

CHROM. 10,220

CHROMATOGRAPHIC BEHAVIOUR OF INORGANIC IONS ON CHITOSAN THIN LAYERS AND COLUMNS

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(Received May 27th, 1977)

SUMMARY

The chromatographic behaviour of 14 inorganic ions has been investigated on thin layers and columns of chitosan eluting with alkaline solutions containing amine ligands. Separations of most of these ions have been achieved. The R_M vs. $p[L]$ trends for some of the ions are reported and discussed.

INTRODUCTION

In a previous paper¹ the behaviour of some nucleic acid constituents and of many phenols was studied on chitosan thin layers. On the basis of the results achieved, and in view of the chelating properties of chitosan towards some metal ions², it was of interest to carry out a similar study for inorganic ions. There have been no previous studies of the behaviour of inorganic ions on thin layers; Muzzarelli² studied the behaviour of some ions on chitosan columns, but did not carry out any separations of mixtures. Thus, besides the separations achieved on thin layers, we have also tried to effect the same separations on chitosan columns. As in the case of the organic compounds¹, the data obtained on powdered chitosan thin layers are compared with those obtained on microcrystalline cellulose and on microcrystalline cellulose impregnated with chitosan formate.

EXPERIMENTAL

The layers were prepared as previously described¹. Since chitosan is gelatinized in acid solutions, neutral or alkaline solutions were employed as eluents. The solutions of the different metal ions were prepared by dissolving the corresponding salts in water, and if necessary, by neutralizing with ammonia. The layers were developed by the ascending technique at 25° and the migration distance was 11 cm unless stated otherwise. The inorganic ions were detected with 8-hydroxyquinoline and then with ammonium sulphide. Sr(II) and Ba(II) were detected with a solution of sodium rhodizonate.

The chitosan used in the column chromatography was obtained as previously

described¹, but had a larger particle size (50–100 mesh) than that (100–200 mesh) used in thin-layer chromatography (TLC) in order to obtain a greater eluent flow-rate.

Columns (cross section 0.94 cm²) filled with 2.5 g of chitosan were used. The eluent flow-rate was 4 ml/min. The concentration of the ions was 20 mg/ml and the sample volume placed on the columns was usually 0.1 ml.

RESULTS AND DISCUSSION

Thin-layer chromatography

Table I lists the R_F values of 14 metal ions eluted with a 0.5 M NH_4NO_3 + 0.5 M NH_3 solution on layers of (a) microcrystalline cellulose, (b) microcrystalline cellulose impregnated with chitosan formate and (c) microcrystalline cellulose mixed with powdered chitosan. It can be seen that the mixed layers of powdered chitosan exhibit a stronger retention and a better selectivity than the other two layers. This is in agreement with the results previously observed for organic compounds¹, and demonstrates the possibility of employing chitosan in the TLC of inorganic ions.

TABLE I

R_F VALUES FOR INORGANIC IONS ON THIN LAYERS OF (a) MICROCRYSTALLINE CELLULOSE, (b) MICROCRYSTALLINE CELLULOSE IMPREGNATED WITH CHITOSAN FORMATE AND (c) MICROCRYSTALLINE CELLULOSE MIXED WITH POWDERED CHITOSAN

For Hg(II), a standard solution of HgCl_2 was employed. e.s. = Elongated spot.

Ion	Eluent				Amount (μg)
	0.5 M NH_4NO_3 + 0.5 M NH_3			0.5 M NH_4NO_3 + 1 M NH_3	
	(a)	(b)	(c)	(c)	
Pb(II)	0.00	0.00	0.00	0.00	0.5
Co(II)	0.80	0.57	0.16	0.37	0.5
Cu(II)	0.87	0.66	0.19	0.43	0.6
Cd(II)	0.87	0.66	0.19	0.45	0.5
Ni(II)	0.96	0.76	0.30	0.59	0.5
Ag(I)	0.91	0.80	0.48	0.67	1.0
Tl(I)	0.79	0.73	0.55	0.55	0.8
Hg(II)	0.86	0.78	0.55	0.64	2.0
Mn(II)	e.s.	e.s.	e.s.	e.s.	1.0
Ca(II)	0.92	0.94	0.74	0.74	2.0
Ba(II)	0.95	0.85	0.74	0.74	2.0
Sr(II)	0.95	0.87	0.75	0.75	4.0
Zn(II)	0.89	0.89	0.77	0.86	0.5
Mg(II)	0.93	0.95	0.79	0.79	0.6

As regards the chromatographic behaviour of the individual ions on the chitosan exchanger (see column 4 of Table I), ions, such as those of Co(II), Cu(II), Ni(II) and Cd(II), which can form complexes with ammonia and which therefore bind to the primary amino group of chitosan, are strongly retained. On the other hand, ions such as those of the alkaline earths, which do not form complexes with ammonia, have much larger and approximately constant R_F values. The behaviour of

Mn(II) is peculiar; it is oxidized in air in an alkaline medium to manganic hydroxide and gives a brown elongated spot.

