# CHROMATOGRAPHIC STUDIES ON SULPHUR COMPOUNDS

## PART II. CONSIDERATIONS FOR QUANTITATIVE STUDIES OF THE FORMATION AND REACTION OF POLYTHIONATES*

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Before commencing with a description of the methods used in dealing with these problems, it is necessary to consider what advantages are gained and what limitations are set by performing a chromatographic separation prior to analysis**.

The advantage of an initial separation of a complex and labile thionate mixture before analysis is evident when viewed in the light of the classical methods for the determination of these compounds. The polythionates themselves are so similar in their chemical properties that their analysis by classical methods is lengthy, involved, and is liable to give erroneous results except in the hands of experienced research workers[1]. A recent scheme allows the determination of tri-, tetra- and hexa-thionate, in the presence of each other, and has been further extended to include other sulphur oxy-acids at the same time[2]. However, other work[3] suggests the presence of polythionates with more than six sulphur atoms per molecule in many of the reaction mixtures previously studied. Owing to the analytical methods, the presence of these acids would merely augment the amounts of the lower polythionates in the result.

In order that the true value of previous results may be appreciated, the methods of analysis will now be briefly reviewed. The following reactions were made use of by KURTENACKER AND GOLDBACH[4] for the estimation of tri-, tetra- and penta-thionates:

(1) Reaction with alkali sulphites. This causes degradation of higher thionates to trithionate and thiosulphate[5–7].

$$S_{x+3}O_6^{2-} + xSO_3^{2-} \longrightarrow S_3O_6^{2-} + xS_2O_3^{2-} \qquad (1)$$

The excess sulphite is complexed with formaldehyde, and the thiosulphate titrated with iodine solution[8–11].

(2) Treatment with alkali cyanide solution converts higher thionates to sulphate, thiocyanate and thiosulphate[12–15].

$$S_{x+3}O_6^{2-} + (x + 2)CN^- + H_2O \longrightarrow SO_4^{2-} + xCNS^- + 2\,HCN + S_2O_3^{2-} \qquad (2)$$

* For Part I see ref. 29.

** The material in this paper is of such a form that for reasons of continuity the experimental details will be set out under separate headings in the following papers of this series, whilst the main reactions are considered in this paper.

Again the liberated thiosulphate is titrated with iodine.

(3) Reduction with alkali sulphide[16–19].

$$S_nO_6^{2-} + S^{2-} \longrightarrow (n - 3)S + 2\ S_2O_3^{2-} \quad (3)$$

As in cases (1) and (2), the liberated thiosulphate is estimated.

From the values of the iodine consumption for these three reactions, simultaneous equations may be set up and solved, by which the concentration of tri-, tetra- and penta-thionate may be determined.

Hexathionate will react with all the above reagents according to eqns. (1), (2), and (3), and another distinguishing reaction is required for its estimation. GOEHRING, FELDMANN AND HELBING[1] utilized the hydrolysis of thionates under controlled conditions; the thiosulphate liberated is titrated with iodine. With excess dilute alkali the overall reactions are represented by[20]:

$$S_6O_6^{2-} \longrightarrow S_5O_6^{2-} + S \quad (4)$$

$$2\ S_5O_6^{2-} + 6\ OH^- \longrightarrow 5\ S_2O_3^{2-} + 3\ H_2O \quad (5)$$

$$4\ S_4O_6^{2-} + 6\ OH^- \longrightarrow 5\ S_2O_3^{2-} + 2\ S_3O_6^{2-} + 3\ H_2O \quad (6)$$

Trithionate is unaffected by this treatment, but is decomposed by strong alkali[21]

$$2\ S_3O_6^{2-} + 6\ OH^- \longrightarrow S_2O_3^{2-} + 4\ SO_3^{2-} + 3\ H_2O \quad (7)$$

With such a scheme, analysis of mixture of tri- to hexa-thionates can be made; however, if higher polythionates are present, the only reliable value is trithionate, which is obtained from reactions (2) and (3). Because of the problems inherent in such schemes, some workers[22,23] have refrained from estimating the concentration of each thionate, but from the ratio of the iodine consumption for reactions (1) and (2) have calculated the average number $n$, the number of sulphur atoms per molecule. While this method is reliable when higher polythionates are present, the information is of limited use.

Both methods suffer from a major drawback when applied to solutions containing colloidal sulphur, a commonly occurring component of polythionate reaction mixtures. Colloidal sulphur reacts readily with sulphite to form thiosulphates, thus increasing the iodine titration figures for reaction (1) and hence affecting the calculated values of $n$.

