographic behaviour of the phenols, on changing counter ion of the resin, cannot be attributed to the

TABLE I

R_F VALUES OF PHENOLS ON THIN LAYERS OF AG 1-X4 IN THE CHLORIDE, ACETATE AND HYDROXIDE FORMS

Eluents: (1) methanol; (2) 1 *M* acetic acid in methanol; (3) 10 *M* acetic acid in methanol.

Phenol	*AG 1-X4 (Cl^-), eluent 1*	*AG 1-X4 (CH_3COO^-)*			*AG 1-X4 (OH^-), eluent 1*
		Eluent 1	*Eluent 2*	*Eluent 3*	
Phenol	0.65	0.61	0.61	0.68	0.45
Guaiacol	0.68	0.67	0.69	0.78	0.60
Hydroquinone	0.45	0.36	0.40	0.45	e.s.
Catechol	0.46	0.34	0.39	0.52	0.12
Resorcinol	0.40	0.27	0.33	0.41	0.14
Orcinol	0.42	0.32	0.35	0.43	0.19
Pyrogallol	0.26	0.08	0.19	0.29	0.01
Phloroglucinol	0.19	0.09	0.15	0.18	0.01
Pyrocatechuic acid	0.01	0.00	0.12	0.30	0.00
Gallic acid	0.00	0.00	0.06	0.19	0.00
o-Cresol	0.61	0.60	0.60	0.70	0.57
m-Cresol	0.61	0.60	0.61	0.71	0.54
2,6-Dimethylphenol	0.69	0.68	0.69	0.78	0.67
2,3-Dimethylphenol	0.60	0.60	0.61	0.72	0.55
3,4-Dimethylphenol	0.62	0.61	0.61	0.73	0.56
3,5-Dimethylphenol	0.61	0.60	0.61	0.72	0.56
m-Nitrophenol	0.48	0.34	0.42	0.57	0.04
o-Nitrophenol	e.s.*	0.07	0.59	0.77	0.01
p-Nitrophenol	0.09	0.04	0.35	0.53	0.01
2,5-Dinitrophenol	0.03	0.00	0.08	0.59	0.00
2,4-Dinitrophenol	0.00	0.00	0.00	0.26	0.00
2,6-Dinitrophenol	0.00	0.00	0.00	0.32	0.00
Picric acid	0.00	0.00	0.00	0.00	0.00
m-Aminophenol	0.43	0.40	0.60	0.95	0.27
o-Aminophenol	0.46	0.44	0.66	0.96	0.33
p-Aminophenol	0.84	0.64	0.70	0.97	0.41
5-Aminosalicylic acid	0.00	0.00	0.08	0.65	0.00
4-Aminosalicylic acid	0.00	0.00	0.03	0.37	0.00
3-Hydroxyanthranilic acid	0.00	0.00	0.00	0.50	0.00
2-Aminophenol-4-sulphonic acid	0.00	0.00	0.00	0.00	0.00
4-Amino-2-nitrophenol	0.43	0.40	0.53	0.88	0.02
2-Amino-5-nitrophenol	0.24	0.14	0.25	0.39	0.00
2-Amino-4-nitrophenol	0.10	0.02	0.28	0.55	0.00
2-Amino-4,6-dinitrophenol	0.01	0.00	0.01	0.30	0.00
2-Amino-3,4,6-trichlorophenol	0.12	0.00	0.42	0.61	0.00
p-Chlorophenol	0.56	0.44	0.55	0.66	0.29
m-Chlorophenol	0.55	0.40	0.52	0.64	0.15
o-Chlorophenol	0.55	0.37	0.53	0.65	0.10
p-Bromophenol	0.52	0.40	0.46	0.60	0.21
o-Bromophenol	0.51	0.35	0.43	0.58	0.08
3,4-Dichlorophenol	0.49	0.30	0.40	0.58	0.06
3,5-Dichlorophenol	0.49	0.21	0.40	0.58	0.04
2,4-Dichlorophenol	0.48	0.19	0.39	0.58	0.03
2,3-Dichlorophenol	0.48	0.15	0.38	0.56	0.03
2,5-Dichlorophenol	0.48	0.10	0.39	0.58	0.02
2,6-Dichlorophenol	e.s.	0.06	0.54	0.70	0.01

(Continued on p. 242)

