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SEPARATION OF PRIMARY AROMATIC AMINES ON ALGINIC ACID AND CARBOXYMETHYLCELLULOSE COLUMNS

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SUMMARY

The chromatographic behavior of primary aromatic amines in columns of alginic acid and carboxymethylcellulose was investigated extensively. Aqueous solutions of mineral acids and organic acids, water and aqueous–organic solvents were used as eluents.

The relation between the data obtained on a thin layer and those obtained in a column was examined.

INTRODUCTION

The column chromatographic separation of primary aromatic amines has mostly been studied using Celite, silica gel and Teflon-6 as adsorbents[1–3]. Ion-exchange chromatography in this field seems only to have been used by TOMPSETT[4], who takes advantage of the high affinity of polystyrene resins for aromatic substances to separate a group of primary aromatic amines from other substances, present in biological materials, on strong cation exchangers and, more recently, on anion exchangers[5]. We have consequently considered it useful, as a logical extension of research already begun in this sector[6], to undertake a systematic investigation of the behavior of aromatic amines on columns of weak cation exchangers.

The study was limited to the use of alginic acid and carboxymethylcellulose, two exchangers that had already been successfully employed in thin-layer chromatography in the separation of the same aromatic amines[6]. We have thus been able to study any relationship which might exist between the data obtained by the two different chromatographic techniques and to verify whether separations obtainable on thin layers could be reproduced on a larger scale on a column of the same adsorbent.

EXPERIMENTAL

The alginic acid employed was obtained as described in a preceding investigation[7], but had a larger particle size (50–150 mesh) than that used in TLC (*viz.* passed mesh sizes greater than 150) so as to have a greater eluent flow rate. Columns having

a cross-section of 0.94 cm^2 and filled with 4 g of exchanger were used. 2 g of the exchanger in the acid form, obtained by treatment of CMCNa (ref. 8) with 1 *M* HCl and successive washing with water until the chloride ions disappeared, were used for the carboxymethylcellulose columns.

The eluent flow rate was found to be greater in the case of alginic acid columns (1 ml every 30 sec) than in the case of CMC columns (1 ml every 60 sec). In the case of the alginic acid, this rate remained practically constant whereas it decreased in the CMC columns to half its initial value.

The amine solutions were prepared by dissolving the sample in the same solvent as was used in the column (water; 0.1 *M* HCl; 1 *M* acetic acid and 1 *M* monochloroacetic acid in water and in 50% isopropyl alcohol). The amine concentration used was 1 g/l with the exception of the following:

(a) *o*- and *m*-nitroaniline (0.5 g/l), *p*-nitroaniline (0.3 g/l) in all solvents excepting water;

(b) arsanilic acids, *p*-aminoacetophenone and *o*-nitroaniline (0.25 g/l), *m*- and *p*-nitroaniline (0.2 g/l) in water.

The volume of solution introduced into the column was in the majority of cases 0.05 ml, as can be seen from the quantity of amine employed (see Table I).

In the case of 4-aminosalicylic acid, a solution having a concentration of 5 g/l (1 ml of which was introduced in the column so as to obtain the maximum quantity reported in Table II) was also employed.

Amine solutions that decompose in air (*e.g.* phenylenediamines and *p*-aminodimethylaniline) were prepared immediately before use.

The elution curves were obtained by colorimetry, using *p*-dimethylaminobenzaldehyde to reveal the aromatic amines[6]. The total quantity of amine eluted was determined spectrophotometrically after diazotization with nitrous acid followed by coupling with N-(1-naphthyl)ethylenediamine[9]. Fig. 1 shows the calibration curves of some of the amines. To obtain reproducible results, the spectrophotometric mea-