Evidently separation prior to analysis offers a sound method of analysing such compounds; WEITZ AND SPOHN[3] suggested the fractional precipitation of the insoluble benzidine salts for this purpose, and the method has been used to advantage by several Russian workers[24–26]. The benzidine polythionate fractions are analysed for sulphur content by oxidation to sulphate (followed by barium precipitation). Such methods give a semi-quantitative estimation of the amount of each polythionate in the mixture.

Only scant attention has been paid to the application of partition chromatography to this problem. WOOD[27] successfully separated thionates by means of paper electrophoresis, and applied the separation to the detection of thionates in gelatines

as used in photography. Using this technique, WOOD verified KURTENACKER AND CZERNOTSKY'S[28] observations on the production of higher thionates in the arsenic-catalysed decomposition of acidified hydrochloric acid; also a solvent is cited for the paper chromatographic separation of the thionates, using methyl cellosolve as the major organic component.

Further qualitative separations of the polythionates by POLLARD, MCOMIE AND JONES[29], BIGHI, TRABANELLI AND PANCALDI[30] and SCOFFONE AND CARINI[31] have been described. None of these workers has applied these separations to the quantitative analysis of these compounds, although applications of the Pollard, McOmie and Jones' solvent have been made by BLASIUS *et al.*[32-35] in studying Wackenroder's solution and the stability of polythionates, using $^{35}S$. Careful examination of reproducibility of each of these methods convinced us that the method of POLLARD, MCOMIE AND JONES[29] was most suited for adaptability as a quantitative micro-method.

Once separated, the analysis of any thionate species is limited to determining the amount or concentration of a solution* of a single characterized substance. Only one reaction or one physical property of the "solution" need be applied to the measurement of this amount instead of the many required by the older methods. There can be no chance of interference due to colloidal sulphur of higher thionates, for dealing with which the classical methods were inadequate. In the case of higher polythionates (those with more than six sulphur atoms), the method must at least be as good as benzidine fractionation. If these compounds do not form discreet zones on the chromatogram (*i.e.* no quantitative separation), it is nevertheless true to say that portions of the zone of higher thionates which have travelled furthest on the chromatogram will contain the components with the highest sulphur number. So that a fractionation can be performed by separating portions of the zone and subjecting these portions to analysis.

In some respects, another advantage is the small quantity of material required for the separation; this factor commends the method for use in the analysis of small samples from kinetic experiments, with no appreciable reduction of the reaction volume. However, small sample volume becomes a necessary condition of chromatographic methods of analysis rather than a chosen desirability, and in many ways adds difficulties and not advantages to the analyst, and becomes the most severe limitation of the method. The other major limitation is that the concentration of the sample solution should not be too high. The reason for these limitations is that, should they be exceeded, conditions are reached when the size of the zones is such that a quantitative separation is no longer obtained. This condition is termed overloading and is especially important when using the rear-phase technique[25].

Two cases of overloading may be distinguished, although, practically, the effects will combine. These may be called, (i) volume overloading and (ii) column overloading; they will be considered in detail.

## VOLUME OVERLOADING

This limitation is not of importance in ordinary paper chromatography, since in this process the initial zone applied to the chromatogram is dried before elution. However,

* By solution is meant the material of a chromatographed thionate zone.

such a procedure is not possible with column or rear-phase chromatography owing to the nature of these techniques. Thus, the discussion which follows applies only to the latter methods.

When the sample solution is applied to a column or rear-phase chromatogram, the mobile and static phases for partition have already been prepared by frontal analysis of the solvent mixture, and the column should be homogeneous and at equilibrium (Fig. 1a). If an aqueous solution is now applied to the column, the equilibrium of the column is disturbed at this point and desorption of the less strongly adsorbed components of the solvent mixture will occur, the vacated adsorption sites being filled with water. Since adsorption is a function of concentration, the sufficient addition of water to the top of the column would result in the complete washing out of static and mobile phases (see Fig. 1b).

