CHROM. 3835

QUANTITATIVE THIN-LAYER CHROMATOGRAPHY ON LIQUID ANION EXCHANGERS

PART I. AN INVESTIGATION INTO SOME OF THE PARAMETERS INVOLVED IN THE DIRECT DENSITOMETRIC DETERMINATION OF ZINC

R. J. T. GRAHAM, L. S. BARK AND D. A. TINSLEY
Department of Chemistry and Applied Chemistry, The University, Salford 5, Lancs. (Great Britain)
(Received September 28th, 1968)

SUMMARY

A study has been made of some of the parameters involved in the direct densitometric determination of zinc on cellulose layers impregnated with the liquid ion exchanger Primene JM-T hydrochloride. The chromogenic reagent PAN was employed to locate the zinc spots.

Of the factors associated with the instrument (the Joyce-Loebl Chromoscan) the most important has been shown to be the setting of the baseline control. The most important factor concerned with the chromatographic system is shown to be the difficulty of distributing the chromogenic reagent uniformly over the layer.

In spite of these problems it has been shown that an accuracy of about 5 % can be achieved at the 1 μg level.

INTRODUCTION

Inorganic ions, previously separated by thin-layer chromatography, have been quantitatively determined in a number of ways. These are *in situ* methods[1–4], including spot area measurements[1–3], radiochemical methods[3,4] and by spectroscopy involving transmitted light[3], as well as by methods involving the removal of the inorganic species from the layers prior to their quantitative determination[5–7].

The layers used for these investigations have been limited to laboratory-prepared silica gel layers[1–3], precoated silica gel layers[4] and cellulose layers[5–7], so that the investigations have been limited to normal adsorption and normal partition techniques.

We have already reported the results of our investigations into the qualitative separations of metal ions by reversed-phase thin-layer chromatography on a neutral organo-phosphorus substrate[8–10] and on layers impregnated with long-chain amines in the form of their hydrochlorides[11–13]. Because of this, and also because of the absence of direct quantitative data obtained from such reversed-phase thin-layer chromatographic systems we decided to investigate the possible applications of

quantitative investigations in one of our reversed-phase systems, namely the system Primene JM-T hydrochloride–hydrochloric acid[12,13].

Early *'in situ'* methods of qualitative inorganic TLC analysis[1–4] were carried out on systems which gave R_F values of low reproducibility whereas the system investigated here has been shown to yield highly reproducible R_F values. This fact is of considerable importance because our quantitative studies were carried out using a direct densitometric method. It has been shown by BLANK and coworkers[14] and THOMAS *et al.*[15] that the precision of quantitative results obtained by this method can be affected by the R_F values of the sample scanned. This phenomenon has also been investigated by DALLAS[16], who investigated a number of other parameters which are of importance in the precision direct densitometry of coloured substances on silica gel thin layers.

In addition to the problems considered by DALLAS[16] further problems arise in the direct densitometry of colourless species chromatographed by reversed-phase systems. In order to assess these additional effects, we have examined the parameters affecting the densitometry of a standard red dye on cellulose layers (the support medium for the impregnants in our reversed-phase systems) as well as those affecting the precision of the results obtained for the element zinc in the chosen system.

EXPERIMENTAL

Chromatography

(a) *Chromatography of the standard dye.* Cellulose (15 g MN 300 HR) was slurried with water (90 ml) and the coated plates were allowed to air dry overnight.

A solution of the standard red food dye (Red 10 B) was applied to the layers and eluted with *n*-butanol–water–glacial acetic acid (20:12:5, v/v/v)[17] by our sandwich chamber technique[18]. When the solvent front had reached the appropriate point (14.0 cm $\pm$ 0.5 cm) the layers were removed from the chamber, dried and scanned.

(b) *Reversed-phase system.* Cellulose (15 g MN 300 HR) was slurried with a solution of the amine hydrochloride in chloroform (70 ml of 0.3 *M*) which had been prepared under the standard conditions previously described[12,13]. A zinc(II) chloride solution (1 μl of a 1 mg/ml solution) was applied to the layers using a Hamilton syringe (1 μl capacity).

The elution of the chromatograms with the hydrochloric acid eluent, in our sandwich chamber[18], the drying of the plates and the visualisation of the zinc spot with the chromogenic reagent PAN have already been described.

Direct densitometry

The instrument used throughout the work was the "Chromoscan" recording and integrating densitometer with a thin-layer attachment*. The instrument may be used for both reflectance and transmission densitometry, but in the work reported here we were concerned only with its use as a reflectance densitometer. The main features of the instrument have been discussed by DALLAS[16].

In scanning the standard spots a 10 mm $\times$ 1 mm slit aperture was used throughout. Unless otherwise stated each standard spot was scanned twenty-five times and the integrator counts quoted represent the mean counts.

* Available from Joyce Loebl and Co., Gateshead on Tyne, England.