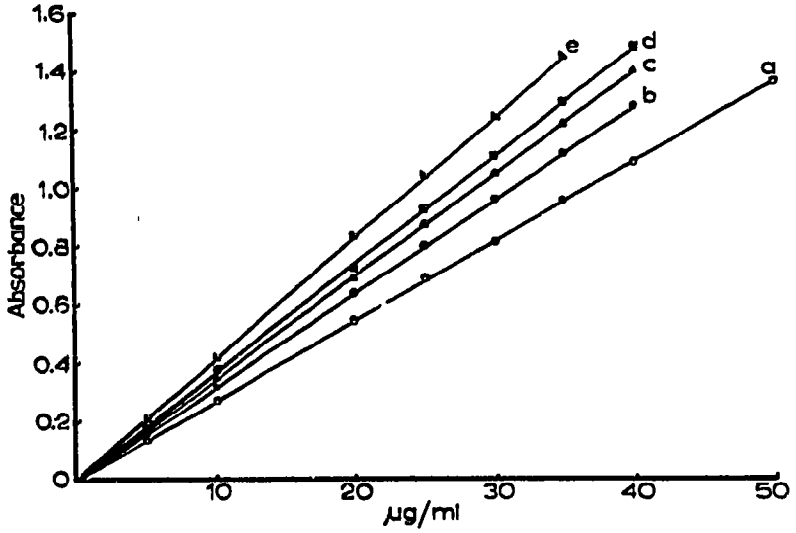

Fig. 1. Calibration curves for some aromatic amines. (a) *o*-Nitroaniline, (b) *m*-nitroaniline, sulfanilamide and *p*-aminohippuric acid; (c) *m*-aminobenzoic acid, (d) *p*-aminobenzoic acid, (e) *p*-nitroaniline.

surements must be made at least 60 min after the addition of the coupling reagent.

As 4-aminosalicylic acid does not react with N-(1-naphthyl)ethylenediamine, it was determined indirectly by taking advantage of the formation of molybdenum blue[10].

RESULTS AND DISCUSSION

Alginic acid columns

Elution with 1 M acetic acid. In thin-layer chromatography of aromatic amines and if 1 *M* acetic acid is used as eluent the diamines show a greater affinity for alginic acid than the monoamines, as indicated by their R_F value ($\leq$ 0.06). This characteristic is enhanced in a column, permitting the quantitative separation of the diamines from the monoamines.

o-Phenylenediamine, although in fact it has the same R_F value as the naphthylamines (0.06), is completely and distinctly separated from the latter. It should also be noted that the naphthylamines, while having the lowest R_F values of the monoamines, give an elongated elution curve which develops over a volume range of about 100 ml (see Fig. 2). For this reason they do not lend themselves to quantitative determination.

It is possible to deduce the following from the monoamine elution curves shown in Fig. 2:

In general the resolving power of a column is less with respect to that of a thin

