

THE
SPECTROCHEMICAL DETERMINATION
OF
CERTAIN MINOR TRACE ELEMENTS
IN
PLANT MATERIAL

- by -

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S U M M A R Y

1. A study has been made of three organic complexing reagents, namely dithizone, oxine and cupferron with a view to developing suitable methods for the separation and concentration of Zn, Co, Ni, Pb, Cu, Mn, Ti, V and Mo prior to the spectrometric determination of their concentrations. In particular the influence of pH on the chloroform extraction of dithizonates, oxinates and cupferrates from aqueous tartrate and citrate solutions and the separation of iron by oxine extraction has been investigated.
2. The development of a method for the chemical concentration and spectrographic determination of Zn, Co, Ni, Pb, Ti, V and Mo is described and the possibility of determining Mn and Cu flame photometrically as part of the analysis scheme is demonstrated. A specially designed slide rule for the calculation of spectrographic results is described.
3. A direct reading spectrometric method for the determination of Zn, Pb and Cu in plant material is presented.
4. The analysis of plant ash for zinc using the 2138.6A spectrum line has been studied and a simple, rapid analysis method is described.

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CHAPTER I

I N T R O D U C T I O N

The importance to agriculture of a knowledge of trace element distribution and availability for both plant and animal nutrition is generally recognised. Because of the complex nature of the combined functions of trace elements it is desirable that the concentrations of as many of the important nutritional trace elements as possible be investigated and if a comprehensive picture is to be obtained, a great number of samples have to be analysed.

Spectrographic methods of analysis have the advantages of speed, sensitivity and the ability to determine the concentrations of a number of elements simultaneously and are thus particularly well suited to research into problems related to the trace element content of plants.

The investigation described in this thesis was initiated with a view to providing rapid and accurate spectrochemical methods of analysis of plant material for certain minor trace metals. The elements considered being zinc, cobalt, nickel, lead, titanium, vanadium, molybdenum, copper and manganese. Methods were sought which would be suitable for use on a routine basis for the analysis of a large number of samples.

The essentials in emission spectrochemical analysis are firstly, an excitation source which normally consists of an electric arc, spark or flame into which the sample is introduced in such a way as to cause the emission of radiation characteristic of its constituent elements, a spectrometer or spectrograph which separates the radiation frequencies to form a spectrum which is recorded photographically or electrically and finally, some means of interpreting from the spectrum the concentrations of the elements present in the sample. Emission spectrographic analysis procedures can thus be divided into three main parts:-

- (1) Preparation of the sample in a suitable form for spectrochemical analysis.
- (2) Excitation of the sample under selected conditions and the recording of the spectrum produced.
- (3) Interpretation of the spectrum recorded.

Each of these steps in the analysis has its own peculiar problems, which depend to a large extent on the nature of the sample and, in the development of the methods which are described here, each has been given attention.

(1) SAMPLE PREPARATION

Spectrochemical methods are normally used to best advantage, from the point of view of speed, when a minimum of sample preparation is necessary. In the analysis of plant material this ideal is approached in the case of the so-called "major" elements, which can be determined satisfactorily using the ashed sample without any further chemical treatment, other than dilution with a buffer mixture to overcome the effect of variation of matrix composition and to suppress the emission of cyanogen bands. Such a method for the determination of calcium, strontium, barium, magnesium, aluminium, iron, sodium, potassium, copper and manganese has been developed by Strasheim and Keddy.⁽¹⁾ Of the elements studied, copper and manganese can thus be satisfactorily determined directly on the sample ash and to this list zinc can be added, since a method of this type in which the 2138.6A spectrum line is used has been found satisfactory for the determination of this element and is described in Chapter V of this thesis.

In analysis for most of the minor elements, however, it is normally considered necessary to separate them from the major constituents of the sample and thus bring about their concentration before the spectrographic step in the analysis. This has the disadvantage, on the one hand, of prolonging the analysis procedure until, as Wark⁽²⁾ has remarked, "The spectrograph will slip from its dominant role to a minor role as a final instrument of determination in a chain of chemical manipulations." On the other hand, chemical concentration and separation from unwanted sample constituents makes possible the determination of elements present in traces too small to allow of their direct determination, while effects of variation in matrix composition are almost entirely eliminated and a wide variety of samples can be tackled without restandardisation.

A number of chemical concentration procedures for the determination of minor trace metals in plants have been proposed. Both precipitation methods and solvent extraction methods have proved successful.

In the well established method developed by Mitchell and his associates⁽³⁾, concentration is effected by precipitation by means of oxine (8-hydroxyquinoline), tannic acid and thionalide (β amino-naphthalide of thioglycolic acid) at pH 5.1 to 5.2. The aluminium in the sample acts as carrier in the precipitation. The precipitate is allowed to stand overnight, is filtered off, washed and, after being dried, ashed at 450°C. The ashed trace element concentrate is then mixed with carbon powder and burned in a d.c. arc. It is claimed that the elements Co, Ni, Mo, Sn, Pb, Zn, Ti, V, Cr, Ga, Ge, Be, Ag and Au can be satisfactorily determined using this method. Iron which is precipitated with the other elements is used as internal standard and its concentration must be determined colorimetrically on an aliquot of the sample solution and allowance made for its concentration. Alternatively, as in the procedure adopted by Strasheim and Camerer⁽⁴⁾, the iron concentration may be adjusted to a predetermined value by the addition of a suitable quantity of standard solution.

Methods in which sodium pyrrolidine dithiocarbamate is used to precipitate the trace metals at pH 4.8 followed by chloroform extraction as originally proposed by Stetter and Exler⁽⁵⁾ have been successfully applied by Scharrer and Judel⁽⁶⁾ and by Strasheim, Eve and Fourie⁽⁷⁾ to the analysis of plant materials. Pb, Mo, V, Co, Ni, Mn and Zn have been determined satisfactorily by this method.