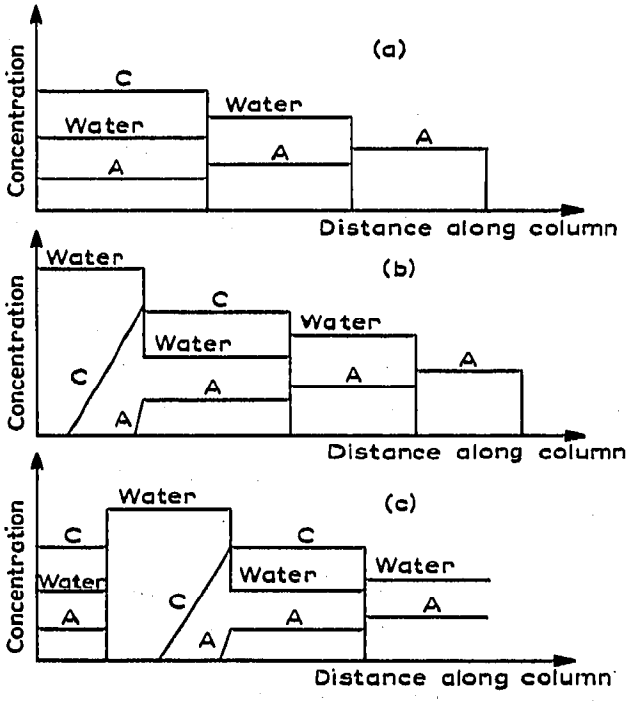


Fig. 1. Assume solvent mixture composed of A, C, and water. (a) Chromatogram showing frontal analysis of solvent mixture when at equilibrium. (b) Application of aqueous solution of solutes. Equilibrium now disturbed. A and C will be replaced by more strongly adsorbed water. Formation of water zone. (c) Fresh solvent mixture added to commence elution of solutes.

Fig. 1c shows the state of affairs when more solvent has been added to elute the mixture of solutes. The solutes themselves, being only of low concentration, cause only slight disturbance of the column equilibrium and their effect is neglected in comparison with that of the water added. This process has caused a "water zone", and zones do not disperse rapidly but travel a considerable distance down the support before completely diffusing into the surrounding solvent. They may be seen when the eluting solvent is coloured with an indicator, as white zones on a coloured background, clearly showing the washing-out processes outlined above. The water zone travels with a velocity similar to that of the solvent front (as would be expected) and a strongly adsorbed solute is soon left behind which may then undergo partition with the eluting

solvent. In this case there is little disturbance. However, for a solute which is only weakly adsorbed on to the cellulose from aqueous solutions, it will travel much further in association with the water in which it was originally dissolved. During this period, only those portions of the zone at the rear of the water zone will be subjected to the partitioning processes. If the water concentration is too great, such an elongated diffuse zone will cause annulment of separation between two adjacent solutes. From the earlier paper of this series[29], the thionates which will be interfered with in this way are those of highest sulphur number, *i.e.* hexa- and higher thionates.

Another and equally important aspect of this problem is that when a solution is applied to dry filter paper it spreads out owing to capillary action until equilibrium is reached and the thickness of the solution impregnating the paper is constant over the zone. Experiments have shown that for Whatman No. 1 filter paper there is an approximately linear relationship between the volume applied to the paper and the area of zone produced, and 5 $\mu$l of solution produces an area of 1 cm$^2$ (see Fig. 2).

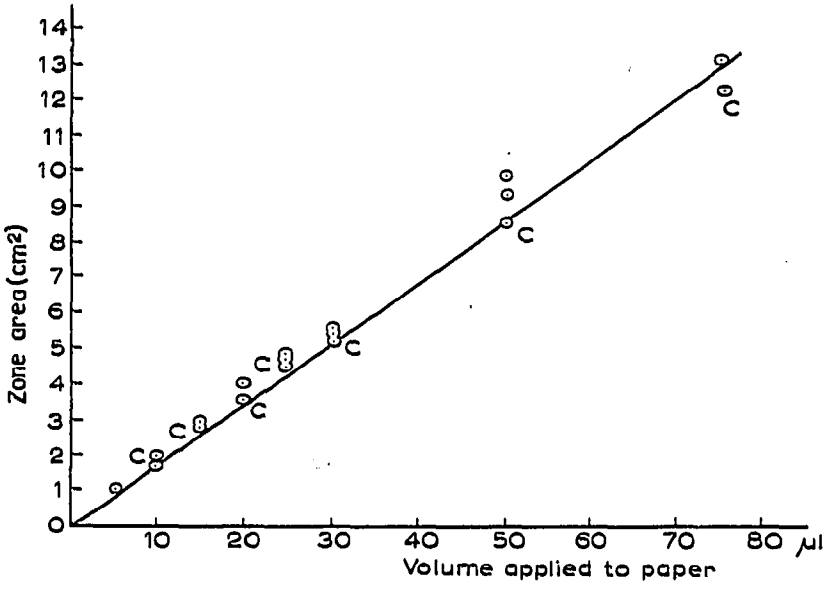


Fig. 2. Area of a zone produced for various volumes of solution applied to the chromatogram. The line is drawn through points corresponding to circular zones (C).