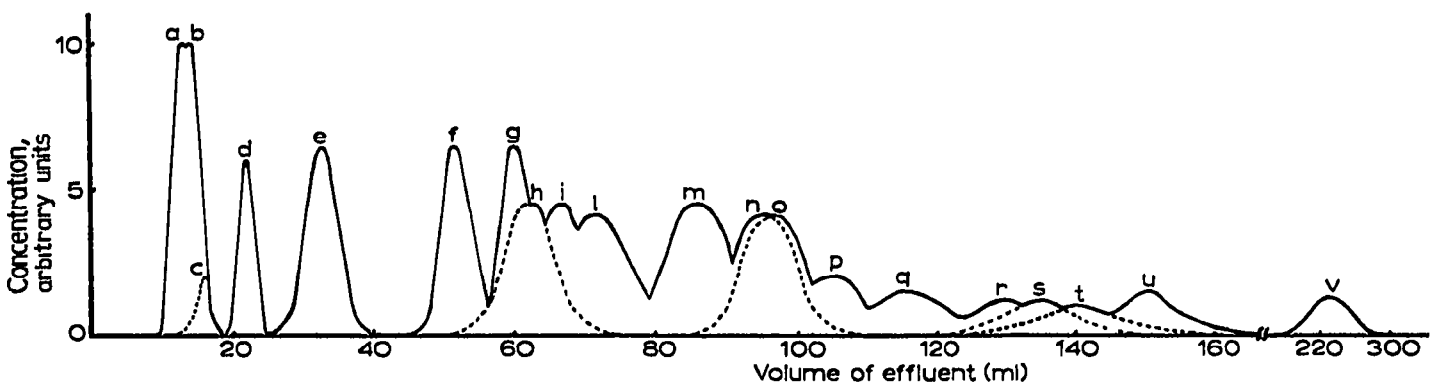

Fig. 2. Elution curves for aromatic amines on an alginic acid column with 1 *M* acetic acid as eluent. (a) Sulfanilic, methanilic and orthanilic acids, (b) *o*-arsanilic acid, (c) *o*-nitroaniline, (d) *p*-nitroaniline, (e) *p*-arsanilic acid; (f) 4-aminosalicylic acid, (g) *o*-aminobenzoic acid, (h) sulfanilamide; (i) *p*-aminoacetophenone; (l) 5-aminosalicylic acid, (m) *p*-aminobenzoic acid, (n) *o*-chloroaniline; (o) *p*-aminohippuric acid; (p) *m*-nitroaniline; (q) aniline; (r) *o*- and *p*-toluidine; (s) *m*-aminobenzoic acid, (t) *o*-anisidine and *o*-aminophenol, (u) *m*- and *p*-aminophenol, (v) α- and β-naphthylamine.

layer when the R_F values are high (of the order of 0.5 or greater), while it is greater when the R_F values are low. In this last case, separations not foreseeable from the R_F values are sometimes possible, as for example in the separation of *o*-chloroaniline (R_F = 0.13) and *p*-aminobenzoic acid (R_F = 0.12) from a group of amines (such as toluidines, aminophenols, *o*-anisidine and *p*-aminobenzoic acid) having R_F values ranging from 0.08 to 0.10.

TABLE I

SEPARATION OF THE ISOMERS OF SOME AROMATIC AMINES ON AN ALGINIC ACID COLUMN WITH 1 *M* ACETIC ACID AS ELUENT

Compound used	*Weight of compound placed on column (μg)*	*Volume range of the eluate (ml)*	*Recovery of base (%)*[a]
o-Nitroaniline	10	13–19	93 ± 4
p-Nitroaniline	30	19–25	95 ± 3
m-Nitroaniline	30	95–115	92 ± 4
o-Aminobenzoic acid	50	54–68	94 ± 3
p-Aminobenzoic acid	50	74–98	94 ± 3
m-Aminobenzoic acid	50	120–150	91 ± 4
o-Arsanilic acid	50	10–18	95 ± 3
p-Arsanilic acid	50	25–40	94 ± 3
p-Arsanilic acid	100	22–45	96 ± 3
4-Aminosalicylic acid	50	42–60	93 ± 4
5-Aminosalicylic acid	50	60–80	94 ± 4

[a] The reported data are the means of several determinations.

Contrary to what might be predicted from the thin-layer data, the separation of amines containing sulfonic groups ($R_F = 0.96$) from *o*-arsanilic acid ($R_F = 0.82$) and from *o*-nitroaniline ($R_F = 0.55$), is not possible.

The possibilities of separating isomeric aromatic amines on alginic acid are illustrated in Table I where data on the volume range of the eluate, the quantity of substance employed, and the percentage base recovered are reported. It can be seen from these data that a good separation among the isomers can be obtained, though, in the case of nitroanilines, the separation of the *ortho*- from the *para*-isomer is critically bound to the quantity of substance indicated in Table I. In fact, 250 μg of *o*-nitroaniline are eluted between 10 and 22 ml (recovery of the base 85%, calculated by direct spectrophotometric determination of the colored solution at 285 or 415 mμ) whereas 150 μg of *p*-nitroaniline pass between 10 and 32 ml (recovery of the base 92%, calculated by direct spectrophotometric determination at 385 mμ).

