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CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOR OF PURINES AND PYRIMIDINES ON LAYERS OF WEAK AND STRONG CATION EXCHANGERS

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SUMMARY

The chromatographic behavior of seven purines, fourteen pyrimidines and three nucleosides on thin layers of alginic acid, sodium carboxymethylcellulose and Dowex 50-X4 (H^+ and Na^+) was investigated.

The eluents used were water, buffer solutions and mineral acid solutions. The possible separations are discussed. The retention mechanisms of these compounds, of eight pyridines and of eight primary aromatic amines are correlated and explained, on both cellulose- and polystyrene-based ion exchangers

The applicability of the relation: $-n \log a_{H^+} = R_M +$ constant is discussed when using eluents with high protonic activity.

Finally, as regards the retention of the bases by Dowex 50-X4 (H^+), the influence of interactions between the sulfonic groups of the resin and the polar groups of the compounds is discussed.

INTRODUCTION

Ion-exchange chromatography has been widely used in the study of the behavior of natural purines and pyrimidines. As regards the techniques employed, chromatography on cation- and anion-exchange columns[1-8] has been the most used. On thin layers much work has been performed on anion exchangers[9,10] while only a short communication concerns cation exchange, on cellulose polyphosphate layers[11]. Therefore we thought it useful to study the chromatographic behavior of natural purines and pyrimidines on weak and strong cation exchangers in order to compare the data obtained on thin layers with those on columns.

This work has been extended to many synthetic purines and pyrimidines and some nucleosides in order to obtain a complete picture of the chromatographic behavior of these classes of compounds. Moreover, in order to explain the complex mechanism determining the retention of these bases, we thought it useful to compare their chromatographic characteristics with those of several pyridines and primary aromatic amines. We chose pyridines because of their structural analogy with pyrimi-

dines, and aromatic amines because of the large adsorption of these compounds on polystyrene-based ion exchangers. These latter compounds have already been studied by us on weak cation-exchange layers[12].

Besides thin-layer chromatography (TLC) we have also employed high-potential electrophoresis.

EXPERIMENTAL

The solutions were obtained by dissolving most compounds in 0.01 *M* HCl. 2-Amino-6-chloropurine, guanine, α-naphthylamine and *p*-nitroaniline were dissolved in 3 *M* HCl; hypoxanthine and 4-aminouracil in water; xanthine and 2-amino-4,6-dihydroxypyrimidine in a 0.01 *M* solution of NH_3. All solutions were stable for at least a week. The concentration of solutions was 1–3 μg/ml.

The solutions of xanthine and 2-amino-4,6-dihydroxypyrimidine were neutralized before their application to alginic acid thin layers in order to prevent the gelatinization of the exchanger.

Detection

In most cases the compounds were detected by means of UV light ($\lambda = 254$ nm). When exposure to UV light was inadequate, a solution of 5% *p*-dimethyl-aminobenzaldehyde (PDAB) in glacial acetic acid was used. 2,4-Lutidine and 2,4,6-collidine were detected with Dragendorff's reagent.

Preparation of the layers

Layers having a thickness of 300 μ were used. The layers were obtained with a Chemetron automatic apparatus using the following mixtures: (1) alginic acid[13]: 4 g of the exchanger and 1 g of cellulose (No. 123, Carl Schleicher and Schüll) in 40 ml of water; (2) sodium carboxymethylcellulose (CMCNa) (No. 132, Carl Schleicher and Schüll), capacity 1.18 mequiv./g): 4.5 g in 50 ml of water; (3) Dowex 50-X4 (H^+ and Na^+): 2 g of the exchanger (200–400 mesh) and 6 g of microcrystalline cellulose (Merck) in 40 ml of water.

The use of microcrystalline cellulose in the preparation of Dowex 50-X4 thin layers was necessary for the following reasons: (a) detection of the compounds by means of UV light is possible only when the ratio Dowex: cellulose is $< 1:3$; (b) on Dowex/non-microcrystalline cellulose at the above-mentioned ratios, the elution rate is very fast and we obtain very elongated spots, which are not suitable for analytical purposes or for the study of the retention mechanism.

The Dowex 50-X4 (H^+) was repeatedly rinsed with water and methanol and then dried at room temperature before use. Dowex 50-X4 (Na^+) was obtained by neutralization of the acid form with NaOH.

The use of microcrystalline cellulose was impossible in the case of alginic acid since the layers had poor mechanical properties.

The chromatographic measurements were carried out at 25° using the DBGM Cryobox Desaga Chamber for constant-temperature TLC.

Electrophoretic measurements

The electrophoretic measurements were made with a Camag apparatus for high-

potential electrophoresis, at a temperature of 18°. The electroosmotic flow was measured with hydrogen peroxide.

RESULTS AND DISCUSSION

Alginic acid

The R_F values of seven purines, fourteen pyrimidines, three nucleosides, eight pyridines and eight primary aromatic amines when eluted with water and mixtures of 1 *M* acetic acid and HCl at different pH values are shown in Table I. Water allows the separation of natural purines and pyrimidines with an $-NH_2$ group (adenine, guanine, cytosine, 5-methylcytosine) from those without amino groups. Among the latter the separation of hypoxanthine from xanthine and of these two from uracil and thymine is possible. As regards pyrimidines it is interesting to note the different behavior of the following isomers: 5-aminouracil ($R_F = 0.00$), 2-amino-4,6-dihydroxypyrimidine ($R_F = 0.31$) and 4-aminouracil ($R_F = 0.52$). Among nucleosides it is possible to separate guanosine from the others.

