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QUANTITATIVE THIN-LAYER CHROMATOGRAPHY ON LIQUID ANION EXCHANGERS

PART II. A COMPARISON OF A SPOT REMOVAL METHOD WITH AN *in situ* DIRECT DENSITOMETRIC METHOD

R. J. T. GRAHAM, L. S. BARK AND D. A. TINSLEY

*Department of Chemistry and Applied Chemistry,
The University, Salford 5, Lancs. (Great Britain)*

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SUMMARY

The quantitative determination of metal ions separated by thin-layer chromatography using Primene JM-T hydrochloride as the stationary phase and hydrochloric acid solutions as the mobile phase has been investigated. A spot removal technique used in conjunction with absorptiometric analysis is comparable in accuracy with a direct densitometric technique. The latter, however, permitted a larger range of metal ions to be investigated than did the former. It is also quicker. No loss of accuracy of the latter technique occurs when mixtures are investigated. A mixture of Zn(II)-Cd(II) (1:1, w/w) was analysed with an accuracy of $\pm 4\%$ at the $1 \mu\text{g}$ level. A similar accuracy is reported for the analysis of Co(II) in an Fe(III)-Co(II) (99:1, w/w) mixture.

INTRODUCTION

While radiochemical methods have been successfully applied to the quantitative determination of metal ions separated by thin-layer chromatography¹⁻³, the rather specialised nature of the techniques has limited their application in this field. Likewise methods involving spot area measurements^{1,4,5} have been of limited application in inorganic thin-layer chromatography.

GAGLIARDI and co-workers have successfully determined a number of metals quantitatively⁶⁻¹⁰. Their method was one of removal of the inorganic species from the layers by suction, their separation from the chromatographic substrate by elution chromatography followed by the spectrophotometric determination of the inorganic species in a suitable complex form. Metals investigated by these workers include barium⁶, as its xylenolphthalein-bis-imido-diacetic acid complex and nickel⁷, uranium⁸, cobalt⁹ and bismuth¹⁰, all as their morpholine dithiocarboxylic acid morpholinium complexes.

Recently, we have investigated the factors governing precision in the applica-

tion of direct densitometry to the quantitative determination of zinc when this metal ion was separated by a reversed-phase thin-layer chromatographic technique¹¹. The stationary phase used was Primene JM-T hydrochloride (0.3 M), and the mobile phase was aqueous hydrochloric acid. The main cause of imprecision was found to be the irregular distribution of the chromogenic reagent over the layer (20 cm × 20 cm), which resulted in the accuracy of the technique being of the order 4–5%.

In this paper we report our investigations into a comparison between the quantitative determination of metal ions separated by the above liquid–liquid thin-layer technique using a spot removal method, followed by absorptiometric analysis, and the direct densitometric technique.

EXPERIMENTAL

(a) Preparation of the metal ion solutions

Solutions of the metal ions Co(II), Cu(II), Zn(II), Cd(II), Pb(II), Bi(III), and UO_2^{2+} were prepared as previously described¹² to give a range of concentrations of each metal ion solution (1–5 $\mu\text{g}/\mu\text{l}$). In addition to these solutions, two test mixture solutions were prepared. The first of these was a mixture containing 1 $\mu\text{g}/\mu\text{l}$ of each Zn(II) and Cd(II). The second was a Fe(III)–Co(II) mixture containing 99 $\mu\text{g}/\mu\text{l}$ of Fe(III) and 1 $\mu\text{g}/\mu\text{l}$ of Co(II).

(b) Preparation of the layers

Cellulose (15 g MN 300 HR) was slurried with Primene JM-T hydrochloride (0.3 M in chloroform) and the mixture was used to prepare the chromatographic layers in the manner previously described^{12,13}.

(c) Application of the metal ions

Three methods of applying the standard metal ion solutions to the layers were initially investigated:

- (i) The application of a fixed volume of solutions of different concentrations.
- (ii) The single application of different volumes of a solution of fixed concentration.
- (iii) The method of building up the sample on the layer to the required concentration by the successive application of sample, with the drying of the sample spot between each successive application, e.g. a 3 μg spot could be obtained by applying three successive 1 μl samples of solutions of 1 $\mu\text{g}/\mu\text{l}$ concentration.

In each case the samples were applied to the layer with a Hamilton microsyringe (1 μl capacity).

