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CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOUR OF PRIMARY AROMATIC AMINES ON ANION-EXCHANGE THIN LAYERS*

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SUMMARY

The use of polystyrene-based anion exchangers with aqueous eluents for the separation of primary aromatic amines has been investigated, and extended with satisfactory results to microcrystalline cellulose, cellulose-based anion-exchangers and sodium CM-cellulose layers.

Interesting separations of the amines have also been effected electrophoretically on AG 1-X4 layers.

The use of water–organic solvent mixtures on polystyrene-based cation exchangers is discussed.

INTRODUCTION

Primary aromatic amines can be separated with difficulty on polystyrene-based cations exchangers in aqueous solutions and also by elution with concentrated solutions of mineral acids[1], owing to the high affinity of such exchangers towards compounds that contain one or more aromatic nuclei[2]. Separations of the aromatic amines have been effected on layers and columns of cellulose-based weak cation exchangers[3,4] and on papers impregnated with such exchangers[5]. On Porapak Q, a polystyrene-based resin without ion-exchange groups, the retention of the aromatic amines, when eluted with aqueous solutions[1], is still high, but it is noticeably influenced by the protonation of the $-NH_2$ group and therefore by the pH of the eluent.

Such an effect, as already pointed out by Zaika[6] for purines and pyrimidines on Amberlite XAD-2 columns, is used to separate aromatic amines by thin-layer chromatography. As resins such as Porapak Q and Amberlite XAD-2 cannot be used in thin-layer chromatography with aqueous eluents, owing to their hydrophobic characteristics, we used polystyrene-based anion exchangers. On such exchangers it is possible to study the influence of the affinity between the exchanger matrix and the amines on their chromatographic behaviour at different pH values when the ion-exchange process is lacking.

In order to obtain a complete picture of the possibilities of using the affinity between the matrix of the resin and the amines from an analytical point of view, the behaviour of the amines was also studied on microcrystalline cellulose and on

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cellulose-based anion exchangers (Cellex D). Anion-exchange layers have been used also for electrophoretic measurements.

To complete the investigation, we considered it interesting to study the chromatographic behaviour of the amines on polystyrene-based cation exchangers eluted with water–organic solvent mixtures. With such eluents, already used by us in thin-layer chromatography[7] and by Gilmer and Pietrzyk[8] in column chromatography, the affinity between the exchanger and the amine is decreased, with a consequent increase in the possibilities of separating the compounds.

EXPERIMENTAL

Solutions were obtained by dissolving the amines in a 1:1 (v/v) mixture of isopropanol and 0.1 *M* hydrochloric acid and stored in dark bottles. Fresh solutions were used for those amines which easily decompose (phenylenediamines and *o*- and *p*-aminophenols). The amount of each amine on the layer was between 0.5 and 2 μg, except for *o*-chloroaniline and *o*-bromoaniline, when the amount was 15 μg. The amines were detected with a solution of 5% N,N-dimethyl-*p*-aminobenzaldehyde (pDAB)[7] in a 5:1 (v/v) mixture of ethanol and glacial acetic acid.

AG 1-X4, Dowex 50-X4, Cellex D and sodium carboxymethylcellulose (CMCNa) layers were prepared as described in previous papers[1,7,9]. The microcrystalline cellulose (E. Merck, Darmstadt, G.F.R.) layers were prepared with 8 g of the product in 40 ml of water.

The chromatographic and electrophoretic measurements were carried out as described in a previous paper[1]. The migration distance was 11 cm unless otherwise stated.

RESULTS AND DISCUSSION

Polystyrene-based anion exchangers (AG 1-X4)

The R_F values of 31 aromatic amines on AG 1-X4 (CH_3COO^-) layers when eluted with solutions at different pH values are shown in Table I, together with the R_F values obtained on AG 1-X4 (ClO_4^-) with 1 *M* acetic acid as eluent. The chromatographic characteristics of the amines on AG 1-X4 (Cl^-) layers are virtually the same as those on AG 1-X4 (ClO_4^-).

From the results in Table I, it can be seen that, for a given compound, the R_F value increases as the pH of the eluent decreases. An exception is given by those amines whose R_F values remain virtually unchanged with changes in pH, because at pH $\geqslant$2.37 such amines are not protonated or are protonated only to a negligible extent. As regards the relationship between the pH of the eluent and the pK_a values of the amines, it can be stated that:

(a) for isomers 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) and therefore it is possible to predict *a priori* the pH of the eluent at which the best separation of the isomers is achieved;

(b) for the different amines, the R_F and pK_a sequences are generally in agreement, with the exception of *o*-phenylenediamine and *o*- and *p*-aminophenols. *o*-Phenylenediamine has higher R_F values and *o*- and *p*-aminophenols have lower R_F values than those predicted on the basis of their pK_a values.