In addition, in this case the height of the column (that is, the quantity of exchanger used) is also a determining factor in that with 3 g of alginic acid, there is a partial overlap of the elution curves of the two nitroanilines even with the quantities reported in Table I.

The feasibility of a clean separation of 4-aminosalicylic acid from *m*-aminophenol should also be noted as it can be used for the purification of the acid. From the data reported in Table II, it can be seen that it is possible that considerable quantities of acid are used, notwithstanding the reduced dimensions of the column adopted by us, as the *m*-aminophenol is completely held back, even if present in a quantity less than 1% with respect to the 4-aminosalicylic acid.

TABLE II

SEPARATION OF *m*-AMINOPHENOL FROM 4-AMINOSALICYLIC ACID ON AN ALGINIC ACID COLUMN WITH 1 *M* ACETIC ACID AS ELUENT

Compound used	*Weight of compound placed on column (μg)*	*Volume range of the eluate (ml)*	*Recovery of base (%)*[a]
m-Aminophenol	50	140–165	90 ± 5
4-Aminosalicylic acid	50	42–60	93 ± 4
4-Aminosalicylic acid	1000	32–75	97 ± 2
4-Aminosalicylic acid	5000	26–90	98 ± 2

[a] The reported data are the means of several determinations.

Elution with 0.1 M hydrochloric acid. The great affinity of diamines for alginic acid can be considerably reduced by using hydrochloric acid as eluent, analogous to that which is observed on a thin layer. The elution curves in Fig. 3 show that the diamines are rapidly eluted with 0.1 *M* hydrochloric acid. On the column, *p*-aminodimethylaniline is eluted together with the *o*-phenylenediamine even though these two amines have considerably different R_F values on thin layers (respectively 0.36 and 0.63). Furthermore, *o*-phenylenediamine is incompletely separated from the *meta*- and *para*-isomers, in contrast to the clean separation observed on thin layers (*m*- and *p*-phenylenediamine have R_F values of 0.28 and 0.27, respectively).

The elution curve for the naphthylamines, which presents a much widened maximum when eluted with 1 *M* acetic acid, is shown in Fig. 3, as is that of aniline, which can be separated from 4-aminodiphenylamine and from benzidine.

Elution with 1 M monochloroacetic acid. The use of monochloroacetic acid is less efficient in the separation of monoamines as compared with acetic acid in that it lowers their affinities for alginic acid. For example, aniline which shows a considerable affinity for the exchanger with acetic acid, is now eluted between 20 and 30 ml. In practice all monoamines are eluted between a minimum of 9 ml and a maximum of 35

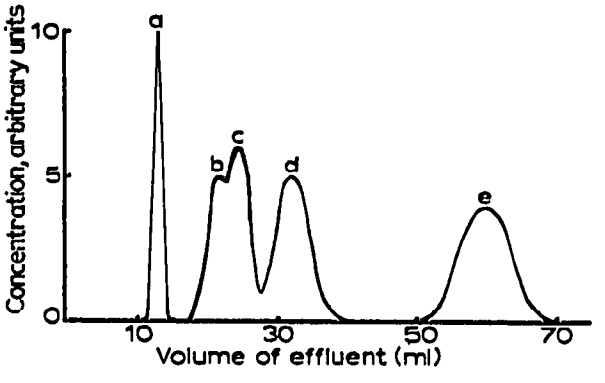

Fig. 3. Elution curves for aromatic amines on an alginic acid column with 0.1 *M* HCl as eluent. (a) Aniline; (b) *p*-aminodimethylaniline; (c) *α*- and *β*-naphthylamine, *o*-phenylenediamine, N-phenyl-*p*-phenylenediamine; (d) *m*- and *p*-phenylenediamine, (e) benzidine.

ml. However, the use of monochloroacetic acid for diamines and in particular for the separation of *o*-phenylenediamine from the *meta*- and *para*-isomers is interesting. The *ortho*-isomer elutes between 46 and 65 ml and the other two between 95 and 120 ml.

