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## THIN-LAYER CHROMATOGRAPHY OF METAL IONS ON CELLULOSE IMPREGNATED WITH THIOCYANATE SALTS OF LIQUID ANION EXCHANGERS

## I. EXPERIMENTAL PARAMETERS INVOLVED IN THE SYSTEM AMBERLITE LA-2-THIOCYANATE/AQUEOUS AMMONIUM THIOCYANATE

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## SUMMARY

A selection of metal ions (Ag(I), Cu(II), Cd(II), Bi(III) and Mn(II)) have been chromatographed on supports impregnated with the liquid anion exchanger, Amberlite LA-2-thiocyanate, formed from the free amine by an indirect method via the chloride form or by a direct method, *i.e.* with thiocyanic acid, using aqueous ammonium thiocyanate as the mobile phase. Cellulose is preferred to silica gel as the support. The  $R_F$  values are independent of the method used to convert the free amine to the salt form. The  $R_F$  values are shown to vary with the impregnation coefficient of the stationary phase and with the composition of the mobile phase. A high degree of reproducibility of  $R_F$  values is obtainable in the system studied.

## INTRODUCTION

Using thiocyanate media as mobile phases, BRINKMAN *et al.*<sup>1</sup> have chromatographed six metal ions on substrates (silica gel thin layers and paper) impregnated with the thiocyanate salts of long-chain (secondary, tertiary and quaternary) amines. From their preliminary results, these authors concluded that further work on these systems was justified. In contrast, others<sup>2,3</sup> have examined a wider range of metal ions on paper impregnated with tertiary amines: tri-*n*-butylamine, tri-*n*-octylamine and tri-isooctylamine, only. For a given mobile phase, little difference in behaviour of the metal ions was observed using these three tertiary amines. This illustrates, as observed by BRINKMAN *et al.*<sup>1</sup>, that the character of the amine group rather than the nature of the substituent hydrocarbon chain is the primary factor governing the chromatographic behaviour of the metal ions in the systems investigated<sup>1-3</sup>. None of these works, however, included a systematic investigation of the experimental

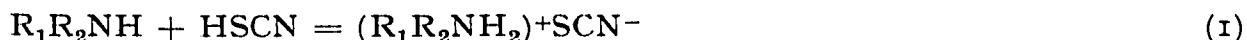
conditions necessary to produce a chromatographic system from which the highly reproducible  $R_F$  values necessary for the unambiguous characterisation of the metal ions separated could be obtained.

For these reasons, before making a systematic investigation into liquid ion-exchange chromatography of metal ions using aqueous thiocyanate media, we investigated a number of chromatographic parameters likely to affect the behaviour of the metal ions in the systems studied. Our object in this work was to establish conditions which give a high degree of reproducibility of  $R_F$  values that are essential in order to gain insight into the mechanisms of the chromatographic system, characterise metal ions according to their  $R_F$  value, and develop systems which can be used for the quantitative determination of the ions separated<sup>4,5</sup>.

Before beginning our experimental work, it was pertinent to consider which type of long-chain amine would be most suitable for an investigation into the parameters which govern the system. Primary and quaternary amines which show the extremes of ion exchange character, as exemplified by BRINKMAN *et al.*<sup>1</sup>, were considered to be unsuitable. We therefore selected a secondary amine—Amberlite LA-2, converted to the thiocyanate form, as the impregnant because we think that results obtained using this anion exchanger of intermediate strength would show a close affinity with the results expected from the use of both primary amines and quaternary compounds.

#### EXPERIMENTAL

The exact molecular weight of the amine—Amberlite LA-2 (370) was determined by a method described earlier<sup>6</sup>. This enabled us accurately to prepare solutions of the amine in chloroform (0.1–0.5 *M*). From these, solutions of the thiocyanate salts were prepared by one of the two following methods: (a) By an indirect method in which the amine hydrochloride was first formed by the method described by BRINKMAN AND DE VRIES<sup>7</sup>. Conversion to the amine hydrochloride was shown to be 100%. The amine hydrochloride was treated with an equal volume of ammonium thiocyanate solution (3 *M*) and the mixture was allowed to stand overnight, after which the aqueous phase was separated from the organic phase. This procedure was repeated. The amine thiocyanate was then washed with three equal volumes of distilled water and filtered through a pad of anhydrous magnesium sulphate to form a clear solution. (b) By a direct method in which thiocyanic acid (1.5 *M*) was prepared by passing potassium thiocyanate (2 *M*) through the cation resin 'Zeo-carb 226' in the hydrogen form<sup>8</sup>. The acid solution obtained was shaken with a solution of Amberlite LA-2 in chloroform. The phases were separated and the procedure repeated, so forming the anion exchanger according to eqn. 1.



