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CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOUR OF PRIMARY MONO- AND DIAMINES ON LAYERS OF WEAK AND STRONG ION EXCHANGERS

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SUMMARY

The chromatographic behaviour of thirty primary aliphatic amines on Dowex 50 X4 (Na^+ and H^+), sodium carboxymethylcellulose and Rexyn 102 (Na^+) thin layers has been studied using HCl, buffer and salt solutions as eluents. Some interesting separations are reported; the affinity sequences on these exchangers are compared with those observed in column chromatography.

The retention mechanism has been investigated by applying the $R_M/ - \log a_{HCl}$ relationship and the activity coefficients in the "mobile phase" are calculated. The factors underlying the ion-exchange selectivity of these amines are discussed.

INTRODUCTION

Primary amines, particularly biogenic ones, have been studied on ion exchangers only by column chromatography[1–6]. With this technique, it has been necessary to use two exchangers with different matrices[2] or aqueous–organic eluents[5,6] in order to separate these compounds, owing to their strong retention on polystyrene-based ion exchangers. With thin-layer chromatography, however, the behaviour of the amines can be studied on a single exchanger without adding organic solvents to the aqueous eluent.

Dowex 50 X4, Rexyn 102 and sodium carboxymethylcellulose (CMCNa) were used as ion exchangers, and CMCNa and alginic acid were used for electrophoretic work.

EXPERIMENTAL

Solutions were obtained by dissolving the amines in 0.01 *M* HCl to give concentrations of 1–3 mg/ml.

Detection

The amines were detected by spraying a solution of 1% ninhydrin in a 5:1 (v/v) mixture of pyridine and glacial acetic acid. When alkaline solutions were used as eluents, the pyridine: acetic acid ratio was 1:5.

Preparation of the layers

The Dowex 50 X4 (H^+ and Na^+), CMCNa and alginic acid layers were prepared as described in a previous paper[7]. The layers of Rexyn 102 (Na^+) and AG1-X4 were prepared by mixing 2 g of each resin with 6 g of microcrystalline cellulose in 40 ml of water. All measurements were carried out at 25°. The migration distance was 11 cm unless otherwise stated.

Electrophoretic measurements

The electrophoretic measurements were carried out as previously described[7].

RESULTS AND DISCUSSION

Dowex 50 X4 (H^+)

The R_F values of 30 amines (arbitrarily divided into three groups) on Dowex 50 X4 (H^+) layers with different HCl concentrations in the eluent are reported in Table I.

TABLE I

R_F VALUES OF PRIMARY MONO- AND DIAMINES ON THIN LAYERS OF DOWEX 50 X4 (H^+)

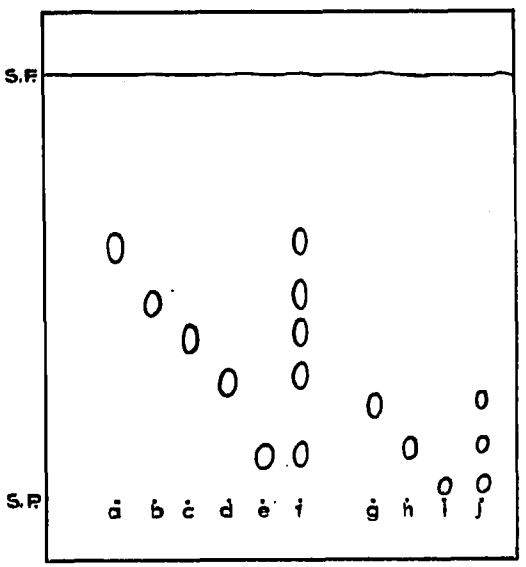
Hydrochloric acid solutions as eluent.