TABLE I *(continued)*

Phenol	*AG 1-X4 (Cl^-), eluent 1*	*AG 1-X4 (CH_3COO^-)*			*AG 1-X4 (OH^-), eluent 1*
		Eluent 1	*Eluent 2*	*Eluent 3*	
β-Naphthol	0.39	0.35	0.39	0.54	0.24
α-Naphthol	0.38	0.34	0.38	0.51	0.23
1,5-Naphthalenediol	0.16	0.10	0.14	0.21	0.04
2-Hydroxy-1-naphthaldehyde	e.s.	0.20	0.53	0.77	0.01
7-Amino-2-naphthol	0.23	0.21	0.35	0.90	0.13
1-Amino-7-naphthol	0.23	0.20	0.34	0.89	0.11
5-Amino-1-naphthol	0.24	0.20	0.32	0.89	0.11
4-Hydroxydiphenylamine	0.32	0.30	0.33	0.55	0.26
3-Hydroxydiphenylamine	0.27	0.25	0.30	0.47	0.18
2,4-Dinitro-4'-hydroxydiphenylamine	0.36	0.31	0.35	0.63	0.23
2,4-Dinitro-4'-hydroxydiphenyl-amine-3'-sulphonic acid	0.00	0.00	0.00	0.00	0.00
4-Hydroxyazobenzene	0.36	0.28	0.36	0.63	0.04

* e.s. = elongated spot.

different Van der Waals' radii of the counter ions, as in this instance the lowest R_F values should be observed on the exchanger in the acetate form; the interactions between the phenols and the counter ion are not correlated, at least in methanol, with the establishment of π-bonds. A more reliable assumption therefore seems to be that based on the different electronegativities of the counter ions. The sequence of the R_F values on the exchanger in the three forms and the stronger retention of those phenols with marked acidic characteristics by the exchanger in the hydroxide form support the assumption of the establishment of hydrogen bonds between the phenols and the counter ion.

For the exchanger in the acetate form and overall in the hydroxide form, however, in our opinion also an acid–base reaction of the following type cannot be excluded:

$$R^+X^- + ArOH \rightleftarrows R^+ArO^- + HX \qquad (1)$$

where ArOH is the phenol, R^+ the resin with a quaternary amino group and X^- the counter ion. Reaction 1 was confirmed by the presence of acetic acid in the methanol solution of 3,4-dichlorophenol after passage through a column of AG 1-X4 (CH_3COO^-).

As regards the phenol–counter ion interactions, the R_F sequences of the phenols on eluting with methanol are very interesting (see Table I).

In fact, while on the exchanger in the hydroxide form the R_F sequence agrees with the acid–base characteristics of the phenols (the greater the pK_a, the smaller is the R_F value), on the exchanger in the acetate form and overall in the chioride form there is no correlation between the R_F and pK_a of the compound.

On AG 1-X4 (Cl^-), the alkyl and dialkylphenols, the halogenated phenols, α- and β-naphthol and the polyhydroxybenzenes are retained more than phenol, despite their pK_a values, which in some instances are higher and in others lower than that of phenol. Of the above compounds, only the polyhydroxybenzenes can give rise to hydrogen bonds with two or more counter ions and therefore they may be

expected to be retained more than phenol. Considering phenol ($R_F = 0.65$), in fact, it should be noted that on the introduction of a second hydroxyl group the R_F values decrease to the range 0.40–0.46 (depending on the position of the substituent in the ring) and that the introduction of a third hydroxyl group decreases the R_F values to the range 0.19–0.26.

The stronger retention of the other phenols cannot be explained on the basis of additional interactions due to hydrogen bonds and can therefore be ascribed to Van der Waals' interactions with the exchanger matrix. The lack of retention of the same phenols on Dowex 50-X4 and Amberlite XAD-2 on eluting with methanol disagrees with such an assumption; it must be borne in mind, however, that the interactions of the phenols with the exchanger matrix may be increased by the establishment of hydrogen bonds with the counterion of the resin.

Influence of the eluent

As regards the role played by the eluent in the adsorption of the phenols, interesting indications can be drawn from a study of the chromatographic behaviour of these compounds on an exchanger in one form using different types of eluent. We used AG 1-X4 (CH_3COO^-), as the retention of the phenols is very close to that observed on AG 1-X4 (Cl^-), but, contrary to the chloride form, it is possible to employ solutions that contain acetic acid as eluent. The number of phenols that may give rise to exchange reactions is noticeably decreased by the presence of acetic acid and therefore the adsorption process can be observed.