The anion exchanger was washed three times with deionised water and filtered through a pad of anhydrous magnesium sulphate. This conversion was found to be 98%. A concentrated solution of ammonium thiocyanate (9 *M*) in deionised water was prepared. The exact molarity of this solution was found by titrating a dilute solution (5 ml made up to 250 ml with deionised water) against silver nitrate (0.1 *M*) using

ferric alum indicator. Calculated volumes of the standardised solution were used to prepare the stock solutions of ammonium thiocyanate (0.1–7.0 *M*), used as the mobile phases.

Cellulose (15 g of MN-300-HR) or silica gel (30 g of Merck G) were slurried with a solution (70 ml), of the appropriate molarity, of the impregnant in chloroform, and the resulting suspension was used to prepare the chromatolayers (5 × 20 × 20 cm) at an applied thickness of 0.3 mm using a Shandon apparatus. They were dried in air for 20 min to allow the chloroform to evaporate.

Solutions of 1  $\mu$ l containing 1  $\mu$ g of five metal ions (Ag(I), Cu(II), Cd(II), Bi(III) and Mn(II)) were applied to the layers by the technique previously described<sup>9</sup>. The chromatograms were eluted with solutions of ammonium thiocyanate of the appropriate molarity in our double saturation chamber (*i.e.* our polythene bag technique<sup>9,10</sup>) at a constant temperature of 25 ± 0.1°, until the solvent front had travelled a fixed distance (12.5 ± 0.5 cm) from the point of application of the metal ions. The eluted layers were then dried in an air oven at 120° for 20 min and sprayed either with PAN (1-(2-pyridylazo)-2-naphthol) solution (0.1% in ethanol). Then they were exposed to ammonia vapour to visualise Mn(II) or with Dithizone (0.1% in chloroform) to visualise the remaining ions. *R<sub>F</sub>* values were computed in the usual way.

## RESULTS AND DISCUSSION

### Choice of support

Silica gel and cellulose were investigated as possible supports for the impregnant

TABLE I

A COMPARISON OF THE *R<sub>F</sub>* VALUES OBTAINED USING SILICA GEL OR CELLULOSE AS SUPPORTS FOR THE IMPREGNANT AMBERLITE LA-2-THIOCYANATE OR USING AMBERLITE LA-2 THIOCYANATE PREPARED EITHER BY THE INDIRECT METHOD OR BY THE DIRECT METHOD

a = results obtained from silica gel impregnated with Amberlite LA-2-thiocyanate (0.1 *M*) prepared by the indirect method; b = results obtained from cellulose impregnated with Amberlite LA-2-thiocyanate (0.1 *M*) prepared by the indirect method; c = results obtained from cellulose impregnated with Amberlite LA-2-thiocyanate (0.1 *M*) prepared by the direct method.

Metal ion	Method	Molarity of ammonium thiocyanate								
		0.1	0.5	1	2	3	4	5	6	7
Ag(I)	a	0.00	0.03	0.08	0.32	0.62	0.76	0.83	0.89	0.91
	b	0.00	0.02	0.08	0.30	0.58	0.74	0.85	1.00	1.00
	c	0.00	0.02	0.09	0.32	0.57	0.78	0.86	1.00	1.00
Cu(II)	a	0.00	0.02	0.07	0.17	0.34	0.54	0.74	0.82	0.88
	b	0.00	0.02	0.04	0.13	0.24	0.42	0.64	0.72	0.81
	c	0.00	0.02	0.05	0.12	0.20	0.42	0.60	0.75	0.83
Cd(II)	a	0.00	0.04	0.04	0.14	0.25	0.39	0.58	0.70	0.81
	b	0.00	0.00	0.00	0.04	0.08	0.18	0.30	0.44	0.58
	c	0.00	0.00	0.00	0.06	0.11	0.21	0.34	0.43	0.60
Bi(III)	a	0.00	0.00	0.00	0.02	0.06	0.16	0.29	0.47	0.60
	b	0.00	0.00	0.00	0.02	0.04	0.06	0.18	0.32	0.49
	c	0.00	0.00	0.00	0.02	0.05	0.08	0.18	0.28	0.48
Mn(II)	a	0.78	0.97	0.95	0.92	0.91	0.91	0.93	0.95	0.97
	b	0.81	0.70	0.58	0.48	0.46	0.41	0.42	0.44	0.46
	c	0.80	0.68	0.56	0.48	0.44	0.42	0.42	0.46	0.49

(prepared by the indirect method). The  $R_F$  values (the mean of at least 6 determinations) obtained are shown in Table Ia and b. Each  $R_F$  value was reproducible to  $\pm 0.04 R_F$  units for the silica gel system and  $\pm 0.02$  for the cellulose system.

