A NEW METHOD FOR QUANTITATIVE PAPER CHROMATOGRAPHY

SIMON DITTRICH

Facultad de Quimica, Montevideo (Uruguay)*

(Received January 21st, 1963)

The high efficiency of paper chromatography in qualitative work explains the numerous attempts to find methods for quantitative estimation of small samples separated by this technique.

Visual comparison[1] of the colour of the spot, allows semi-quantitative analysis, and other authors have carried out spot size measurements[2] or direct colorimetric determinations[3–10].

More accurate results are obtained by making colorimetric measurements after elution[11–12], and "retention analysis" is a good method of quantitative estimation without elution[13–14].

In the present work an indirect quantitative determination is described: the spots are detected by iodine vapour[15–16], eluted, and the amount of fixed halogen is determined.

Iodine vapour was first used by BRANTE[15] to detect nitrogenous substances on paper chromatograms. At a later date, MARINI-BETTOLO AND GUARINO[16] showed the possibilities of iodine as a detector of non-nitrogenous substances.

In our experiments we have established the proportionality between the amount of substance and the iodine fixed by the spot.

The knowledge of the amount of iodine fixed by a certain quantity of substance enables us to give a numerical value to the sensitivity, which is defined as the number of μg of iodine fixed by one μg of substance, and values for various substances are given in Table I. These values vary greatly with experimental conditions, for example in the case of the amino acids, the sensitivity is greater if the chromatogram is dried at 160° (toasting) before being exposed to the iodine vapours.

In general we have noticed that the presence of moisture in the iodine chamber increases the sensitivity but that too high a humidity causes spot diffusion. The work was therefore carried out in an atmosphere at 76 % relative humidity stabilized with a saturated solution of KCl.

In some cases (benzedrine, atropine, etc.), the colour sensitivity improves if the chromatogram is left some minutes in the air after the exposure. In this way the paper-fixed iodine is rapidly lost and the contrast between the spot and the paper increases.

EXPERIMENTAL

Apparatus and reagents

Detection chamber. An ordinary desiccator of 15–20 cm diameter, with two small dishes at the bottom, one for the iodine crystals and the other for the KCl saturated solution, makes a good chamber.

* Avda. Gral. Flores 2124, Montevideo, Uruguay.

TABLE I

SENSITIVITY OF COMPOUNDS TO IODINE VAPOUR

Substance	*Sensitivity (in μg)*	*Solvent*	*Drying temperature (°)*	*Exposure (h)*
Alanine	2.1	Butanol–acetic acid	160	24
Arginine	1.8	Butanol–acetic acid	160	24
Glycine	1.9	Ethanol–NH_4OH	160	24
Tryptophan	1.9	Ethanol–NH_4OH	160	24
Leucine	1.8	Ethanol–NH_4OH	160	24
Glucose	0.5	Ethanol–NH_4OH	160	24
Pyramidon	5.6	Butanol–acetic acid	120	3
Creatinine	3.7	Butanol–acetic acid	120	3
Ephedrine	1.8	Butanol–acetic acid	120	5
Sulphathiazole	1.6	Butanol–acetic acid	120	18
Benzedrine	1.6	Butanol–acetic acid	120	18
Atropine	4.3	Butanol–acetic acid	120	18
Brucine	2.9	Butanol–acetic acid	120	18
Novocaine	4.6	Butanol–acetic acid	120	18
Pilocarpine	1.9	Butanol–acetic acid	120	18
Codeine	1.7	Butanol–acetic acid	120	18
Malonic acid	2.0	Ethanol–NH_4OH	120	2
p-Aminobenzoic acid	1.4	Ethanol–NH_4OH	120	5
Ascorbic acid	2.7	Butanol–acetic acid	120	2
Thiamine–HCl	0.5	Propanol–HCl	80	20

Stand for the paper cut-outs. A grill of a size suited to the desiccator was made with 4 mm diameter glass rod (Fig. 1).

Micropipettes. These can be easily made with capillary tubes marked so that they deliver 0.005 ml. If the same pipette is used for the sample solution and the standard solution, it is unnecessary to know the exact volume used.

Chromatographic paper. This should be large enough for duplicates of the sample solution together with three or four spots of the reference solution to be chromatographed.

Before spotting the paper is divided up into 2 cm wide strips, marked in pencil, which run perpendicular to the line along which the samples are placed.

The paper must be handled by touching only the edges with clean fingers.

5–10 % KI solution.

1 % soluble starch solution.

0.001 N (approx.) sodium thiosulphate. Prepared by dilution of a more concentrated solution.

Standard reference solutions. 0.5–5 % of the substance in an appropriate solvent. The concentration range chosen must be near that of the sample solution and all solutions must be freshly prepared.

EEL or a similar type photocolorimeter. A calibration curve is made with a solution of iodine in 5 % KI.

