

QUANTITATIVE ESTIMATION OF PAPER CHROMATOGRAMS BY DIRECT PHOTOMETRY

II. MEASUREMENT OF PAPER CHROMATOGRAMS IN A SPECTROPHOTOMETER BY A SINGLE READING REFLECTION METHOD*

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INTRODUCTION

The most important methods of evaluating paper chromatograms by direct photometry involve measurement of:

(i) *Maximum colour density of the spot*²⁻⁸. The principle of this method is to determine the transmittance (or reflectance) at the centre of the spot by a single reading.

(ii) *Whole area of the spot by a single reading*⁹⁻¹⁷. This method is similar, except that a beam of light of relatively large area is employed. This is restricted to the size of the spot by means of a mask.

(iii) *Whole area of spot by scanning*^{8, 18-25}. This method is similar to the second method in that the whole of the area of the spot is explored, but differs from it in that a large number of readings are taken over different areas of the spot. Automatic scanning with pen recorder would be the most logical way of carrying out this method²⁶. Commercial instruments for this purpose usually, however, employ a white light source, and filters, with consequent errors.

The most important factors determining the choice of a particular method are accuracy, instrumental convenience, and speed. Chromatographed spots are seldom perfect in shape. Ideally, those run on wide sheets of paper should be circular or slightly elliptical, while those run on narrow strips should be rectangular. In practice, however, spots seldom approach these ideal shapes and invariably suffer from all manner of distortions. Furthermore, it is often difficult in practice to prepare closely reproducible chromatograms; many factors may influence the shape and size of a spot and the distribution of material in a spot. The best methods of quantitative estimation are those which give nearly the same result regardless of the size, shape, and regularity of the spot. It might be expected that the second and third methods cited above would be capable of more accurate results, since the whole of the spot is explored.

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The first method has, however, much to recommend it on the grounds of instrumental convenience and speed. It may readily be carried out in a spectrophotometer without any special apparatus. Many spectrophotometers produce a beam of light of small cross-sectional area and are not readily adapted to the second method referred to above. It is true they may be adapted to the third method, that employing scanning, but only with the aid of additional apparatus.

In order to obtain reasonably accurate results with the first method it is very important:

- (i) To obtain closely reproducible chromatograms possessing spots of good shape.
- (ii) To remember that readings taken over small areas are particularly subject to variations on account of the non-homogeneity of the paper. The resulting optical non-uniformity of filter paper has been shown by the present writers¹ to be less apparent by reflected light than by transmitted light. It is suggested therefore, that in this case, in which only a single reading is taken on a small area of paper, estimation would be expected to be more accurate by reflected than by transmitted light.

The work which will now be described, was carried out using the Hilger Uvispek Model 700 (together with the maker's diffuse reflection attachment) but without the use of any other supplementary apparatus. Only one reading was taken on the centre of each spot, on an area of approximately 3 mm \times 3 mm. The method is, no doubt, somewhat less accurate than a scanning method, but is exceptionally rapid and convenient.

EXPERIMENTAL

Introduction

Copper and nickel were chromatographed together but the quantitative estimation was limited in this work to copper. The substances for analysis were dissolved in 3 *N* HCl because solutions of this acid concentration produced uniform initial spots.