The results of the preliminary work led us to adopt method c(i) for the subsequent preparation of the calibration curves for both absorptiometry and densitometry.

(d) Elution of the chromatograms

The elution of the chromatograms with aqueous hydrochloric acid as the mobile phase was carried out as previously described^{12,13} in a sandwich chamber¹⁴.

(e) Visualisation of the metal ions

Following the elution of the chromatograms, they were dried at 120° for 15 min.

They were then sprayed with PAN (0.1 % in ethyl alcohol) and exposed to ammonia vapour to generate the metal-PAN complex.

(f) *Quantitative analysis*

Absorption analysis. A standard area of substrate surrounding each spot was demarcated by means of a template and the substrate in this area was loosened from the plate. A similar area of background substrate was removed to act as a blank.

A porous polythene* support was inserted into a microchromatography column*. This arrangement was attached to a vacuum line and the loosened substrate was sucked into the column thereby simultaneously removing the standard area from the plate and transferring it into a chromatography column ready for subsequent elution from the cellulose.

The packed columns were fixed in a rack and the complex/reagent was eluted from the cellulose by means of chloroform. The eluate was made up to a standard volume.

The optical densities of the extracted complexes were measured against a solution prepared from the extracted blank using an EEL absorptiometer** with a filter appropriate to the metal ion-PAN complex being investigated.

Direct densitometric analysis. The metal ions were determined with a "Chromoscan" instrument filled with a thin-layer attachment***. The general operating conditions necessary to obtain precision in the direct densitometric analysis of metal ions separated by the liquid-liquid thin-layer chromatographic system used by us have been reported in a previous paper¹², where it was shown that the integral count depended on the choice of the cam used in the instrument.

RESULTS AND DISCUSSION

Of the three methods used for the spotting of the metal ions on to the layers (c(i), (ii), and (iii), the experimental part above), methods c(i) and c(ii) gave satisfactory results whereas c(iii) gave unsatisfactory results probably because of the uneven distribution of the metal ions in the final applied spot. In choosing between the two alternatives c(i) and c(ii), we decided that method c(i), *i.e.* the application of a fixed volume of solutions containing different amounts of the metal ions to the layers, would be more directly appropriate under conditions in which the amount of metal ion present in solutions of unknown concentrations of that metal ion was to be determined. This method of spotting the layers was therefore used for the construction of calibration curves.

Table I shows the % absorption obtained from the solutions of different concentrations of the ions Co(II), Cu(II), Zn(II) and UO_2^{2+} . The calibration curves constructed for these metal ions are shown in Fig. 1.

The importance of pH control in the stabilisation of PAN-metal ion complexes has been stressed by a number of workers¹⁵⁻¹⁷. The method used by us for the generation of the alkaline conditions necessary for the formation of such complexes for the qualitative identification of metal ions has been given¹¹.

* Available from Quickfit and Quartz, Stone, Staffs., England.

** Available from Evans Electro Selenium Ltd., St. Andrews Works, Halstead, Essex, England.

*** Available from Joyce Loebl & Co. Ltd., Princeway, Team Valley, Gateshead on Tyne 11, England.

TABLE I

THE ABSORPTIOMETRIC DETERMINATION OF METAL IONS (% ABSORPTION)

	Weight of metal ion (μg)				
	1	2	3	4	5
Co(II)	0.8	2.1	3.2	4.2	5.2
Cu(II)	1.7	3.4	4.3	5.6	6.8
Zn(II)	5.0	10.0	15.0	17.0	18.5
UO ₂ ²⁺	1.3	3.6	5.1	6.3	8.3