TABLE I

R_F VALUES OF PRIMARY AROMATIC AMINES ON AG 1-X4 (CH_3COO^-) AND AG 1-X4 (ClO_4^-) THIN LAYERS

Eluents: (1) 0.1 *M* acetate buffer; (2) 0.1 *M* acetic acid; (3) 1 *M* acetic acid.

Amine	pK_a*	*AG 1-X4 (CH_3COO^-)*			*AG 1-X4 (ClO_4^-)*
		1	*2*	*3*	*3*
p-Bromoaniline	3.92	0.12	0.39	0.69	0.76
m-Bromoaniline	3.22	0.10	0.26	0.55	0.55
o-Bromoaniline	2.32	0.10	0.16	0.27	0.23
p-Chloroaniline	3.98	0.18	0.49	0.75	0.83
m-Chloroaniline	3.52	0.15	0.33	0.64	0.70
o-Chloroaniline	2.65	0.12	0.22	0.40	0.43
p-Aminophenol	5.49	0.70	0.76	0.84	0.93
o-Aminophenol	4.72	0.46	0.68	0.83	0.93
m-Aminophenol	4.17	0.30	0.63	0.83	0.93
p-Phenylenediamine	6.08	0.79	0.81	0.84	0.94
m-Phenylenediamine	4.88	0.71	0.76	0.84	0.94
o-Phenylenediamine	4.47	0.67	0.74	0.83	0.93
p-Anisidine	5.34	0.74	0.77	0.84	0.93
o-Anisidine	4.52	0.58	0.73	0.83	0.93
m-Anisidine	4.23	0.44	0.68	0.83	0.93
m-Nitroaniline	2.46	0.21	0.20	0.31	0.21
p-Nitroaniline	1.00	0.02	0.03	0.07	0.02
o-Nitroaniline	−0.26	0.04	0.04	0.08	0.03
2,4-Dinitroaniline	−4.53	0.00	0.00	0.00	0.00
2,5-Diaminotoluene	—	0.79	0.81	0.84	0.94
2,6-Diaminotoluene	—	0.73	0.76	0.83	0.94
2,4-Diaminotoluene	—	0.72	0.76	0.83	0.93
2,4-Diaminoanisole	—	0.72	0.76	0.83	0.93
3,4-Diaminotoluene	—	0.67	0.73	0.83	0.93
4-Amino-2-nitrophenol	—	0.22	0.36	0.65	0.80
2-Amino-4-nitrophenol	—	0.01	0.03	0.10	0.30
2-Amino-5-nitrophenol	—	0.00	0.01	0.02	0.05
2-Amino-4,6-dinitrophenol	—	0.00	0.00	0.00	0.00
4-Nitro-*o*-phenylenediamine	—	0.08	0.10	0.16	0.16
2-Amino-4-chlorophenol	—	0.03	0.18	0.41	0.70
2-Amino-3,4,6-trichlorophenol	—	0.00	0.00	0.01	0.01

* Taken from ref. 10.

From a comparison between the results on the exchanger in the perchlorate and acetate forms, it may be noted that for the amines, which are appreciably protonated at the pH of the eluent, higher R_F values are generally obtained on the exchanger in the perchlorate form. The different behaviour of such amines on the same exchanger in the two forms may be ascribed to the exchange process between the acetate ions in the eluent and the perchlorate ions in the resin; this process gives rise to a different pH gradient on the layer. Such an effect has also been shown by means of pH measurements on the two layers, as described in a previous paper[11]. As the curves in Fig. 1 show, the pH variation along the layer is smaller on the exchanger in the perchlorate form than on that in the acetate form.