Then for a circular zone, the diameter $w$ (cm) is given in terms of the volume applied $v$ ($\mu$l) by

$$w = \sqrt{\frac{0.8v}{\pi}}$$

It is evident from the plate theory of MARTIN AND SYNGE[36] that the larger the area of the initial zone, the greater is the number of theoretical plates necessary for separation of a pair of solutes. Since the height equivalent to a theoretical plate has a constant value for the chromatographic system used, it follows that a longer chromatogram is necessary for separation. However, in practical terms, an extended chromatogram does not necessarily give better resolution, owing to diffusion causing the zones to spread.

A plot of $Q_r$ (concentration of solute in plate $r$) against plate number $r$, is usually assumed to be of the Gaussian type, $c$ is the concentration limit of detection, and the points L and L′ where the line $Q = c$ intersects the curve, are the boundaries of the detected zone. Assume the concentration of the initial zone is unit quantity of solute in one plate (Fig. 3).

According to MARTIN AND SYNGE[36], the amount of material in plate $(r + 1)$ is

$$Q_{r+1} = \frac{1}{\sqrt{2\pi r}} \cdot \left(\frac{v}{PV}\right)^r e^{r - v/V} \tag{8}$$

and at the maximum $r = v/V = R$.

Let the plate at L′ be $(n + 1)$

$$Q_{n+1} = \frac{1}{\sqrt{2\pi n}} \cdot \left(\frac{R}{n}\right)^n e^{n-R} = c \tag{9}$$

The diameter of the detected zone, $w$, is given by LL′, which in terms of number of plates will be,

$$w = 2\,(n - R)$$

Let $n - R = S$, that is $w/2$

$$c = \frac{1}{\sqrt{2\pi(S + R)}} \cdot \left(\frac{R}{S + R}\right)^{S+R} e^S \tag{10}$$

This equation has been solved for $c = 0.01$, and for various values of $R$ the following values of $w$ are obtained,

| $R$ | 0 | 50 | 100 | 150 | 200 | 250 |
|---|---|---|---|---|---|---|
| $w$ | 1 | 26 | 34 | 40 | 45 | 46 |

These values are all in terms of the unit $h$. MARTIN AND SYNGE[36] found that for their chromatograms $h = 0.02$ cm and calculations on our chromatograms tend to

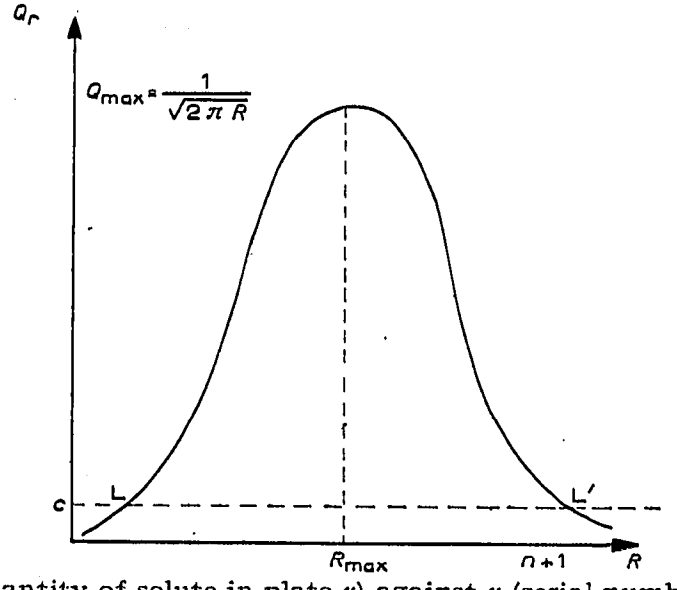


Fig. 3. Graph of $Q_r$ (quantity of solute in plate $r$) against $r$ (serial number of the plate). Symbols as used in the text.

produce a similar value. Since, in practice, more than one plate is filled with solute from the inital zone, the values of $w$ given above are merely the increases in the width of a zone. Notice that this process is due to chromatography and not to diffusion, although since diffusion is always greatest at a steep concentration gradient, its effect would be expected to be greatest at the beginning of the process and become less as the concentration gradients are evened out. At first sight this appears to nullify the statement that diffusion effects make the use of extended chromatograms worthless. However, as the solvent elutes solute down the paper, its progress becomes slower, and hence the longer the chromatogram, the more time there is for diffusion.