Methanol–acetic acid mixtures. Table I gives the R_F values of the phenols on eluting with methanol–acetic acid mixtures on AG 1-X4 (CH_3COO^-) thin layers (columns 2 and 3).

With such eluents, compounds with a protonated amino group are less retained than with methanol alone, while those whose acid–base characteristics are similar to that of phenol are little affected by an increase in the amount of acetic acid in the eluent. The phenols whose pK_a values are much lower than that of phenol move noticeably and to a greater extent than those with less acidic characteristics. The only exceptions are picric acid and by the two phenols with a sulphonic group in the ring, which remain at the starting point independently of the acetic acid concentration in the eluent. The behaviour of these last compounds is correlated with the prevalence of the ion-exchange process over the adsorption process.

The R_F trends of some phenols with increasing percentages of acetic acid in the eluent are reported in Fig. 1. Such trends refer to phenols whose affinities towards the exchanger change from high values (curves 6 and 7) to small values (curves 2 and 3), and are common to most compounds.

In each curve in Fig. 1 there is an increase in the R_F value until a 50% acetic acid content is reached. For higher percentages of acetic acid the trends are different because, for some phenols, constancy or a small increase in the R_F value is observed while for others a decrease is involved. This last trend is observed in for polyhydroxybenzenes, particularly those with a carboxylic group in the ring.

The increase in the R_F values of the phenols may be attributed to the stronger interactions of acetic acid than methanol with the counter ion of the resin. The decrease in the R_F values of polyhydroxybenzenes for acetic acid concentrations in the eluent above 50% must be ascribed to their lower solubility in such eluents.

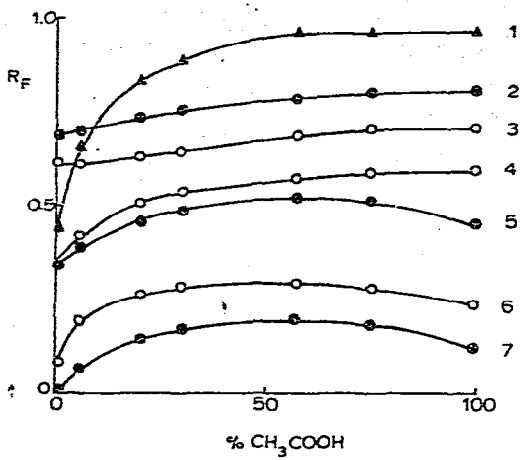

Fig. 1. R_F values of phenols on AG 1-X4 (CH_3COO^-) thin layers *versus* acetic acid content in the eluent. Curves: 1 = *o*-aminophenol; 2 = 2,6-dimethylphenol; 3 = phenol; 4 = *m*-nitrophenol; 5 = catechol; 6 = pyrogallol; 7 = gallic acid.

Dinitrophenols are the only compounds whose trend differs from those reported in Fig. 1. These compounds remain at the starting point up to a 30% acetic acid concentration, and for higher percentages a progressive increase of the R_F values is observed; such an increase is clearly correlated with a smaller degree of dissociation of the phenolic hydroxyl group and therefore with a decrease in the anion-exchange reaction.

1 M acetic in methanol–water mixtures. With increasing amounts of water in methanol, owing to the higher degree of dissociation of the phenolic hydroxyl group, the behaviour of many compounds is remarkably affected by the anion-exchange process. In order to reduce the anion-exchange process and to observe

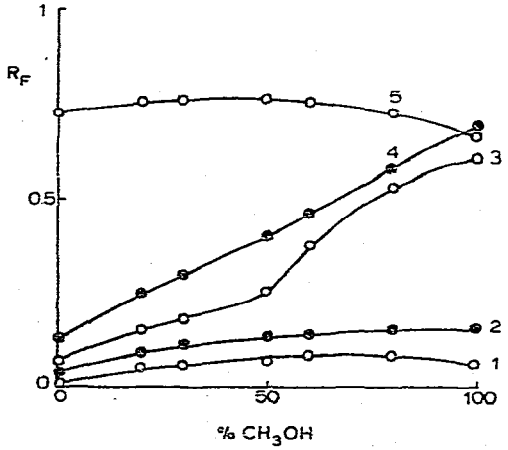

Fig. 2. R_F values of phenols on AG 1-X4 (CH_3COO^-) thin layers *versus* methanol content in the eluent. Curves: 1 = gallic acid; 2 = phloroglucinol; 3 = *o*-cresol; 4 = guaiacol; 5 = *o*-aminophenol.

the adsorption process, a constant amount of acetic acid was added to the water–methanol mixtures.