In all the concentration methods mentioned above iron is concentrated with the other elements. It has been shown by Strasheim, Eve and Fourie that, unless its concentration is kept constant or allowance made for variation in its concentration, analysis results can be seriously affected by the presence of iron in the sample even when instruments of relatively high dispersion (e.g. the Large Hilger Spectrograph) are used. With a low or medium dispersion spectrograph the presence of relatively large concentrations of iron in the sample gives rise to a spectrum so crowded with lines that the quantitative determination of the minor trace metals is exceedingly difficult. A concentration method in which iron is separated from the elements to be determined is therefore very desirable and a major part of the work described here was devoted to the development of such a method.

Procedures in which iron is separated have been proposed by Gorbach and Pohl⁽⁸⁾ and by Doll and Specker⁽⁹⁾ while Wark⁽²⁾ has proposed a method for cobalt, zinc and copper in which these elements are separated from iron and other sample constituents by dithizone extraction from citrate solution at pH8.3.

In the method described by Doll and Specker, intended primarily for soil extract samples, the sample is dissolved in 7N lithium chloride and extracted with isobutylmethylketone. According to the information given in their paper, this should have the effect of separating in the organic phase virtually all the iron together with varying proportions of other elements, including small fractions of the zinc and copper, and leaving most of the important elements in the aqueous phase. Some of the metals, particularly gallium, coextracted with the iron can be recovered in two more extraction steps, but the zinc and copper lost cannot apparently be recovered. The sample solution with iron removed is then extracted with chloroform after the addition of sodium pyrrolidine dithiocarbamate at pH 4.8 and cupferron (ammonium-N-nitrosophenyl hydroxylamine) at pH2. All the elements studied would apparently be concentrated in this scheme (apart from small losses of zinc and copper) together with a number of others but the successful application of it to the analysis of actual plant or soil samples has not been reported.

Van Rooyen⁽¹⁰⁾ has shown that the analysis of plant material, using the method of Gorbach and Pohl, which involves a number of different chemical separations, is quite feasible and this method was therefore made the starting point in the investigations which are recorded in this thesis.

In this method ammonium benzoate is added to the sample solution buffered at pH3.8 to 4 when iron and a number of other elements including titanium and vanadium are precipitated. This precipitate is filtered off, dissolved in 6.5N (or 8N) hydrochloric acid and iron removed by extraction with diethyl ether (or isopropyl ether). The acid solution is then evaporated and the residue returned to the initial filtrate to give a sample

solution from which the iron and some of the tin have been removed. Ammonium tartrate is added and the solution extracted with a chloroform solution of dithizone at pH7 and 9 which separates the zinc, cobalt, nickel, lead, copper and other metals which form dithizone complexes. The other metals to be determined are extracted by a chloroform solution of oxine at pH5 and 6.5 after the decomposition of the tartrate which interferes, by oxidation with hydrogen peroxide.

(2) EXCITATION CONDITIONS.

The correct choice of excitation source conditions is undoubtedly one of the more important factors influencing the accuracy and sensitivity of spectrochemical analysis.

Gorbach and Pohl used a Feussner-type high tension spark in their analysis method but the d.c. arc is generally regarded as being the most sensitive form of spectroscopic source and is therefore commonly used for trace element analysis. The normal d.c. arc with anode excitations has been used successfully by Scharrer and Judel⁽⁶⁾ and by Strasheim, Eve and Fourie⁽⁷⁾. Mitchell⁽³⁾ favours the use of the cathode layer d.c. arc technique of Mannkopff and Peters⁽¹¹⁾ and has obtained very good results using this technique. The results of a general comparison of anode excitation and the cathode layer technique, reported by Ahrens and Liebenberg⁽¹²⁾, tend to throw some doubt on the advantages claimed for the latter. Strasheim and Camerer⁽⁴⁾ compared the two techniques under similar conditions for the analysis of plant material and found only slightly improved sensitivity in the case of cobalt, vanadium and molybdenum, sensitivity being unchanged in the case of the other elements, while the accuracies of the two techniques were reported to be similar. Since it is normally possible to burn more sample in the arc using the anode excitation, these slightly improved sensitivities are likely to prove of small advantage. Because of the strict controls that are necessary with the cathode layer technique it is likely to prove disadvantageous in a routine laboratory where other analyses are regularly being carried out. Therefore, while the cathode layer technique undoubtedly merits further study, especially as the difficulty normally experienced in filling the narrow electrode cavities required

for it has been overcome by the invention of an efficient electrode packing machine⁽¹³⁾, it was initially decided to limit the investigation to anode excitation where the d.c. arc was used.

The requirements of direct reading spectrometric analysis led to the study of other types of source, particularly the interrupted arc source, which has been shown to have certain advantages over the normal d.c. arc and this type of excitation was later used with the spectrographic (photographic) method. Experiments were also carried out with solution techniques with the aim of reducing the number of steps before spectrographic analysis.

(3) INTERPRETATION.

The spectrum of radiation emitted by the sample can be recorded either photographically, as is done in conventional spectrographic analysis, or photoelectrically as in direct reading spectrometry.

When the spectrum is recorded photographically, the procedure to be followed before the element concentrations in the sample can be determined includes a number of time-consuming steps most of which have inherent errors which can be reduced only by strict controls. These include loading the photographic plate into the spectrograph plate holder, unloading and development after exposure, measurement of the density of the spectrogram lines and computation of relative intensities or intensity ratios. The calculation of intensity ratios is normally done graphically by any one of a number of published methods⁽¹⁴⁾⁽¹⁵⁾⁽¹⁶⁾. A study of these methods has been made and a rapid routine method of calculating intensity ratios, using a specially designed slide rule will be described in Chapter III, Section B.4.

Direct Reading Spectrometry.