The main point we must gather is that the width of the initial zone sets a lower limit to the widths of the zones of the chromatographed materials. If this is too great, then a quantitative separation cannot be attained. This means that an accurate method for applying small volumes of solution to the chromatogram is essential if the separation is to be used in a quantitative method of analysis, especially when using the rear-phase technique.

## CONCENTRATION OVERLOADING

The area of a chromatographed zone is strongly dependent upon the concentration of the solution of zone-forming substance applied to the chromatogram.

Equation (10) holds for unit quantity of solute being admitted to the first "plate" of the chromatogram. For a solution of such concentration that an amount $x$ is admitted to the first plate eqn. (10) becomes

$$c = \frac{x}{\sqrt{2\pi(S+R)}} \cdot \left(\frac{R}{S+R}\right)^{S+R} e^{S} \tag{11}$$

or

$$x = \frac{K\,(S+R)^{S+R+0.5}}{R^{S+R} \cdot e^{S}} \tag{12}$$

$$K = c\sqrt{2\pi}$$

The following values of $x/c$ have been obtained for $R = 100$ and various values of $S$.

| $S$ ($h$ units) | 0 | 5 | 10 | 15 | 20 | 30 | 50 |
|---|---|---|---|---|---|---|---|
| $x/c$ | $2.4 \cdot 10$ | $2.65 \cdot 10$ | $3.96 \cdot 10$ | $7.34 \cdot 10$ | $1.57 \cdot 10^2$ | $1.15 \cdot 10^3$ | $9.29 \cdot 10^5$ |

For some time, an experimentally determined linear relationship between the area of a chromatographed zone and the log of the concentration has been used as a basis of analytical methods[37,38]. It appeared likely, therefore, that a plot of $S^2$ *versus* $\log x/c$ should give a straight line, and the result is confirmed (Fig. 4).

Equation of the line is

$$S^2 = 557 \log_{10} \frac{x}{c} - 765$$

$S$ measured in $h$ units.

Now examining eqn. (12), some meaning may be attached to the constants of this equation.

By taking logs and re-arranging

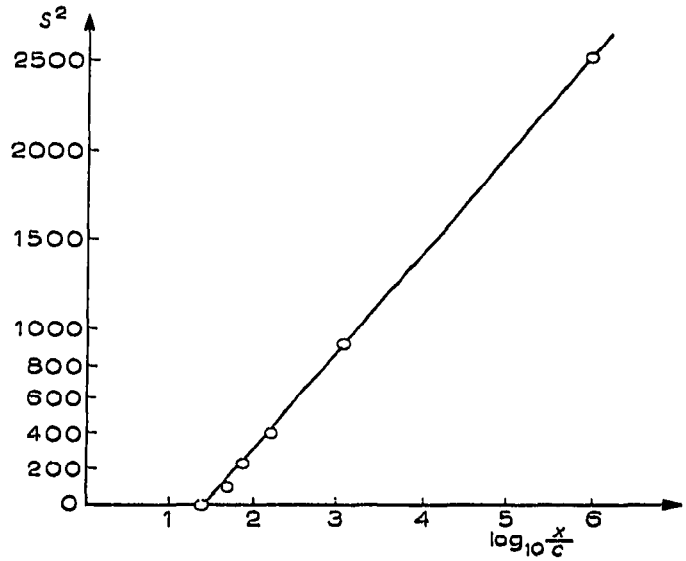


Fig. 4. Graph of $S^2$ ($S \sim$ area of eluted zone) against log $x/c$. Intercept on log $x/c$ axis is 1.36.

$$\log_e \frac{x}{c} = \log_e \sqrt{2\pi R} + (S + R + 0.5)\left(\frac{S}{R} - \frac{S^2}{2R^2} + \frac{S^3}{3R^3}\right) - S \qquad (13)$$

Since $R$ is much larger than $S$ for a normal chromatogram, terms of $(S/R)^4$ or higher are neglected.

$$\log_e \frac{x}{c} = \log_e \sqrt{2\pi R} + \frac{S}{2R} + \frac{S^2}{2R}\left(1 - \frac{1}{2R} + \frac{S}{2R^2} + \frac{2S^2}{3R^2}\right)$$