Elution with aqueous-organic solvents. To complete the picture of the behavior of aromatic amines in a column we deemed it useful to employ aqueous-organic solutions as eluents even though the results obtained on thin layers with eluents of this type were not very satisfactory.

A solution of 1 *M* monochloroacetic acid in 50% isopropyl alcohol was used. The separations obtainable on a column are not as good as those which might have been expected on the basis of the behavior of the amines on thin layers. For example 4-aminosalicylic acid, although it has an R_F of 0.28, elutes in the same volume range as those amines having an $R_F \geq 0.45$, and that is between 9 and 20 ml.

Carboxymethylcellulose columns

Elution with water. Carboxymethylcellulose shows, with respect to alginic acid, a lower affinity for aromatic amines and therefore has fewer possibilities of application in column chromatography. For this reason water was used as the eluent since it has, with respect to other solvents, a smaller leveling capacity[6].

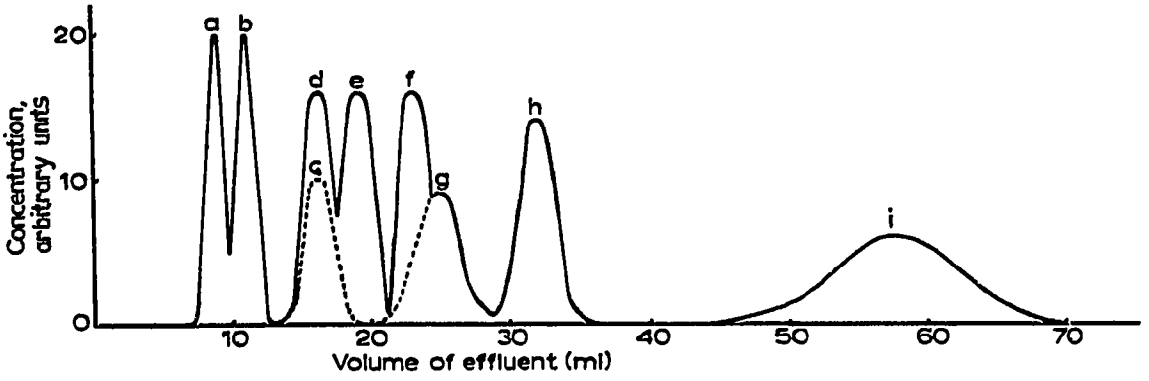

Fig. 4. Elution curves for aromatic amines on a carboxymethylcellulose column with water as eluent. (a) Sulfanilic, methanilic and orthanilic acids; (b) *o*- and *p*-arsanilic acids; (c) *o*-nitroaniline and *o*-aminobenzoic acid, (d) 4-aminosalicylic acid, and *p*-nitroaniline; (e) sulfanilamide, and *p*-aminohippuric acid; (f) *p*-aminoacetophenone; (g) *p*-aminobenzoic acid; (h) *m*-nitroaniline; (i) *m*-aminobenzoic acid.

As the elution curves reported in Fig. 4 show, the resolving power of the column increases considerably for R_F values ≤ 0.2. The clean separation of *m*-aminobenzoic acid ($R_F = 0.14$) from other amines and in particular from *p*-aminobenzoic acid ($R_F = 0.28$) and from *m*-nitroaniline ($R_F = 0.25$) should be noted.

As on alginic acid, it is also possible to separate the isomers of aminobenzoic acid on carboxymethylcellulose, as shown by the data reported in Table III.

The separation of the *ortho*- from the *para*-isomer, however, is critically bound to the quantity of amine indicated in Table III. It should, however, be pointed out that the use of water as eluent presents considerable advantages in that it permits us to obtain amine solutions in the absence of other ions.

