CHROMATOGRAPHIC STUDIES ON SULPHUR COMPOUNDS

PART III. QUANTITATIVE ANALYSIS OF A MIXTURE CONTAINING THIOSULPHATE AND POLYTHIONATES BY PAPER CHROMATOGRAPHY*

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When using a scanning technique for the determination of peak area measurements, in elution chromatography the absorbances of small segments of a zone are recorded and plotted against position as illustrated in Fig. 1. For the following simple treatment, the zone may be considered to be divided into segments, the smaller each segment the closer will be the approximation to reality.

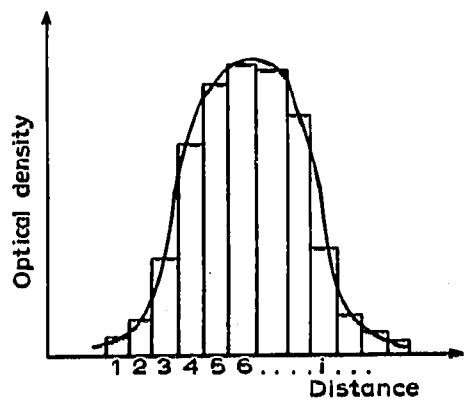


Fig. 1. Area beneath an absorbance section of a zone.

The total absorbance of the zone, I_T, is given by

$$I_T = \Sigma I_i = I_1 + I_2 + \cdots I_n.$$

Subscripts 1, 2 . . . n refer to the numbers of the segments from the start. If the weight of solute in each segment is symbolised by W_i, and if Beer's law is assumed to hold,

$$I_T = k \Sigma W_i = kW_T$$
$$= kW_1 + kW_2 + kW_3 \cdots kW_n,$$

where W_T = total weight of solute in the zone.

If Beer's law does not hold for the regions of higher concentration, then the errors of assuming proportionality between the absorbance of the total zone and

* For Part II of this series, see ref. 2.

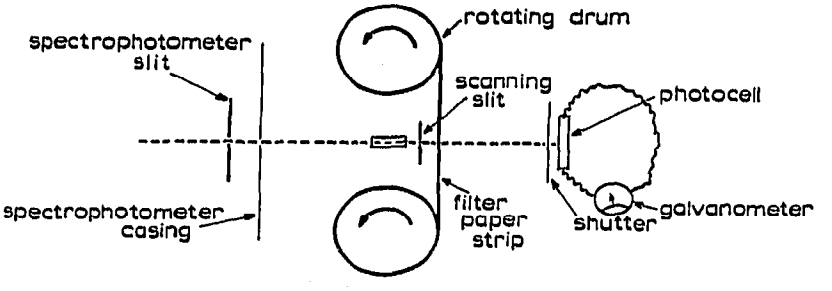
weight of solute therein can only be estimated from an absorbance section. Even with a calibration graph of concentration of solute solution *versus* total absorbance, large errors might be obtained if the zone is distorted owing to interfering substances in the sample solution. Obviously the scanning method offers the best solution of these problems.

To summarise, the method consists in measuring the absorbance of a uniform band of silver sulphide and sulphur on a paper strip by means of a scanning device, the silver sulphide and the sulphur being produced by the decomposition of silver thionates[1] obtained by spraying the chromatogram with silver nitrate solution.

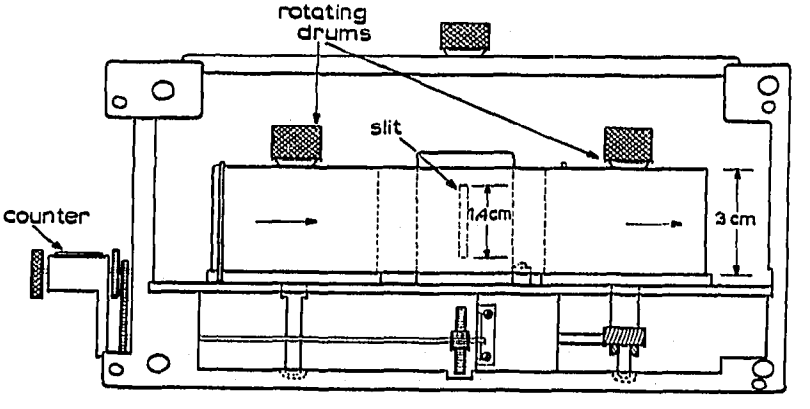
Obviously for the success of such a method, a uniform band of liquid should be applied to the chromatogram, this being done by using a zoning machine, designed to the specification of Part II of this series[2].

THE SCANNING INSTRUMENT

The scanning instrument used consisted of an attachment to the Unicam SP. 500 spectrophotometer. Fig. 2a shows the principle of the attachment, while Fig. 2b shows the attachment diagrammatically. The light beam passes through a slit between two rotatable drums which carry the chromatographic strip. The movement of the strip was hand-operated from outside the cell, and was measured by a geared counter of the cyclometer type. The movement of the strip for one unit on the counter was 0.55 mm,



(a)



(b)

Fig. 2. (a) Principle of the scanning attachment. (b) Diagram of the scanning attachment.

the width of the slit in the scanning instrument was 2 mm, and the height 1.15 cm.