Of the terms containing $S$, only $S^2/2R$ and $2S^2/3R^2$ contribute, hence other terms are very much smaller. If this is so, then an approximate equation is

$$\log_e \frac{x}{c} = \log_e \sqrt{2\pi R} + \frac{S^2}{2R}\left(1 + \frac{2S^2}{3R^2}\right)$$

Solution for $S$ being

$$S = \pm\sqrt{-1 \pm \frac{2}{3}\sqrt{3\chi R^{3/2}}}$$

$\chi = \log x/c - \log \sqrt{2\pi R} > 1$, but at very low concentrations when $S$ is very small compared with $R$, sufficiently accurate to use,

$$S^2 = 2R \log_e \frac{x}{c} - 2R \log_e \sqrt{2\pi R}$$

or

$$S^2 = 4.606\, R\,(\log_{10} \frac{x}{c} - \log_{10}\sqrt{2\pi R}) \qquad (14)$$

Interception with the $\log_{10} x/c$ axis will be at $\log \sqrt{2\pi R}$. This means that a zone for which

$$\frac{x}{c} = \sqrt{2\pi R}$$

will have no detectable spread and the area of the zone after elution for $R$ plates will be equal to initial area, but unfortunately, no account of diffusion is taken here.

Consider the chromatography of a mixture of two solutes forming an original zone of radius, $\rho_z$, which, after elution, yields two zones whose centres are at $R$ and $R'$ plates from the origin. Let $x/c = \chi$, and the two values for the two solutes be $\chi$, and $\chi'$.

Now

$$S^2 = 4.606R\ (\log \chi - \log \sqrt{2\pi R}) \tag{14a}$$

$$(S')^2 = 4.606R'\ (\log \chi' - \log \sqrt{2\pi R'}) \tag{15}$$

$$R\rho_z = \rho_z + S$$

$$R'\rho_z = \rho_z + S'$$

$$R' - R = \rho_z + S + \rho_z + S' + d.$$

The two zones will interfere when $d = 0$ and

$$R' - R = 2\rho_z + S + S' \tag{16}$$

This cannot be solved easily, but it may be assumed that since in general $R' - R$ is small compared to $R$, then $S = S'$.

Thus

$$R' - R = 2(\rho_z + S)$$

$$S = \left(\frac{R' - R}{2}\right) - \rho_z.$$

Substituting

$$\frac{(R' - R - 2\rho_z)^2}{4} = 4.606 \left(\frac{R' + R}{2}\right) \left(\log \chi - \log \sqrt{\frac{2\pi\ (R' + R)}{2}}\right) \tag{17}$$

Thus let $R = 500$, $R' = 600$, $\rho_z = 10$ ($h$ terms), and $x/c = 2 \cdot 10^4$.

In this case, if $h \approx 0.02$ cm, the distance travelled by zone 1 is 10 cm, by zone 2 12 cm, if the diameter of the original zone was 0.2 cm.

Thus eqn. (17) can be used to determine the limiting size of the initial zone for chromatography of solutions of a given concentration, such that overloading does not occur.

It must be pointed out, however, that no account of diffusion has been allowed for these calculations. Diffusion will act towards a lessened resolution of the zones such that the actual limiting concentrations which may be employed in a quantitative separation will be less than those calculated, and the degree by which these are lower will be greater the longer the substances are chromatographed. In a qualitative nature, the effects of spreading of a zone by diffusion are embodied in the equation

$$S_D = \phi \left(\frac{dx}{dR}, t\right)$$

where $S_D$ is spreading due to diffusion; $t$ is period during which zone is chromatographed.

The radius ($\rho_R$) of a chromatographed zone after travelling $R$ plates can thus be expressed as

$$\rho_R = \rho_0 + S_C + S_D$$

($S_C$ = spread due to chromatography)

$$\rho_R = \rho_0 + \sqrt{4.606R \log_{10} \frac{\psi}{\sqrt{2\pi R}}} + \phi\left(\frac{dx}{dR}, t\right) \qquad (18)$$

## METHODS OF ANALYSIS

In general two procedures are available for the analysis of solutes which have been previously chromatographed. The substances may be extracted from the chromatogram after drying, and determined by classical micro-analytical techniques, or the analysis may be accomplished without extraction by an *in situ* method. Such methods have been adequately described elsewhere[39]. On considering these methods with reference to the thionates, it was seen that the major difficulty with hot extraction methods is that there will be considerable decomposition of the species with the formation of a mixture of products. Such methods are not, therefore, of any value unless a controlled reaction leading to a definite product is instigated during the extraction. However, it appeared that elution of the thionates from the chromatogram would not be followed by any subsequent decomposition, since the species are relatively stable in the solvent used for chromatography[35]. It was therefore necessary to find an analytical procedure for the estimation of micro-quantities of thionate present in a solution containing organic solvent and potassium acetate. All the reactions of thionates used by GOEHRING lead to the production of thiosulphate, so that, if one such reaction was chosen, the method involves the determination of thiosulphate.