This characteristic, notwithstanding the low flow rate of the eluent in such columns, could be used to advantage to obtain a solution having a primary aromatic

TABLE III

SEPARATION OF *o*-, *m*- AND *p*-AMINOBENZOIC ACID ON A CARBOXYMETHYLCELLULOSE COLUMN WITH WATER AS ELUENT

Compound used	*Weight of compound placed on column (μg)*	*Volume range of the eluate (ml)*	*Recovery of base (%)*[a]
o-Aminobenzoic acid	12.5	12–20	94 ± 3
p-Aminobenzoic acid	12.5	20–30	94 ± 3
m-Aminobenzoic acid	16.5	45–70	91 ± 4

[a] The reported data are the means of several determinations.

amine composition different from its initial one. A possible application would therefore be in concentration processes.

Elution with 1 M acetic acid. Amines that are held back strongly on the column when eluted with water and do not appear in Fig. 4, can be successively eluted with 1 *M* acetic acid. In this way it is possible to recover them in an interval of 8–30 ml and, at the same time, effect their separation from benzidine and the *meta*- and *para*-isomers of phenylenediamine.

Comparison of column and thin-layer data

The existence of a relation between the R_F value and the volume of the eluent needed to obtain the maximum concentration of an eluted ion in the effluent (V_{max}) has been assumed, even though, recently, it has been observed that results obtained on ion-exchange papers do not necessarily imply analogous results on a column[11]. In the present case considerable differences in the chromatographic behavior of the same amines, when the two techniques were employed, were also observed. It is not to be doubted, however, that a relation does exist between the two types of data. We therefore endeavoured to verify whether the nature of this relation was purely qualitative,

TABLE IV

VOLUME OF EFFLUENT RELATIVE TO THE PEAK OF THE ELUTION CURVE (V_{max}) AND R_F VALUE FOR SOME AROMATIC AMINES ON ALGINIC ACID WITH 1 *M* ACETIC ACID AS ELUENT

Compound used	V_{max}	R_F	$1/(R_F - 1)$
o-Arsanilic acid	14.0	0.82	0.22
o-Nitroaniline	16.0	0.55	0.82
p-Nitroaniline	22.0	0.47	1.13
p-Arsanilic acid	32.5	0.41	1.44
4-Aminosalicylic acid	52.0	0.26	2.85
o-Aminobenzoic acid	60.5	0.23	3.35
Sulfanilamide	62.5	0.22	3.55
p-Aminoacetophenone	67.0	0.21	3.77
5-Aminosalicylic acid	71.5	0.20	4.00
p-Aminobenzoic acid	86.0	0.16	5.25

semiquantitative, or rather quantitative by taking advantage of the equation:

$$V_{max} = V_{int} + \frac{A_l}{A_s} g \left(\frac{1}{R_F} - 1\right) \quad (1)$$

where

V_{int} = interstitial volume of the column;
A_l/A_s = cross sectional areas ratio of mobile and stationary phase on thin layer;
g = weight of the exchanger in column.

This relation was obtained by equating the two expressions that give the molar distribution coefficient K_d (amount of ion per gram of resin/amount of ion per ml of

TABLE V

VOLUME OF EFFLUENT RELATIVE TO THE PEAK OF THE ELUTION CURVE (V_{max}) AND R_F VALUE FOR SOME AROMATIC AMINES ON CARBOXYMETHYLCELLULOSE WITH WATER AS ELUENT

Compound used	V_{max}	R_F	$1/(R_F - 1)$
p-Arsanilic acid	11.0	0.85	0.18
o-Nitroaniline	16.5	0.52	0.92
4-Aminosalicylic acid	16.5	0.50	1.00
p-Nitroaniline	16.5	0.47	1.08
o-Aminobenzoic acid	16.5	0.44	1.27
Sulfanilamide	19.0	0.43	1.32
p-Aminohippuric acid	19.0	0.43	1.32
p-Aminoacetophenone	23.0	0.35	1.86
p-Aminobenzoic acid	25.0	0.28	2.57
m-Nitroaniline	32.0	0.25	3.00
m-Aminobenzoic acid	57.5	0.14	6.15

solution) in a column ($V_{max} = V_{int} + K_d g$) and on ion-exchange papers $\{K_d = 1/(R_F - 1)\ A_l/A_s\}$[12]. From the data reported in Tables IV and V and from the corresponding diagrams in Figs. 5 and 6, one can deduce the following:

Eqn. 1 seems to fit for both exchangers, under the experimental conditions we operated, in the range of R_F values between 0.2 and 0.8 only.