RESULTS AND DISCUSSION

In his study, DALLAS[16] preferred not to use the integrator with which the instrument is provided. We have orientated our study to a consideration of the factors which affect the efficiency of the integrator. These are:

(i) instrument drift;
(ii) linearity of the integrator;
(iii) speed of scanning the spot relative to the chart speed;
(iv) use of cams to adjust the pen deflection;
(v) base line adjustment;
(vi) centring of the spot under the light beam.

Each of these parameters was investigated using the standard red dye on cellulose layers.

(*i*) *Instrument drift.* This was noticeable in readings taken immediately after the instrument was switched on, but became negligible if a period of half an hour was allowed to elapse between first switching on the instrument and scanning the first spot.

(*ii*) *Linearity of the integrator.* The scale deflection, produced by adjustment of the base line control, and the numbers of integral counts per minute produced by this deflection are given in Table I.

TABLE I

LINEARITY OF THE INTEGRATOR ATTACHMENT

Scale deflection (cm)	1	2	3	4	5	6	7	8	9	10
Counts/min	147	338	524	698	862	1047	1275	1460	1658	1849

Fig. 1 shows that a plot of scale deflection *vs.* counts per minute is linear, though the line does not quite pass through the origin. This failure of the line to pass through the origin is not unexpected because the instrument possesses a low background count which may be adjusted by the base line control to 1 count/5 sec.

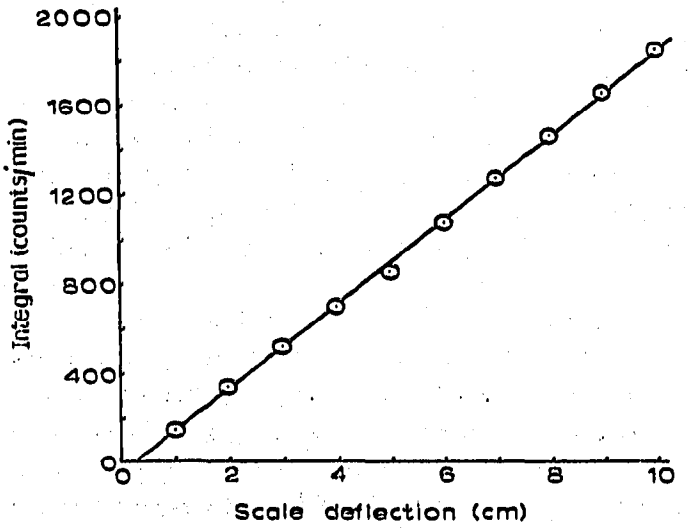

Fig. 1. Integral count (counts/min) *vs.* scale deflection.

(*iii*) *Speed of scanning the spot relative to the chart speed.* The standard spot was scanned using each of the drive ratios 1:2 and 1:4 (specimen speed:chart speed) the chart speed being maintained constant. These results are given in Table II.

TABLE II

THE EFFECT OF THE SPECIMEN/CHART DRIVE RATIO ON REPRODUCIBILITY

Drive ratio	*Mean count*	*Standard deviation* (%)
1:2	207.7	0.48
1:4	413	0.66

From the results, it can be seen that the difference in reproducibility is insignificant. However, the 1:4 ratio scans the spot more slowly and hence allows the instrument more time to respond to changes in the light intensity. Thus for subsequent work we used the 1:4 ratio.

(*iv*) *Use of cams to adjust the pen deflection.* Three cams, A, B and C, are provided with the instrument. These produce linear scale deflections in the ratios 1:1.3:2.4. The standard spot was scanned with each cam in position in turn, and average counts of 170, 215, 413 were obtained for cams A, B, and C, respectively, giving a ratio of 1:1.27:2.42, which confirms the accuracy of the system. For maximum sensitivity in subsequent work a drive ratio of 1:4 and cam C were used.

(*v*) *Base line adjustment.* The standard spot was scanned, resetting the base line control after each scan. A standard deviation of 1.63% was obtained from the mean. This compares unfavourably with a standard deviation of 0.66% for a similar test in which the base line control was not adjusted after its initial setting.

(*vi*) *Centring of the spot under the light beam.* One of the problems associated with the successive scanning of either several spots on the same plate, or several different spots is the question of the correct positioning of the spot under the light beam, particularly as this has to be done visually. This was checked by removing the plate from the carriage after each scan and recentring the spot before commencing the next scan. A standard deviation of 0.83% was obtained compared with 0.66% for rescanning without altering the position of the plate relative to the light beam.

It is interesting to note that our standard deviation for repeat scanning based on integrator read out is smaller than that quoted by DALLAS[16] for his preferred method of planimetry for determining the quantity of sample in the spot. This may be due to our use of a 10 mm × 1 mm slit aperture compared with the 1 mm diameter circular aperture used by this worker resulting in the positioning of the beam being more critical in the latter case.

The foregoing results confirm and compliment the findings of DALLAS that a good degree of precision can be achieved in the densitometry of coloured compounds using the Chromoscan instrument. The greatest source of error was found to be introduced by attempting to adjust the base line between each scan. For this reason we did not attempt to adjust the base line in the work in our attempts to appraise the instrument for work on colourless substances chromatographed by reversed phase systems.