When a direct reading spectrometric method is employed, the amount of time required for interpretation of results is considerably reduced and the inherent errors of the photographic method are avoided. For this reason there has recently been considerable advance in the design and use of direct reading spectrometers and the development of direct reading methods for plant material analysis is highly desirable.

In direct reading spectrometers the photographic

plate of the spectrograph is replaced by a number of exit slits, positioned so as to receive the light from chosen spectrum lines. Behind each of these a photomultiplier tube is placed. Generally the current produced by the photomultiplier, which is directly proportional to the intensity of the light, is used to charge a condenser and the potential between the plates of the condenser is measured on completion of the exposure. Since the charge on the condenser is directly proportional to the intensity of the corresponding spectrum line, the calculation of the element concentrations presents no difficulty.

While direct reading methods have advantages of speed and often of accuracy over conventional spectrographic methods, they can only be used to best advantage when source conditions and sample form are carefully chosen. To increase the amount of light available and to make slit setting less critical, entrance and exit slits are made relatively wide. This means that the effective resolving power of the spectrometer is reduced. In addition, due to the fact that the response of the photomultiplier tube is linear over a wide range, the influence of background illumination becomes relatively more important than with photographic recording, where the response to low intensities of illumination is extremely small. Thus a simple sample spectrum with low background is desirable, particularly when instruments of relatively low dispersion are used.

For precision in direct reading spectrometry exposures should be made for equal lengths of time. This means that the consumption of the sample in the excitation source should be reproducible. This requirement and that of low background intensity militate against the use of the d.c. arc in direct reading spectrometry. Where the rates of volatilisation of the various elements in the arc are similar and constant, this form of excitation may possibly be usable, but as described in Chapter V.b, the direct transfer of a technique developed for a spectrographic analysis method to direct reading can lead to reduced precision.

These facts tend to make direct reading spectrometric analysis largely a separate study. As a result of research into this field a technique has been developed for the direct introduction of organic complex extracts

into a spectroscopic source of sufficiently high energy for trace metal analysis. This technique has been applied to the determination of copper, zinc and lead in plant material and is described in Chapter IV.

All the experimental work described was carried out in the laboratories of the Spectrochemistry Division of the National Physical Research Laboratory, South African Council for Scientific and Industrial Research.

Outline of Thesis.

The arrangement of the material has been made with a view to logical grouping of related subjects of research rather than to the chronology of the experimentation. Thus the studies made with the various organic reagents used are reported together in Chapter II, although it was the needs of the various analysis methods described in later chapters which from time to time necessitated further work with particular reagents.

In Chapter III the development of a concentration scheme and spectrographic analysis method for zinc, cobalt, nickel, lead, titanium, vanadium and molybdenum is described. The method of calculating the results is discussed in some detail and the possibility of using a specially designed slide rule for the calculation is demonstrated. The determination of copper and manganese as part of the analysis scheme was considered and the work done in this connection is also described.

Chapter IV describes the development of a direct reading spectrometric method for the determination of copper, zinc, and lead in plant material.

In Chapter V an account is given of the development of a method for the determination of zinc in plant ash without further chemical treatment.

Finally, in Chapter VI all the results are discussed briefly and suggestions made as to lines along which further research could be carried out.

CHAPTER II

STUDY OF ORGANIC COMPLEXING REAGENTS
AND EXTRACTION METHODS

A number of possible methods are available for the separation and concentration of trace metals. These include precipitation, solvent extraction, ion exchange and electrolytic deposition. Of these, precipitation methods⁽³⁾ and solvent extraction methods⁽²⁾⁽⁵⁾⁽⁶⁾⁽⁷⁾⁽⁸⁾ are well established, a little work has been done on the use of electrolyte deposition⁽¹⁷⁾, and recently, use of ion exchange resins in conjunction with spectrochemical analysis has received some attention⁽¹⁸⁾⁽¹⁹⁾. The work described here was limited to solvent extraction procedures, which have the advantages that they can be both rapid and quantitative for very small concentrations of metals and no carrier or collector element is necessary, so that a high degree of concentration can be achieved.

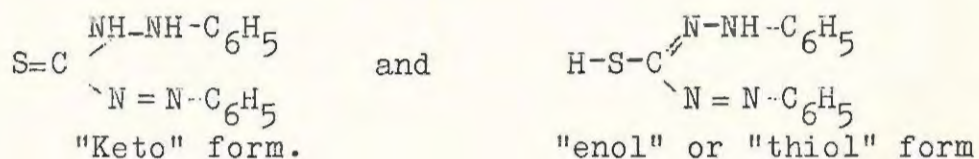
In solvent extraction work it is desirable that the extracting solvent should be readily purified and that its solubility in water should be very low. In addition, from a practical point of view, especially if more than one extraction has to be made, it is advantageous for the solvent to be denser than water. If this condition is fulfilled, successive extracts can easily be tapped from the separatory funnel and no transfer of the sample solution is necessary. A number of different techniques for removing an upper solvent layer from a separatory funnel have been described, but none of these would be completely satisfactory as part of a routine analysis method. If the solvent extraction is used in conjunction with a spectrographic analysis for which the sample is required in the form of a powder, then a solvent which wets graphite is desirable.

Chloroform fulfils these requirements and particular attention was therefore given to organic reagents which form chloroform-soluble metal complexes. In the process of development of a suitable rapid method for the separation and concentration of zinc, cobalt, lead, nickel, titanium, vanadium, molybdenum, copper and manganese, the properties of three organic reagents, namely dithizone (diphenylthiocarbazone), oxine (8-hydroxyquinoline) and cupferron (ammonium-n-nitroso-phenyl-hydroxylamine) were examined in some detail. In particular the effectiveness of the reagents for the extraction of metals from aqueous solutions containing

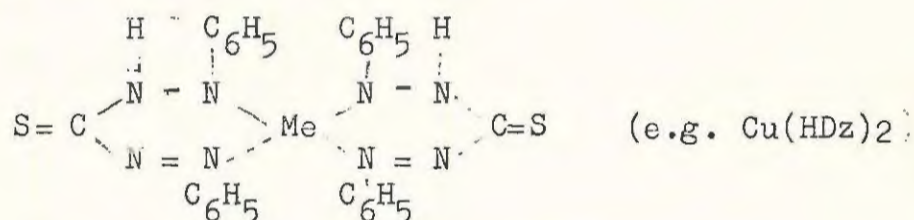
citrate and tartrate was investigated.