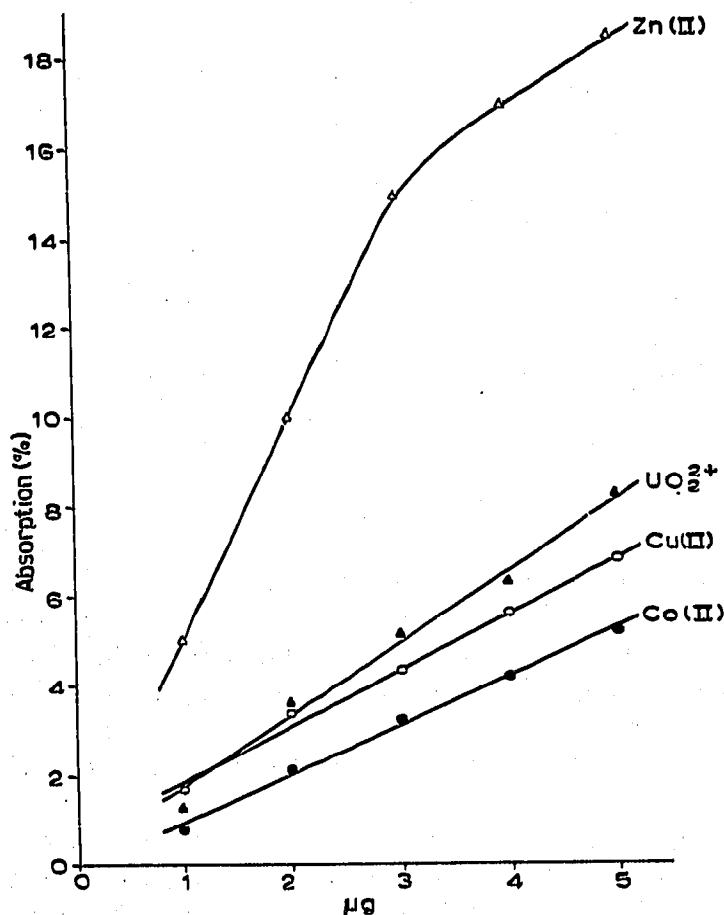


Fig. 1. Absorptiometric calibration curves for metal ions in the range 1.0–5.0 μg of metal ion. ● = Co(II); ○ = Cu(II); Δ = Zn(II); \blacktriangle = UO₂²⁺.

For the PAN-Zn(II) complex, it has been shown that fading of the complex, on exposure to the normal laboratory atmosphere, is negligible for a period of 40 min, *i.e.* ten times larger than is necessary for the densitometric scanning of a chromatoplate (20 cm. \times 20 cm)¹².

However, in the extraction process used by us, elution of Zn(II), Cd(II), Pb(II) and Bi(III) with chloroform resulted in fading of the complex colour in the eluate solution. This we attributed to a lack of stability of the complexes of these metals under these conditions. We therefore pretreated the chloroform by shaking it with a

range of aqueous buffer solutions before using it to extract these metals. No improvement in colour development of the complexes in the eluate was observed.

Next, we attempted pH control of the eluent chloroform using a chloroform-soluble base, triethanolamine. In the case of Zn(II), a coloured eluate suitable for absorptiometric analysis was obtained using triethanolamine (1%) in chloroform as the eluent. No comparable improvement was obtained for Cd(II), Pb(II) or Bi(III). For these three metals, therefore, no absorptiometric data are presented.

Table II shows the mean integrals obtained from the densitometric analysis of the metal ions Co(II), Cu(II), Zn(II), Cd(II), Pb(II), Bi(III) and UO_2^{2+} . The calibration curves constructed from them are given in Fig. 2.

TABLE II

THE DIRECT DENSITOMETRIC DETERMINATION OF METAL IONS
Mean integral values.

	<i>Weight of metal ions (μg)</i>				
	1	2	3	4	5
Co(II)	281	410	559	652	745
Cu(II)	300	477	581	749	850
Zn(II)	200	310	375	418	457
Cd(II)	200	314	411	502	580
Pb(II)	32	72	88	113	135
Bi(III)	85	160	239	287	361
UO_2^{2+}	29	91	193	278	339

With the exceptions of Zn(II) (absorptiometric and densitometric) and Cd(II) (densitometric), the calibration curves show that in the range 1–5 $\mu\text{g}/\mu\text{l}$ metal ions the Beer-Lambert law is obeyed. For Zn(II) and Cd(II) (Figs. 3 and 4) linear densitometric plots are obtained for layer loadings of up to 2 μg of metal ion. These plots fulfil the statistical requirement for a straight line. The standard error of determination at 1 μg for both metals is 4%, *i.e.* this is within the limits of experimental error reported previously for Zn(II)¹².

A comparison of the data obtained from both the absorptiometric and densitometric investigations show that they are comparable in accuracy. However, the latter method is undoubtedly quicker and, as we have shown, can be used for the quantitative determination of a greater number of metals since it avoids the problem of producing the correct pH conditions necessary for the stabilisation of the PAN-metal ion complex in the eluate. For these reasons we consider the densitometric method to be more suitable than the absorptiometric method.