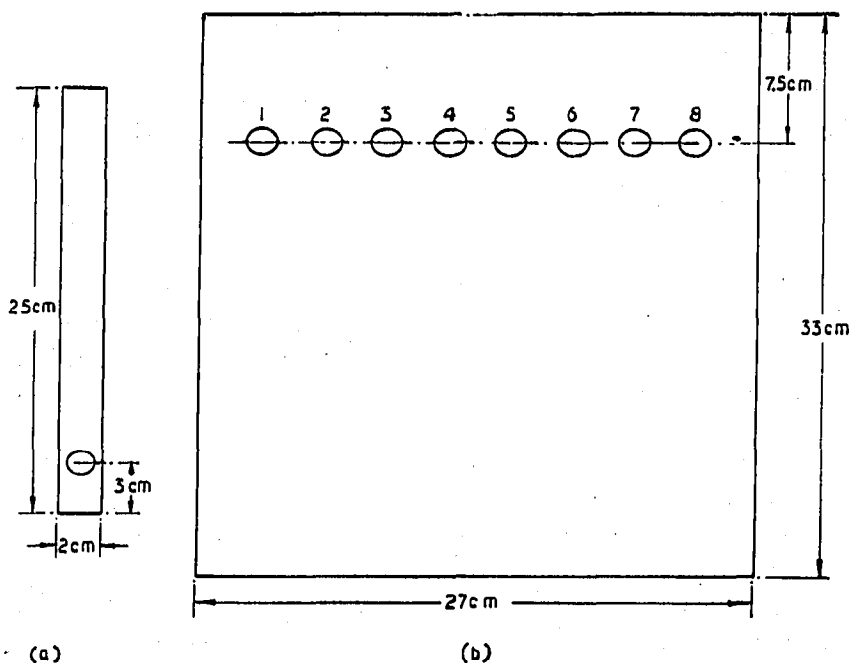


Fig. 1. Details of preparation of chromatograms (a) on narrow strips, and (b) on broad sheets. Number on spot refers to Table II, in which details of the spots are given.

Application of spots to paper

Spots of volume $2.64 \mu\text{l}$ were applied to sheets of Whatman No. 2 paper. Narrow strips were used in preliminary work employing ascending chromatography, as shown in Fig. 1 (a). Broad sheets, enabling several spots to be estimated at the same time, were used in conjunction with descending chromatography, as shown in Fig. 1 (b).

Equilibration of paper in solvent vapour

The paper bearing the initial spots was dried before equilibrating in the solvent vapour. Quite short equilibration periods of about 0.5 h were used for preliminary work with ascending chromatography, which was carried out in gas jars. A period of 24 h, however, was used with descending chromatography, since this was carried out in large tanks.

Eluting solvents

The following solvents for the separation of copper and nickel were investigated by ascending chromatography on narrow strips.

(i) Methyl ethyl ketone, conc. HCl, water solvents, and the solvent methyl ethyl ketone–conc. HCl (90:10)* appeared, at first, to be very promising. A complete separation was produced in less than half an hour. The spot of copper, however, underwent a change in shape which will be termed “straight line formation”. This is a tendency for the initial spot to change into a line-shaped spot at right angles to the direction of flow, during the first few minutes of elution. This line-shaped spot gradually changed back into a round spot, but the time taken for this change varied greatly as a result of small differences in procedure which were difficult to control. It seemed best to avoid this straight line formation altogether, and it was found that this could be done by omitting the usual period of equilibration of the paper in the solvent vapour and replacing it by equilibrating the paper in HCl vapour for half an hour. This procedure produced good separations and well shaped spots.

(ii) Methyl propyl ketone–conc. HCl solvents were found to be very prone to straight line formation. Acetone–conc. HCl solvents appeared less prone to straight line formation, but ran a little too rapidly for closely reproducible chromatography.

(iii) Acetone–butanol–conc. HCl–water solvents. The proportion of each component in this system was varied in turn. The most suitable composition was found to be acetone 50, butanol 46, conc. HCl 10, water 7.5. This solvent produced a good separation and almost perfect rectangular spots, free from straight line formation. It was well suited to ascending chromatography and was used in preference to methyl ethyl ketone–conc. HCl (90:10), because it was found that more reproducible results could be obtained. No equilibration of the paper either in the solvent vapour, or in HCl vapour, was necessary.

For closely reproducible results, descending chromatography on wide sheets is probably preferable and therefore a fourth type of solvent of rather slow rate of flow was investigated, first using the ascending technique in a gas jar and then the descending technique in a large tank.

(iv) Butanol–conc. HCl–water solvents were prepared by shaking butanol with dilute HCl and separating off the organic layer for use. Strong HCl mixtures produced

* Solvent compositions are given by volume.

greater separation than weak HCl mixtures but tended to weaken and discolour the paper. The organic layer of butanol-3 *N* HCl solvent appeared to be the most suitable solvent. This produced a complete separation of copper and nickel in 24 h, and did not weaken or discolour the paper. Elution was preceded by a period of 24 h of equilibration during which the paper was allowed to hang in the solvent vapour.