The ionic strength of the eluent has a large influence on the chromatographic characteristics of the amines. As the ionic strength is increased, a decrease in the R_F values and in the compactness of the spots is observed. The compactness of the spots, and therefore the possibility of the separation of the amines, is also influenced by the pH of the eluent. For instance, when eluting with 0.1 *M* sodium acetate solution, elongated spots are obtained and, in most instances, part of the amine remains at the starting point. With 0.1 *M* acetate buffer solutions, compact spots are obtained and some interesting separations can be achieved, such as those among the

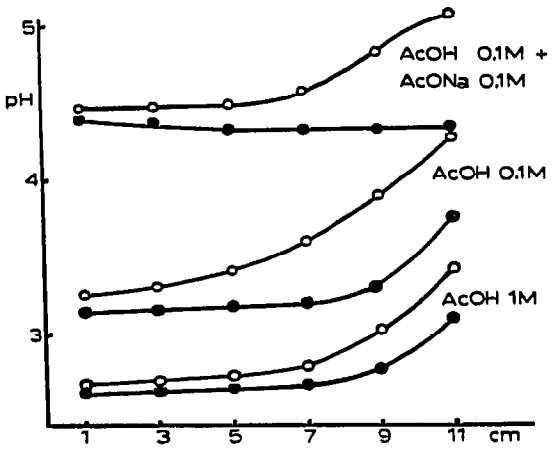

Fig. 1. pH values of (○) AG 1-X4 (CH_3COO^-) and (●) AG 1-X4 (ClO_4^-) suspensions referred to the centre of the strips[11].

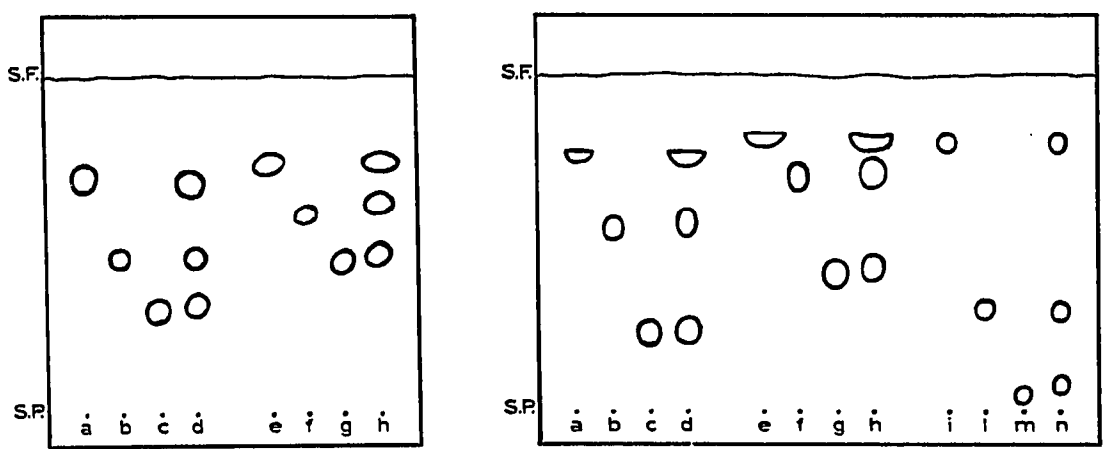

Fig. 2. Thin-layer chromatogram of some aromatic amines on AG 1-X4 (CH_3COO^-). Eluent: 0.1 *M* acetate buffer. (a) *p*-aminophenol; (b) *o*-aminophenol; (c) *m*-aminophenol; (d) mixture of aminophenols; (e) *p*-anisidine; (f) *o*-anisidine; (g) *m*-anisidine; (h) mixture of anisidines.

Fig. 3. Thin-layer chromatogram of some aromatic amines on AG 1-X4 (ClO_4^-). Eluent: 1 *M* acetic acid. (a) *p*-bromoaniline; (b) *m*-bromoaniline; (c) *o*-bromoaniline; (d) mixture of bromoanilines; (e) *p*-chloroaniline; (f) *m*-chloroaniline; (g) *o*-chloroaniline; (h) mixture of chloroanilines; (i) 4-amino-2-nitrophenol; (l) 2-amino-4-nitrophenol; (m) 2-amino-5-nitrophenol; (n) mixture of aminonitrophenols.

three aminophenols and the three anisidines (see Fig. 2). The separations of the three chloro- and bromoanilines and of three aminonitrophenols have been effected with 1 *M* acetic acid as eluent (see Fig. 3). Those amines which are strongly retained with 1 *M* acetic acid as eluent can be displaced with acetic and hydrochloric acid solutions. It is not expedient to employ high concentrations of perchloric acid, as the detection of the spots is made more difficult owing to its corrosive action on the layer. With 1 *M* acetic acid plus 2 *M* hydrochloric acid as eluent, the separation of the three nitroanilines ($R_{F(ortho)}=0.08$; $R_{F(para)}=0.36$; $R_{F(meta)}=0.63$) and of 2,4-dinitroaniline from 2-amino-4,6-dinitrophenol ($R_F=0.03$ and 0.22, respectively) is possible on AG 1-X4 (Cl^-) layers.