Experiments were carried out on the disappearance of colour of an iodine–potassium iodide solution when a solution of sodium thiosulphate is added to it. Some difficulty was found in preparing a reproducible calibration curve for optical density *versus* concentration of iodine solution. This was found to be due to variations in the concentration of potassium iodide; however, when a standardised technique was employed, estimations of standard sodium thiosulphate solutions were possible to less than 2 % relative error.

For the conversion of thionates to thiosulphate, the sulphide degradation

$$S_nO_6^{2-} + S^{2-} \longrightarrow (n - 3)S + 2\ S_2O_3^{2-} \qquad (3)$$

was chosen, since for each mole of thionate, two moles of thiosulphates are produced. After boiling the eluant sample with alkaline sodium sulphide solution, the excess sulphide was removed by addition of freshly precipitated zinc carbonate slurry. The mixture was centrifuged, and made up to a standard volume with standard iodine solution. The optical density was then measured.

Results, however, were most unreliable, even when the utmost care was taken. It is thought that the fault is the solubility of zinc sulphide $7 \cdot 10^{-3}$ g/l at 18°[39], and although being negligible for macro-analysis, introduces considerable error when such small amounts of precipitate are considered.

Several other methods were then tried. They included:

(i) The extraction and oxidation of the thionate-containing section of the paper to sulphate, using potassium chlorate solution. The sulphate was estimated spectrophotometrically using 4-chloro-4'-aminodiphenyl[40]. Unfortunately, this method failed to yield consistent results, probably because of the bisulphite content of the filter paper.

(ii) The alkaline hydrolysis of the polythionate species on the paper to thiosulphate according to the equations[20]

$$4\ S_4O_6^{2-} + 6\ OH^- \longrightarrow 5\ S_2O_3^{2-} + 2\ S_3O_6^{2-} + 3\ H_2O \quad (6)$$

$$2\ S_5O_6^{2-} + 6\ OH^- \longrightarrow 5\ S_2O_3^{2-} + 3\ H_2O \quad (5)$$

$$S_6O_6^{2-} \longrightarrow S_5O_6^{2-} + S \quad (4)$$

while trithionate is decomposed only in concentrated alkaline solution[21]

$$2\ S_3O_6^{2-} + 6\ OH^- \longrightarrow S_2O_3^{2-} + 4\ SO_3^{2-} + 3\ H_2O \quad (7)$$

and titration of the resultant thiosulphate with formaldehyde being used to find the sulphite from the paper. Recovery experiments on chromatographed samples of potassium thiosulphate gave reproducible results with an average recovery of 98 % ± 4 %.

The hydrolysis reactions given above were tested by hydrolysing samples of each thionate with alkali under varying conditions. After adding formaldehyde and neutralising, the solutions were titrated with iodine solution to obtain values of $S_2O_3^{2-}$ produced. Also samples of the hydrolysates were chromatographed to identify the thionate species present.

Trithionate was found to be especially stable to hydrolysis. Boiling with 0.5 *M* caustic soda solution for 2 h only produced about 16 % hydrolysis, while 1 *N* caustic soda was required for quantitative conversion to thiosulphate, the only species observed on chromatographing the hydrolysate. With tetrathionate, standing with 0.5 *N* caustic soda solution was sufficient to cause hydrolysis, and from the alkali consumption, and thiosulphate produced, excellent agreement with equation (6) was obtained, a chromatogram showing only $S_2O_3^{2-}$ and $S_3O_6^{2-}$ to be present. However, on using a large excess of 0.5 *N* alkali, eqn. (6) was no longer obeyed. The results corresponded to $2\ S_4O_6^{2-}$ consuming $4\ OH^-$ with the production of $3\ S_2O_3^{2-}$ to within 2 %. Experiment showed that $S_3O_6^{2-}$ was not hydrolysed under these conditions, and neither did the presence of thiosulphate catalyse the decomposition of $S_3O_6^{2-}$ as it does with higher thionates[34]. Chromatograms showed that $S_3O_6^{2-}$ was present, and it is possible to formulate a stoichiometric equation which represents the alkali consumption correctly,

$$4\ S_4O_6^{2-} + 8\ OH^- \longrightarrow 4\ S_2O_3^{2-} + 2\ S_3O_6^{2-} + SO_4^{2-} + S^{2-} + 4\ H_2O.$$

Since the oxidation of $S^{2-}$ requires twice the concentration of $I_2$ per mole required for the oxidation of thiosulphate, it can be seen that the iodine consumption would also be correct on this basis, and therefore the reaction seems to be a likely one.