Fig. 2 shows some unusual trends of the R_F values with increasing percentages of methanol in the eluent. Such trends can be explained on the basis of interactions of the phenols with the matrix and with the counter ion of the resin. The addition of methanol to the eluent causes a decrease in the interactions between the phenols and the matrix of the exchanger and therefore the retention of these compounds, on eluting with water–methanol mixtures at a high methanol content, can be ascribed mainly to interactions with the counter ion of the resin. Depending on the strength of these last interactions, the trends of curves 1–4 can be obtained.

The trend of curve 1 can also be ascribed to a decrease in the solubility of the phenol in the mobile phase as the concentration of methanol in the eluent is increased. A similar behaviour is shown, in fact, by *o*-aminophenol (curve 5). This compound contains a protonated amino group and is less soluble in methanol than in water or in water–methanol mixtures at low methanol contents; its chromatographic behaviour is therefore affected by its solubility in the eluent.

The influence of this last parameter on the chromatographic behaviour of polyhydroxybenzenes can be confirmed by a comparison of the R_F values obtained on eluting with 10 *M* acetic acid in water and in methanol.

Unlike the other phenols, in fact, hydroquinone, resorcinol and pyrocatechuic acid have the same R_F values in both eluents, whereas pyrogallol, phloroglucinol and gallic acid exhibit lower R_F values on eluting with acetic acid–methanol than with acetic acid–water solutions.

Influence of the exchanger matrix

Table II gives the R_F values of phenols on BD-cellulose layers on eluting with water, water–methanol mixtures, methanol and 1 and 3 *M* acetic acid.

On eluting with water, most phenols remain almost at the starting point ($R_F \leqslant 0.05$), with the exception of cresols ($R_F = 0.09$), phenol ($R_F = 0.10$), polyhydroxybenzenes ($R_F = 0.14$–0.38) and the three aminophenols ($R_F = 0.22$–0.45). These results indicate that only the polyhydroxybenzenes and the aminophenols are less retained than phenol.

As regards the influence of the position of the substituent in the ring on the chromatographic behaviour of dihydroxybenzenes and aminophenols, the following sequence is observed: $R_{F(\text{para})} > R_{F(\text{meta})} \geqslant R_{F(\text{ortho})}$.

The replacement of a hydroxyl group with an amino group involves an increase in the overall R_F value if such replacement occurs in the para and meta positions.

With water–methanol mixtures, as the methanol concentration is increased, an increase in the R_F values of most phenols is observed; the only exceptions are those phenols with marked acidic characteristics (dinitrophenols, and phenols with a carboxylic or a sulphonic group in the ring), which remain at the starting point. With methanol, a general levelling of the R_F values, which in most instances lie in the range 0.75–0.91, is achieved. The chromatographic behaviour of phenols on this exchanger is completely different from that observed on PEI- and DEAE-cellulose under the same experimental conditions[1]. On these two last exchangers, in fact, on eluting with water most phenols exhibit high R_F values and different behaviour of

TABLE II

R_F VALUES OF PHENOLS ON THIN LAYERS OF BD-CELLULOSE

Eluents: (1) water; (2) water–methanol (4:1); (3) water–methanol (1:1); (4) water–methanol (3:7); (5) methanol: (6) 1 *M* acetic acid in water; (7) 3 *M* acetic acid in water.