Microcrystalline cellulose and cellulose-based anion exchangers (Cellex D)

On these layers, the adsorption of the amines is noticeably reduced and such exchangers can therefore be employed for the study of the chromatographic behaviour of those amines with two aromatic nuclei which, on polystyrene-based exchangers, give rise to elongated spots owing to their strong adsorption.

In Table II are listed the R_F values of most of the amines studied on AG 1-X4 (see Table I) and of those with two aromatic nuclei on microcrystalline cellulose and Cellex D layers with 0.5 *M* acetate buffer as eluent. Table II also gives results relative to the CMCNa layers eluted with 0.1 and 0.5 *M* acetate buffer solutions.

The *ortho*- and *meta*-isomers of chloro- and bromoanilines show a chromatographic behaviour similar to that of the corresponding *para*-isomer. The phenylenediamines, which are missing from Table II, have R_F values between those of 2,5- and 2,4-diaminotoluenes. 7-Amino-2-naphthol and 1-amino-7-naphthol show a behaviour analogous to that of 5-amino-1-naphthol.

Most amines on microcrystalline cellulose and Cellex D layers have very similar R_F values, between 0.78 and 0.81. Those amines whose R_F values are lower than 0.78 exhibit different chromatographic behaviour on the two layers in most instances. The amines that are not protonated at the pH of the eluent (and therefore the ion-exchange process is lacking) are less retained on the CMCNa layers than on microcrystalline cellulose under the same experimental conditions.

The different behaviour of the amines on the two exchangers and on microcrystalline cellulose may be ascribed to the differences in the structures of the three celluloses[12,13]. A further demonstration of the structural differences, at least as regards Cellex D with respect to CMCNa and microcrystalline cellulose, is shown by the behaviour of Cellex D towards acidic eluents, particularly 1 *M* acetic acid[7]. Furthermore, on Cellex D layers, when eluting with 0.5 *M* sodium acetate solution, a notable decrease in the R_F values of most amines is observed, unlike the behaviour on CMCNa and microcrystalline cellulose. The different behaviour of numerous amines on Cellex D in comparison with microcrystalline cellulose can be used from an analytical point of view, as the chromatograms in Fig. 4 show. Such separations concern the amines with two aromatic nuclei and are not possible on microcrystalline cellulose.

Electrophoretic measurements on AG 1-X4 layers

The use of these layers in electrophoretic measurements has some advantages over the use of microcrystalline cellulose and cellulose-based cation exchangers.

TABLE II

R_F VALUES OF PRIMARY AROMATIC AMINES ON MICROCRYSTALLINE CELLULOSE (mC), CELLEX D (CH_3COO^-) AND CMCNa THIN LAYERS

Migration distance, 12 cm. Eluents: (1) 0.1 *M* acetate buffer; (2) 0.5 *M* acetate buffer.

Amine	*mC*	*Cellex D*	*CMCNa*	
	2	*2*	*1*	*2*
p-Chloroaniline	n.d.*	0.68	0.70	0.81
p-Bromoaniline	n.d.	0.67	0.69	0.81
p-Aminophenol	0.78	0.79	0.30	0.68
o-Aminophenol	0.78	0.79	0.40	0.70
m-Aminophenol	0.79	0.79	0.75	0.74
p-Anisidine	0.80	0.79	0.38	0.76
o-Anisidine	0.81	0.79	0.77	0.84
m-Anisidine	0.80	0.79	0.84	0.84
2,5-Diaminotoluene	0.81	0.79	0.22	0.62
p-Phenylenediamine	0.81	0.79	0.26	0.66
m-Phenylenediamine	0.80	0.79	0.33	0.68
2,4-Diaminotoluene	0.80	0.79	0.38	0.68
m-Nitroaniline	0.68	0.64	0.78	0.79
p-Nitroaniline	0.54	0.55	0.67	0.69
o-Nitroaniline	0.61	0.61	0.70	0.70
4-Amino-2-nitrophenol	0.63	0.64	0.70	0.71
2-Amino-4-nitrophenol	0.53	0.48	0.64	0.65
2-Amino-5-nitrophenol	0.46	0.38	0.54	0.54
2-Amino-4,6-dinitrophenol	0.46	0.13	0.94	0.75
4-Nitro-*o*-phenylenediamine	0.48	0.47	0.52	0.56
2-Amino-4-chlorophenol	0.64	0.56	0.66	0.66
2-Amino-3,4,6-trichlorophenol	0.33	0.24	0.39	0.39
5-Amino-1-naphthol	0.43	0.33	0.20	0.34
4-Amino-1-naphthol	0.54	0.58	0.70	0.76
4-Amino-4'-methoxydiphenylamine	0.60	0.70	0.14	0.40
4-Amino-2,4'-dinitrodiphenylamine	0.38	0.33	0.37	0.55
4-Aminodiphenylamine	0.70	0.71	0.18	0.42
2-Aminodiphenylamine	0.64	0.48	0.71	0.71
4,4'-Diaminodiphenylamine	0.75	0.80	0.03	0.23
Benzidine	0.46	0.49	0.12	0.27
o-Tolidine	0.43	0.36	0.08	0.21