Table I (column 5) also shows the influence of increasing the ammonia concentration, at the same ionic strength and pH, on the chromatographic characteristics of the ions. It can be seen that the R_F values of the ions which do not give rise to ammonia complexes do not change, while those of the ions which do give rise to complexes noticeably increase. The retention of the former ions can therefore be ascribed to an adsorption process whose extent is essentially correlated with the type of layer employed (see columns 2–4). The case of Tl(I) is particularly illustrative: its R_F value decreases from 0.79 on microcrystalline cellulose to 0.55 on mixed layers of powdered chitosan and does not change on this layer even if the ammonia concentration in the eluent is doubled. The R_F sequence of the bivalent ions which give rise to stable complexes with ammonia can be explained in terms of the corresponding instability constants. Table II shows the good correlation between the sequence of R_F values of these ions and the negative logarithm of their overall instability constants. Except for Cu(II), the R_F values decrease with decreasing instability constant. The stronger retention of Cu(II) can be explained in terms of the high affinity shown by chitosan towards this ion².

TABLE II

R_F VALUES FOR BIVALENT IONS ON POWDERED CHITOSAN LAYERS ELUTED WITH 0.5 M NH_4NO_3 + 1 M NH_3 , AND NEGATIVE LOGARITHMS OF THE OVERALL INSTABILITY CONSTANTS OF THEIR COMPLEXES WITH AMMONIA AT 30° (REF. 3)

<i>Ion</i>	R_F	β_4	β_6
Cu(II)	0.43	12.67	—
Zn(II)	0.86	9.46	—
Ni(II)	0.59	7.95	8.73
Cd(II)	0.45	7.12	5.14
Co(II)	0.37	5.55	5.11

In order to obtain a more complete picture of the chromatographic behaviour of the 14 ions on mixed layers of powdered chitosan we also used buffer solutions containing chloride and acetate instead of ammonium nitrate and an aliphatic amine (isopropylamine, ethanolamine or ethylenediamine) in place of ammonia. The replacement of nitrate by chloride ion does not involve any advantage in the separation of the different ions and produces elongated spots in the case of Ag(I), Tl(I) and Hg(II). Better results were achieved with ammonium acetate since it has been possible, on the basis of the ligand properties of the acetate ion, to separate Cu(II) ($R_F = 0.25$) from Cd(II) ($R_F = 0.39$), to improve the separation between Co(II) ($R_F = 0.29$) and Ni(II) ($R_F = 0.48$) and to separate Mg(II), which runs with the solvent front, from the alkaline-earth ions and from Zn(II) ($R_F = 0.86$ – 0.88). With 0.5 M isopropylammonium nitrate + 0.5 M isopropylamine as eluent, in contrast to the behaviour with 0.5 M ammonia buffer, Cu(II), Cd(II), Co(II) and Ni(II), but not Pb(II), remained at the application point. The other ions, including those of the alkaline earths and Tl(I) which do not give rise to stable complexes with isopropylamine, are more strongly retained; for example, the R_F values of Zn(II), Ba(II) and Tl(I) are 0.30,

0.56 and 0.37, respectively. The only exception is shown by Ag(I) which exhibits a higher R_F value (0.71) than that obtained by eluting with the ammonia buffer. The behaviour of Tl(I) and of the alkaline-earth metal ions can be ascribed mainly to a liquid-liquid partition process involving the aqueous organic eluent.

Elution with 0.5 *M* ethanolamine + 0.5 *M* 2-hydroxyethylammonium nitrate (Table III, column 2) gave interesting results: Cu(II) can be separated from Cd(II); Zn(II) from Mg(II) and from the alkaline-earth metal ions; and a compact spot, which can be used for analysis, is obtained in the case of Mn(II). The stability of ethanolamine complexes of Mn(II) accounts for the compactness of the spot, the oxidation of the metal ion being prevented. The behaviour of Co(II) is just the opposite; the presence of ethanolamine causes partial oxidation to Co(III) by air and therefore an elongated spot is obtained. The smaller retention of Cu(II) with respect to Cd(II) and, overall, to Zn(II) must be ascribed to the high stability of its complexes with ethanolamine, complexes such as $\text{Cu}(\text{NH}_2\text{CH}_2\text{CH}_2\text{OH})_2(\text{OH})_2$ being formed⁴ ($\beta = 19.9$).

