Area Methods of Paper Chromatography: Part I—Determination of Alkali Metals & Sugars*

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A study has been made of the relation between spot area and spot content of alkali metals and sugars, separated on one-dimensional paper chromatograms. The usefulness of this relationship in the estimation of the components from mixtures of sugars has been discussed. Waxed capillary pipettes have been used with advantage for the quantitative spotting of solutions.

THE areawise estimation of substances, separated on the filter-paper chromatogram, has been described by a number of investigators. Fisher, Parsons and Morrison[1] observed a linear relation between the spot area and the logarithm of spot content (of amino acid or sugar). Using this relationship, Fisher and Holmes[2] were able to estimate amino acids with an error of ± 5 per cent in samples containing 1-2 μg. of α-amino nitrogen. Kalyankar, Krishnaswamy and Sreenivasaya[3] successfully employed a similar relationship for the determination of organic acids. In a detailed study of the chromatographic estimation of C_2-C_7 fatty acids, Reid and Lederer[4] reported regression equations involving the logarithm of spot content and spot area, and found that fatty acids could be estimated from these equations with an accuracy of ± 2-5 per cent.

In the present paper, an account is given of the application of the area method to two groups of substances, viz. the alkali metals and sugars, convenient methods for the estimation of which were required in connection with work on marine animals and plants. Since the alkali metals are difficult to estimate accurately by the conventional chemical methods, it was felt desirable to attempt their chromatographic estimation. The quantitative chromatography of sugars has been extensively studied but details are not available regarding the accuracy of the area method, excepting for the work of Fisher *et al.*[1] on xylose and arabinose.

Estimation of alkali metals

The alkali chlorides were separated on the filter-paper chromatogram by a method described elsewhere[5]. For the quantitative spotting of solutions, waxed capillary pipettes of the type described by Wigglesworth[6] were used, each addition of 2 cu. mm. of solution being made after the previous one had evaporated. The capacities of the waxed

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TABLE 1 — ESTIMATION OF ALKALI METALS

[*Linear regression of* $\log_{10}$ (*spot area*) *on spot content; range of spot content of alkali chloride, 3·33-10·66 µg.; No. of concn. for each alkali metal, 40; area in one hundredths of a sq. in. and spot content in µg.*]

Alkali metal (as chloride)	R_f		Regression		Standard error (S.E.) of (b)	(S.E. × 100)/(b)
	Mean	Standard deviation	Coeff. (b)	Constant (a)		
K	0·09	0·01	0·0403	0·8812	0·00427	10·6
Na	0·18	0·02	0·0358	1·3147	0·00413	11·5
(NH_4)	0·29	0·03	0·0577	0·9764	0·00448	7·8

TABLE 2 — ESTIMATION OF ALKALI METALS

[*Linear regression of spot content on* $\log_{10}$ (*spot area*); *range of spot content of alkali chloride, 3·33-10·66 µg.; No. of concn. for each alkali metal, 40; spot content in µg. and area in one hundredths of a sq. in.*]

Alkali metal (as chloride)	Regression coeff. (b)	Standard error (S.E.) of (b)	(S.E. × 100)/(b)	Deviation of predicted from actual values of spot content	
				Standard deviation	Error*
K	17·489	1·836	10·5	1·45	20·8
Na	18·273	2·093	11·5	1·52	21·8
(NH_4)	14·030	1·088	7·8	1·15	16·4

* (Standard deviation × 100)/(Mid-pt. of range)

TABLE 3 — ESTIMATION OF SUGARS

[*Linear regression of* $\log_{10}$ (*spot area*) *on* $\log_{10}$ (*spot content*); *range of spot content of sugar, 18·75-93·75 µg.; No. of concn. for each sugar, 10; area in sq. cm. and spot content in µg.*]

Sugar	R_f range	Regression coeff. (b)	Standard error (S.E.) of (b)	(S.E. × 100)/(b)
Lactose	0·050-0·060	0·795	0·026	3·2
Maltose	0·066-0·069	0·792	0·025	3·1
Dextrose	0·140-0·150	0·778	0·026	3·3
Galactose	0·120-0·150	0·812	0·032	3·9
Arabinose	0·200-0·220	0·793	0·043	5·4

capillary pipettes relative to that of a standard constriction pipette[7] were estimated by titrating sodium chloride solutions delivered from the pipettes with silver nitrate, using potassium chromate as indicator. The capacity of the constriction pipette was determined by measuring 20 volumes of water from the pipette into a tared wax-lined micro-tube (250 cu. mm. capacity) and weighing the water thus pipetted out. The spots of silver sulphide were traced on to sq. in. graph paper.

The results of the area estimations are given in Tables 1 and 2.