From the data obtained with acidic eluents we note an increase in the R_F values of most bases as the pH decreases, with the exception of uracil, thymine, 4-aminouracil, *p*-nitroaniline and, in a more limited pH range, xanthine and 2-amino-4,6-dihydroxypyrimidine. This behavior may be utilized for analytical purpose by selecting an eluent with a suitable pH value. As regards the behavior of natural purines and pyrimidines the possibility should be noted, with respect to the trend obtained on eluting with water, of separating at pH = 2 (among the bases containing $-NH_2$ groups) adenine and guanine from cytosine and 5-methylcytosine.

Dowex 50-X4 (H^+)

The data in Table II show that only uracil and thymine have high R_F values which do not increase with the acid concentration in the eluent in the 0.25–1.0 *M* HCl range. In contrast to alginic acid, on this exchanger the separation of adenine from guanine and of adenosine from the remaining nucleosides is possible, when eluting with 2 *M* HCl. It is interesting to note that the affinity sequence of natural purines and pyrimidines on this layer is similar to that found on Dowex 50-X4 (H^+) columns by WALL[5] and COHN[6]. Nucleosides, in contrast, show a sequence (adenosine > cytidine = guanosine) which is different from that found on a column[6] (adenosine > cytidine > guanosine). This difference may be ascribed to the presence of microcrystalline cellulose in the layer. The different behavior of uracil and thymine on this exchanger, not observed on cellulose-based ion exchangers, is ascribed to the greater affinity of the polystyrene matrix of the resin for the pyrimidine containing the methyl group. Such behavior has been observed by COHN[14].

When eluting with water all the bases remain at the origin, with the exception of 4-aminouracil ($R_F = 0.13$), thymine ($R_F = 0.55$) and uracil ($R_F = 0.62$), and therefore the separation of these from the others is possible.

CMCNa and Dowex 50-X4 (Na^+)

On CMCNa we observe for most bases a lower retention than that on the other exchangers when eluting with water (see Table III). However, 4,5-diaminopyrimidine and 2,4,6-triaminopyrimidine are strongly retained and their separation from all the

TABLE I

R_F VALUES OF PURINES, PYRIMIDINES, NUCLEOSIDES, PYRIDINES AND PRIMARY AROMATIC AMINES ON THIN LAYERS OF ALGINIC ACID

Water and acetic acid + hydrochloric acid solutions as eluents. Acetic acid concentration, 1 mole/l. n.d. = not determined.

Substance	*Water*	*pH of acetic acid and hydrochloric acid solutions*					
		2.55	*2.20*	*2.00*	*1.75*	*1.45*	*1.20*
Purine	0.06	0.12	0.16	0.23	0.31	0.47	0.60
2-Aminopurine	0.00	0.05	0.09	0.14	0.24	0.37	0.49
Guanine	0.00	0.06	0.10	0.15	0.25	0.38	0.50
2-Amino-6-chloropurine	0.00	0.06	0.10	0.15	0.24	0.37	0.50
Adenine	0.00	0.05	0.10	0.15	0.24	0.37	0.51
Hypoxanthine	0.17	0.24	0.28	0.31	0.39	0.48	0.58
Xanthine	0.40[a]	0.53[a]	0.53[a]	0.53[a]	0.55[a]	0.58[a]	0.61[a]
Pyrimidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Aminopyrimidine	0.01	0.07	0.14	0.21	n.d.	n.d.	n.d.
Isocytosine	0.00	0.07	0.14	0.22	0.32	0.48	0.60
2-Amino-4,6-dihydroxy-pyrimidine	0.31	0.44	0.44	0.45	0.47	0.54	0.63
2-Amino-4,6-dimethyl-pyrimidine	0.00	0.06	n.d.	n.d.	n.d.	n.d.	n.d.
Cytosine	0.00	0.07	0.14	0.22	0.32	0.48	0.60
5-Methylcytosine	0.00	0.07	0.14	0.21	0.31	0.48	0.60
Uracil	0.79	0.81	0.81	0.81	0.82	0.82	0.82
Thymine	0.79	0.80	0.81	0.81	0.81	0.82	0.82
4-Aminouracil	0.52	0.58	0.58	0.58	0.60	0.60	0.61
5-Aminouracil	0.00	0.09	0.11	0.17	0.25	0.45	0.60
4,5-Diaminopyrimidine	0.00	0.05	0.10	0.16	0.26	0.43	0.58
2,4-Diamino-6-chloropyrimidine	0.01	0.07	0.10	0.16	0.26	0.43	0.59
2,4,6-Triaminopyrimidine	0.00	0.02	0.04	0.06	0.11	0.22	0.35
Adenosine	0.00	0.09	0.14	0.22	0.32	0.49	0.61
Guanosine	0.13	0.20	0.24	0.30	0.38	0.49	0.59
Cytidine	0.00	0.08	0.16	0.23	0.34	0.50	0.61
Pyridine	0.00	0.12	0.23	0.32	0.44	0.62	n.d.
2-Aminopyridine	0.00	0.09	0.18	0.26	0.37	0.53	0.68
4-Picoline	0.00	0.12	0.23	0.31	0.44	0.63	0.74
2,4-Lutidine	0.01	0.14	0.26	0.36	0.49	0.66	0.77
2,4,6-Collidine	0.01	0.16	0.31	0.42	0.56	n.d.	n.d.
Nicotinic acid	0.24	0.26	0.32	0.43	0.56	0.71	n.d.
Nicotinamide	0.01	0.09	0.16	0.24	0.33	0.50	0.64
Pyridoxine	0.00	0.12	0.23	0.33	0.44	n.d.	n.d.
Aniline	0.00	0.10	0.18	0.26	0.38	0.56	0.68
p-Toluidine	0.00	0.09	0.18	0.26	0.38	0.55	0.68
p-Nitroaniline	0.34	0.49	0.50	0.50	0.52	0.53	0.55
p-Chloroaniline	0.00	0.08	0.15	0.22	0.33	0.50	0.64
p-Bromoaniline	0.00	0.08	0.15	0.22	0.33	0.56	0.63
p-Aminobenzoic acid	0.08	0.17	0.20	0.28	0.38	0.53	0.66
p-Aminohippuric acid	0.11	0.16	0.18	0.28	0.39	0.55	0.68
α-Naphthylamine	0.00	0.06	0.11	0.17	0.24	0.40	0.53