No.	Substance	Concentration of HCl (M)			
		0.5	1	2	4
1	Methylamine	0.22	0.38	0.59	0.78
2	Ethylamine	0.20	0.35	0.53	0.73
3	Ethanolamine	0.30	0.48	0.66	0.82
4	Propylamine	0.17	0.30	0.46	0.65
5	Isopropylamine	0.22	0.37	0.54	0.73
6	*sec.*-Butylamine	0.16	0.30	0.45	0.65
7	Isobutylamine	0.14	0.23	0.38	0.58
8	*n*-Butylamine	0.10	0.19	0.32	0.52
9	Isoamylamine	0.07	0.14	0.25	0.41
10	Hexylamine	0.03	0.07	0.12	0.24
11	1,2-Diaminoethane	0.02	0.06	0.20	0.55
12	1,2-Diaminopropane	0.02	0.05	0.18	0.52
13	1,3-Diaminopropane	0.03	0.07	0.24	0.59
14	1,4-Diaminobutane	0.02	0.06	0.20	0.56
15	1,5-Diaminopentane	0.01	0.03	0.15	0.49
16	1,6-Diaminohexane	0.00	0.02	0.09	0.36
17	1,7-Diaminoheptane	0.00	0.01	0.06	0.25
18	1,8-Diaminooctane	0.00	0.00	0.03	0.18
19	Agmatine	0.01	0.03	0.08 0.19	0.34 0.56
20	Benzylamine	0.06	0.10	0.16	0.28
21	2-Phenylethylamine	0.03	0.06	0.10	0.18
22	1-Phenylethylamine	0.05	0.09	0.14	0.25
23	Amphetamine	0.03	0.07	0.11	0.20
24	Tyramine	0.05	0.09	0.14	0.23
25	Octopamine	0.09	0.16	0.24	0.41
26	3-Hydroxytyramine	0.06	0.11	0.19	0.30
27	Noradrenaline	0.13	0.22	0.34	0.49
28	Histamine	0.01	0.03	0.13	0.47
29	Tryptamine	0.00	0.00	0.02	0.04
30	Naphthylethylenediamine	0.00	0.00	0.01	0.03

The aliphatic monoamines are retained less than the diamines and those amines that contain one or more aromatic rings in the side-chain. The chromatographic characteristics of each group are correlated with both the number of carbon atoms and the structure of the side-chain. With the aliphatic monoamines, a gradual decrease in the R_F value is observed when changing from methylamine to hexylamine; an increase in the R_F value occurs when a hydrogen atom in the side-chain is replaced by a hydroxyl group. Such differences can be used from an analytical point of view, as the chromatogram in Fig. 1 shows. An increase in the R_F value is also observed when changing from a linear to a branched side-chain with the same number of carbon atoms (*e.g.*, compare isopropylamine and isobutylamine with propylamine and butylamine).

With the diamines, owing to the introduction of a second NH_2 group, a decrease in the influence of the non-polar part of the molecule is observed. In fact, the chromatographic characteristics of the first four diaminoalkanes are very similar, with the exception of 1,3-diaminopropane, the R_F values of which are higher than those of the preceding diamines. From 1,4-diaminobutane onwards a decrease in the R_F values is observed as the number of CH_2 groups present increases.

Agmatine is unusual as it gives two spots at HCl concentrations of 2 *M* and above. The two spots may be caused by the partial hydrolysis of agmatine to give 1,4-diaminobutane, as the R_F values of 1,4-diaminobutane and those of the agmatine spot with the higher R_F value are the same. For amines with an aromatic ring in the side-chain, a decrease in R_F value as the number of CH_2 groups present increases is