Both 0.5 *N* and 1.0 *N* caustic soda solution caused rapid hydrolysis of penta-

thionate to thiosulphate, the only species detected chromatographically. The results agreed with equation (5).

With regard to hexathionate, the results could not be interpreted on the basis of any stoichiometric eqn. (4), such as that quoted by GOEHRING *et al.*[20] and from the chromatograms it appeared that numerous side reactions were occurring. The hydrolysis could not therefore be used to estimate hexathionate.

It would seem then that although the method would be of use in the analysis of $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$ and $S_5O_6^{2-}$, it is likely to fail with higher thionates. These results tend to agree with the results obtained by BLASIUS *et al.*[34,35] concerning the stoichiometry of such degradation reactions. It is just these higher thionates which are the main interest in the investigations for which the analytical method was required.

All these proposed methods having been found inadequate for ready and accurate analysis of thionates from chromatograms, attention was focused upon *in situ* methods. Such methods involve measuring the area, optical density, or some other property of the developed zone which bears a simple relationship to the concentration of solute present.

Area measurement requires that the zone should be sharply defined. Preliminary experiments were carried out on trithionate zones, which were developed with ammoniacal silver nitrate solution, the excess silver nitrate being removed with sodium thiosulphate solution. Measurement of the area of the zone was found to be only semi-quantitative. This was mainly due to the difficulty of delimiting the zone boundaries. A major factor in such systems is the sensitivity of the spray reagent[41]. Thus, whereas it is possible that area measurements of zones may afford a method of estimation of solutes, in most cases it is only semi-quantitative.

Measurement of the absorbance of zones using scanning techniques proved much more promising. The measurement of the total absorbance of a small zone was rejected as this would severely limit the volume of solution (not more than 5 $\mu$l). The other method was to measure the absorbance of a section of a uniform band. This necessitates the application of a uniform band to the chromatogram, and a suitable machine was designed for this purpose. In designing the machine, care was taken in its specification to adhere to the ideas reported in this paper concerning (a) volume and (b) concentration overloading.

Because of the space needed for these considerations and a description of the machine, details and developments are described in the following paper.

## SYMBOLS

$A$ = area of cross-section of column = $A_I + A_L + A_S$.
$A_I$ = area of cross-section of inert support.
$A_L$ = area of cross-section of mobile (liquid) phase.
$A_S$ = area of cross-section of static phase.
$\alpha$ = partition coefficient = $\frac{\text{g solute/ml of stationary phase}}{\text{g solute/ml of mobile phase}}$ at equilibrium.
$c$ = concentration limit of detection.
$d$ = distance between centres of gravities of two spots.
$h$ = H.E.T.P. (height equivalent of a theoretical plate).

$Q_r$ = concentration of solute in plate $r$.
$r$ = serial number of "plate", measured from top of chromatogram.
$R$ = $\frac{\text{displacement of position of maximum concentration of solute}}{\text{simultaneous displacement of liquid surface of developing fluid}}$.
$\rho_0$ = radius of zone at commencement of elution.
$\rho_R$ = radius of chromatographed zone after travelling $R$ plates (diffusion).
$S$ = radius of zone.
$S_C$ = spreading due to chromatography.
$S_D$ = spreading due to diffusion.
$t$ = time period of chromatographic development.
$v$ = volume of solvent used in development of chromatogram.
$V$ = $h(A_L + A_S)$.
$w$ = diameter of zone.

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SUMMARY

This paper begins with a short review of previous analytical methods for polythionates, and goes on to consider the limitations of the chromatographic method which must be taken into account when the method is to be used for quantitative measurements. A discussion on overloading follows in which two types of overloading are defined due to high volume and high concentration, respectively. In this case of concentration overloading, the treatment leads to an equation which may be used to determine the conditions for interference of two zones whose centres have a given separation.

The actual analytical methods applied are then described. Many macro-analytical methods for the analysis of thionates were adopted to semi-micro scales in the search for a method of estimation which could be coupled with the chromatographic separation. The final method adopted for the estimation of the species was an *in situ* method.

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