Recently, the quantitative determination of the PAN-Co(II) complex using the Chromoscan was reported¹⁸. PAN complexes of Fe(III), Co(II), Ni(II) and Cu(II) were precipitated from aqueous solution (pH 6–7), the complexes were extracted into organic solvents (chloroform, ether or benzene) and the extracted complexes were separated on silical gel thin layers using benzene-chloroform-methanol-water (20:75:12:0.5) as the eluent. A calibration curve was constructed for only one of these metals, Co(II). This calibration curve was comparable with ours for this metal

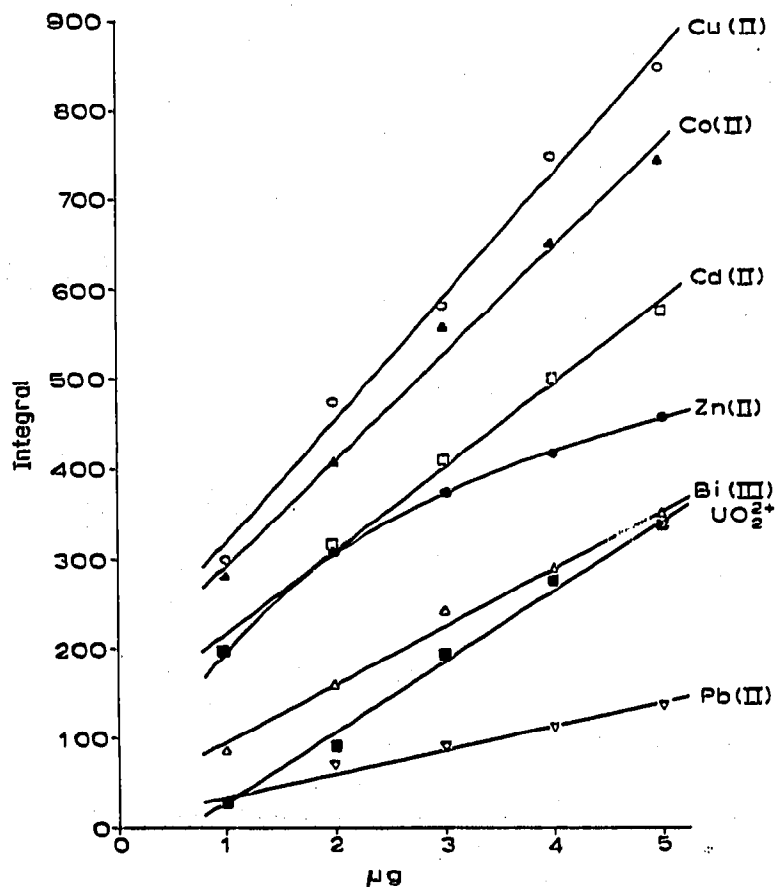


Fig. 2. Densitometric calibration curves for metal ions in the range 1.0-5.0 µg of metal ion. ▲ = Co(II); ○ = Cu(II); ● = Zn(II); ▽ = Pb(II); □ = Cd(II); △ = Bi(III); ■ = UO₂²⁺.