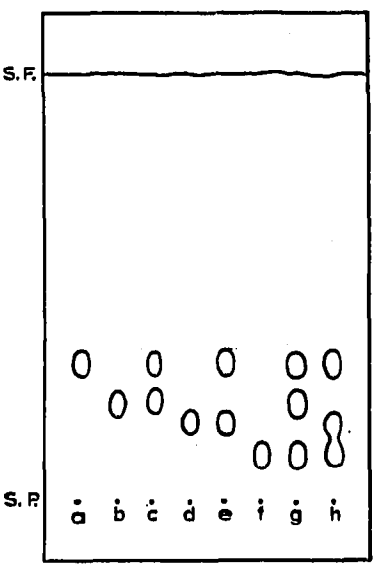


Fig. 1. Thin-layer chromatogram of some aliphatic mono- and diamines on Dowex 50 X4 (H^+). HCl concentration: 2 *M*. Migration distance: 14 cm. (a) Ethanolamine; (b) ethylamine; (c) propylamine; (d) butylamine; (e) hexylamine; (f) mixture; (g) 1,3-diaminopropane; (h) 1,5-diaminopentane; (i) 1,8-diaminooctane; (j) mixture.

Fig. 2. Thin-layer chromatogram of some amines containing an aromatic ring in the side-chain on Dowex 50 X4 (H^+). HCl concentration: 2 *M*. Migration distance: 14 cm. (a) Noradrenaline; (b) octopamine; (c) mixture of (a) and (b); (d) 3-hydroxytyramine; (e) mixture of (a) and (d); (f) tyramine; (g) mixture of (a), (b) and (f); (h) mixture of (a), (d) and (f).

observed. The replacement of a hydrogen atom by a hydroxyl group in the ring and/or in the aliphatic part of the side-chain results in a decrease in the retention. The greatest increase in R_F value is observed when a hydroxyl group is introduced into the aliphatic part of the side-chain, as shown by the chromatogram in Fig. 2.

Dowex 50 X 4 (Na^+), CMCNa and Rexyn 102 (Na^+)

With acetate buffer as eluent, the characteristics of the strong exchanger in the sodium form are practically the same as those of the exchanger in the acid form, apart from a lower retention of the amines when eluting with the same eluent concentration. It should be noted that the affinity sequence of the Dowex for the monoamines is the same as that found by PERRY AND SCHROEDER[2] on Amberlite CG-120 columns. On CMCNa layers, the behaviour of the monoamines is very similar, with the exception of

TABLE II

R_F VALUES OF PRIMARY MONO- AND DIAMINES ON THIN LAYERS OF DOWEX 50 X4 (Na^+), CMCNa AND REXYN 102 (Na^+)

Acetate buffer solutions as eluent.

No.	Substance	*Acetate buffer concentration*			
		Dowex 50 X4 (Na^+)	*CMCNa*		*Rexyn 102 (Na^+)*
		1 M	*0.1 M*	*0.5 M*	*0.2 M*
1	Methylamine	0.53	0.49	0.81	0.34
2	Ethylamine	0.50	0.49	0.81	0.48
3	Ethanolamine	0.60	0.44	0.78	0.32
4	Propylamine	0.42	0.51	0.83	0.55
5	Isopropylamine	0.50	0.51	0.83	0.61
6	*sec.*-Butylamine	0.38	0.52	0.83	0.61
7	Isobutylamine	0.34	0.52	0.83	0.61
8	*n*-Butylamine	0.28	0.52	0.83	0.58
9	Isoamylamine	0.22	0.52	0.83	0.60
10	Hexylamine	0.11	0.52	0.83	0.54
11	1,2-Diaminoethane	0.20	0.05	0.41	0.03
12	1,2-Diaminopropane	0.20	0.06	0.47	0.03
13	1,3-Diaminopropane	0.19	0.06	0.47	0.03
14	1,4-Diaminobutane	0.18	0.08	0.53	0.05
15	1,5-Diaminopentane	0.15	0.08	0.54	0.05
16	1,6-Diaminohexane	0.11	0.09	0.56	0.05
17	1,7-Diaminoheptane	0.06	0.09	0.57	0.06
18	1,8-Diaminooctane	0.04	0.09	0.58	0.05
19	Agmatine	0.04	0.06	0.46	0.03
20	Benzylamine	0.14	0.37	0.72	0.43
21	2-Phenylethylamine	0.08	0.38	0.73	0.38
22	1-Phenylethylamine	0.12	0.40	0.76	0.51
23	Amphetamine	0.11	0.38	0.73	0.43
24	Tyramine	0.08	0.32	0.65	0.31
25	Octopamine	0.20	0.32	0.65	0.30
26	3-Hydroxytyramine	0.12	0.23	0.56	0.21
27	Noradrenaline	0.25	0.24	0.57	0.20
28	Histamine	0.11	0.06	0.46	0.15
29	Tryptamine	0.03	0.19	0.46	0.23
30	Naphthylethylenediamine	0.02	0.16	0.31	0.10

ethanolamine, which is the most strongly retained owing to the presence of a hydroxyl group in the side-chain.