There are two possible ways of using such an instrument, either the total absorbance of a small zone is measured, or an absorbance section of a large band may be determined (Fig. 3). With the first method, a primary requisite is that the diameter of the zone should be less than the height of the slit, *i.e.*, with the apparatus at hand,

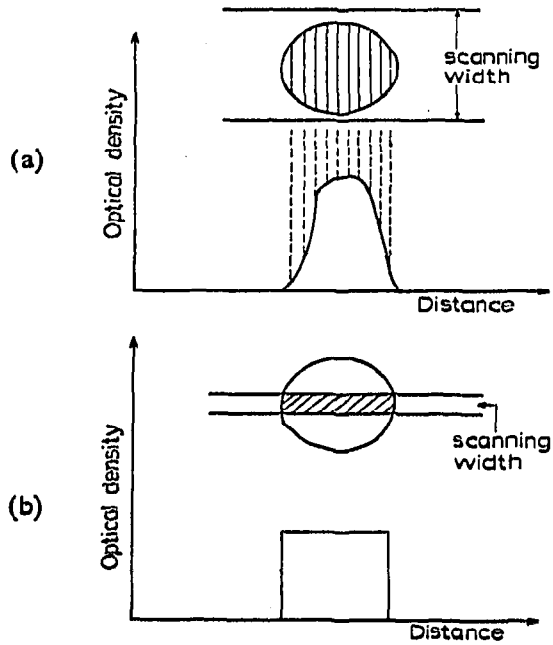


Fig. 3. Scanning methods. (a) Total absorbance of a zone is measured. (b) Absorbance of a section (partial area of a zone) is measured.

less than 1.15 cm in diameter. This means that the volume of solute solution applied to the chromatogram may not exceed 5 μl, if the initial zone is to have such a diameter; owing to spreading of the zones during chromatography[2], it would be necessary to use much smaller volumes. The accurate measurement and delivery of such small volumes is difficult. The Agla-micrometer syringe, an instrument widely used for such purposes, allows the measurement of volumes with an error of $\pm$ 0.05 μl. Thus, while the relative error is only 0.5 % for 10 μl, for 1 μl it is 5 %.

The imposition of a small volume must simultaneously restrict the concentration of solute which may be chromatographed, if previous concentration of the solute solution is not possible or undesirable. This introduces further errors. Owing to variations in the chromatographic paper, its absorbance is not constant; the variations are small but appreciable—this may be referred to as base-line instability. Variations of $\pm$ 0.01 absorbance units were found to be normal in the system studied; for a substance having a peak height of one absorbance unit, this error requires least consideration. However, it comes into prominence for dilute zones and, coupled with volume errors, is sufficient to ensure that this method of working gives results which are only semi-quantitative.

The absorbance *versus* distance plot obtained by this method gives a curve which is partially influenced by the shape of the zone and is thus not a true absorbance cross-section. (Fig. 3 shows the absorbance cross-section and the type of curve likely

to be found on scanning a circular zone of uniform cross-section). The area under the curve will be proportional to the concentration of the zone-forming solute if Beer's law is obeyed, whereas if the absorbance cross-section is taken, this will only be true if the zone has uniform concentration or, in the case of a band, has identical absorbance cross-section throughout its length.

Since the utilization of such small zones of diameter in order that the absorbance of the entire zone may be measured is liable to give inaccurate results, the problem is to produce a uniform band of solute on the chromatogram. The procedure in this case is shown in Fig. 4a, which depicts part of a developed paper chromatogram including a zone which has been applied to the paper as a band. The ends of the band (shaded) will not have uniform cross-sections, and may not be used for scanning. Any portion of the middle section of the band of suitable width, *i.e.*, greater than the height of the slit in the scanning attachment, may be chosen for absorbance measurement. The width normally used is 3 cm; this is cut out and scanned as in Fig. 4b. It gives an absorbance cross-section as shown in Fig. 4c.

If V is the volume per cm of solution applied to the chromatogram on the starting line, and c is the concentration of the solute, then the weight, W, of the solute in the section of the zone scanned is given by

$$W = cV\alpha,$$

where α = the width of zone scanned = slit height.