Phenol	*Eluent*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Phenol	0.10	0.19	0.39	0.64	0.90	0.30	0.40
Guaiacol	0.05	0.14	0.31	0.54	0.86	0.16	0.31
Hydroquinone	0.38	0.44	0.63	0.77	0.91	0.56	0.73
Catechol	0.20	0.30	0.50	0.68	0.87	0.40	0.57
Resorcinol	0.22	0.33	0.53	0.71	0.89	0.45	0.58
Orcinol	0.14	0.20	0.47	0.68	0.88	0.31	0.48
Pyrogallol	0.33	0.41	0.56	0.68	0.85	0.51	0.65
Phloroglucinol	0.34	0.44	0.61	0.76	0.88	0.55	0.69
Pyrocatechuic acid	0.04	0.05	0.10	0.16	e.s.*	0.36	0.56
Gallic acid	0.04	0.04	0.09	0.14	e.s.	0.38	0.57
o-Cresol	0.09	0.13	0.30	0.56	0.88	0.12	0.21
m-Cresol	0.09	0.14	0.32	0.58	0.89	0.11	0.25
2,6-Dimethylphenol	0.00	0.09	0.19	0.48	0.86	0.00	0.12
2,3-Dimethylphenol	0.01	0.04	0.15	0.43	0.83	0.06	0.12
3,4-Dimethylphenol	0.01	0.04	0.19	0.48	0.86	0.08	0.15
3,5-Dimethylphenol	0.02	0.06	0.26	0.53	0.88	0.06	0.13
m-Nitrophenol	0.01	0.06	0.24	0.49	0.82	0.09	0.17
o-Nitrophenol	0.00	0.03	0.18	0.34	0.77	0.05	0.14
p-Nitrophenol	0.00	0.02	0.15	0.30	0.79	0.08	0.17
2,5-Dinitrophenol	0.00	0.00	0.00	0.01	0.08	0.00	0.06
2,4-Dinitrophenol	0.00	0.00	0.00	0.00	0.03	0.00	0.03
2,6-Dinitrophenol	0.00	0.00	0.00	0.00	0.02	0.00	0.03
Picric acid	0.00	0.00	0.00	0.00	0.01	0.00	0.00
m-Aminophenol	0.32	0.43	0.62	0.69	0.77	0.89	0.90
o-Aminophenol	0.22	0.34	0.52	0.63	0.75	0.89	0.90
p-Aminophenol	0.45	0.50	0.66	0.70	0.77	0.95	0.95
5-Aminosalicylic acid	0.00	0.05	0.05	0.06	0.04	0.80	0.89
4-Aminosalicylic acid	0.00	0.03	0.03	0.04	0.03	0.16	0.39
2-Aminophenol-4-sulphonic acid	0.01	0.01	0.01	0.02	0.02	0.67	0.80
4-Amino-2-nitrophenol	0.03	0.11	0.27	0.43	0.68	0.64	0.83
2-Amino-5-nitrophenol	0.01	0.07	0.20	0.41	0.69	0.10	0.21
2-Amino-4-nitrophenol	0.01	0.05	0.15	0.33	0.65	0.28	0.55
2-Amino-4,6-dinitrophenol	0.00	0.00	0.00	0.01	0.01	0.00	0.04
2-Amino-3,4,6-trichlorophenol	0.00	0.01	0.04	0.26	0.67	0.00	0.02
p-Chlorophenol	0.04	0.07	0.26	0.53	0.83	0.09	0.17
m-Chlorophenol	0.02	0.06	0.26	0.53	0.83	0.09	0.17
o-Chlorophenol	0.02	0.06	0.26	0.52	0.82	0.08	0.18
p-Bromophenol	0.02	0.05	0.21	0.52	0.83	0.06	0.12
o-Bromophenol	0.02	0.06	0.23	0.53	0.83	0.04	0.14
3,4-Dichlorophenol	0.01	0.02	0.10	0.42	0.83	0.03	0.06
3,5-Dichlorophenol	0.01	0.02	0.09	0.38	0.82	0.03	0.06
2,4-Dichlorophenol	0.00	0.02	0.09	0.38	0.82	0.03	0.08
2,3-Dichlorophenol	0.00	0.02	0.09	0.38	0.81	0.03	0.06
2,5-Dichlorophenol	0.00	0.01	0.09	0.39	0.82	0.03	0.09
2,6-Dichlorophenol	0.00	0.01	0.08	0.04	0.77	0.03	0.09
β-Naphthol	0.00	0.01	0.08	0.29	0.72	0.01	0.03
α-Naphthol	0.00	0.01	0.04	0.24	0.69	0.00	0.02
1,5-Naphthalenediol	0.01	0.03	0.11	0.31	0.70	0.03	0.05
2-Hydroxy-1-naphthaldehyde	0.00	0.00	0.03	0.17	0.64	0.01	0.03