* n.d. = not determined.

In particular, the influence of the electroosmotic flow is greatly decreased owing to the strong adsorption of the polystyrene matrix towards the amines, especially if the amine is deprotonated, and therefore a better reproducibility of the measurements can be achieved. Furthermore, the resolution of amine mixtures is favoured by the retention capacity of the layer, which has a greater effect with the deprotonated form of the amine than the protonated form, unlike the behaviour on cation exchangers, where the retention capacity of the layer is higher the greater is the degree of protonation of the amine[3].

In Table III are listed the migration distances of some amines, divided for

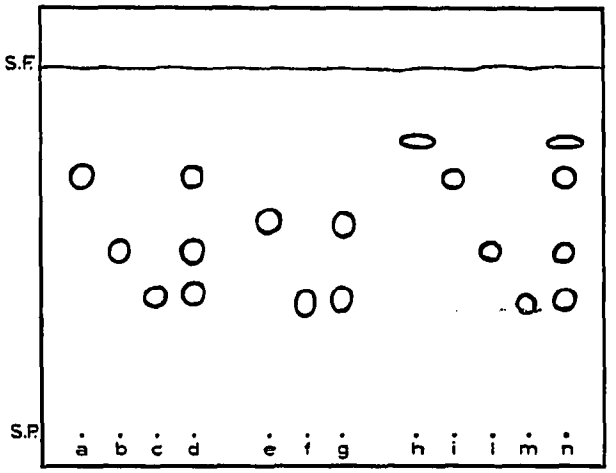

Fig. 4. Thin-layer chromatogram of some aromatic amines on Cellex D (CH_3COO^-). Eluent: 0.5 *M* acetate buffer. (a) 4-aminodiphenylamine; (b) benzidine; (c) *o*-tolidine; (d) mixture of the three amines; (e) 4-amino-1-naphthol; (f) 5-amino-1-naphthol; (g) mixture of aminonaphthols; (h) 4,4′-diaminodiphenylamine; (i) 4-aminodiphenylamine; (l) 2-aminodiphenylamine; (m) 4-amino-2,4′-dinitrodiphenylamine; (n) mixture of diphenylamines.

TABLE III

MIGRATION DISTANCES OF PRIMARY AROMATIC AMINES ON AG 1-X4 (CH_3COO^-) THIN LAYERS WITH 1 *M* ACETIC ACID AS ELECTROLYTE

Electric potential, 1000 V. Migration time, 60 min. *o*-Nitroaniline, 2,4-dinitroaniline, 2-amino-5-nitrophenol, 2-amino-4,6-dinitrophenol and 2-amino-3,4,6-trichlorophenol remain at the start. The migration distances of aminophenols, anisidines and phenylenediamines are $\geqslant$125 mm. Benzidine and *o*-tolidine give rise to elongated spots.

Amine	*Migration distance (mm)*	*Amine*	*Migration distance (mm)*
p-Chloroaniline	96	4-Amino-2-nitrophenol	76
p-Bromoaniline	76	2-Amino-4-chlorophenol	42
m-Chloroaniline	68	*m*-Nitroaniline	27
m-Bromoaniline	53	4-Nitro-*o*-phenylenediamine	13
o-Chloroaniline	37	2-Amino-4-nitrophenol	9
o-Bromoaniline	29	*p*-Nitroaniline	2
7-Amino-2-naphthol	36	4-Amino-2,4′-dinitrodiphenylamine	38
1-Amino-7-naphthol	32	2-Aminodiphenylamine	35
5-Amino-1-naphthol	22	4-Aminodiphenylamine	30
4-Amino-1-naphthol	9	4-Amino-4′-methoxydiphenylamine	30

convenience into four groups, with 1 *M* acetic acid as supporting electrolyte. Some amines, as noted above Table III, remain at the starting point, while others, which are considerably protonated at the pH of the eluent, have migration distances greater than 125 mm and their separation from the others is therefore possible.