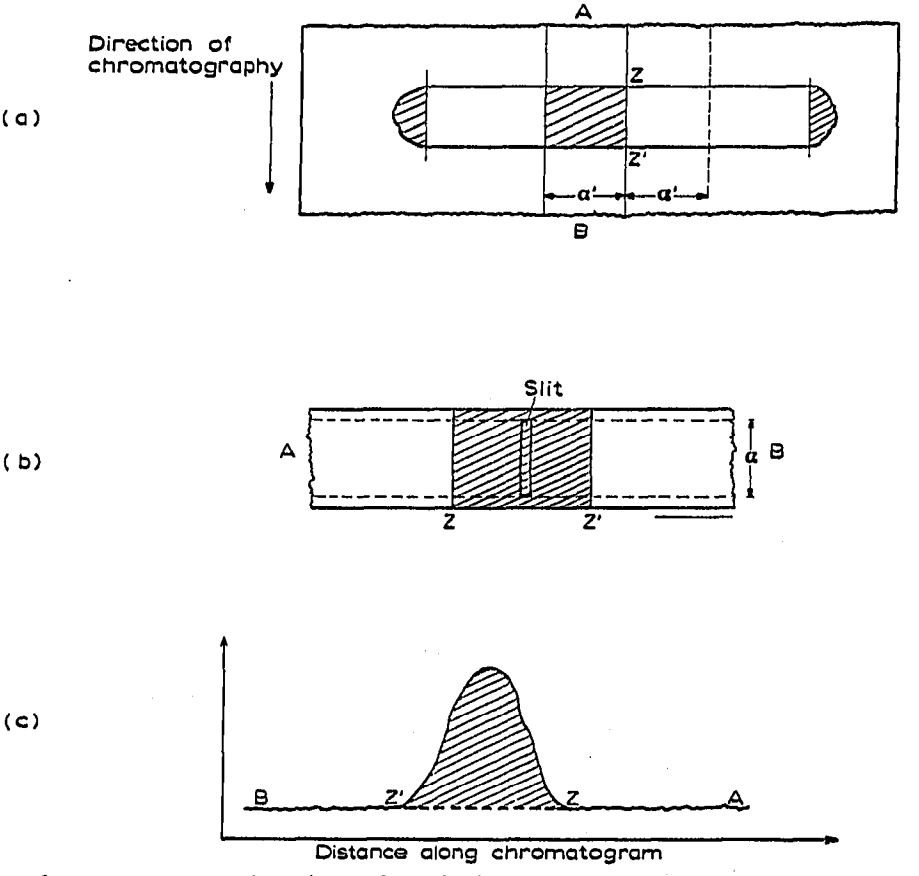


Fig. 4. (a) Part of a chromatogram showing a band. (b) Section of a chromatogram cut for scanning. (c) Optical density section.

If the shaded area in Fig. 4c is A sq. cm, and if Beer's law is obeyed,

$$W = k_1 A \alpha,$$

where k_1 is a constant.

Thus, the concentration is given by

$$c = \frac{k_1 A \alpha}{V \alpha} = \frac{k_1 A}{V}.$$

If V is kept constant,

$$c = k_2 A, \text{ where } k_2 = k_1/V.$$

Thus, it is seen that the criterion for success is that V, the volume of solution applied per cm, should be constant. This was found impossible to achieve manually, and therefore the zoning machine was used.

Several zoning machines have been described in the literature[3-5], but none have been used for applying to chromatograms in the stage of elution; hence it was necessary to design such a machine. The machine used is shown in Figs. 5 and 6 and, diagrammatically, in Fig. 7b. The block B supports an Agla micrometer syringe A, which was filled with the solution to be applied to the chromatogram. A rubber band was fastened round the syringe plunger and looped over the block B so that the plunger was kept in contact with the metal micrometer piston D. B was mounted on a

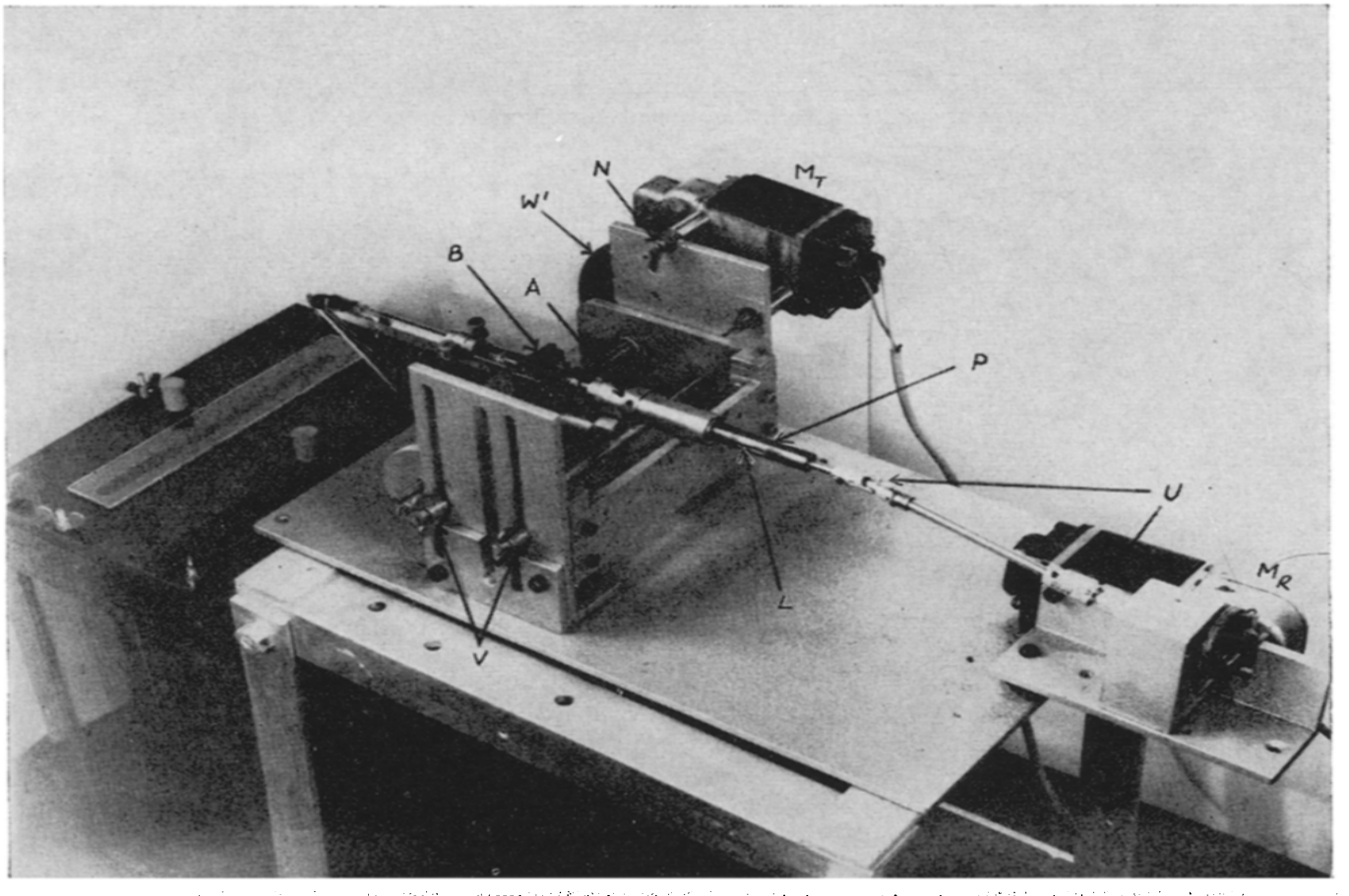


Fig. 5. The zoning apparatus (rear view).