The retention of the diamines decreases as the number of CH_2 groups present increases (Table II). With amines with an aromatic ring in the side-chain, an increase in the affinity for the exchanger is observed when a hydroxyl group is introduced into the aromatic ring. The increase in the affinity is negligible, however, if a hydroxyl group is introduced into the aliphatic part of the side-chain (unlike the results on Dowex).

On sodium polymethacrylate (Rexyn 102), the separation of methylamine from the other monoamines (with the exception of ethanolamine) can be achieved. The amines with an aromatic ring in the side-chain have a very similar behaviour to that observed on CMCNa layers. A good separation can be obtained, however, between the isomers 1-phenylethylamine and 2-phenylethylamine.

The affinity sequence on Rexyn 102 layers is different from that found by PERRY AND SCHROEDER[2]. This difference may be caused by the presence on the layer of microcrystalline cellulose which evidently has a considerable influence on the chromatographic behaviour of the amines.

Influence of the pH of the eluent

Because of the acid–base characteristics of the amines, it is necessary to use alkaline solutions as eluents in order to achieve deprotonation of at least part of them. As almost all of the amines ran with the solvent front both on CMCNa and Rexyn 102 (Na^+) layers with a 0.1 *M* solution of Na_2CO_3, we used solutions with a lower pH. Of the eluents used, the best results were obtained with 0.08 *M* $NaHCO_3$ and 0.01 *M* Na_2CO_3 solution as eluent. Some useful separations obtained with this eluent on CMCNa and Rexyn 102 (Na^+) layers are reported in Table III.

TABLE III

SEPARATIONS OBTAINED ON CMCNa AND REXYN 102 (Na^+) THIN LAYERS

Elution with 0.01 *M* Na_2CO_3 + 0.08 *M* $NaHCO_3$.

Amine	R_F *value*	
	CMCNa	*Rexyn 102 (Na^+)*
1,2 Diaminoethane	0.70	
1,2-Diaminopropane	0.70	
1,3-Diaminopropane	0.31	
1,4-Diaminobutane[a]	0.12	
Benzylamine	0.55	0.45
2-Phenylethylamine	0.39	0.28
Octopamine	0.69	0.39
Tyramine	0.37	0.20
Noradrenaline	0.68	0.35
3-Hydroxytyramine	0.43	0.18
Histamine	0.43	0.18
Tryptamine	0.21	0.11

[a] 1,4-Diaminobutane can be replaced with any of the homologous C_5–C_8 diamines (R_F range = 0.08–0.03).

Electrophoretic measurements

The electrophoretic measurements were carried out on alginic acid and on CMCNa layers using 1 M acetic acid and 0.05 M acetate buffer, respectively, as electrolytes.

On both exchangers, the mobility of the monoamines is greater than that of the other amines. Such behaviour, which cannot be predicted on the basis of the charge of the corresponding ions, is due to the greater retention characteristics of the two exchangers towards the diamines. It is interesting to note the good separation among the amines with an aromatic ring in the side-chain and containing different numbers of hydroxyl groups in the ring (Fig. 3). Fig. 3 also shows the separation of histamine, tryptamine and naphthylethylenediamine.