This solvent was finally chosen as the most suitable one for estimation by a single reading reflection method. The details of the method are now summarised:

2.64 μl spots containing Cu^{2+} and Ni^{2+} in 3 *N* HCl were placed on the paper 7.5 cm from the shorter edge of the paper. After drying, the sheets were placed in an empty tank lined with paper, with the paper dipping into a trough. The organic layer of butanol-3 *N* HCl was used both for saturation and for elution. 250 ml of the solvent were placed in the tank, in such a way as to wet the paper lining the tank. The lid was then closed and equilibration was allowed to take place for 24 h. 40 ml of the solvent were then placed in the trough through a hole in the lid. The chromatogram was eluted for 24 h, by which time the solvent had run off the end of the paper. The papers were then removed and allowed to dry in a current of air for 2 h before development in rubeanic acid. The resulting spots were of good "circular" shape. Their dimensions are given in Table I.

TABLE I
DIMENSIONS OF CHROMATOGRAPHED SPOTS
Solvent butanol-3 *N* HCl; time of elution 24 h.

Concentration g Cu^{2+}/l (vol. 2.64 μl)	Distance run by copper spot cm	Concentration g Ni^{2+}/l (vol. 2.64 μl)	Distance run by nickel spot cm
1.0	7.2-9.1	1.0	4.4-6.0
0.5	7.4-9.2	0.5	4.5-6.1
0.25	7.5-9.0	0.25	4.7-6.1

Development

After drying the chromatograms the portion of paper containing the copper spots was cut out and developed, by the method previously described¹, *viz.*:

- (i) Hanging in ammonia vapour for half a minute,
- (ii) Immersing in 0.1 % alcoholic rubeanic acid for 1 minute with agitation,
- (iii) Hanging again in ammonia vapour for half a minute, and
- (iv) Washing in alcohol for half a minute.

The paper was then allowed to dry in a current of air at 20° for at least 5 minutes before measuring in the spectrophotometer.

Photometric evaluation

This was extremely simple. Each of the spots was cut out by a corkborer or with scissors. The blank spot was placed in the standard compartment of the diffuse reflection attachment, and each of the spots was placed in turn in the measure compartment. A background of six sheets of white filter paper was used in both compartments¹. It was important to centre the spot in the measure compartment so that the centre of the spot coincided with the centre of the beam of light measuring approxi-

TABLE II

ESTIMATION OF RELATIVE ERROR AT DIFFERENT CONCENTRATIONS

Temperature of elution 23°, time of elution 24 h, volume of spot 2.64 μ l. Reflection readings on chromatographed spots of copper.

Number and designation of spot (see Fig. 1b for position of each spot on paper)	Copper concentration g Cu ²⁺ /l	Nickel concentration g Ni ²⁺ /l	% Reflectance at centre of copper spot	Corresponding value of K/S	Measured concentration g Cu ²⁺ /l	% Relative error
1	1.00	—	32.3	0.709	—	—
5	0.60	—	42.5	0.389	—	—
7	0.20	—	64.3	0.099	—	—
3	0.10	—	73.8	0.047	—	—
2	0.80	0.5	37.5	0.521	0.77	-3.8
4	0.50	0.5	47.2	0.295	0.49	-2.0
6	0.25	0.5	58.8	0.144	0.27	+4.0
8	0	0	—	—	—	—