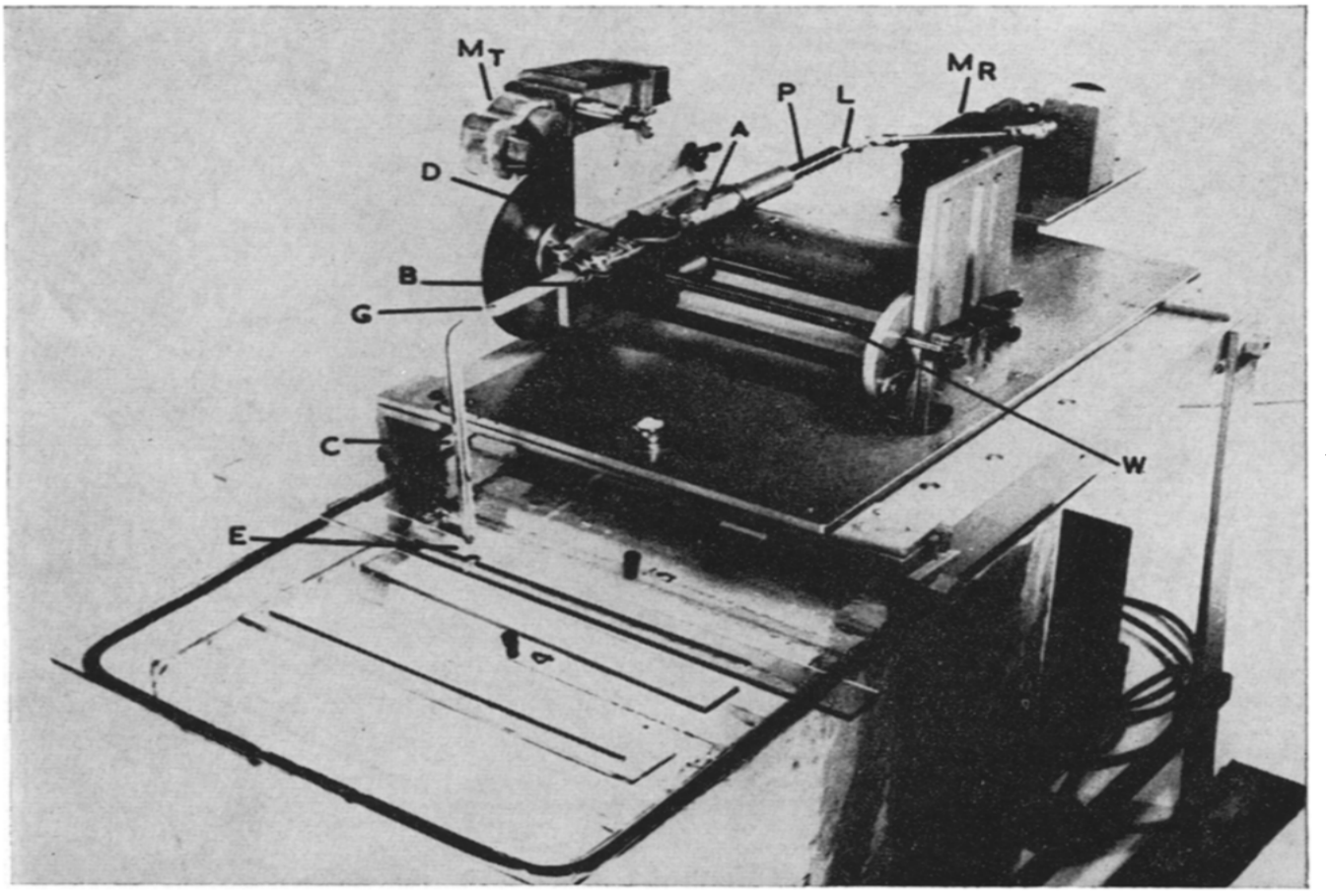


Fig. 6. The zoning apparatus (front view).

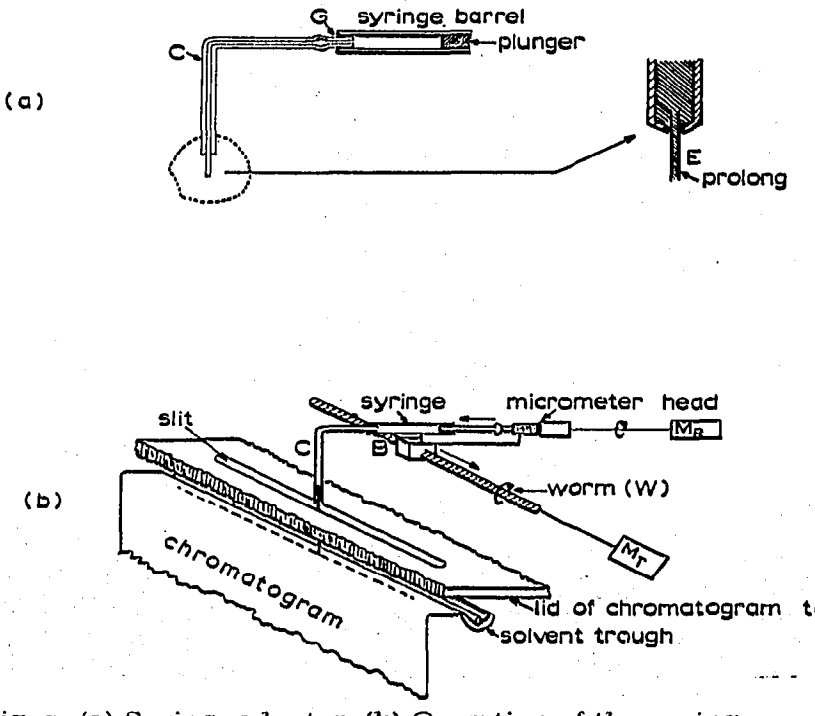


Fig. 7. (a) Syringe adaptor. (b) Operation of the zoning apparatus.