A. DITHIZONE
(DIPHENYLTHIOCARBAZONE).

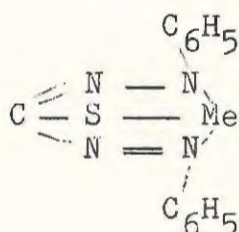
Dithizone, which was first prepared by Fischer⁽²⁰⁾ is one of the more important organic reagents which form organo-metal complexes of chelate type. Structural formulae attributed to it are:-



The following formulae were postulated by Fischer for the dithizone-metal compounds:-



where Me is a divalent metal atom, and



Irving and Bell⁽²¹⁾, however, have shown by experiments involving the replacement of the hydrogen of the sulphhydryl group of the thiol form by a methyl group, that only this hydrogen can be replaced by a metal.

Dithizone reacts with the following metals to form coloured complexes: Mn, Fe, Co, Ni, Cu, Zn, Pd, Ag, Cd, In, Sn, Te, Pt, Au, Hg, Tl, Pb, Bi, Po. (Only the divalent forms of Fe, Sn, Pt react). Most of these compounds are soluble in chloroform and provided conditions are properly chosen, these metals can be extracted from aqueous solution with chloroformic dithizone. Dithizone extraction has been applied extensively to the separation and concentration of metals for colorimetric and polarographic analysis and in recent years a number of publications⁽²⁾⁽⁸⁾ have dealt with the application of dithizone extraction methods to the concentration of trace metals for spectrographic analysis.

A considerable amount of information regarding the

extractability of metals with dithizone and the influence of pH and presence of other substances in solution has been published, and a large proportion of this information has been collected together by Sandell⁽²²⁾.

In the case of zinc, the information published is apparently somewhat conflicting⁽²³⁾⁽²⁴⁾⁽²⁵⁾. Marsten and Dewey⁽²⁶⁾ have investigated the extraction of cobalt from citrate and acetate solutions with a large excess of dithizone. Apart from the work of Koroleff recently reported by Sandell⁽²²⁾, there has been little systematic study of the extraction of nickel by dithizone. The extraction of copper has been systematically studied by Geiger⁽²⁷⁾, but he unfortunately studied only acid solutions containing chloride and perchlorate. In addition it has been reported by Yasumutsu Uzumasa and Isao Mujashito⁽²⁸⁾ that citrate and tartrate interfere with the quantitative extraction of copper dithizonate by carbon tetrachloride. Biefield and Patrick⁽²⁹⁾ have shown that lead can only be quantitatively extracted at pH9.5 or above from solutions containing cyanide and citrate ions, whereas this metal is completely extracted at pH8 from tartrate solutions and at pH7 from solutions containing cyanide and no citrate.

Generally, from the information published, it is evident that the extraction equilibrium is profoundly affected by the nature and concentration of other substances present, the concentration and volume of extractant and the method of extraction. It was therefore felt that, if dithizone extraction was to be used as part of the scheme of analysis, the extraction should be investigated under the analysis conditions.

1. The Influence of pH on the Extraction of Zinc, Cobalt, Nickel and Copper from Tartrate Solutions using Dithizone.

In the analysis method described by Gorbach and Pohl⁽⁸⁾ ammonium tartrate is added to the sample solution after the removal of the iron and the metals which form dithizonates are extracted with a chloroform solution of dithizone, first at pH7 and then at pH9. It was felt that it was likely that extraction at a single pH value would suffice to extract all the elements together, thus shortening the method a little. The influence of pH on the extraction of zinc, cobalt, nickel and copper from tartrate solutions was therefore investigated.

Experimental

(i) Apparatus

Beckman model H-2 pH meter.

Beckman D.U. Spectrophotometer with photomultiplier and flame photometry attachment.

(ii) Standard Solutions of the Metals

These were prepared from the corresponding analytical grade salts (i.e. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) dissolved in double distilled water. The dilute solutions used were prepared by dilution from more concentrated solutions and kept in polythene bottles.

(iii) Dithizone

During the course of this investigation, two commercial brands of dithizone were used (Hopkin and Williams and Eastman Kodak). Solutions were initially prepared on the assumption that the dithizone was moderately pure, but a determination of the purity by extractive titration against standard silver nitrate in 0.1N sulphuric acid indicated that the Hopkin and Williams product was in fact only 47% diphenylthiocarbazone and in all work after this the Eastman Kodak product was used. This was found to be 96% pure.

Solutions of the required strength were prepared by dissolving weighed amounts of dithizone in redistilled chloroform.

(iv) Procedure

10 ml of an 8.2 per cent (0.546 M) Tartaric acid solution (corresponding to 10 ml of 10% ammonium tartrate, as required by the method of Gorbach and Pohl) were run into a 200 ml separatory funnel. Sufficient N ammonia solution was added to give the desired pH. (This was determined previously). 10 ml of a 10 ppm solution of the metal was then pipetted into the funnel and the mixture diluted to 100 ml. A measured volume of dithizone solution in chloroform was added and the contents of the funnel shaken vigorously for two minutes. The chloroform layer was separated and the pH of the aqueous phase was measured with the pH meter.

In the estimation of cobalt, nickel and copper the chloroform layer was evaporated and the metal complexes destroyed by evaporation with 5 ml nitric acid containing a few drops of perchloric acid. The residue was dissolved

in water containing a few drops of N HCl and after diluting to 10 ml in a measuring flask, the metal content was determined flame photometrically, measurements being made with an oxy-hydrogen flame at the following wave lengths:- Co, 345.4 m μ ; Ni, 352.5 m μ ; Cu, 327.4 m μ .