TABLE III

R_F VALUES FOR INORGANIC IONS ON MIXED LAYERS OF POWDERED CHITOSAN, AND NEGATIVE LOGARITHMS OF THE OVERALL INSTABILITY CONSTANTS OF THEIR COMPLEXES WITH ETHYLENEDIAMINE AT 25° (REF. 3)

Ca(II), Sr(II) and Ba(II) have the same R_F value (0.69) when eluted with 0.5 *M* ethanolamine + 0.5 *M* 2-hydroxyethylammonium nitrate, and run with the solvent front with the other eluent. n.d. = Not determined.

Ion	Eluent					β_2	β_3
		0.5 <i>M</i> Ethanolamine + 0.5 <i>M</i> 2-hydroxyethyl ammonium nitrate		2-Aminoethylammonium nitrate (<i>M</i>)			
		0.05	0.10	0.25	0.50		
Pb(II)	0.03	0.60	0.00	0.00	0.00	—	—
Co(II)	e.s.	0.65	0.80	0.91	n.d.	10.66	13.96
Cu(II)	0.39	0.94	0.95	0.98	0.98	20.13	—
Cd(II)	0.15	0.08	0.19	0.38	0.60	10.02	12.09
Ni(II)	0.39	0.80	0.88	0.93	0.96	14.08	19.11
Ag(I)	0.40	0.00	0.00	0.00	0.00	7.84	—
Tl(I)	0.50	0.57	0.60	0.61	0.57	—	—
Hg(II)	0.61	0.71	0.84	0.89	0.93	—	—
Mn(II)	0.14	0.49	0.52	0.55	0.65	4.79	5.67
Zn(II)	0.36	0.25	0.40	0.56	0.73	10.81	12.98
Mg(II)	0.77	0.95	0.95	0.96	0.93	—	—

The chromatographic behaviour of the ions when eluted with different concentrations of 2-aminoethylammonium nitrate is also shown in Table III (columns 3–6). With respect to the behaviour with the other eluents, a general increase of the R_F values is observed (see column 6) with the exception of Ag(I) which remains at the starting point notwithstanding the stability of its complexes with ethylenediamine. The strong retention of Ag(I) must be ascribed to the presence of chitosan, since similar behaviour is not observed on microcrystalline cellulose layers. It should be

noted that Cu(II) runs with the solvent front even in 0.05 M 2-aminoethylammonium nitrate (see column 3) and that, under these elution conditions, it can be easily separated from the other metal ions which form stable complexes with ethylenediamine. The sharp separation of Cu(II), Co(II), Ni(II), Zn(II) and Mn(II) is also noteworthy. As with ammonia buffer as eluent, the R_F values of the different bivalent ions decrease as the negative logarithm of the overall instability constants of their complexes decrease (see Table III). This behaviour is particularly evident when eluting with 0.05 M 2-aminoethylammonium nitrate. The only exception is Mn(II), which is less strongly retained than Cd(II) and Zn(II), notwithstanding its β_2 value which is smaller than those of the other two ions. This behaviour can be ascribed to the weak affinity of Mn(II) towards the amino groups of chitosan.

$R_M/p[L]$ relation. Since the R_F values of the different ions change with changing ligand concentration in the eluent, we tried to find a quantitative relation between R_M and $p[L]$.

In Fig. 1 are shown the R_M vs. $p[\text{NH}_3]$ trends of some mono- and bivalent ions. Linear relations between R_M and $p[\text{NH}_3]$ are evidently of the type:

$$R_M = \text{constant} + y \cdot p[L]$$

Such a relation is quite similar to that found between R_M and pH or $p[M^{n+}]$ in cation-exchange reactions⁵. The slopes of the straight lines in Fig. 1 are: 0 for Tl(I), 0.8 for Ag(I) and 1.47–1.64 for Ni(II), Cu(II), Co(II) and Cd(II). These values show that Tl(I) is not appreciably complexed with ammonia, while the complexation reaction which accounts for the chromatographic behaviour of Ag(I) and of the bivalent ions involves one and two molecules of ammonia, respectively.