worm W. This worm was rotated by a D.C. motor M_T, which caused horizontal traverse of the syringe, while at the same time a second motor M_R caused rotation of the micrometer head and piston of the syringe, thus ejecting the solution through the adaptor C on to the paper chromatogram. After investigating different power units to drive the motors, the syringe delivered 6.13 μl in 107 sec; the maximum possible traverse used was 12 cm.

The motor M_R is coupled to the syringe head in such a way that the transverse motion of the syringe does not interfere with the rotation or place a torque upon the syringe. This is allowed by the combined action of the universal joints U. Since, as the micrometer head turns, the distance between it and M_R increases, it was necessary to incorporate the Teflon piston P, lubricated with oil, which maintained a rotational torque on the brass sleeving by means of the locating pin L, which is free to slide in a slit. The micrometer head is detached from the coupling by means of the Allen screw A_1 (see Fig. 5).

In order that the machine could be used with different chromatogram tanks, the slots V were cut to allow vertical displacement of the syringe. The motor M_T may be disconnected from the gear wheel W, by loosening the nut N. This is necessary at the beginning and end of a zoning operation. The syringe fittings are clamped into place by means of the brass block B, fitted with Allen screws.

Some difficulty was encountered initially in maintaining a liquid contact between the end of the tube C and the chromatographic paper. Since the chromatogram cannot be supported in a rigid manner and stretches during elution, no fixed alignment can be made between the height of the tip of tube C and the paper. Because of this, the delivery tube occasionally lost contact with the paper with consequent formation of a liquid drop and loss of uniformity in application of the solution.

To obviate this drawback, it was found necessary to introduce the thin glass canulus E (Fig. 7a). At the start of a run the canulus was pushed into C so that there is no contact with the paper. The motor M_R was set going and, as the solution slowly expelled from the syringe, so the canulus slowly emerged from its sheathing until it approximated to the surface of the paper. At this moment M_T was switched on and the end of the canulus touched the paper surface while undergoing vertical transverse. During the continued journey of the syringe the canulus rests upon the paper, delivering with a uniform flow and no drop formation. The tube C was fixed at an acute angle to the paper in the direction of motion to avoid any puncturing of the paper, which was sometimes found to occur when the tube was held in an upright position. When it was desired to conclude the application, the joint G was turned to raise the "stylus" from the paper, and both motors switched off. The slit in the lid of the chromatography tank was then covered with a glass plate, and the solutions allowed to chromatograph[6].

It was soon found that the opening of this slit for the time necessary to carry out the application of samples resulted in a substantial loss of equilibration inside the tank marring the chromatographic separation. In further experiments, the slit cover was replaced by another cover bearing a small hole in it, 0.5 cm diameter, through which the adaptor could pass. This was then removed over the slit at the same speed as the syringe; in this way only a very small outlet for the vapours in the tank was permitted, and the equilibrium was not seriously disturbed.

The further procedure was as illustrated in Fig. 4. Calibration graphs for $S_2O_3^{2-}$,

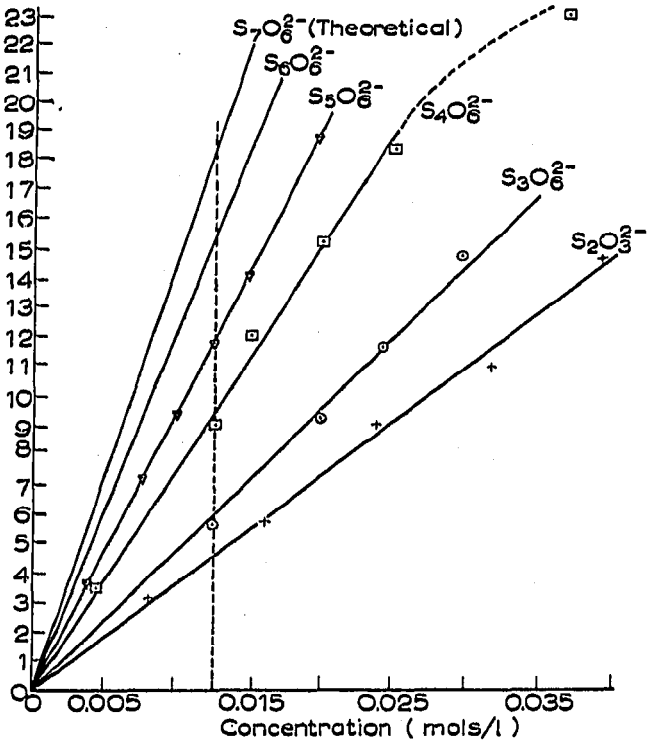


Fig. 8. Variation of area beneath absorbance section with concentration of applied solution