[a] Elongated spot.

others is therefore possible. It is interesting to note on this exchanger the clear separation of isocytosine from cytosine and 5-methylcytosine.

With acetate buffer as eluent, the R_F values are similar to those obtained when

TABLE II

R_F VALUES OF PURINES, PYRIMIDINES, NUCLEOSIDES, PYRIDINES AND PRIMARY AROMATIC AMINES ON THIN LAYERS OF DOWEX 50-X4 (H^+)

Hydrochloric acid solutions as eluents. n.d. = not determined.

Substance	*Normality of acid*					
	0.25	*0.5*	*0.75*	*1.0*	*2.0*	*4.0*
Purine	0.06	0.10	0.15	0.18	0.33	0.57
2-Aminopurine	0.02	0.04	0.06	0.08	0.16	0.42
Guanine	0.04	0.08	0.10	0.13	0.23	0.47
2-Amino-6-chloropurine	0.04	0.07	0.10	0.13	0.23	0.46
Adenine	0.02	0.04	0.06	0.08	0.16	0.42
Hypoxanthine	0.11	0.19	0.23	0.27	0.39	0.61
Xanthine	0.13[a]	0.25	0.31	0.33	0.48	0.64
Pyrimidine	0.09	0.16	0.23	0.27	0.44	0.69
2-Aminopyrimidine	0.07	0.14	0.17	0.23	0.37	0.61
Isocytosine	0.07	0.13	0.16	0.22	0.35	0.60
2-Amino-4,6-dihydroxypyrimidine	0.11	0.16	0.20	0.24	0.38	0.59
2-Amino-4,6-dimethylpyrimidine	0.06	0.10	n.d.	n.d.	n.d.	n.d.
Cytosine	0.08	0.14	0.18	0.25	0.38	0.62
5-Methylcytosine	0.08	0.14	0.18	0.25	0.38	0.60
Uracil	0.65	0.67	0.67	0.68	0.70	0.81
Thymine	0.61	0.62	0.63	0.63	0.66	0.77
4-Aminouracil	0.21	0.23	0.26	0.29	0.40	0.59
5-Aminouracil	0.22	0.35	0.40	0.47	0.64	0.76
4,5-Diaminopyrimidine	0.03	0.06	0.09	0.12	0.30	0.54
2,4-Diamino-6-chloropyrimidine	0.02	0.04	0.06	0.08	0.18	0.36
2,4,6-Triaminopyrimidine	0.00	0.00	0.02	0.03	0.11	0.41
Adenosine	0.06	0.10	0.15	0.19	0.34	0.57
Guanosine	0.11	0.17	0.23	0.29	0.46	0.69
Cytidine	0.10	0.17	0.23	0.29	0.46	0.70
Pyridine	0.06	0.10	0.16	0.19	0.33	0.57
2-Aminopyridine	0.03	0.06	0.09	0.11	0.20	0.43
4-Picoline	0.05	0.08	0.12	0.15	0.26	0.52
2,4-Lutidine	0.03	0.06	0.09	0.11	0.20	0.43
2,4,6-Collidine	0.02	0.04	0.06	0.08	0.15	0.37
Nicotinic acid	0.07	0.12	0.18	0.21	0.36	0.62
Nicotinamide	0.07	0.12	0.18	0.21	0.36	0.62
Pyridoxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aniline	0.04	0.07	0.11	0.14	0.28	0.43
p-Toluidine	0.01	0.01	0.03	0.07	0.16	0.26
p-Nitroaniline	0.01	0.02	0.04	0.08	0.17	0.33
p-Chloroaniline	0.01	0.01	0.03	0.05	0.11	0.20
p-Bromoaniline	0.00	0.01	0.01	0.03	0.09	0.12
p-Aminobenzoic acid	0.03	0.05	0.08	0.09	0.20	0.35
p-Aminohippuric acid	0.09	0.16	0.22	0.24	0.39	0.58
α-Naphthylamine	0.00	0.01	0.02	0.02	0.05	0.10

[a] Elongated spot.

eluting with water, with the exception of 4,5-diaminopyrimidine, 2,4,6-triaminopyrimidine, cytosine, 5-methylcytosine, adenine and 2-aminopurine, which are at least partly protonated at the acetate buffer pH.

The R_F values of those bases whose retention is independent of the eluent represent a direct measurement of the adsorption of the compounds on the cellulose matrix of the exchanger.

TABLE III

R_F VALUES OF PURINES, PYRIMIDINES, NUCLEOSIDES, PYRIDINES AND PRIMARY AROMATIC AMINES ON THIN LAYERS OF CMCNa AND DOWEX 50-X4 (Na^+)

Water and acetate buffer as eluents. n.d. = not determined.