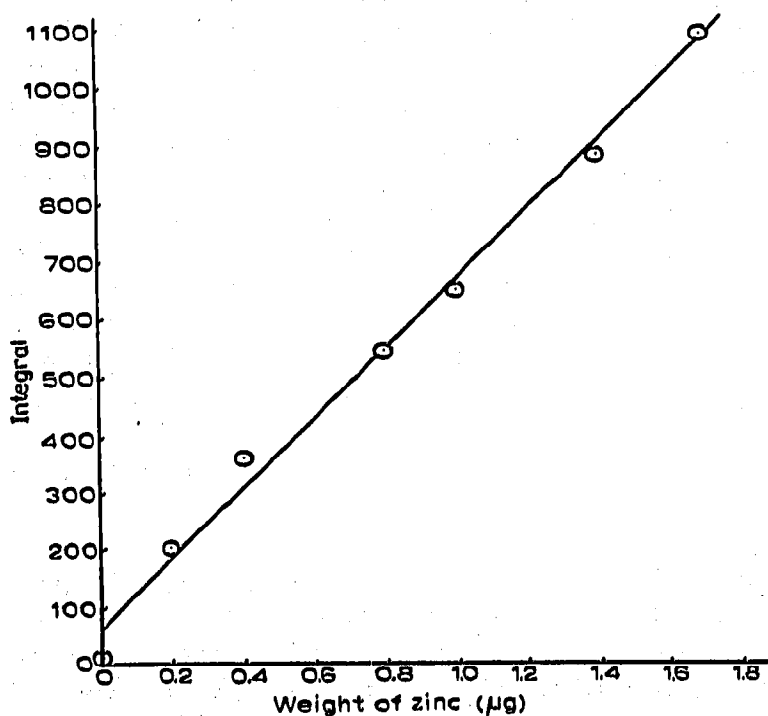


Fig. 3. Densitometric calibration curve for zinc in the 0.0-2.0 µg range.

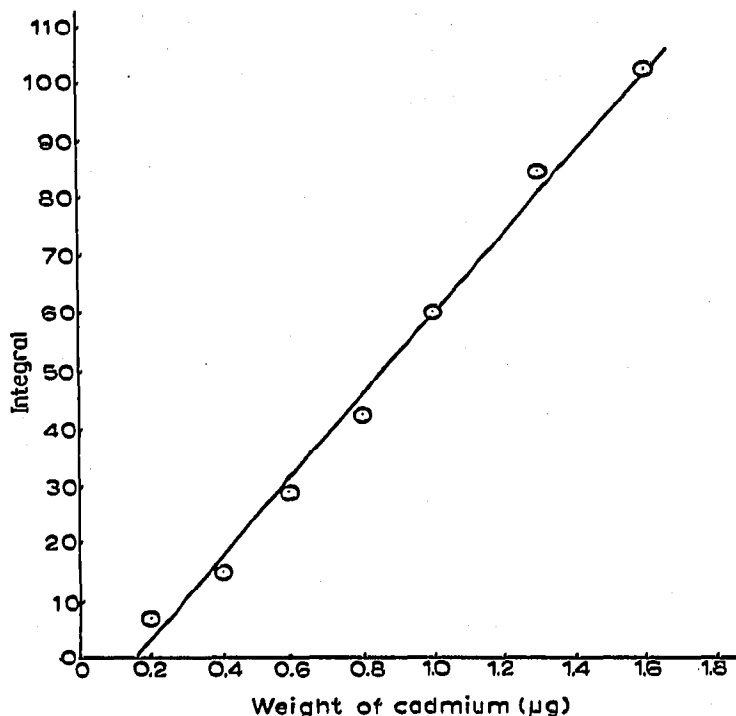


Fig. 4. Densitometric calibration curve for cadmium in the 0.0–2.0 μg range.

ion thus stressing the importance of the densitometric method for the quantitative determination of metal ions.

The problem of uneven spraying as one factor which may cause loss of precision in the densitometric technique appears, at first sight, to have been overcome by these workers, who report an error of 1.2 % in their determination. However, this % error is based on the determination of a single metal. Furthermore, the metals chromatographed by these workers are those which are stable in the pH range 6–7, *i.e.* they are those which we found to be amenable to our extraction technique as a pre-requirement to absorptiometric analysis. The lack of stability of the PAN complexes Zn(II), Cd(II), Pb(II) and Bi(III) in chloroform, as was shown by our attempted extraction of these elements, suggests that the preformed complexes of these elements could not be successfully chromatographed in the eluent system used by FLORET AND MASSA¹⁸, in which the major component was chloroform. Furthermore, the slightly acidic silica gel layers, over which these workers exercised no pH control, would also contribute to the breakdown of the PAN complexes of Zn(II), Cd(II), Pb(II) and Bi(III). These factors impose limitations, absent from our method, on the usefulness of the technique proposed by FLORET AND MASSA¹⁸.

We have tested the usefulness of our system by reference to two mixtures of the metal ions studied.