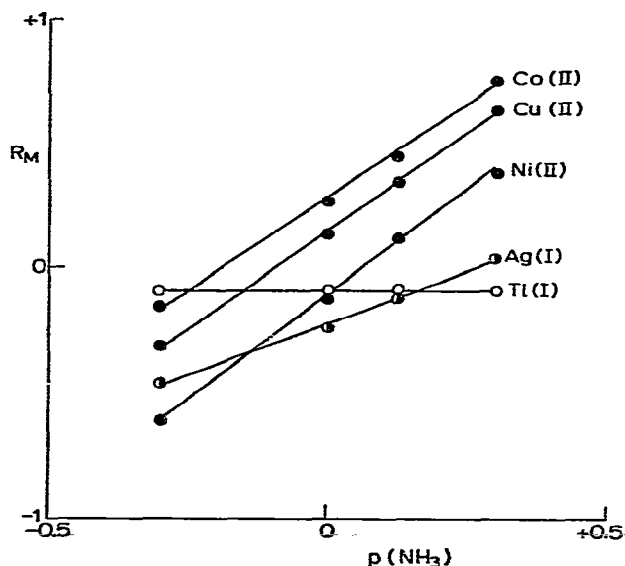
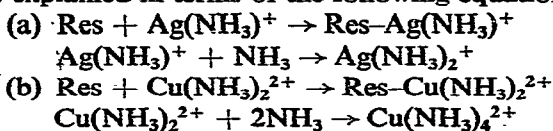


Fig. 1. R_M vs. $p[\text{NH}_3]$ curves for mono- and bivalent ions on powdered chitosan thin layers with ammonia buffer (0.5 M NH_4NO_3 + 0.5 M NH_3) as the eluent.

Since the coordination number of the latter ions with ammonia is higher than one or two, and keeping in mind that at these ammonia concentrations the percentages of the ions in the uncomplexed form are negligible, the experimental data can be explained in terms of the following equations:



$\text{Ag}(\text{NH}_3)_2^+$ and $\text{Cu}(\text{NH}_3)_4^{2+}$ cannot form bonds with the amino groups of chitosan since the two metal ions are exhibiting their highest coordination number in these complexes. Equations similar to those shown above for Cu(II) can also be postulated for Cd(II), Ni(II) and Co(II), even though we cannot exclude the possibility that the four-coordinate complex could also be bound to the resin since the highest coordination number of these ions is 6.

Similar trends to those in Fig. 1 are obtained in the cases of Ag(I) and Zn(II) when ammonia is replaced by isopropylamine, the slopes of the straight lines being 0.93 and 1.48, respectively.

Column chromatography

The chitosan columns, if used with alkaline eluents only, exhibit a constant effluent flow-rate. On the basis of the results achieved in TLC, we used different concentrations of ammonia buffer and 2-aminoethylammonium nitrate as the mobile phases. The column behaviour of the same ions was studied with the exception of Pb(II), Mn(II) and Hg(II).

Elution with 0.5 M ammonia buffer gave different results to those obtained in TLC in that all the ions were eluted between 10 and 65 ml of eluent. On decreasing the ammonia buffer concentration (for instance to 0.25 M), Cu(II), Cd(II), Ni(II), Co(II) and Ag(I) were more strongly retained than the other ions but gave elongated elution curves covering a range of ca. 150 ml. With 0.01 M ammonia buffer (Fig. 2), Zn(II) is well separated from Mg(II), Ca(II), Sr(II), Ba(II) and Tl(I), and all of these ions are separated from Cu(II), Co(II), Ni(II), Cd(II) and Ag(I) which are strongly retained on the column. If the column is then eluted with 0.01 M 2-aminoethylammonium nitrate, Cd(II) and Ag(I), which are not eluted, can be separated from Cu(II), Co(II) and Ni(II) (see Fig. 2). The elution of Cd(II) is possible using 0.5 M

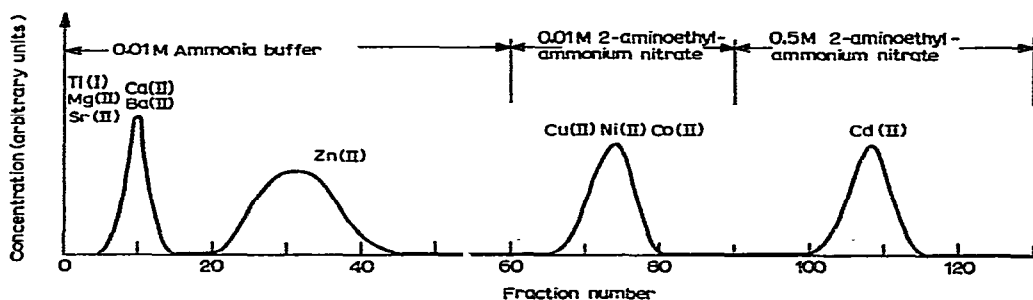


Fig. 2. Elution curves for inorganic ions on a chitosan column. One fraction = 2 ml.

2-aminoethylammonium nitrate as mobile phase (see Fig. 2), while Ag(I) can be eluted with 0.5 M ammonia buffer. In this way it is possible to recover Ag(I) within an interval of 16–48 ml and, at the same time, effect its separation from all of the other ions.

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