Substance	*Eluents*				
	CMCNa		*Dowex 50-X4 (Na^+)*		
	Water[a]	*0.5 M acetate buffer*	*Water*[a]	*0.1 M acetate buffer*	*0.5 M acetate buffer*
Purine	0.88	0.88	0.48	0.36	0.47
2-Aminopurine	0.67	0.71	n.d.	0.04	0.17
Guanine	0.61	0.62	n.d.	0.08	0.22
2-Amino-6-chloropurine	0.63	0.63	n.d.	0.08	0.21
Adenine	0.56	0.63	n.d.	0.03	0.09
Hypoxanthine	0.82	0.83	0.39	0.38	0.39
Xanthine	0.81[b]	0.72[b]	0.52[b]	0.26[b]	0.30[b]
Pyrimidine	n.d.	n.d.	n.d.	n.d.	n.d.
2-Aminopyrimidine	n.d.	n.d.	n.d.	0.13	0.34
Isocytosine	0.75	0.76	0.48	0.06	0.20
2-Amino-4,6-dihydroxypyrimidine	0.93	0.84	0.60	0.59	0.60
2-Amino-4,6-dimethylpyrimidine	n.d.	n.d.	n.d.	0.02	0.08
Cytosine	0.56	0.70	0.47	0.02	0.13
5-Methylcytosine	0.54	0.71	0.38	0.02	0.11
Uracil	0.84	0.92	0.62	0.62	0.62
Thymine	0.86	0.93	0.58	0.56	0.58
4-Aminouracil	0.81	0.80	0.51	0.51	0.52
5-Aminouracil	0.88	0.88	0.58	0.35	0.54
4,5-Diaminopyrimidine	0.08	0.71	0.08[b]	0.00	0.05
2,4-Diamino-6-chloropyrimidine	0.71	0.72	0.29	0.05	0.11
2,4,6-Triaminopyrimidine	0.00	0.44	0.00	0.00	0.04
Adenosine	0.84	0.83	n.d.	0.09	0.23
Guanosine	0.80	0.80	0.44	0.33	0.43
Cytidine	0.81	0.82	n.d.	0.08	0.24
Pyridine	—	n.d.	—	0.01	0.07
2-Aminopyridine	—	0.75	—	0.00	0.04
4-Picoline	—	n.d.	—	0.01	0.06
2,4-Lutidine	—	n.d.	—	0.00	0.05
2,4,6-Collidine	—	n.d.	—	0.00	0.05
Nicotinic acid	—	0.95	—	0.72	0.78
Nicotinamide	—	0.93	—	0.16	0.40
Pyridoxine	—	0.86	—	0.01	0.09
Aniline	—	n.d.	—	0.02	0.09
p-Toluidine	—	n.d.	—	0.01	0.04
p-Nitroaniline	—	0.75	—	0.10	0.10
p-Chloroaniline	—	n.d.	—	0.02	0.05
p-Bromoaniline	—	n.d.	—	0.00	0.03
p-Aminobenzoic acid	—	0.96	—	0.20	0.25
p-Aminohippuric acid	—	0.96	—	0.62	0.65
α-Naphthylamine	—	0.58	—	0.01	0.02

[a] The R_F values refer to solutions that were originally neutral or alkaline.
[b] Elongated spot.

On Dowex 50-X4 (Na^+) the R_F values, relative to water as eluent, are the same as those obtained on the acid form of the exchanger for those compounds which are protonated in the starting solutions. This behavior is anomalous if compared with that of the same bases on CMCNa, and this may be explained by supposing that, when applying the solution to the layer, a bond between the sulfonate group of the resin and the protonated form of the base is formed; such a bond being stronger than the one concerning the carboxyl group bond of CMCNa. In fact, when neutralizing the solutions of the bases, we obtain R_F values different from those found on Dowex in the acid form. The purines, pyrimidines and nucleosides, whose R_F values are not reported, were not sufficiently soluble in water for their detection to be possible.

Since on Dowex 50-X4 (Na^+) we can exclude, with the exception of 4,6-diaminopyrimidine and 2,4,6-triaminopyrimidine, the influence of the ion-exchange process on the retention mechanism, the R_F values are a direct measurement of the adsorption by the exchanger matrix.

5-Methylcytosine is more strongly adsorbed than cytosine or isocytosine, and this is similar to the behavior of thymine and uracil.

In acetate buffer the R_F values of hypoxanthine, 2-amino-4,6-dihydroxypyrimidine, uracil, thymine and 4-aminouracil are similar to those obtained by eluting with water. The behavior of these compounds shows that, even at the acetate buffer pH, their retention is due to an adsorption process; a further confirmation of this is given by their R_F values which do not vary as the buffer concentration increases.

From an analytical point of view the behavior of purines and pyrimidines on Dowex 50-X4 (Na^+) allows some interesting separations which are not possible on the same exchanger in the acid form. It is particularly interesting to note the differences in the R_F values, eluting with 0.5 M acetate buffer, between adenine and 2-aminopurine and among isocytosine, 2-aminopyrimidine and 2-amino-4,6-dihydroxypyrimidine.

As regards pyridines, good chromatographic separation between nicotinic acid, nicotinamide and pyridoxine is possible. In the case of aromatic amines it is interesting to note the separation of *p*-aminohippuric acid from *p*-aminobenzoic acid, and of these two from all the others.