A Zn(II)–Cd(II) mixture was chosen because we have shown it to be amenable to determination by the densitometric technique but not by the absorptiometric technique. This separation is of importance because cadmium may be present to the extent of 0.5 % of crude zinc. The results are given in Table III and in Figs. 5 and 6. To obviate the variations in the integral counts from plate to plate caused by uneven spraying we used the technique of internal standards previously described by us¹². On

TABLE III

THE DIRECT DENSITOMETRIC DETERMINATION OF A MIXTURE OF Zn(II)-Cd(II) (1:1, w/w)

	Standard solutions				Test solution (added)	Test solution (found)	% error
	0.50	0.75	1.00	1.25			
Zn(II) (μg)	0.50	0.75	1.00	1.25	1.00	0.97	-3.0
Mean integral	420	608	743	901		724	
Cd(II) (μg)	0.5	0.75	1.00	1.25	1.00	1.03	+3.0
Mean integral	117	168	208	248		213	

one plate we applied one spot (1 μl volume) of each of four standard solutions of different concentrations of Zn(II) together with two separate spots of our test mixture. After chromatography and visualising the metal ions, the layer was scanned and the results obtained were used to determine the concentration of Zn(II) in the test solution. The procedure was repeated with a second plate except that standard Cd(II) samples were applied to the layers. The results of scanning this plate were used to determine the amount of Cd(II) in the test solution.

The results (Table III) confirm the accuracy of the technique as being $\pm 4\%$ at the 1 μg level for the single metal ions. More particularly they show that there is no loss in accuracy when mixtures are analysed rather than the individual ions when the elements concerned are present in a 1:1 ratio.

Secondly we attempted the analysis of one metal in the presence of a fairly large excess of another. For this we chose as our test mixture Fe(III)-Co(II) (99:1). This mixture was chosen because of the importance of cobalt as an alloying metal in many steels.

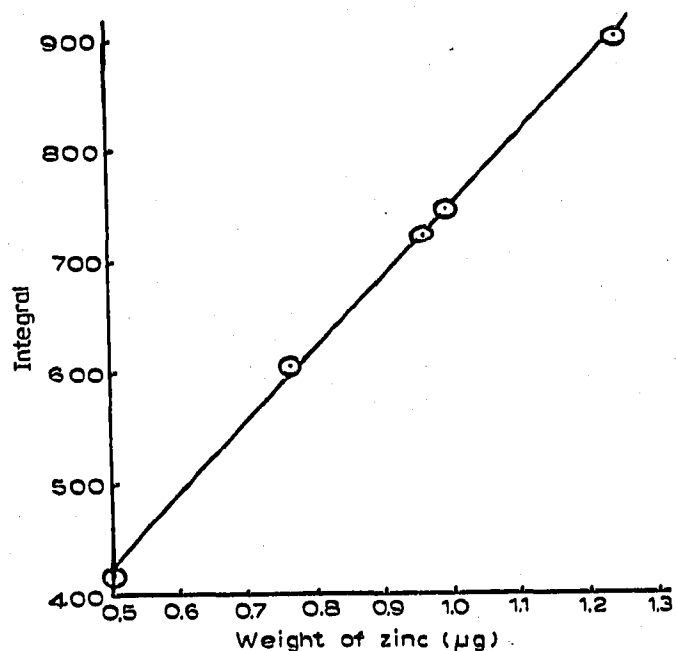


Fig. 5. Densitometric determination of zinc in a zinc-cadmium (1:1, w/w) mixture.

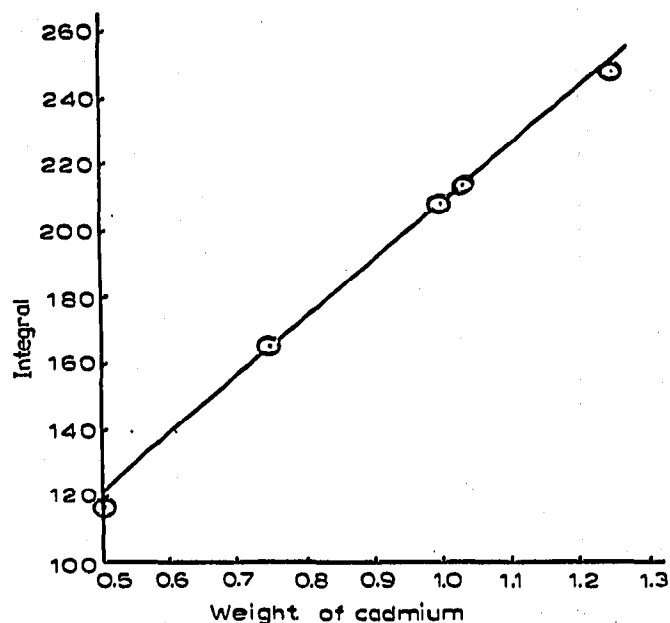


Fig. 6. Densitometric determination of cadmium in a zinc-cadmium (1:1, w/w) mixture.