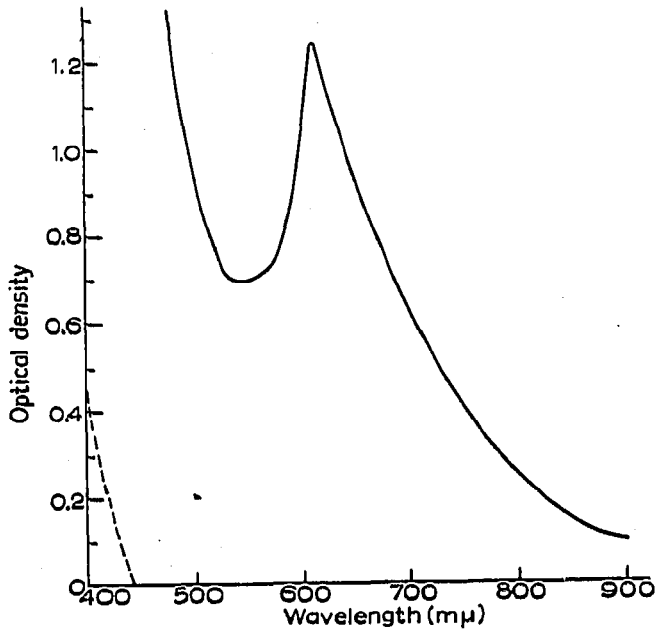


Fig. 9. Absorption spectra of silver sulphide and sulphur on filter paper. —— AgS + S stain from tetrathionate; – – – blank filter paper. Spectrophotometer slit width = 0.07 cm.

$S_3O_6^{2-}$, $S_4O_6^{2-}$, $S_5O_6^{2-}$, and $S_6O_6^{2-}$ were obtained (Fig. 8) using scans made at 600 mμ, since a maximum in the absorption spectra occurs at this wavelength (Fig. 9). The spectra of silver sulphide alone, as obtained by scanning a zone from thiosulphate, and the spectra of silver sulphide plus sulphur, obtained from tetrathionate, show no differences either in shape or position of the maximum. A typical separation of $S_3O_6^{2-}$, $S_4O_6^{2-}$ and $S_5O_6^{2-}$ is shown in Fig. 10. The results show that the method will allow estimation of thionates, the values being subject to an error of about 4%. This large error stems from two causes:

(i) the impossibility of standardising the developing technique to more than a qualitative degree;

(ii) there seems to be no possibility of obtaining absorbance measurements with respect to a comparative standard during the scanning;

(iii) there are variations in absorbance due to irregularities in the filter paper.

The development of the chromatograms involves spraying them with silver nitrate solution, heating before an electric fire to decompose the silver thionates

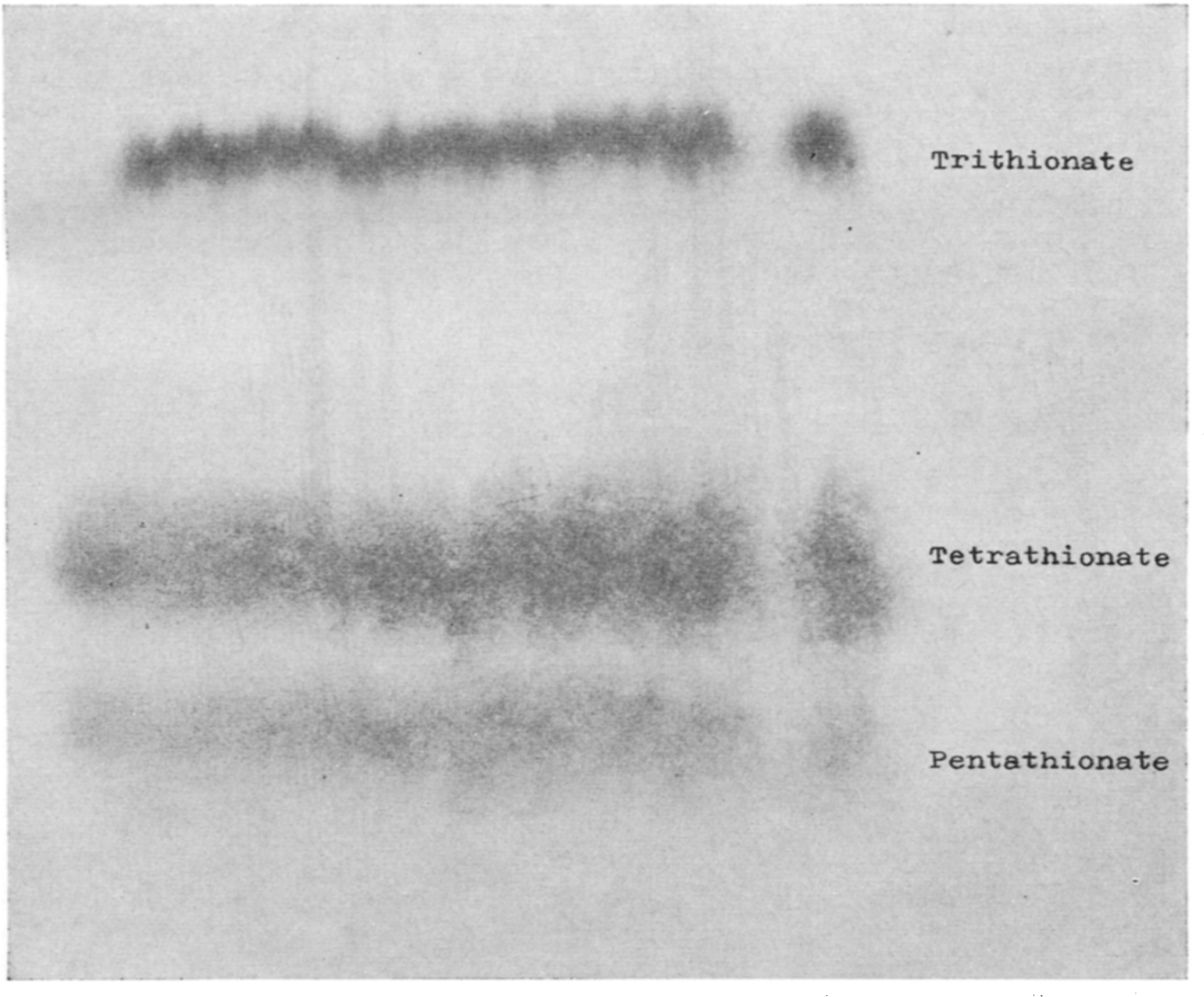


Fig. 10. Separation of tri-, tetra- and penta-thionate zones.