Electrophoretic measurements

The electrophoretic measurements were carried out on layers of alginic acid, CMCNa and Dowex 50-X4 (Na^+) using 1 M acetic acid and 0.1 M acetate buffer as electrolytes. The best results were obtained on alginic acid and are reported in Table IV. It is interesting to note the separation of the compounds containing two or three amino groups: 2-amino-4,6-dimethylpyrimidine from 2-aminopyrimidine and these two compounds from all the others. In the case of the pyridines the separation of nicotinic acid, nicotinamide and pyridoxine from the others is possible.

There is no correlation between the migration distances and the pK_a values. From this we deduce that the mobility of these substances is greatly influenced both by the adsorption on the layer and by the ion-exchange process.

Retention mechanism

In order to ascertain the influence of the ion-exchange process on the retention mechanisms on alginic acid and Dowex 50-X4 (H^+) and to determine the charge of the

TABLE IV

MIGRATION DISTANCES (mm) AND pK_a OF PURINES, PYRIMIDINES, NUCLEOSIDES, PYRIDINES AND PRIMARY AROMATIC AMINES ON THIN LAYERS OF ALGINIC ACID.

Electric potential, 1100 V; electrolyte, 1 *M* CH_3COOH. n.d. = not determined.

Substance	*Time (min)*		pK_a[6,16,17]
	30	*60*	
Purine	24	47	2.4
2-Aminopurine	15	30	3.7
Guanine	15	29	3.3
2-Amino-6-chloropurine	15	29	—
Adenine	16	32	4.1
Hypoxanthine	20	37	1.9
Xanthine	16[a]	30[a]	0.8
Pyrimidine	30	n.d.	1.3
2-Aminopyrimidine	34	75	3.5
Isocytosine	27	52	4.0
2-Amino-4,6-dihydroxypyrimidine	19	32	1.3
2-Amino-4,6-dimethylpyrimidine	29	62	4.9
Cytosine	26	52	4.6
5-Methylcytosine	25	49	4.6
Uracil	21	40	<1
Thymine	21	40	~0
4-Aminouracil	17	34	0.8
5-Aminouracil	22	36	—
4,5-Diaminopyrimidine	23	48	6.0
2,4-Diamino-6-chloropyrimidine	18	33	3.6
2,4,6-Triaminopyrimidine	9	22	6.8
Adenosine	13	25	3.5
Guanosine	14	25	2.2
Cytidine	16	31	4.1
Pyridine	48	120	5.2
2-Aminopyridine	39	78	6.7
4-Picoline	41	108	6.0
2,4-Lutidine	41	97	6.7
2,4,6-Collidine	40	88	7.5
Nicotinic acid	27	56	2.0
Nicotinamide	29	56	3.4
Pyridoxine	29	57	5.0
Aniline	32	55	4.6
p-Toluidine	26	47	5.1
p-Nitroaniline	18	35	1.0
p-Chloroaniline	23	40	4.0
p-Bromoaniline	21	36	3.9
p-Aminobenzoic acid	18	34	2.2
p-Aminohippuric acid	17	29	—
α-Naphthylamine	13	22	4.0
H_2O_2	25	52	

[a] Elongated spot.

ion involved in the exchange process, the following relationship[15] was applied:

$$-n \log a_{H^+} = R_M + \text{constant.} \quad (1)$$

Alginic acid. By applying eqn. 1 to most bases we obtain lines whose slopes are between 0.9 and 1, which is in accordance with an ion-exchange mechanism involving monovalent cations. For the other bases curvilinear trends are obtained (see Fig. 1),

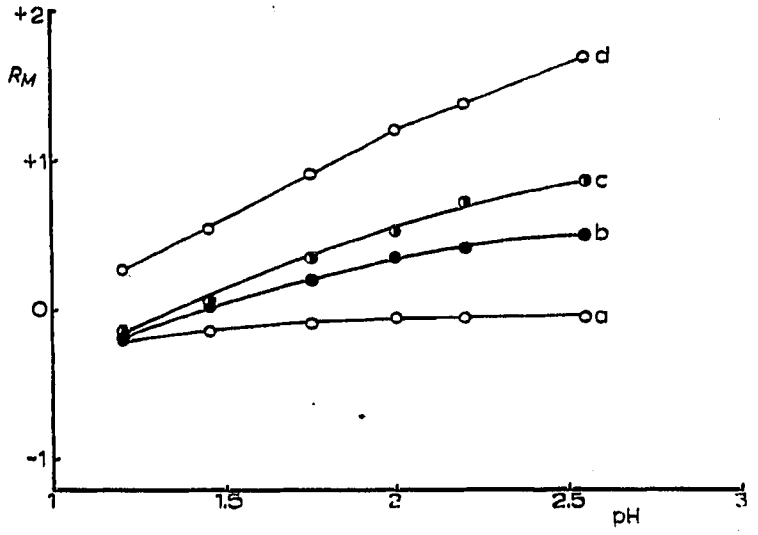

Fig. 1. R_M values *vs.* pH for some purines and pyrimidines on alginic acid thin layers. Eluents: 1 *M* acetic acid + hydrochloric acid solutions. (a) Xanthine; (b) hypoxanthine; (c) purine; (d) 2,4,6-triaminopyrimidine.

which are attributable to the different protonation of these bases in the pH range explored. In fact the bases whose pK_a values are <1 show a trend similar to curve (a) of Fig. 1 and those whose pK_a values are between 1.9 and 2.4 are similar to curves (b) and (c) of the same figure.