In the case of zinc the excess dithizone in the chloroform phase was removed by extraction with 0.04 per cent sodium sulphide as described by Sandell⁽³⁰⁾ and the zinc content determined photometrically after dilution to 50 ml. with pure chloroform.

Results

Nickel

Because, of the metals studied, least was known about the extraction of nickel, a more thorough study was made of the effect of varying excesses of dithizone in the extraction of this metal than the others.

The results for nickel are shown in Figure 1. Curve 1 shows the effect of varying pH on the extraction of nickel using 20 ml of .0047 per cent dithizone (0.01% weight to volume, prepared from the 47% commercial brand). This represents a 6.8 per cent excess of the reagent. (Calculated on the assumption that a primary dithizonate is formed). Curve 2 shows the extraction with 20 ml of 0.0096 per cent dithizone, i.e. 120 per cent excess. Curve 3 represents the results obtained when extractions were made with 14 ml of 0.0096 per cent dithizone to give a 54 per cent excess. Curve 4 shows the extraction with the same excess of reagent (54 per cent) obtained by using 20 ml. of 0.007 per cent dithizone.

Cobalt

Figure 2 shows the results obtained for the extraction of cobalt. Extractions were made with two dithizone solutions, 0.0096 per cent and 0.0047 per cent, using 20 ml. of extractant which gave excesses of reagent of 7.2 per cent and 121 per cent respectively.

Zinc and Copper

In Figure 3 the results obtained for the extraction of zinc and copper are shown. In each case 20 ml of 0.0047 per cent dithizone were used for the extraction.

Discussion

Koltoff and Sandell⁽³¹⁾, in a preliminary study of dithizone extraction, derived a simple expression for the pH extraction relationship, viz:-

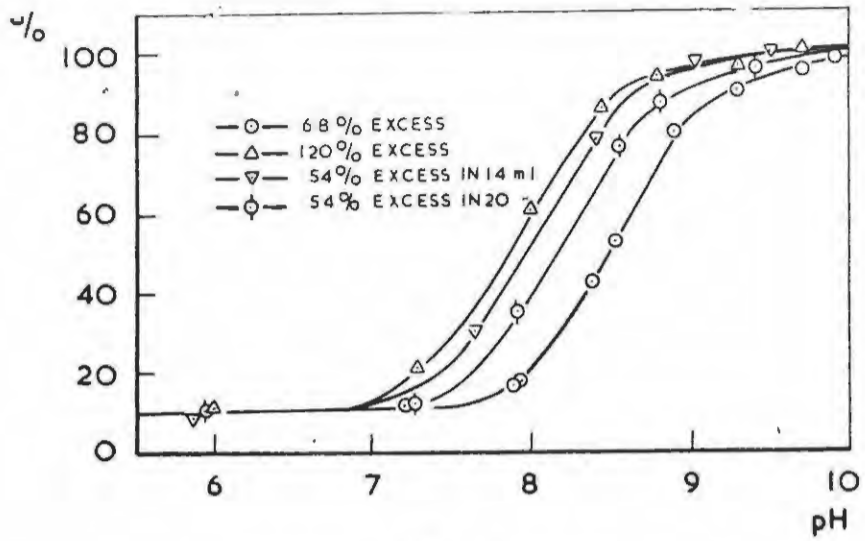


Fig. 1
pH-Extraction relationship for nickel.

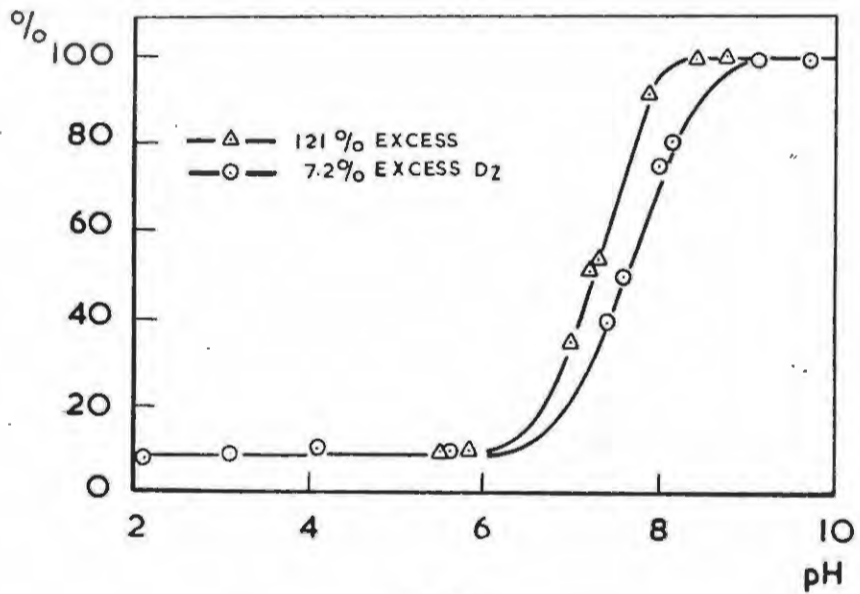


Fig. 2
pH-Extraction relationship for cobalt.