It can be seen that, whilst the  $R_F$  values are higher on the silica gel than on the cellulose support, the  $R_F$  value trends on the two supports are similar, suggesting that each substrate is completely covered with the impregnant so that we can regard the system as one in which the distribution of the solutes is between the thiocyanate mobile phase and the liquid anion exchanger impregnant. In the event of incomplete coverage of the layers with the impregnant, we would not expect the observed comparability of the results because of a competitive interaction between the solutes and the supposedly inert support<sup>11-13</sup>.

Similar observations concerning the higher  $R_F$  values for impregnated silica gel layers compared with impregnated cellulose layers when each bore the same impregnation coefficient of the same impregnant (Primene JM-T-hydrochloride or tri-*n*-butylphosphate) have been reported<sup>6,10</sup>. Two possible reasons exist for this behaviour. Firstly a higher weight:volume ratio of substrate to impregnant exists in the case of silica gel compared with cellulose; this effectively reduces the impregnation coefficient of the former<sup>10</sup>. Secondly, it has been shown that the impregnant is more firmly held on the former<sup>10</sup>. This results in a less effective uptake of the impregnant from the slurring solvent by the silica gel during the preparation of the plates. These two effects are superimposable.

We confirmed the observation of BRINKMAN *et al.*<sup>1</sup> that the solvent front on the silica gel layers was fairly irregular. On cellulose layers, however, we found that the solvent front was uniform except at very high molarities of the mobile phase. Two other disadvantages were noticed when silica gel was used as a substrate which was not apparent when cellulose was used. Firstly, the presence of iron in the silica gel resulted in an interaction between the iron and the mobile phase, causing the iron to be precipitated on the layers as a red thiocyanate complex. This colour could possibly interfere with the identification of many metal ions which form red complexes with PAN or other chromogenic reagents. Secondly, the mechanical stability of the layers formed from silica gel as a support for the stationary phase was observed to be very inferior to that of cellulose layers. When silica gel layers were placed in the eluent, the silica gel at the bottom of the plate often flaked off, increasing the elution time considerably. This also leads to irregular solvent fronts. Often normal movement of the eluent caused the silica gel to flake, ruining the plate. This is in accordance with the findings of other workers<sup>6,10</sup>.

For these reasons, preference was given to the choice of cellulose as a support for our subsequent investigations.

#### *Mode of formation of the thiocyanate form of the liquid anion exchanger*

Table Ib and c shows the results obtained when the amine is converted to the liquid anion exchanger, *i.e.* the salt form, by the indirect and direct methods. The former method suffers from the disadvantage that in its final form the exchanger may not be present exclusively as the thiocyanate salt but as a mixture of the thiocyanate and the chloride forms<sup>14</sup>. The disadvantage inherent in the latter process is the instability of free thiocyanic acid which necessitated its preparation afresh on each occasion it is used.

From Table Ib and c it appears that the  $R_F$  values, within the limits of experimental error, are largely independent of the method used for the preparation of the ion exchanger. In subsequent work, we therefore decided to adopt the simpler, more convenient method of formation of the liquid anion exchanger from the free amine, namely the indirect method.

In conjunction with this work it is significant to note that neither WAKSMUNDSKI AND PRZESZLAKOWSKI<sup>2</sup> nor PRZESZLAKOWSKI<sup>3</sup> appear to have preformed the anion exchanger from the free amine prior to the impregnation of the paper. In spite of this, they discuss their results in terms of an anion exchange mechanism between anionic thiocyanate complexes of the metal ions and the thiocyanate salt form of the amine<sup>3</sup>. This discrepancy between their chromatographic practice and the theoretical discussion of their results can be reconciled only if one assumes the *in situ* formation of the amine salt during the development of their chromatograms. For this to happen, however, the presence of protons in the system are essential. Since ammonium thiocyanate is the salt of a strong acid and a weak base, it is reasonable to suppose that some degree of hydrolysis of the salt will occur in aqueous solution with the formation of thiocyanic acid, thus enabling *in situ* formation of the anion exchanger to occur.

We consider that this is likely to be so, for the pH range of the aqueous ammonium thiocyanate varies from pH 5.8 (0.1 M) solution to pH 5.2 (7.0 M) solution, *i.e.* all the solutions used by us and by the above workers have an acid pH.