In the case of 2,4,6-triaminopyrimidine the curvilinear trend (see curve (d) of Fig. 1) is not due to partial protonation, owing to the high value of its basicity constant. Such a trend may be considered as the result of two straight lines whose slopes are 0.87 and 1.18, respectively. These values may be explained by supposing that the base, as the acid concentration in the eluent increases, changes from the monoprotonated form into the diprotonated one.

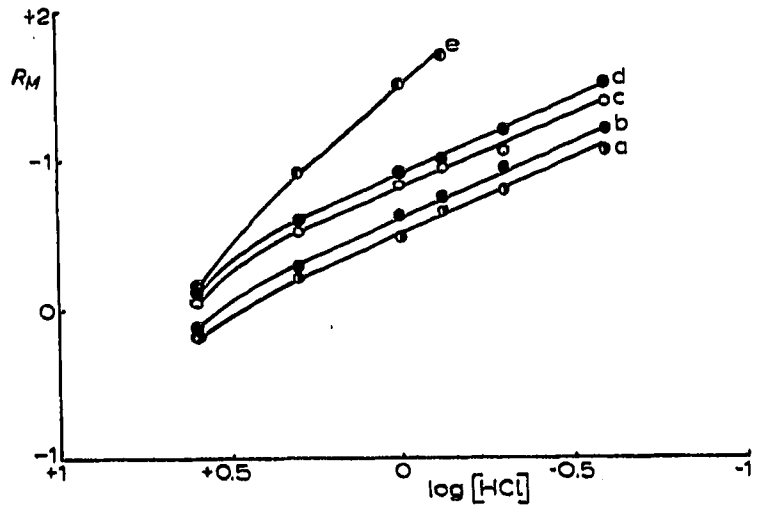

Fig. 2. R_M values *vs.* log[HCl] for some bases on Dowex 50-X4 (H^+) thin layers. Eluents: hydrochloric acid solutions. (a) Cytosine; (b) adenosine; (c) guanine; (d) 2-aminopyridine; (e) 2,4,6-triaminopyrimidine.

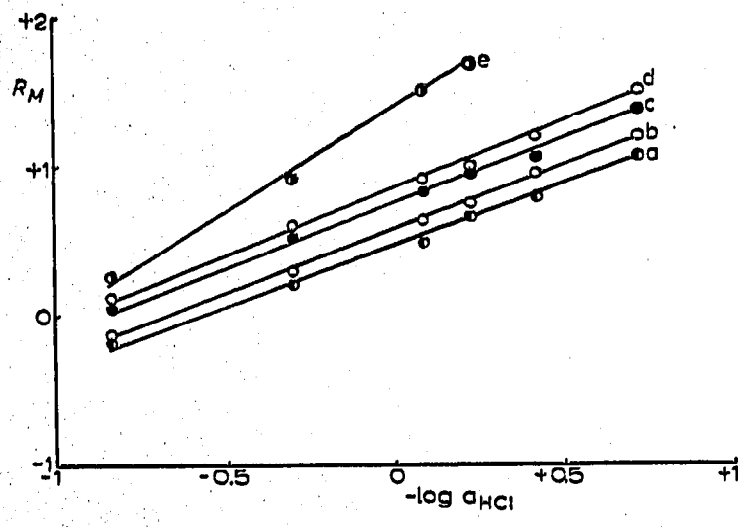

Fig. 3. R_M values *vs.* $-\log a_{HCl}$ for some bases on Dowex 50-X4 (H^+) thin layers. Eluents: hydrochloric acid solutions. (a) Cytosine; (b) adenosine; (c) guanine; (d) 2-aminopyridine; (e) 2,4,6-triaminopyrimidine.

Dowex 50-X4 (H^+)

ALBERTI *et al.*[18] and CERRAI *et al.*[19] applied eqn. 1 by plotting the logarithm of the concentration instead of the logarithm of the activity. Such approximation gives fairly good results on exchangers in the sodium salt form when eluting with neutral salt solutions; in this case, however, the slope values deduced from the $R_M/\log C_{salt}$ graph are lower than those from the $R_M/\log a_{salt}$ graph[20].

On cation exchangers in the acid form, plotting R_M as a function of $\log C_{HCl}$, we obtain a linear trend until $C_{HCl} < 2M$ (see Fig. 2). For $C_{HCl} > 2M$ we observe a deviation from the linearity observed by NELSON *et al.*[21] for the progression of $\log K_d/\log C_{HCl}$ values in the case of inorganic ions. If the concentration is replaced by the activity we observe a linear trend in the whole hydrochloric acid concentration range. The curvilinear trend of the $R_M/\log C_{HCl}$ graph for $C_{HCl} > 2\ M$ may there-