As regards the amines reported in Table III, among the separations that can be predicted on the basis of the migration distances, we effected the following: *p*-,

m- and *o*-bromoanilines; *p*-bromoaniline and *p*-, *m*- and *o*-chloroanilines; 7-amino-2-naphthol, 5-amino-1-naphthol and 4-amino-1-naphthol; 4-amino-2-nitrophenol, 2-amino-4-chlorophenol, *m*-nitroaniline, 4-nitro-*o*-phenylenediamine and *p*-nitroaniline. While some of these separations are also possible by thin-layer chromatography (see Fig. 2), separations of the aminonaphthols are possible only electrophoretically, as elongated spots are observed in TLC.

With 0.1 *M* acetate buffer as supporting electrolyte, the amines reported in Table III have migration distances of less than 11 mm, while the anisidines, the aminophenols and particularly the phenylenediamines have migration distances greater than 11 mm.

The separation of the three aminophenols for a migration time of 60 min (migration distances at 800 V: *para*=62 mm; *ortho*=36 mm; *meta*=15 mm) is very interesting; such a separation is not possible either on microcrystalline cellulose or on CMCNa under the same experimental conditions[3].

Water–organic solvent mixtures

In Table IV are given the R_F values of some aromatic amines on Dowex 50-X4 (H^+) layers with water–ethanol and water–dimethyl sulphoxide mixtures as eluents, together with the pK_a values. Such amines are the only ones that move from the starting point, even if only to a small extent in some instances. It should be noted that the amines with pK_a values below about 1 move appreciably and can therefore be separated from the others.

As regards the influence of the acid–base characteristics of the organic solvent on the chromatographic behaviour of the amines, it can be noted that, on changing from ethanol to the more basic dimethyl sulphoxide, a significant decrease in the retention capacity of the layer occurs. The use of such a technique from an analytical

TABLE IV

R_F VALUES OF PRIMARY AROMATIC AMINES ON DOWEX 50-X4 (H^+) THIN LAYERS WITH DIFFERENT WATER–ORGANIC SOLVENT MIXTURES AS ELUENT

Amine	pK_a*	*Ethanol (%)*			*80% dimethyl sulphoxide*
		95	*80*	*60*	
2-Amino-4-nitrophenol	–	0.00	0.00	0.00	0.04
o-Chloroaniline	2.65	0.00	0.00	0.00	0.08
m-Nitroaniline	2.46	0.00	0.00	0.00	0.08
o-Bromoaniline	2.32	0.00	0.00	0.00	0.09
2-Amino-5-nitrophenol	–	0.08	0.22	0.09	0.62
p-Nitroaniline	1.00	0.13	0.30	0.15	0.65
2-Amino-4,6-dinitrophenol	–	0.41	0.53	0.26	0.82
2-Amino-3,4,6-trichlorophenol	–	0.60	0.67	0.36	0.94
o-Nitroaniline	−0.26	0.68	0.82	0.54	0.82
2,4-Dinitroaniline	−4.53	0.68	0.82	0.55	0.85
Methanilic acid	3.75	–	0.66	–	–
Sulphanilic acid	3.24	–	0.70	–	–
Orthanilic acid	2.48	–	0.90	–	–

* Taken from ref. 10.

point of view is limited to a restricted number of amines and does not have great advantages in comparison with the separations obtained by us on AG 1-X4 with aqueous eluents. The good separation of the three nitroanilines should, however, be noted. Such a technique can be employed successfully with aromatic amines that contain sulphonic groups, as shown by the R_F values of the three aminobenzenesulphonic acids (see Table IV). To complete this investigation, we also used mixtures of ethanol and pyridine in the proportions 9:1, 8:2, 6:4 and 3:7 on Dowex 50-X4 (H^+) layers. With these eluents, most amines move from the starting point, but give rise to elongated or double spots. Furthermore, the selectivity of the layer is noticeably decreased so that, for instance, the chloro- and bromoanilines cannot be separated, as their R_F values differ by, at most, 0.09.

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