Retention mechanism

Applying the relationship

R_M + constant = $-n \log a_{AcONa}$

linear plots for all the amines on CMCNa layers were obtained. The slope of the lines is unity for the monoamines, tryptamine and naphthylethylenediamine, and about 1.7 for the other amines. This seems to suggest that the retention mechanism is essentially an ion-exchange process. On Dowex 50 X4(Na^+), when eluting with buffer solutions, linear plots with a slope of unity are also obtained for the aliphatic monoamines. The other amines are retained too strongly, so that it is not possible to obtain a reliable plot of R_M as the sodium acetate activity changes.

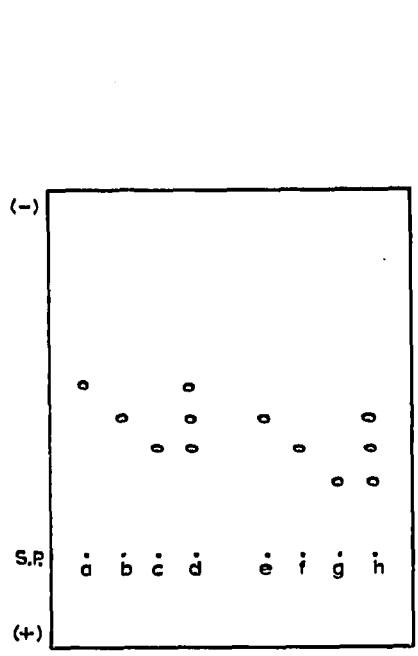
On Dowex 50 X4 (H^+), however, curvilinear plots are obtained (Fig. 4). The plot of R_M against $-\log[HCl]$ departs from linearity for $[HCl] > 2\ M$, at which concentrations a large increase in the hydrochloric acid activity is obtained.

The plot of R_M against $-\log a_{HCL}$ shows the opposite effect. Such behaviour, which has already been observed for purines and pyrimidines, although to a lesser extent[7], may be explained by assuming that the hydrochloric acid activity in the "mobile phase" is different from that in the eluent. In order to support such an assumption, the following should be considered: (a) the aliphatic mono- and diamines are not adsorbed on layers of polystyrene-based anion exchanger (AG1-X4), and (b) the aliphatic monoamines exhibit linear plots with a slope of unity on Dowex 50 X4 (Na^+) layers. In Fig. 4 are shown two arbitrary straight lines with slopes of 1 and 1.7, drawn through the point common to the two experimental curves. The value of 1.7 found for the diamines on CMCNa can be considered to be reliable for the slopes of the divalent ions[8].

On the basis of these results, it is possible to obtain "new activity coefficients" for the hydrochloric acid concentrations investigated. Such values, reported in Table IV, are useful when the $R_M/-\log a_{HCl}$ relationship must be studied on these layers. In this connection, it is interesting to note that by using these activity coefficients and the R_F values of the purines and pyrimidines reported in a previous paper[7], linear plots are obtained with slopes that agree with those predicted for mono- and divalent ions.

With these new activity coefficients, linear plots are not obtained, however, for amines with an aromatic ring in the side-chain. The deviation from linearity is due to the adsorption of these compounds by the exchanger matrix. In fact, on AG1-X4 layers these amines are strongly retained over the whole range of HCl concentrations

used. It follows, therefore, that the deviation from linearity of the plot of R_M against HCl activity in the "mobile phase" may be an indirect measure of those adsorption processes which occur in the absence of ion exchange.