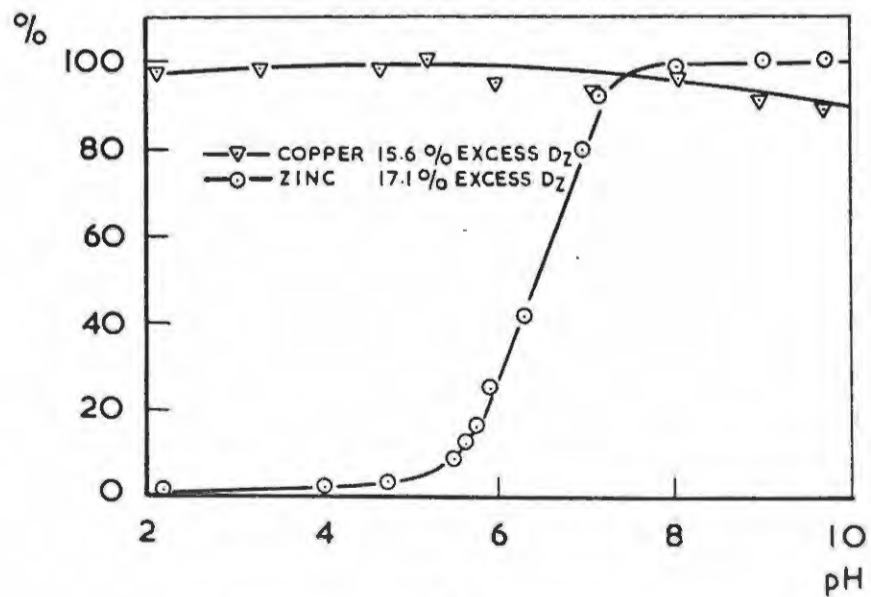


Fig. 3
pH-Extraction relationship for copper and zinc.

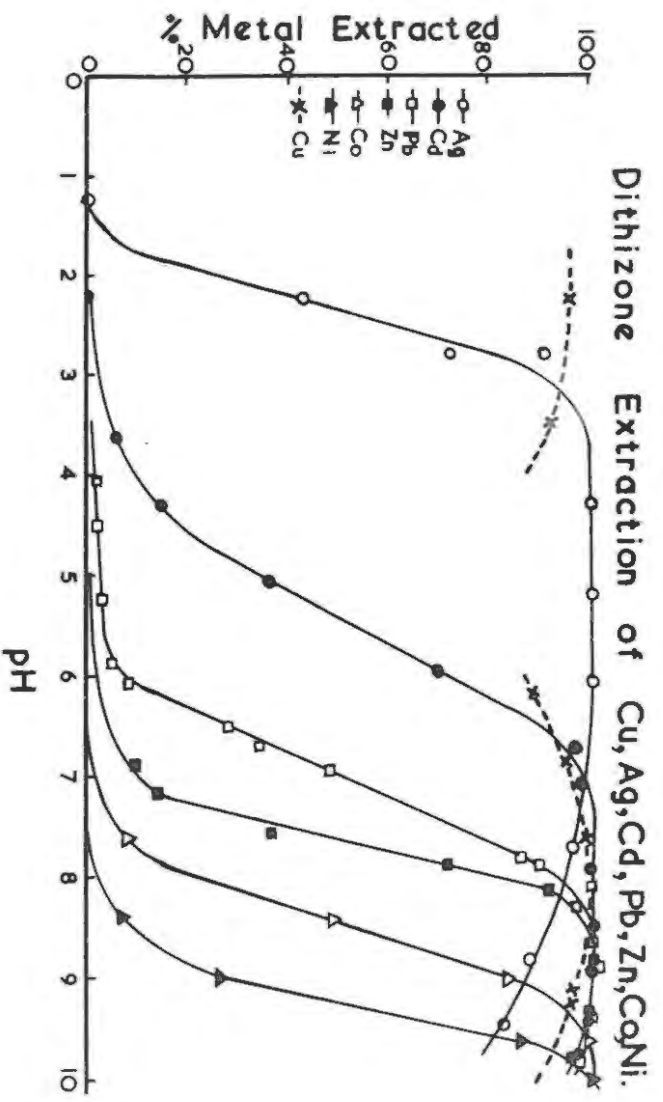


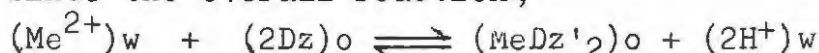
Figure 4.

$$\frac{[\text{Me Dz}'_2]_o}{[\text{Me}^{2+}]_w} = \frac{K [\text{Dz}]_o^2}{[\text{H}^+]_w^2} \quad (2.1)$$

where K is referred to as the "extraction constant."

The subscripts "o" and "w" indicate the organic and aqueous phases respectively. Dz' represents the "dithizonate radical", dithizone which has lost a hydrogen ion by dissociation or reaction with the metal and Me represents a bivalent metal.

Since the overall reaction,



involves the transfer of dithizone and dithizonate from one phase to the other, i.e. the reaction actually proceeds by the steps,

- (1) $(\text{Dz})_o \rightarrow (\text{Dz})_w$
- (2) $(\text{Dz})_w \rightarrow \text{H}^+ + \text{Dz}'^-$
- (3) $\text{Me}^{2+} + 2\text{Dz}'^- \rightarrow \text{MeDz}'_2$
- (4) $(\text{MeDz}'_2)_w \rightarrow (\text{MeDz}'_2)_o$

so that the full equilibrium expression is (32)

$$\frac{[\text{MeDz}'_2]_o}{[\text{Me}^{2+}]_w} = \frac{P_m \cdot K_d^2}{K_m \cdot P_d^2} \cdot \frac{[\text{Dz}]_o^2}{[\text{H}^+]_w^2} \quad (2.2)$$

where P_m = Distribution coefficient of the metal complex between the phases. (organic phase/aqueous phase)

P_d = Distribution coefficient for the dithizone.

K_m = Dissociation constant of the metal complex.

K_d = Dissociation constant of dithizone.

Thus from equation (2.1)

$$K = \frac{P_m K_d^2}{K_m P_d^2} \quad (2.3)$$

and the "extraction constant" is dependent on the dissociation constants and distribution coefficients of dithizone and dithizonate.