The experimental procedure adopted was similar to that reported above for Zn(II) and Cd(II), *i.e.* a single spot of each of four different standard solutions of Co(II) together with two spots of the test mixture were applied to the layer, chromatographed, visualised and scanned. The results shown in Table IV and Fig. 7 indicate that the $\pm 4\%$ error is not exceeded even when one of the metals is present in a large excess over the other.

One possible disadvantage of this system lies in the fact that the layer is grossly overloaded with respect to one of the metal ions (in this case Fe(III)). In our investigation we found that Fe(III), normally of R_F 0.00 at the $1\ \mu\text{g}$ level, streaked so that the leading edge of the streak was found at an R_F value of 0.33. The R_F value of Co(II), however, was 0.72¹¹ so that the Co(II) spot was well clear of the Fe(III) and hence interference from the Fe(III) did not occur. This observation indicates that it should be possible to determine Co(II) in an even larger excess of Fe(III) before the Fe(III)

TABLE IV

THE DIRECT DENSITOMETRIC DETERMINATION OF Co(II) IN A MIXTURE OF Fe(III)-Co(II) (99:1, w/w)

	<i>Standard solutions</i>				<i>Test solution (added)</i>	<i>Test solution (found)</i>	<i>% error</i>
Co(II) (μg)	0.75	1.00	1.25	1.50	1.00	0.96	-4.0
Mean integral	102	136	169	201		130	

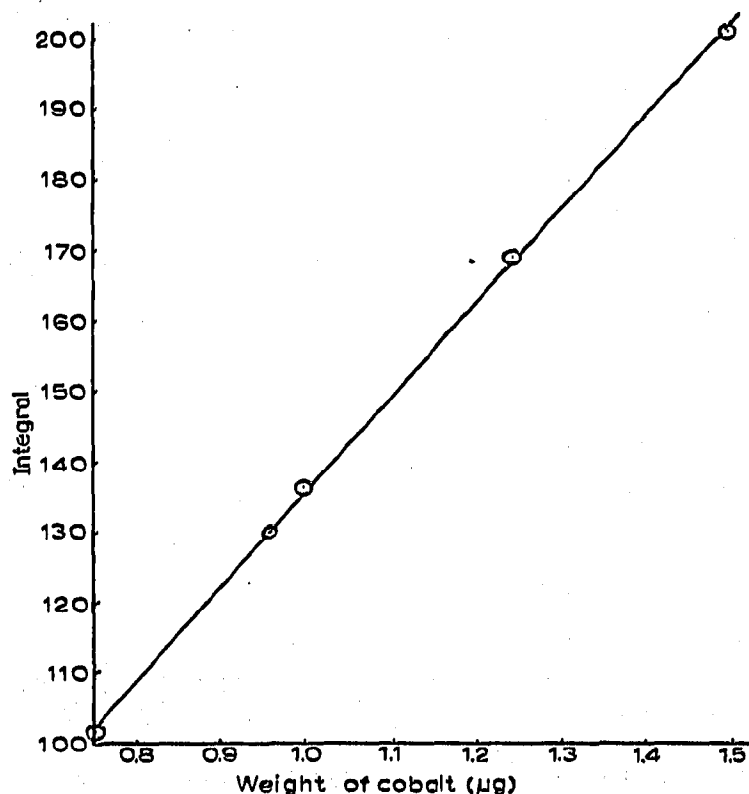


Fig. 7. Densitometric determination of cobalt in an iron-cobalt (99:1, w/w) mixture.

interferes with the accuracy of the technique as a result of the overlap of the Fe(III) streak with the Co(II) spot.

CONCLUSION

The quantitative determination of metal ions, which have been separated on thin layers of cellulose impregnated with Primene JM-T hydrochloride using hydrochloric acid as a mobile phase, is possible using either the spot removal technique in conjunction with absorptiometric analysis or the *in situ* direct densitometric technique.

The two techniques are of comparable accuracy provided that the PAN-metal ion complex can be stabilised to permit its absorptiometric analysis. The direct densitometric technique could be applied to those metal ions the PAN complexes of which were unsufficiently stable to permit their extraction from the layers. It also had the advantage of speed over the spot removal method.

The analysis of mixtures of metal ions is possible without loss of accuracy.

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