Procedure

When preparing the chromatogram special care must be taken in measuring the sample volume, which must be placed at the starting line and equidistant from the guide lines.

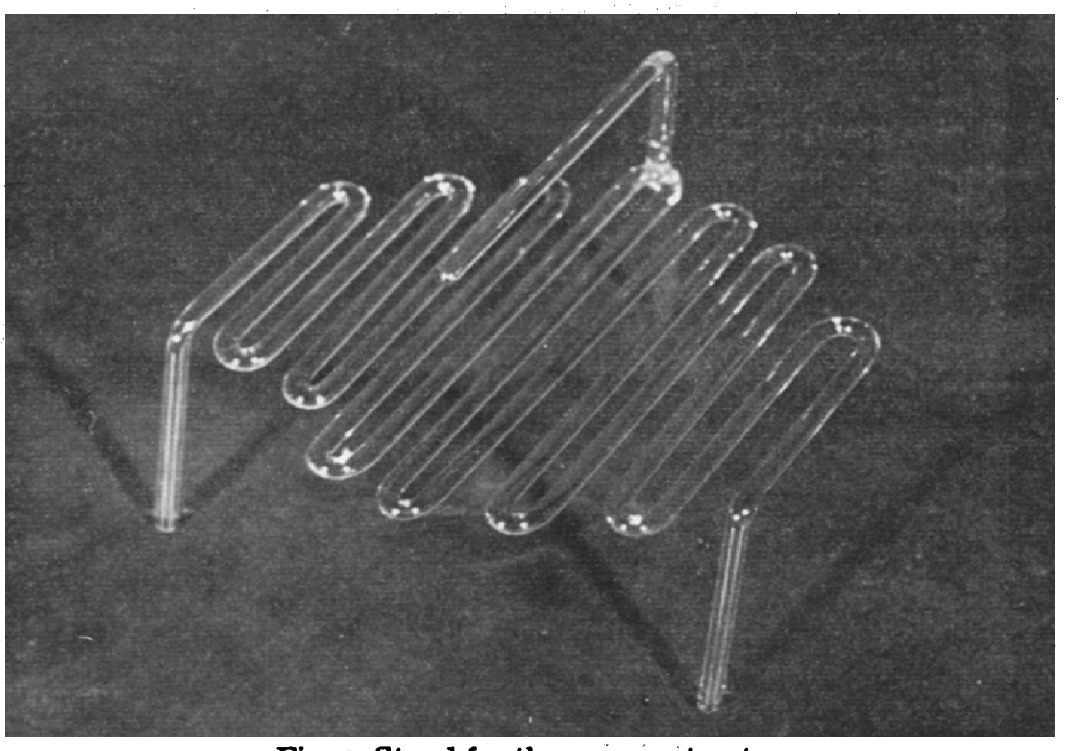

Fig. 1. Stand for the paper cut-outs.

After the chromatogram has been developed the solvent is removed by heating in an oven. The temperature used varies with the solvent mixtures and with the substance; for example propanol–HCl mixture must not be heated to more than 80°; on the other hand, to sensitize some substances like alanine, one must reach 160°. Usually the chromatogram is heated for 15–20 min at 120°.

When dry the chromatogram is placed in the iodine chamber for the qualitative detection of the spots on the paper. After their appearance, the chromatogram is removed from the iodine chamber, placed on a clean surface and two parallel lines are drawn with a pencil near the upper and lower edges of the spots. In this way one obtains a series of identical squares, which are properly numbered, cut out and finally placed back on the glass stand in the iodine chamber for the completion of the reaction.

These cut-outs are best handled with metal tweezers. The exposure time varies from 2–20 h depending on the sensitivity and the size of the sample. When all the spots stand out clearly against the paper the reaction is usually complete.

Each spot is individually eluted from the paper by KI solution contained in 50–100 ml beakers.

For the iodometric titration, the elution is carried out with 5 ml of 10 % KI, three drops of 1 % starch solution are then added and the titration is carried out with 0.001 *N* (approx.) sodium thiosulphate.

The elution for the colorimetric measurement is made with exactly 10 ml of 5 % KI, and the colour is developed with five drops of 1 % starch solution.

Either the thiosulphate or the colorimetric data enable us to calculate in a relative way the μg of iodine fixed in each cut-out by comparison with the calibration curve drawn for the series of reference samples.

RESULTS

In order to illustrate the scope of this method some results are given in Table II and Fig. 2. In alle cases ascending chromatograms on Whatman No. 1 paper were run and development times varied between 60 and 150 min.