The V_{int} values obtained from Figs. 5 and 6 (7 ml in both cases) and, therefore, the values of the corresponding ratios A_l/A_s (0.6 for alginic acid and 0.7 for carboxy-

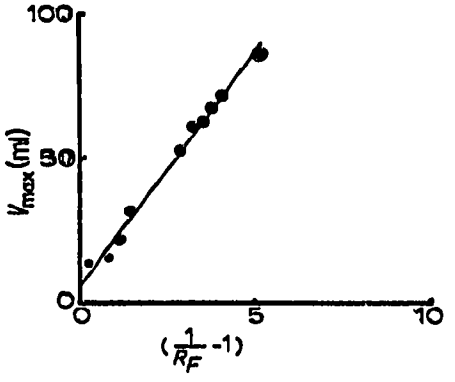

Fig. 5. Relationship between V_{max} and $1/(R_F - 1)$ for some aromatic amines on alginic acid with 1 *M* acetic acid as eluent.

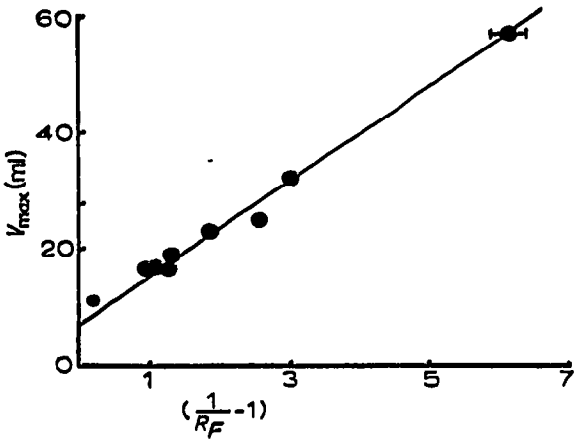

Fig. 6. Relationship between V_{max} and $1/(R_F - 1)$ for some aromatic amines on carboxymethylcellulose with water as eluent.

methylcellulose) seem reliable even if considerably different from those found for sulfonic resins (0.3–0.4)[13].

The A_l/A_s values obtained from the same diagrams (3.95 for alginic acid and 3.27 for carboxymethylcellulose) are comparable with those determined experimentally using ion-exchanger papers[14] and thin layers of Dowex 50 X4[15].

The fact that eqn. 1 is only fit to a limited extent (in the R_F range mentioned above) can be explained by bearing in mind that, for R_F values > 0.8, the solvent front influences the chromatographic behavior of compounds[12] considerably and that, for R_F values < 0.2, the enormous increase in the percentage error in the determination of these R_F values must also be taken into consideration. Another factor which cannot be neglected is the variation of the A_l/A_s ratio along the layer. It can therefore be concluded that, notwithstanding the great limitations indicated, eqn. 1 is on the whole verified and can serve as a convenient reference point for the transposition of a compound studied on layers of weak cation exchangers to a column.

CONCLUSIONS

The examination of the behavior of primary aromatic amines on chromatographic columns has permitted the realization of important separations both among isomers and among groups of amines having different acid–base characteristics, such as diamines from monoamines.

We have also been able to establish that the recovery of various amines is on the whole satisfactory and could therefore be used to advantage for preparative purposes. Finally, it has been verified that a quantitative relation exists between the data obtained on a thin layer and that obtained on a column, although this only applies to a definite R_F range.

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