From the above it is clear that *in situ* formation of the amine salt probably does occur; but when it does, it must result in the formation of a concentration gradient of the mobile phase over the stationary phase. Such behaviour is not consistent with a high degree of reproducibility of  $R_F$  values, and this must be regarded as a real weakness in the interpretation of the data obtained by WAKSMUNDSKI AND PRZESZLAKOWSKI<sup>2</sup> and PRZESZLAKOWSKI<sup>3</sup>. Such a weakness is obviated in our work by our making the liquid anion exchanger before the preparation of the chromatolayers.

#### *The variation of the $R_F$ values with variations in the composition of the mobile phase*

In general, we see that an increase in the  $R_F$  values (Table Ia, b and c) of most of the metal ions occurs with an increase in the molarity of the ammonium thiocyanate in the mobile phase. This pattern, however, is not observed in the case of Mn(II) which shows an initial decrease in values with an increase in the molarity of the mobile phase with a slight increase in the values at high molarities of the mobile phase. The overall patterns of the  $R_F$  values obtained by us agree with those obtained by other workers<sup>1-3</sup>.

#### *The effect of the variation of the amine-salt concentration on the thin layers*

It has been shown that the  $R_M$  values obtained from reversed-phase thin-layer chromatograms are linearly related to the cross sectional area ( $A_s$ ) of the stationary phase according to eqn. 2 (refs. 6, 11, 12).

$$R_M = \log_{10} A_s + \text{constant} \quad (2)$$

If the  $\log_{10} A_s$  term is directly related to the log of the concentration of the

TABLE II

$R_F$  AND  $R_M$  VALUES OF SOME METAL IONS ON CELLULOSE LAYERS IMPREGNATED WITH VARYING CONCENTRATIONS OF AMBERLITE LA-2-THIOCYANATE WITH AMMONIUM THIOCYANATE (4.0 M) AS THE ELUENT

		Loading of Amberlite LA-2-thiocyanate (molarity with respect to $-NH$ )							
		0.0	0.1	0.15	0.2	0.25	0.3	0.4	0.5
Cd(II)	$R_F$	1.00	0.19	0.15	0.13	0.11	0.10	0.06	—
	$R_M$	—	+0.630	+0.728	+0.826	+0.908	+0.954	-1.279	—
Cu(II)	$R_F$	1.00	0.43	0.34	0.28	0.24	0.19	0.10	—
	$R_M$	—	+0.122	+0.288	+0.410	+0.501	+0.630	+0.954	—
Ag(I)	$R_F$	1.00	0.74	0.69	0.62	0.58	0.53	0.45	0.37
	$R_M$	—	-0.454	-0.341	-0.213	-0.140	-0.052	+0.087	+0.231
Time of run (min)		45	70	85	100	120	150	170	200

stationary phase in the solvent used to slurry the cellulose then the latter should also be linearly related to the  $R_M$  values<sup>15</sup>.

The results in Table II show that an increase in the impregnation coefficient of the stationary phase results in a decrease in the  $R_F$  values. For the three metals studied, the plots of  $R_M$  vs. the log of the impregnation coefficient are linear, giving a series of parallel lines. This suggests that the variation in  $R_M$  values with the variation in the impregnation coefficient is a property of the impregnant rather than one of a particular ion. After a given plate loading (0.25 M) there is a break in the parallel lines and the  $R_M$  value plots show an upward trend. At loadings above this point, the metal ion spots become more diffuse, the time taken for the solvent front to travel a fixed distance increases and the solvent front becomes very ragged. All these features have been previously reported for reversed-phase thin-layer chromatography of metal ions chromatographed on thin layers of cellulose impregnated with Primene JM-T-hydrochloride using hydrochloric acid as the mobile phase<sup>6</sup>. It is of interest to note that similar alterations in the shapes of the  $R_M$  vs. impregnation coefficient curves have also been observed in reversed-phase paper chromatography when tri-*n*-butylamine was the impregnant<sup>2,3</sup>. In these investigations, however, the authors studied very high impregnation coefficients and found a downward  $R_M$  trend at ca. 25% impregnation coefficient. This corresponds approximately to an impregnation coefficient of 1.5 M.

In an earlier paper we have shown that the void spaces in filter paper result in an approx. 5-fold higher degree of impregnation compared with thin-layer systems<sup>16</sup>. We have also shown that the molar volume of the impregnant, as well as its molar concentration, is of importance in governing the points at which deviation from linearity occurs in chromatographic systems<sup>15</sup>. Considering both these facts we find that the point of deviation in our system and that observed by WAKSMUNDSKI AND PRZESZLAKOWSKI<sup>2</sup> and PRZESZLAKOWSKI<sup>3</sup> become reconciled.