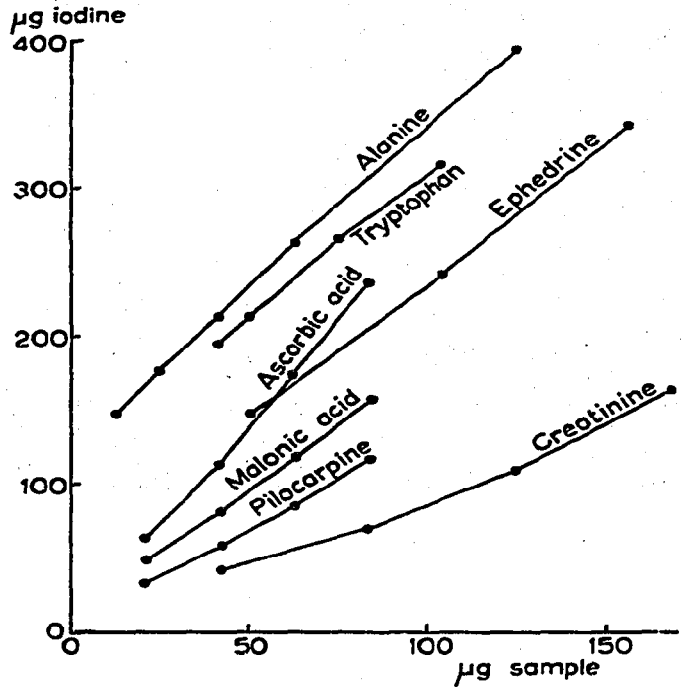

Fig. 2. Examples of calibration curves. Each point is an average of two spots.

The number of µg of fixed iodine and sample indicated are not absolute values and they serve only for comparison purposes.

DISCUSSION

As this method allows the determination of very different substances due to the lack of specificity of iodine vapour, great care has to be taken over the chromatographic separation to avoid interferences. However, it has the advantage that the apparatus and the reagents employed are in common use.

The reproducibility of the results is very good and in the duplicate determinations the normal deviation from the mean is less than 5 %. The best results correspond to the more sensitive substances and the sensitivity has to be more than 1 in order to obtain good results with small samples.

Thin-layer chromatography offers new possibilities for this method.

ACKNOWLEDGEMENT

The author thanks the Central Directive Council of the University for granting him a full-time fellowship which made this work possible.

TABLE II

RESULTS

Substance	µg of		Solvent	Drying	Exposure (h)	Method
	Sample	Iodine				
Alanine	13	151	Butanol–acetic acid	30 min at 160°	20	Iodometric
	25	178				
	42	215				
	63	266				
	126	392				
Tryptophan	42	198	Ethanol–NH_4OH	20 min at 160°	20	Colorimetric
	51	216				
	75	267				
	105	315				
Ascorbic acid	21	66	Butanol–acetic acid	15 min at 120°	2	Colorimetric
	42	116				
	63	174				
	84	237				
Creatinine	42	45	Butanol–acetic acid	15 min at 120°	2	Colorimetric
	84	73				
	126	110				
	168	163				
Malonic acid	21	50	Ethanol–NH_4OH	15 min at 120°	5	Colorimetric
	42	83				
	63	119				
	84	158				
Pilocarpine	21	35	Butanol–acetic acid	15 min at 120°	3	Colorimetric
	42	60				
	63	87				
	84	118				
Ephedrine	52	149	Butanol–acetic acid	15 min at 120°	5	Iodometric
	105	242				
	157	342				

SUMMARY

A general method is described which allows a quantitative estimation of those substances that are detectable by iodine vapour on paper chromatograms. The quantity of iodine fixed by the substance is determined after elution with KI solution, either by iodometry or colorimetry.

REFERENCES

1 K. Miyaki, K. Satake and M. Hayashi, *J. Pharm. Soc. Japan*, 71 (1951) 249.
2 R. Fisher, K. Parson and G. Morrison, *Nature*, 161 (1948) 764.
3 B. M. Bull, J. W. Hahn and V. H. Baptist, *J. Am. Chem. Soc.*, 71 (1949) 550.
4 R. Fisher and R. Holmes, *Biochem. J.*, 44 (1949) 54.
5 L. S. Fosdick and R. Q. Blackwell, *Science*, 109 (1949) 314.
6 R. H. Mueller and D. L. Clegg, *Anal. Chem.*, 21 (1949) 1123.
7 R. J. Block, *Anal. Chem.*, 22 (1950) 1327.
8 A. Lacourt, *Mikrochim. Acta*, (1957) 269.
9 H. C. Ehrmantraut and A. Weinstock, *Biochim. Biophys. Acta*, 15 (1954) 589.
10 H. R. Roberts, *Anal. Chem.*, 29 (1957) 1443.
11 A. Flood, E. Hirst and J. Jones, *Nature*, 160 (1947) 86.
12 L. Naftalin, *Nature*, 161 (1948) 763.
13 Th. Wieland and E. Fischer, *Naturwiss.*, 35 (1948) 29.
14 E. R. Reichl and J. E. Löffler, *Mikrochim. Acta*, (1954) 226.
15 G. Brante, *Nature*, 163 (1949) 651.
16 G. B. Marini-Bettolo and S. Guarino, *Experientia*, 6 (1950) 309.