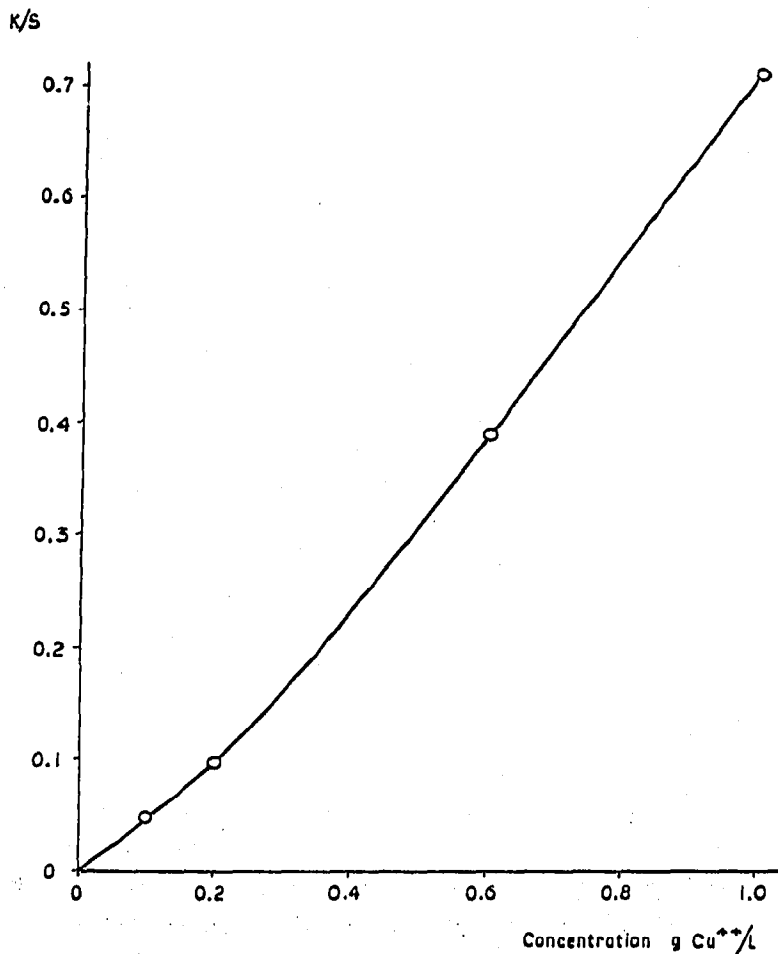


Fig. 2. Calibration curve for chromatographed copper.

mately 3 mm × 3 mm. The reflectance at 660 m μ was then measured, using a slit width of 0.2 mm¹.

A typical set of results is shown in Table II. Seven spots containing metals and one blank spot were placed on the paper. In all cases their true concentrations were known. Of these, four spots which contained only copper, were treated as standards and were used to draw the calibration curve shown in Fig. 2. Three spots, which

TABLE III
EFFECT OF VARYING NICKEL CONCENTRATION
(AND OF THE PRESENCE OF OTHER METALS) ON ESTIMATED CONCENTRATION OF COPPER
Temperature of elution 23°; time of elution 24 h; volume of spot 2.64 μ l.

Number of spot (see Fig. 1b for position of each spot on paper)	Copper concentration g Cu ²⁺ /l	Nickel concentration g Ni ²⁺ /l	Concentration of other metals g ion/l	% Relative error (from mean)
1	0.5	4.00	0	-3.6
2	0.5	2.00	0	-3.6
5	0.5	1.00	0	+1.7
6	0.5	0.50	0	+1.7
4	0.5	0.25	0	+1.7
2	0.5	0	0	-3.6
7	0.5	0	{ Hg ²⁺ 2.0 Cd 1.0 Bi 1.0 }	+3.6
8	0	0	0	—

contained both copper and nickel were treated as unknowns, and their copper concentration was determined by interpolation from the calibration curve. This enabled the error in each estimation to be established.

The blank spot contained only 3 N HCl. After elution and development, the area of the chromatogram where a spot would have appeared if a metal had been present, was cut out and placed in one compartment of the reflection attachment. The spot which was being measured was placed in the other compartment. Both the blank and the spot were backed by six sheets of filter paper, as already described¹.

A second sheet contained seven spots of identical copper concentration; of these six were of widely varying nickel concentration, and one contained three other metals in relatively large concentrations. One blank spot was also placed on the paper. This sheet was eluted in the same tank simultaneously with the other sheet referred to above. The copper concentration of each spot was estimated by interpolation from the calibration curve drawn from the spots selected as standards on the other sheet. These results are shown in Table III.

The relative errors here are no greater than those shown in Table II. It is clear that the estimated concentration of copper is not affected by the presence of relatively large concentrations of nickel. The presence of Hg²⁺, Cd, and Bi also do not appear to affect the estimated concentration of copper.

DISCUSSION

The method described above, in which the maximum colour density of the spot is measured by reflection in a spectrophotometer, is both rapid and convenient. It is

suggested that, providing that spots of good shape can be obtained, this method is capable of producing reasonably accurate results of perhaps $\pm 5\%$ even with spots of quite small volume, *viz.* 2.64 μl . The present writers have also evaluated small spots of copper on narrow strips of paper by scanning using transmitted light. The lack of uniformity of the paper was more apparent by transmitted light and it was not found possible to obtain more accurate results than those obtained with the single reading reflection method described.

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SUMMARY

A method of estimating copper in the presence of relatively large quantities of nickel is described. A single reflection reading on the densest part of the spot was taken in a spectrophotometer fitted with a diffuse reflection attachment. No other photometric apparatus was required. The method is both rapid and convenient and gives reasonably accurate results, providing that spots of good shape are used.

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