The comparability of the present results with those obtained for the Primene JM-T-hydrochloride-hydrochloric acid system<sup>6</sup> confirms that the point of deviation from linearity occurs as a result of polymerisation of the stationary phase<sup>4</sup>.

From Fig. 1, it appears that there is no particular advantage to be gained

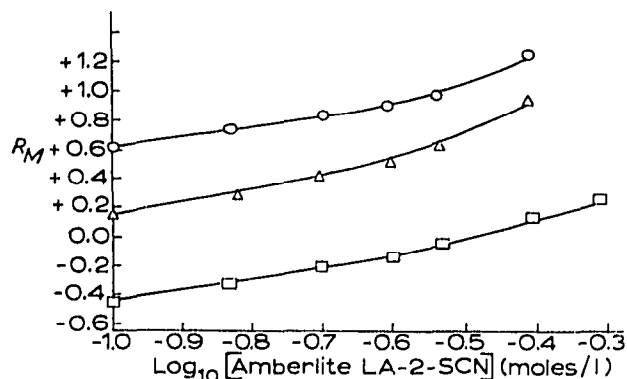


Fig. 1.  $R_M$  values of metal ions *vs.*  $\log_{10}$  concentration of Amberlite LA-2-thiocyanate in the slurring solvent.  $\circ$ — $\circ$  = Cd(II);  $\triangle$ — $\triangle$  = Cu(II);  $\square$ — $\square$  = Ag(I).

from using impregnation coefficients greater than 0.1  $M$  with respect to the amine functional group, even though higher impregnation coefficients lower the  $R_F$  values. Therefore for further work on the system Amberlite LA-2-thiocyanate–aqueous ammonium thiocyanate, we used an impregnation coefficient of 0.1  $M$ .

#### Reproducibility of the $R_F$ values

The results quoted in the foregoing sections were the mean of at least 6 determinations suggesting that some degree of reproducibility of  $R_F$  values is obtainable in the system Amberlite-LA-2-thiocyanate–aqueous ammonium thiocyanate. However, it was considered that before we could use the  $R_F$  values obtained in the system either for the characterisation of metal ions or for a discussion of the mechanisms involved in the separation of the metal ions in the chromatographic system investigated, it was necessary to re-assess the degree of reproducibility of the  $R_F$  values of the metal ions once the experimental conditions discussed above had been standardised. Table III shows the degree of reproducibility of the  $R_F$  values obtained for Mn(II). This ion was chosen because the preliminary experiments had shown that the  $R_F$  values obtained for it lay in the region of meaningful  $R_F$  values, *i.e.* 0.1–0.9 (ref. 16).

TABLE III

THE CHROMATOGRAPHIC BEHAVIOUR OF MANGANESE(II) IONS IN CELLULOSE LAYERS IMPREGNATED WITH AMBERLITE LA-2-THIOCYANATE (0.1  $M$ ) PREPARED BY THE INDIRECT METHOD WITH VARYING CONCENTRATIONS OF AQUEOUS AMMONIUM THIOCYANATE AS THE MOBILE PHASE

	Concentration of $NH_4SCN$ (molarity)						
	1	2	3	4	5	6	7
$R_F$ (Mn(II))	0.58	0.47	0.44	0.41	0.42	0.44	0.46
S.D. ( $\times 10^{-2}$ )	1.1	0.96	0.89	0.67	0.73	0.81	0.95
Average length of spot (cm)	2.1	1.6	1.4	1.5	1.5	1.7	1.9
Time of run (min) ( $\pm 5$ min)	50	65	75	85	100	115	135
No. of $R_F$ values determined	120	120	120	120	120	120	120

Assuming a Gaussian distribution of  $R_F$  values about the mean, then 68% of the  $R_F$  values should lie within the standard deviation and 95% of the  $R_F$  values should lie within twice the standard deviation. From these results it may be concluded that the reproducibility of the system is of the order of  $\pm 0.02 R_F$  units because the standard deviation is of the order 0.007–0.01. This was confirmed by the fact that on all subsequent plates the standard manganese spot was never more than 0.02  $R_F$  units above or below the value shown in Table III.

#### CONCLUSIONS

In the reversed-phase thin-layer chromatography of metal ions on supports impregnated with the liquid anion exchanger Amberlite LA-2-thiocyanate using aqueous ammonium thiocyanate as the mobile phase we find that: (a) cellulose is to be preferred to silica gel as a support for the impregnant, (b) the  $R_F$  values are independent of the method used to convert the free amine to the appropriate salt form, and (c) the  $R_F/R_M$  values vary with the degree of impregnation of the support with the stationary phase and with the composition of the mobile phase. It is possible to obtain a high degree of reproducibility of  $R_F$  values in the system.

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