TABLE II *(continued)*

Phenol	*Eluent*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
7-Amino-2-naphthol	0.02	0.04	0.15	0.42	0.62	0.59	0.81
1-Amino-7-naphthol	0.02	0.05	0.18	0.46	0.63	0.62	0.81
5-Amino-1-naphthol	0.01	0.03	0.12	0.34	0.58	0.40	0.71
4-Hydroxydiphenylamine	0.00	0.01	0.04	0.26	0.72	0.04	0.05
3-Hydroxydiphenylamine	0.00	0.01	0.04	0.24	0.71	0.00	0.02
2,4-Dinitro-4′-hydroxydiphenylamine	0.00	0.00	0.00	0.03	0.38	0.00	0.00
2,4-Dinitro-4′-hydroxydiphenylamine-3′-sulphonic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Hydroxyazobenzene	0.00	0.00	0.01	0.22	0.60	0.00	0.00

* e.s. = elongated spot.

isomers is not observed. Further, the dihydroxybenzenes and 1,5-naphthalenediol have R_F values that are similar to or lower than those of phenol and α- or β-naphthol, in contrast to their behaviour on BD-cellulose. From a comparison between DEAE- and BD-cellulose, the influence of the exchanger matrix on the retention of the compounds can be seen. The benzoylation of the hydroxyl groups of cellulose seems to be the parameter that determines the retention of most phenols; the functional group, on the contrary, plays an important role only with those phenols with marked acidic characteristics. As the retention on BD-cellulose is ascribed to its matrix, it is interesting to compare the data on such an exchanger with those on Dowex 50-X4 (H^+)[2]; on the latter exchanger, in fact, the retention was found to be strongly correlated with the presence of the polystyrene matrix on eluting with water or water–methanol mixtures at low methanol contents.

The correlations between R_F values and methanol percentages on the two exchangers are similar, but on Dowex 50-X4 (H^+) the R_F values, on eluting with methanol, are higher than those on BD-cellulose. Further, on the polystyrene-based exchanger, the chromatographic behaviour of the polyhydroxybenzenes and 1,5-naphthalenediol with respect to phenol and α- or β-naphthol is different from that on BD-cellulose.

Such differences may be explained by assuming that, with this last exchanger, the adsorption mechanism is determined other than by the interactions of the compound with the aromatic ring of the exchanger and also by the establishment of hydrogen bonds between the phenolic hydroxyl group and the oxygen atoms of the benzoyl group. Both interactions cause greater selectivity towards the phenols of this exchanger with respect to Dowex 50-X4 (H^+).

The data obtained on eluting with 1 and 3 *M* acetic acid agree with the formation of hydrogen bonds between the phenols and the exchanger matrix. With these eluents, in fact, the phenols run more than with water–methanol mixtures (see Table II). Such a difference may be explained on the basis of the lesser weakening of the hydrogen bonds between the phenolic hydroxyl group and the benzoyl group of the exchanger caused by methanol than by acetic acid.

Analytical applications

AG 1-X4. On this exchanger, many separations can be effected by eluting with

water, methanol or water–acetic acid, methanol–acetic acid and water–methanol–acetic acid mixtures in different proportions.

The separation of the three aminophenols was effected on AG 1-X4 (CH_3COO^-) by eluting with 1 *M* acetic acid in methanol or in 1:1 (v/v) water–methanol. In both instances the migration distance was 14 cm.

With 1 *M* acetic acid in methanol, the following separations were achieved: 2-amino-4,6-dinitrophenol, 2-amino-4-nitrophenol, 4-amino-2-nitrophenol and 2-amino-3,4,6-trichlorophenol; and 1,5-naphthalenediol, 2-hydroxy-1-naphthaldehyde and α- or β-naphthol.

With two-dimensional chromatography in methanol and 1 *M* acetic acid in methanol, the following compounds were separated: phenol, guaiacol, *o*-nitrophenol, *p*-nitrophenol, *m*-nitrophenol, 2,5-dinitrophenol and 2,6-dinitrophenol.

BD-cellulose. Owing to the unusual R_F sequence observed on this exchanger, the analytical applications are different from those on the other ion exchangers studied. In particular, it is possible to separate the polyhydroxybenzenes (with the exception of orcinol) and the three aminophenols from all of the other compounds by eluting with 4:1 or 1:1 (v/v) water–methanol. The polyhydroxybenzenes, furthermore, can be separated from the aminophenols by two-dimensional chromatography in 4:1 (v/v) water–methanol and 1 *M* acetic acid .

Interesting separations were effected by eluting with 1 or 3 *M* acetic acid: 4-amino-2-nitrophenol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol and 2-amino-4,6-dinitrophenol; and 4- and 5-aminosalicylic acid.

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