When other complexing agents such as tartrate or citrate are present in the aqueous solution not all of the metal in the aqueous phase is in the form Me^{2+} ; but $[\text{Me}^{2+}]$ is dependent on the dissociation constant of the complex and the concentration of the complexing agent. If the concentration of the latter is relatively high it may be assumed to be constant and

$$[\text{Me}^{2+}]_w = \bar{Z}[\text{Me}]_w \times \text{constant} \quad (2.4)$$

where $\bar{Z}[\text{Me}]_w$ represents the total concentration of metal in the aqueous phase in all its forms. Hence when tartrate or citrate is present (2.1) becomes

$$\frac{[\text{Me Dz}'_2]_o}{[\text{Me}^{2+}]_w} = \frac{K'[\text{Dz}]_o^2}{[\text{H}^+]} \text{----- (2.5)}$$

where K' is a constant for a particular concentration of the complexing agent.

Strictly speaking, activities rather than concentrations should be used and the value of the extraction constant is therefore affected by variation in the ionic strength of the solution.

This simple treatment of dithizone extraction equilibria has been criticised by Oosting⁽³³⁾ who has proposed more general equations governing the extraction and by Irving and Williams⁽³⁴⁾. An exacting proof of any such equation would require the extraction to be made under rigidly controlled conditions which could not be maintained in a routine analysis method. Using the results obtained here, however, it was possible to prove the practical applicability of the equation(2.1).

Points on the steep part of each curve were selected for the calculation of the extraction constant. Due to the relative inaccuracy of the methods used for the determination of the lower metal concentrations the lower parts of the curves could not be expected to give good results. Where possible points were selected at pH values below 8, as in more alkaline solutions the position becomes somewhat complicated by the increasing solubility of dithizone in the aqueous phase[as noted by Biefield and Patrick⁽²⁹⁾.]

Calculated values of K for the extraction of nickel, cobalt and zinc are shown in Tables I, III and V, while Tables II and IV illustrate the use of the equation (2.1) for predicting the effect of varying Dz concentration.

TABLE I
Calculation of K for Extraction of Nickel

Curve	pH	% Extracted	K
1	8.5	51.6	.55x10 ⁻⁸
1	8.0	20.0	.57x10 ⁻⁸
2	8.0	60.0	1.01x10 ⁻⁸
3	8.0	52.2	1.27x10 ⁻⁸
4	8.0	40.5	0.91x10 ⁻⁸

As the steep portion of curve 1 lies in the pH region above 8, values for K from this curve were neglected.

Mean K for curves 2,3,4, = 1.06x10⁻⁸.

TABLE II

Calculation of points on the curves assuming
that $K = 1.06 \times 10^{-8}$

Curve	% Extracted	Calculated pH	pH found
1	70	8.72	8.79
1	40	8.19	8.34
2	70	8.21	8.17
2	40	7.76	7.68
3	70	8.29	8.27
3	40	7.88	7.81
4	70	8.37	8.45
4	40	7.96	8.00

TABLE III

Calculation of K for the Extraction
of Cobalt

Curve	pH	% Extracted	K
1	8.0	71.5	3.40×10^{-7}
1	7.5	44.5	3.56×10^{-7}
2	8.0	96.5	3.11×10^{-7}
2	7.5	90.6	3.01×10^{-7}
2	7.0	35.0	2.26×10^{-7}

Mean K, 3.07×10^{-7}

TABLE IV

Theoretical Calculation of Position of Curve 2 from
Curve 1 - Cobalt K (from curve 1), 3.48×10^{-7}

% Extract	Calculated pH	pH Found
40	7.00	7.07
70	7.36	7.50

TABLE V

Calculation of K for the Extraction of Zinc

pH	% Extracted	K
7.0	79.5	5.38×10^{-5}
6.5	53.0	5.50×10^{-5}

Mean K, 5.4×10^{-5}

Conclusions

At pH 9.5, using only a small excess of reagent, over 90 per cent extraction was obtained in each case. It was therefore concluded that zinc, nickel, cobalt, copper and, from the results reported by Biefield and Patrick, lead may be quantitatively extracted from tartrate solutions at this pH. Dithizone extractions of these metals from tartrate solutions for spectrographic analysis were therefore carried out at pH 9.5 only.

The results demonstrated in curves 3 and 4 of Figure 1 for nickel indicates, with support from the theoretical calculation, that more efficient extraction may, within certain limits, be obtained by using the same weight of dithizone in a smaller volume of chloroform. These results also show the importance of stating all the experimental conditions when reporting work of this nature.

For nickel and cobalt the expression (2.1) may be used to predict the position of the pH-extraction curve for other dithizone extractions and volumes of extractant after K has been determined. Above pH 8, however, due to increasing solubility of dithizone in the aqueous phase, the relationship cannot be expected to apply.

2. The Influence of pH on the Dithizone Extraction of Zinc, Lead, Cobalt, Nickel, Copper, Cadmium and Silver from Citrate Solutions.

As the development of suitable analytical methods progressed, it was found that citrate was more efficient than tartrate in retarding the precipitation of iron and aluminium hydroxides. The dithizone extraction of zinc, lead, cobalt, nickel, copper, cadmium and silver from solutions containing citrate was therefore investigated. (The latter two elements were used as internal standards in the spectrographic analysis of plant material).

Experimental

(i) Apparatus: As described in Section 1, above.

(ii) Solutions

Pb, prepared from analytical grade lead nitrate
Cd, prepared from analytical grade cadmium sulphate and
Ag, prepared from analytical grade silver nitrate. The rest of the metal solutions were prepared as described in Section 1.

(iii) Dithizone

0.02% (weight to volume) in pure chloroform.

(iv) Procedure

The procedure followed was similar to that described in the previous section.

Measured volumes of ammonium citrate, standard metal solution, water and hydrochloric acid or ammonia were run into a 200 ml. separatory funnel so as to give 100 ml. of aqueous solution of the required pH. This was then agitated vigorously with 20 ml. of dithizone solution for two minutes, after which the chloroform phase was separated and the pH of the aqueous phase determined.