Colourless substances chromatographed by reversed-phase thin-layer chromatography

The following additional factors must also be considered for colourless substances chromatographed by reversed phase thin-layer chromatography namely:

(a) effect of the stationary phase;
(b) treatment of the layers after elution and before spraying;
(c) method of application of the chromogenic reagent to the layer;
(d) stability of the coloured complex.

(*a*) *Effect of the stationary phase.* The most probable effect contributing to lack of precision from this source is the non-uniform distribution of the stationary phase over the cellulose support. However, it has been shown by DUNCAN[10] that the slurry method of incorporating an impregnant into the support gives a reasonably uniform distribution of the impregnant over the layer.

Even so, we scanned a number of impregnated layers and it was observed that the base line, once initially adjusted remained constant when the same plate was scanned in several different positions across its width. From this we concluded that errors due to the method of impregnation will be minimal and that the presence of the impregnant is unlikely to affect any results obtained from impregnated layers.

(*b*) *Treatment of the layer after elution and before spraying.* The need to heat the plates in order to remove the hydrochloric acid eluent before spraying with the chromogenic reagent, PAN, has been discussed by us[8–13]. Such treatment caused a darkening of the cellulose layer, and in particular a very dark zone immediately behind the solvent front. The lower edge of this zone was uneven and extended to a maximum distance of 0.5 cm behind the front. The scanning of eluted, dried chromatograms resulted in marked variation in the integral count in the region of this zone. However, scans between the lower end of this zone and the point of application of the samples yielded a fairly uniform base line.

From this, it can be seen that the technique will lead to imprecise results when attempts are made to scan spots immediately behind the solvent front, even when the upper, as well as the lower, borders of the spots are clearly demarcated. When the upper end of the spot merges with the solvent front, further imprecision is introduced consequent upon the lateral zone spreading of the spot.

At this juncture we would like to add a further note concerning the phenomenon of lateral zone spreading. It is our opinion that this can lead to marked imprecision in all densitometric determinations, whether of coloured or colourless species, in normal or in reversed-phase systems when the sample migrates with the solvent front, or when demixion occurs during the run. When this latter happens, those samples which run with the β etc. fronts, *i.e.* in polyzonal thin-layer chromatography, also show lateral migration of the spots and hence attempts at a quantitative analysis of such samples by direct methods will yield imprecise results. This parameter, as a contributory factor to imprecision in densitometric determinations, was not commented on by DALLAS[10], probably because his use of a single component mobile phase obviated the need for him to consider this factor, but it is a factor which must be constantly borne in mind by anyone attempting to use the technique.

(*c*) *Method of application of the chromogenic reagent to the layer.* Chromogenic reagents may be applied to thin layer chromatograms as follows:

(i) by dipping the layer, face downwards into the reagent;

(ii) by drawing a filter paper through the reagent and then impressing the reagent-loaded paper against the layer;

(iii) by direct spraying of the reagent onto the surface of the layer.

Both (i) and (ii) were tried as a means of visualising zinc in our reversed-phase system. When the layers were dipped into the alcoholic solution of PAN, the layers became separated from the glass plate. Blotting the layers with filter paper similarly damaged the layers. Thus we were forced to rely on method (iii) and the results obtained using this method are given in Table III.

TABLE III

IMPRECISION CAUSED BY UNEVEN SPRAYING OF THE PLATES

Eight spots of Zn(II) standard solution were applied across each plate.

Plate number	*Standard deviation* (%)
1	4.3
2	3.2
3	5.2
4	3.9

Thus we see that the greatest source of error in the direct densitometry of colourless substances will be associated with the application of the chromogenic reagent to the layer. To some extent, however, errors from this source can be mitigated by spotting a sample of known concentration on either side of the unknown sample because we observed that the average difference between any two adjacent spots is 2.5% compared with 5.2% maximum error for a whole plate.

(*d*) *Stability of the coloured complex.* The stability of the zinc–PAN complex was investigated by counting a 1 μg spot at regular time intervals after removal of the plate from the ammonia vapour. The results are shown in Table IV.

TABLE IV

STABILITY OF A ZINC–PAN SPOT

Time (min)	0	10	20	30	40
Integral	266	264	262	264	260

As all the spots on a plate can be counted in less than 5 min, the results in Table IV show that fading of the spots will not have a significant effect on the accuracy of our results.

CONCLUSIONS

Direct densitometry affords a quick and reasonably accurate method of quantitatively assessing thin-layer chromatograms. The major source of error in the evaluation of colourless compounds is seen to be the unevenness of the application of the

chromogenic reagent. Other sources of error may be associated with the instability of the coloured complex formed and the effects of demixion on the shape of the spots.

ACKNOWLEDGEMENT

We thank Messrs. Charles Lennig & Co. Ltd., London for the gift of the Primene JM-T used in this investigation.

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