to sulphide and sulphur, washing in concentrated thiosulphate solution to remove excess silver, then in distilled water, and finally drying. Of these processes, that which is least readily standardised is spraying. The results of spraying vary considerably with the experience of the operator. The most even spraying was obtained by using a fine, light spray directed from a distance of a few feet on to the paper. As saturation is reached, a sheen may be seen on holding the chromatogram obliquely, all chromatograms were sprayed until this sheen appeared evenly all over the paper. By this means, gross variations were ruled out.

When using the spectrophotometer for measuring the absorbance of solutions, it is usual to use two cells which may be placed alternatively in the light beam. One of these contains pure solvent and the other is the solution whose absorbance it is desired to measure. For any given measurement, the blank cell is put into the beam first, and the width of the slit in the spectrophotometer adjusted until a certain voltage is produced by the detector. With the same setting of the slit width, the blank cell is replaced by that containing the solution, and the absorbance is measured, which is thus relative to that of the solvent. Not only does this comparison method nullify any effects due to light absorption by the solvent at certain wavelengths, it also ensures that any variations in the light source do not influence the measured absorbance. When scanning a chromatogram, it is not possible to do this, however, since once the scan is commenced, it may not be interrupted and the construction of the instrument does not permit simultaneous measurement of two absorbance sections. The time taken to complete a scan of a single zone is not less than 15 min, and it was found that quite considerable variation in intensity of the light source sometimes occurred over such periods. In order to reduce the extent of these effects, a blank strip was inserted in the scanning attachment such that a marked position interrupted the light beam. The slit width was then adjusted to bring the voltage to a certain value and the actual scan of the zone was then carried out using this slit width. After a scan, the blank was again inserted to check the variation in light intensity and, if necessary, the slit width readjusted. With a blank standardised at absorbance 0.153, the slit width varied between 0.15 and 0.20 mm over a period of months. No assessment can be made of the variations encountered during an actual scan, although it was sometimes observed that the baseline on one of the sides of the zone was higher than the other. However, it is thought that a large part of the error in the results obtained is due to this cause.

In an attempt to eliminate the variation in absorbance due to irregularities in the filter paper, translucing agents were used. Paraffin oil[7], Nujol[8], methyl salicylate[9], and a mixture of Nujol and *n*-amyl alcohol[9] have all been reported as reducing absorption and these were investigated. The strips of filter paper were soaked in the various organic solvents, and dried between filter paper. Using a constant slit width of 0.035 cm, the strips were scanned at 600 mμ. The maximum deviations in absorbance were calculated. In Table I these results are shown and can be compared.

From these results it was seen that a strip soaked in Nujol alone for 5 min and then dried between filter papers reduced the absorption of the paper to the greatest degree. Next a tetrathionate scan on plain filter paper with the scan of the same strip was obtained after immersion in Nujol. In both cases the absorbance of the filter paper background was adjusted to 0.180 by variation of the slit width. The third scan was carried out on a different background absorbance (see Table II).

TABLE I

COMPARISON OF VARIOUS TRANSLUCING AGENTS AT A CONSTANT SLIT WIDTH OF 0.035 CM

Agent	*Method of application*	*Optical density*
Plain		0.78 ± 0.015
Nujol	Soak for 5 min and dry between filter paper	0.04 ± 0.015
Nujol 60 amyl alcohol 40	Soak for 5 min and dry between filter paper	0.180 ± 0.08
Methyl salicylate	Soak for 5 min and dry between filter paper	0.112 ± 0.02
Amyl alcohol	Soak for 5 min and dry between filter paper	0.82 ± 0.017
Nujol*	Soak for 5 min, hang for 1 h, and dry between filter paper	0.096 ± 0.018
		0.083 ± 0.031
Nujol*	Soak for 5 min, hang for 3 h, and dry between filter paper	0.065 ± 0.032
Nujol*	Soak for 5 min, hang for 8 h, and dry between filter paper	0.095 ± 0.031
Nujol*	Soak for 5 min, leave wet for 24 h, and then dry	0.136 ± 0.024

* Slit width = 0.067 cm.

From these results it was seen that although Nujol did reduce the background, it also reduced the size of peak obtained by a factor of 4. Thus it is preferable to scan the untreated strip.

On referring to Fig. 8, it is seen that the method is most sensitive for higher thionates and least sensitive for thiosulphate. Consideration of why this is also allows

TABLE II

COMPARISON OF TRANSLUCING AGENTS FOR RELATIVE SENSITIVITY

	Area (sq. in.)	*Slit width (cm)*	*Background absorbance*
Plain	20.14	0.138	0.180
Nujol	5.13	0.066	0.180
Nujol	4.99	0.077	0.050

some conclusions regarding the slopes of the straight lines in the figure to be drawn, and the theoretical prediction of the curve for $S_7O_6^{2-}$ and so on can be made.