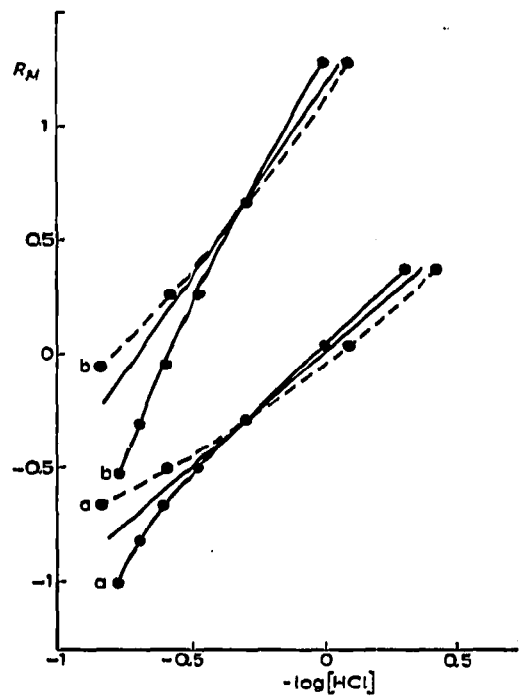


Fig. 3. Electrophoresis on CMCNa thin layers of (a) 2-phenylethylamine; (b) tyramine; (c) 3-hydroxytyramine; (d) mixture of (a), (b) and (c); (e) histamine; (f) tryptamine; (g) naphthylethylene diamine; (h) mixture of (e), (f) and (g). Potential 800 V. Time = 30 min. Electrolyte: 0.05 *M* acetate buffer.

Fig. 4. Plots of R_M values against — log [HCl] (continuous line) and against — log a_{HCl} (dashed line) on Dowex 50 X4 (H^+) thin layers. (a) Ethanolamine; (b) 1,2-diaminopropane.

TABLE IV

MEAN ACTIVITY COEFFICIENTS OF HYDROCHLORIC ACID IN SOLUTION AND IN THE "MOBILE PHASE"

HCl concentration (M)	*Activity coefficient*	
	In solution[a]	*In the "mobile phase"*
0.5	0.757	0.87
1.0	0.809	0.90
2.0	1.009	1.00
3.0	1.316	1.12
4.0	1.762	1.21
5.0	2.38	1.40
6.0	3.22	1.64

FACTORS UNDERLYING ION-EXCHANGE SELECTIVITY

In order to characterize the factors that affect the chromatographic behaviour of the amines, we think it useful to sum up the most important characteristics obtained from the experimental results:

(a) the aliphatic monoamines and diamines show opposite behaviours on CMCNa and Dowex 50 X4 (Na^+) when using the same solutions as eluents;

(b) no appreciable differences are obtained on Dowex 50 X4 (H^+) and (Na^+) layers when eluting with different solutions or with different concentrations of acid or salt in the eluent;

(c) on CMCNa layers, ethanolamine is the most strongly retained of the monoamines;

(d) on microcrystalline cellulose and AGl-X4 layers, the most strongly retained of the amines with an aromatic ring in the side-chain are those which contain one or more hydroxyl groups in the aliphatic part of the side-chain or in the aromatic ring (Table V).

On the basis of such characteristics, a choice is possible between the two factors that generally produce the selectivity in the ion-exchange processes, *i.e.*, the interactions of the side-chain of the organic ion with (*i*) the solvent and (*ii*) the matrix of the resin[10].

In this case, the interactions with the solvent are negligible with respect to those with the matrix of both CMCNa and Dowex 50 X4. With Dowex points (a), (b) and (d), and with CMCNa points (a), (c) and (d), support this assumption. Points (c) and (d), in particular, are completely opposed to the characteristics that can be predicted on the basis of the influence of the interactions between the side-chain of the amine and the solvent. It should also be noted that with amino acid cations on polystyrene exchangers the adsorption by the matrix of the resin is the determining factor[10].

TABLE V

R_F VALUES OF THOSE AMINES RETAINED ON MICROCRYSTALLINE CELLULOSE AND AG1-X4 THIN LAYERS

Eluent: 1 *M* HCl.

Substance	R_F *value*	
	Microcrystalline cellulose	*AG1-X4*
Benzylamine	0.92	0.89
2-Phenylethylamine	0.93	0.83
1-Phenylethylamine	0.94	0.88
Amphetamine	0.93	0.88
Tyramine	0.84	0.58
Octopamine	0.84	0.59
3-Hydroxytyramine	0.79	0.54
Noradrenaline	0.77	0.54
Tryptamine	0.70	0.24
Naphthylethylenediamine	0.70	e.s.[a]

[a] e.s. = elongated spot.

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