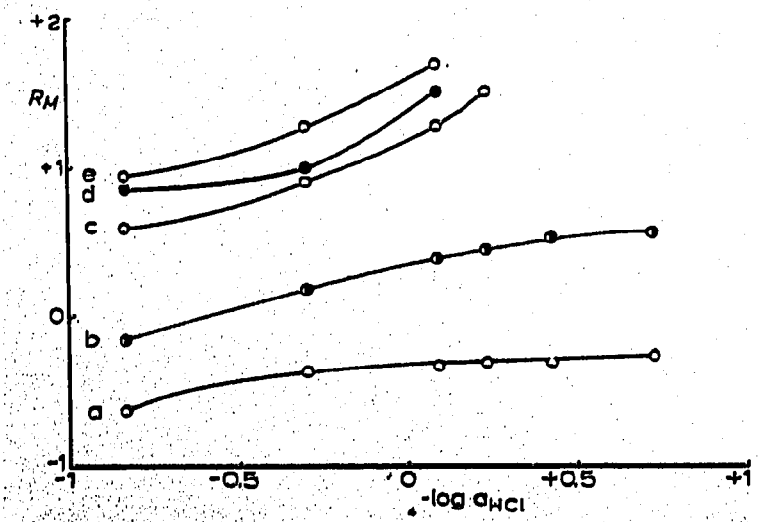

Fig. 4. R_M values *vs.* $-\log a_{HCl}$ for some bases on Dowex 50-X4 (H^+) thin layers. Eluents: hydrochoric acid solutions. (a) Uracil; (b) 4-aminouracil; (c) *p*-chloroaniline; (d) *p*-bromoaniline; (e) *a*-naphthylamine.

TABLE V

VOLUME RANGE OF THE ELUATE (V_r) AND VOLUME OF EFFLUENT RELATIVE TO THE PEAK OF THE ELUTION CURVE (V_{max}) OF SEVERAL COMPOUNDS

Porapak Q (80–100 mesh), 1.0 × 17-cm column with water and hydrochloric acid solution as eluents. Effluent rate = 3 ml/min. n.d. = not determined.

Substance[a]	H_2O		*0.25 N HCl*	
	V_r *(ml)*	V_{max} *(ml)*	V_r *(ml)*	V_{max} *(ml)*
Purine	[b]	[b]	10–20	14
Guanine	10–60	25	10–20	15
Hypoxanthine	20–40	27	10–20	15
2-Amino-6-chloropurine	20–50	35	10–20	15
Adenine	60–150	95	10–30	18
2-Amino-4,6-dihydroxypyrimidine	10–20	15	10–20	15
5-Aminouracil	10–25	15	10–20	15
4,5-Diaminopyrimidine	10–30	16	10–20	15
Uracil	10–30	16	10–20	15
Cytosine	10–40	18	10–20	15
Isocytosine	10–55	26	10–25	16
Pyrimidine	80–170	125	10–30	18
2,4-Diamino-6-chloropyrimidine	470–800	n.d.	10–70	40
Cytidine	15–70	35	10–30	17
Guanosine	100–200	146	10–65	30
Adenosine	300–600	455	10–60	30
Pyridine	[b]	[b]	10–30	18
4-Picoline	[b]	[b]	10–40	20
2-Aminopyridine	170–350	272	20–50	30
p-Aminohippuric acid	15–95	55	10–60	32
p-Aminobenzoic acid	[b]	[b]	15–70	35
Aniline	[b]	[b]	35–85	50
p-Toluidine	[b]	[b]	10–90	55
p-Nitroaniline	[b]	[b]	35–140	95
p-Chloroaniline	[b]	[b]	70–170	115
p-Bromoaniline	[b]	[b]	330–500	n.d.

[a] The samples are dissolved in water or in hydrochloric acid when eluting with water or hydrochloric acid, respectively. The amount of compound used is 20 μg with the exception of 4,5-diaminopyrimidine (10 μg) and 2-amino-6-chloropurine, 2,4-diamino-6-chloropyrimidine and 2-aminopyridine (30 μg).

[b] In this case the substance is strongly retained when eluting with water.

fore be ascribed to the sharp increase of the activity of the hydrochloric acid at these concentrations.

However, the slope values we deduce from the $R_M/\log a_{HCl}$ graph (see Fig. 3) are lower than those from the linear trends of the $R_M/\log C_{HCl}$ plots; in both cases the slope values are in accordance with those found for monovalent cations.

The slope of the line for 2,4,6-triaminopyrimidine (1.40) indicates that this compound behaves like a divalent ion (see curve (e) of Fig. 3).

In other cases we observe curvilinear trends similar to those of Fig. 4. For most bases (see curves (a) and (b) of Fig. 4) such trends are attributable to their acid–base characteristics (likewise found on alginic acid) while for *p*-chloroaniline, *p*-bromoaniline, and α-naphthylamine (curves (c), (d) and (e) of Fig. 4) they may be attributed to the strong adsorption of these compounds by the matrix of the resin (see

Table V). Such adsorption predominates over ion exchange and determines the retention of these substances by the exchanger.

Correlation between the R_F values and pK_a

In order to understand the influence of the acid–base characteristics on the retention of the different classes of compounds on (both cellulose- and polystyrene-based exchangers) we tried to correlate the R_F values of the bases with their pK_a values and to ascertain if the sequence of these values was the same both inside each class and among the different classes.

Alginic acid. The sequences of R_F and pK_a values inside each class are in agreement only for the bases whose pK_a values are $\leqslant 3.3$; such agreement, however, is not found at low pH values of the eluent, as the data in Table I show. The difference in the R_F values tends to decrease owing to the almost complete protonation of the bases as the acidity increases; for the same reason there is no differentiation in the whole pH range among the R_F values of the bases whose pK_a is >3.3

As regards pyridines, 2,4-lutidine and 2,4,6-collidine show anomalous behavior; in fact there is an increase in the R_F values as the pK_a increases. Such behavior may be ascribed to the increase of the steric hindrance of the nitrogen atom in the ring due to the introduction of one or two methyl groups in the α-position.