BASSETT AND DURRANT[1] showed that the silver thionates decompose according to:

$$Ag_2S_3O_6 \longrightarrow Ag_2S + 2\ SO_3 \quad (1)$$

$$Ag_2S_4O_6 \longrightarrow Ag_2S + 2\ SO_3 + S \quad (2)$$

$$Ag_2S_5O_6 \longrightarrow Ag_2S + 2\ SO_3 + 2\ S \quad (3)$$

while silver thiosulphate decomposes as follows:

$$2\ S_2O_3^{2-} \longrightarrow S^{2-} + 2\ SO_3^{2-} + S \quad (4)$$

It is evident at once that the stain produced for tetrathionate should be twice as dense as that for thiosulphate. Also, if the absorptions due specifically to Ag_2S, M_2SO_4, and S are a, b and c, respectively, then the absorbances of the thionates should be in the ratio:

$$S_3O_6^{2-} : S_4O_6 : S_5O_6 = 1 : 1 + \frac{c}{a + 2b} : 1 + \frac{2c}{a + 2b}.$$

It may be shown that if, for an absorbance such as those measured, the absorbance is increased in a certain ratio at all points on the curve, then the area under that curve will be increased in the same ratio.

It is therefore to be expected that the areas obtained from thionate zones corresponding to a given concentration should bear a simple ratio to one another. This is in fact the case. In Fig. 8 the ordinate at concentration 0.0125 moles/l intersects the curves at the A values given in Table III.

TABLE III

AREAS OF THIONATE ZONES

	$S_2O_3^{2-}$	$S_3O_6^{2-}$	$S_4O_6^{2-}$	$S_5O_6^{2-}$
A	4.5	6	9.4 (9)	11.8 (12)
Ratio to $S_2O_3^{2-}$	1	1.33	2	2.66
Ratio to $S_3O_6^{2-}$		1	1.5	2

This would suggest that $c/(a + 2b) = 0.5$, and these ratios are thus directly correlated to eqns. (1), (2), (3), and (4). Evidently, for any given higher thionates (assuming the decomposition of the silver salt is similar) the appropriate ratio may be found by adding on the requisite number of contributions due to sulphur atoms to the value of 1 for $S_3O_6^{2-}$. In this way the theoretical curves for $S_6O_6^{2-}$ and $S_7O_6^{2-}$ have been drawn. The theoretical curve and experimental curve are coincident. This procedure has considerable advantages when dealing with substances such as higher thionates, in which case it is usually difficult to obtain specimens suitable for preparation of calibration curves.

An intriguing application of this analysis method so far neither tested nor exploited is the possibility of recording a continuous "picture chromatogram" of the

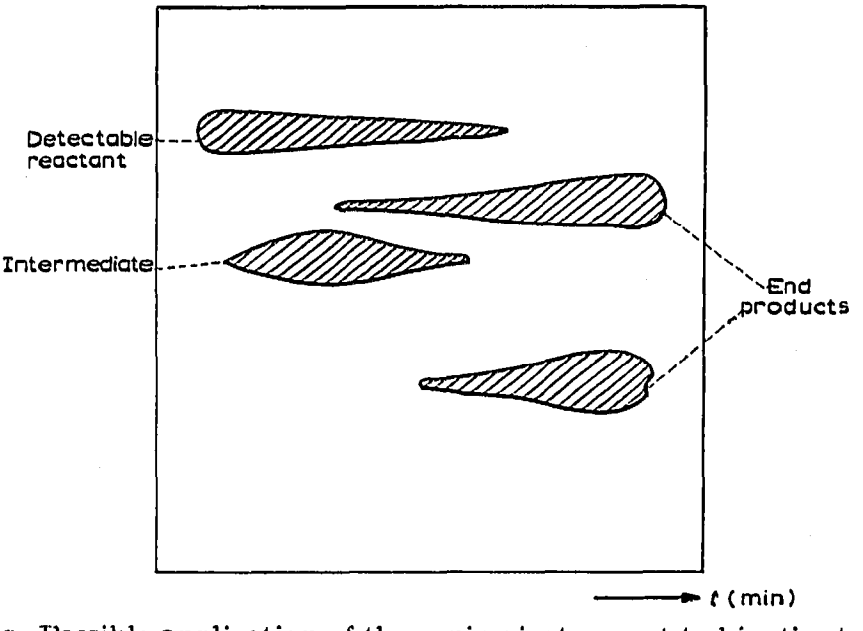


Fig. 11. Possible application of the zoning instrument to kinetic studies.

progress of a slow reaction. The automatic zoning machine takes about 15 min to traverse 10 cm and continuously applies solution to the chromatogram during this time. Given a homogeneous reaction in solution with a velocity such that it would be useful to study a 15 min period of the reaction, then by setting the reaction mixture in the syringe and zoning as described previously, the chromatogram produced would give a complete record of the kinetics of the reaction during this time. It has already been noted that for reactions involving thionates, reaction ceases as soon as the solution is applied to the paper[6]. A diagram of a chromatogram illustrating this application in the case of a hypothetical reaction is shown in Fig. 11. The exact times at which certain products are formed may be calculated, and by the method outlined above, the concentration of any component at any desired time may be found.

ACKNOWLEDGEMENT

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SUMMARY

The "band" analysis method for the quantitative determination of thiosulphate, tri-, tetra-, penta-, and hexa-thionates has now reached a stage of development where it can be used to estimate the concentration of these ions in unknown mixtures.

The method should be very useful in following the course of reactions involving the species, as by using it their appearance and disappearance can be followed.

The main advantage of this method over classical methods is that each species is identified unequivocally and analysed separately.

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