Sugars

One per cent aqueous solutions of lactose, maltose, dextrose, galactose and arabinose were used. The chromatograms were obtained by a method similar to the one described elsewhere[8] excepting that multiple development was not employed. The spots were traced on to sq. mm. graph paper. The waxed capillary pipette used was calibrated by weighing 10 volumes of water delivered from the pipette into a tared wax-lined micro-tube (250 cu. mm. capacity). Two sizes of filter paper (28 × 4 cm.; 28 × 2 cm.) were used. But the regression coefficients of area on spot content were not affected to any significant extent by the size of the paper strip.

The results of area estimations are given in Tables 3 and 4.

Discussion

From Table 1 it is seen that for the same concentration of alkali chloride the spots, in descending order of magnitude of area, are those due to sodium, ammonium and potassium respectively. Employing the silver nitrate-fluorescein technique of detection, Chakrabarthy and Burma[9] had observed a similar effect with solutions of sodium, potassium and lithium chlorides.

The regression coefficients of $\log_{10}$ (area) on $\log_{10}$ (spot content of sugar) are almost identical (Table 3); this is remarkable, considering the heterogeneity of the group of sugars tested, which includes two disaccharides, two hexoses and a pentose. A comparison of the regression coefficients in Table 4 by the use of the "t" test[10] shows that the value for the individual sugar is not significantly altered by admixture with a second sugar.

TABLE 4 — ESTIMATION OF SUGARS

[*Linear regression of* $\log_{10}$ (*spot content*) *on* $\log_{10}$ (*spot area*); *spot content in* μ*g. and area in sq. cm.*]

Sugar	Range of spot content μg.	No. of concn.	Regression coeff. (b)	Standard error (S.E.) of (b)	(S.E. × 100) / (b)	Deviation of predicted from actual values of spot content	
						Standard deviation μg.	Error*
Lactose	18·75-93·75	10	1·277	0·040	3·1	2·88	5·1
Maltose	18·75-93·75	10	1·267	0·037	2·9	1·72	3·1
Dextrose	18·75-93·75	10	1·289	0·042	3·3	2·84	5·0
Galactose	18·75-93·75	10	1·229	0·052	4·2	3·41	6·1
Arabinose	18·75-93·75	10	1·247	0·067	5·4	4·11	7·3
Lactose (in lactose + arabinose)	16·92-49·47	8	1·150	0·171	14·8	2·35	6·8
Arabinose (in lactose + arabinose)	14·37-42·09	9	1·426	0·197	13·8	2·09	7·3
Maltose (in maltose + galactose)	13·59-39·84	8	1·380	0·204	14·8	3·22	12·9
Galactose (in maltose + galactose)	16·69-51·46	8	1·241	0·112	9·0	2·48	7·6

* $\frac{(\text{Standard deviation} \times 100)}{(\text{Mid-pt. of range})}$

The accuracy of the area method is limited by the error involved in the measurement of small volumes. In this connection, the waxed capillary pipettes would appear to possess certain advantages over devices like the micrometer syringe[11,12] normally used in quantitative work. The use of paraffin minimizes drainage errors, and it is possible to use the same pipettes for delivering equal volumes of standard and test solutions. A knowledge of absolute volumes is necessary only when it is required to find the range of applicability of the method in terms of absolute concentrations of the test substances. For routine work, however, where it is preferable to run standards side by side with the unknowns for every set of estimations[1,2,4], it is enough if the precaution is taken to deliver identical " volumes " of the standard and test samples from the same waxed capillary pipette.

In many biological investigations, it is often sufficient to work with a method which combines reasonable accuracy with adaptability to serial determinations. The area method of paper chromatography would seem to satisfy the requirements of simplicity and utility. The chromatographic apparatus is such as can easily be assembled in any biological laboratory, the pipettes are easy to construct and can be stocked in large numbers, and the spot-area measurements are fairly reproducible and obviate losses of substances which would be involved in elution and further analysis by conventional methods. It is thus evident that the area method is full of possibilities especially in laboratories where the emphasis may not always be on specialized, elaborate or even normal chemical equipment.

Summary

An areawise estimation of alkali metals and sugars, separated by one-dimensional paper chromatography, is described.

For identical concentrations of alkali chlorides, the spots in descending order of magnitude of area are those due to sodium, ammonium and potassium respectively. At the 3-11 μg. level of sodium chloride or potassium chloride, the regression of spot content on $\log_{10}$ (spot area) is linear. The standard deviation of the estimated values of spot content from the actual values is 1·5 μg, and the error (= S.D. × 100/mid-pt. of range) is 21-22 per cent.

In the 0-100 μg. range of sugar content, the coefficients of linear regression of $\log_{10}$ (spot area) on $\log_{10}$ (spot content) do not vary significantly for lactose, maltose, dextrose, galactose and arabinose. These sugars can be estimated by the area method with an error of $\pm$3-7 per cent when they are present singly and $\pm$7-13 per cent when they are present in binary mixtures.

The use of waxed capillary pipettes for the quantitative spotting of solutions has been found to add to the advantages and convenience of the paper-chromatographic technique.

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