We observe a deviation from the general behavior, among pyrimidines, in the case of 2,4-diamino-6-chloropyrimidine and, among aromatic amines, in the case of *p*-chloroaniline, *p*-bromoaniline and α-naphthylamine. The strong retention of the first three compounds may be ascribed to the presence of a chlorine or bromine atom in the molecule and of α-naphthylamine to the presence of two aromatic rings in the molecule. Such behavior, usual on polystyrene-based ion exchangers, seems quite common on cellulose-based ion exchangers, despite the differences in the polarities of the two matrices.

In order to correlate the behavior of the different classes, we tried to minimize the influence of the acid–base characteristics of the compounds by considering the R_F values obtained by eluting with solutions at pH = 1.45 and 1.20, and the bases which, at such pH values, are completely (or almost completely) protonated. From these data we obtain the following sequence for the retention of the bases by the exchanger: purines > pyrimidines > aromatic amines > pyridines. Such a sequence is the same as the one observed on microcrystalline cellulose with water and 1 *M* acetic acid as eluents; this may be explained by considering that on cellulose-based exchangers the compounds with the largest number of polar groups or centers are the most strongly retained. The sequence for the number of polar centers is as follows: purines > pyrimidines > pyridines = aromatic amines.

The stronger retention of aromatic amines in comparison with those of pyridines may be ascribed to the fact that the bonding of the $-NH_3^+$ group to the carboxyl group of the exchanger is more likely than the bonding of the protonated nitrogen of the pyridine ring.

Dowex 50-X4 (H^+ and Na^+). On this exchanger, in the sodium salt form, the acid–base characteristics determine the chromatographic behavior of those purines, pyrimidines and pyridines whose pK_a values are between 2 and 5. It is interesting to note that behavior on this exchanger is similar to that observed on alginic acid for the bases having $pK_a \leqslant 3.3$. Aromatic amines, on the contrary, have a behavior independent of their acid–base characteristics, in accordance with their high affinity for polystyrene-based ion exchangers.

The aromatic amines containing a carboxyl group are retained less than the others owing to the anionic nature of this group.

For the bases having $pK_a > 5$, the similarity of their R_F values is due to complete protonation at the acetate buffer pH.

When the exchanger is in the acid form, the differences in the chromatographic behavior of the bases are attributable to their different affinities for the exchanger, since, at the hydrochloric acid concentration of the eluent, most bases are completely protonated. Therefore the R_F value is a direct measurement of the affinity of the base for the exchanger.

Pyridines containing methyl groups in the α-position (2,4-lutidine and 2,4,6-collidine) show a behavior different from that observed on alginic acid. On this latter exchanger, steric hindrance plays an important role in the behavior of these bases; whereas on Dowex 50-X4 (H^+) its importance is secondary to the increase in the affinity of these bases for the exchanger as the number of methyl groups increases.

Among aromatic amines, with reference to aniline, the introduction into the ring of a chlorine or bromine atom in the *para* position increases the affinity of the compound for the exchanger more than the introduction of other functional groups.

The sequence of affinities of the different classes for the exchanger is as follows: aromatic amines > purines > pyridines > pyrimidines. Since on polystyrene-based ion exchangers the interactions between the compounds and the matrix of the resin determine the retention mechanism, we tried to see if it was possible to explain this sequence on the basis of such interactions. We carried out a series of measurements on a Porapak Q column (a polystyrene resin without sulfonic groups) eluting with water and with 0.25 *M* HCl. It was necessary to use a column since Porapak Q is not suitable for TLC when eluting with aqueous solutions.

From the data in Table V the following deductions may be made. (1) The presence of polar groups in the molecule decreases the affinity of the different bases for the resin, with the exceptions of 2,4-diamino-6-chloropyrimidine and aromatic amines without any carboxyl group (whose affinity, on the contrary, notably increases). (2) The protonation decreases the affinity of the bases for the resin. Such behavior may be explained both by the formation of a cation and by a decrease in the aromatic characteristics of the base. (3) The introduction of a ribose molecule into the purine and pyrimidine nucleus increases the affinity of the corresponding nucleosides for the resin, as found by ZAIKA[22]. Such an increase seems to be contrary to observation (1) and may be due to a decrease of the overall polarity of the molecule, although we cannot exclude the possible influence of the increased size of the molecule. The smaller retention of nucleosides in comparison with that of the corresponding bases, both on alginic acid and microcrystalline cellulose, favours the first hypothesis.

The affinity sequence of the protonated bases for Porapak Q (aromatic amines > puridines > pyrines = pyrimidines) is different from that observed on Dowex 50-X4 (H^+) thin layers, with the exception of aromatic amines. The strong retention of aromatic amines may therefore be due to the large influence of the interactions between these bases and the matrix of the resin. The data in Table V, however, do not explain the differences in the retention observed on thin layers among purines, pyrimidines and pyridines. It is therefore probable that the behavior of these classes of compounds on Dowex 50-X4 (H^+) thin layers is due to the presence of sulfonic groups in the resin and, therefore, to the interactions between these groups and polar

groups in the bases. The presence of microcrystalline cellulose in the layers does not affect the sequence of natural purines and pyrimidines since this sequence is the same as that found on Dowex 50-X4 (H^+) columns[5,6].

The stronger retention of adenine in comparison with that of aniline on Dowex 50-X4 (H^+) thin layers (contrary to results on the Porapak Q column) confirms the great influence of the interactions between the sulfonic groups of the resin and the polar groups of the bases. We must point out that, in this case too, the presence of cellulose in the layer is not significant since adenine is more strongly retained than aniline on a Dowex 50-X4 (H^+) column using the same eluents as employed in TLC.

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