Zinc: In the case of zinc a volume of standard solution equivalent to 50 μg of the metal was used. The extraction was carried out using 20 ml. of 0.005% dithizone (weight to volume) and the zinc content of the extract was determined colorimetrically as described above (A.1). Extractions were carried out from solutions containing both 8% and 2% ammonium citrate.

Lead: Lead was extracted from both 8% and 2% ammonium citrate solutions containing 125 μg of the metal with 20 ml. of 0.005% dithizone. The lead content of the metal extract was determined colorimetrically. A mixed colour method was used, transmission measurements being made at 510 $\text{m}\mu$ and 610 $\text{m}\mu$, corresponding to a maximum and minimum in the transmission curve for lead dithizonate. From the reading at 610 $\text{m}\mu$ a correction was made at 510 $\text{m}\mu$ for the presence of excess dithizone. It was necessary to make a blank correction at each pH.

Cobalt and Nickel: 50 μg of each of these metals were extracted in accordance with the concentration method eventually proposed. Extractions were made from 8% ammonium citrate solution with 20 ml. of 0.02% dithizone. The metal content of the extract was determined spectrographically, the extract being evaporated onto graphite and burned in the arc.

Copper: Both 8% and 2% ammonium citrate solutions containing 100 μg of this element were extracted with 20 ml. of 0.01% dithizone and with 20 ml. of 0.02% dithizone. The copper content of the extract was determined flame photometrically as already described (II A.1).

Cadmium. In the extraction of this metal from 2% and 8% ammonium citrate solution 20 ml. of 0.005% dithizone were used and the metal content of the extract determined

colorimetrically by a mixed colour method, the optical density of the diluted extract being determined at 518 m μ and correction made for the excess dithizone by making a blank extraction at each pH value.

Silver. The extraction of silver has apparently not been investigated over a wide pH range previously. Since silver is normally present only in exceedingly low concentrations in plant material, the extraction of this metal was investigated for as low a concentration as could conveniently be determined colorimetrically, namely 30 μ g. 8% ammonium citrate solutions containing 2.5% ammonium chloride and this amount of silver, were extracted with 20 ml. of 0.02% dithizone and the metal content of the extract was determined colorimetrically after removal of most of the excess dithizone by extraction with dilute ammonia solution. Optical density measurements were made at 500 m μ which spectral transmission measurements showed to be the optimum wave length.

Results

The results are shown graphically in Figure 4. In the case of cadmium, lead and zinc no difference was found in the extraction from 2% and 8% ammonium citrate solution and the points are therefore not shown separately.

The extraction curve for silver supports the findings of Friedeberg⁽³⁵⁾. Under the conditions of the experiment, which approximate to those prevailing in the analysis method for plant material, this metal is quantitatively extracted from approximately pH3.5 to pH7, the efficiency of extraction dropping off slowly at higher pH values.

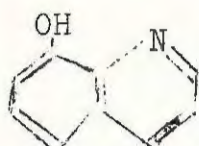
Cadmium appears to be quantitatively extracted between pH7 and pH10 and lead and zinc between pH8.5 and pH10. Under the experimental conditions 98% of the cobalt was extracted at pH9.5 and 95% of the nickel at pH9.75.

The extraction of copper seems to be somewhat retarded in the presence of citrate, particularly in the pH range 4-6, and rather erratic results were obtained for the extraction of this metal. For this reason the extraction curve for copper is shown as a broken line.

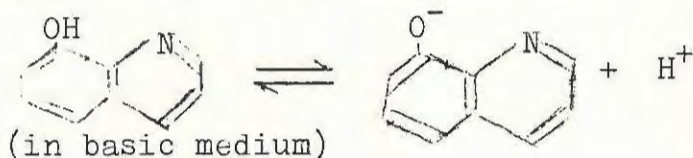
B. OXINE

(8-Hydroxyquinoline)

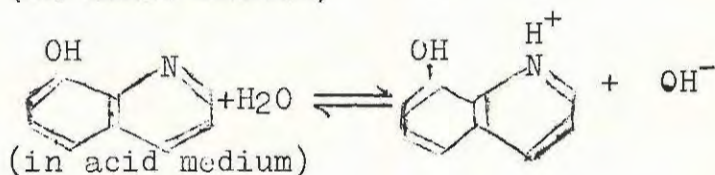
8-Hydroxyquinoline, commonly known as "Oxine", which has the structural formula,



reacts with most metals to form chelate complexes which are sparingly soluble in water but in many cases soluble in chloroform. This compound can ionise in two different ways

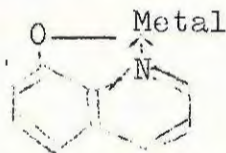


and



and pH therefore has an important influence on the way in which it reacts.

Most of its compounds are thought to have the structure



the number of oxine molecules attached to each metal atom being dependent on the valency and coordination number of the latter.

Because of the fact that different ionic forms are possible and the solubility of oxine in water varies markedly with pH⁽³⁶⁾, the theory of extraction is not as simple as in the case of dithizone.

1. The Extraction of Titanium, Vanadium and Molybdenum from Tartrate Solutions Using Oxine.

In the analysis method proposed by Gorbach and Pohl⁽⁸⁾, a group of trace metals including titanium, molybdenum, vanadium and manganese are extracted from aqueous solution, from which tartrate has been removed, with 0.1% chloroformic oxine, extractions being made at pH5 and pH6.5. The work of Moeller⁽³⁷⁾ who used oxine solutions of similar strength (0.01M=0.145%) indicates that even with repeated extraction the pH ranges of complete extraction are relatively narrow and Gentry and Sherrington⁽³⁸⁾ have recommended the use of

1% oxine which, as they reported, gives more rapid and complete extraction over wider pH ranges.

The latter authors have investigated the oxine extraction of a number of metals including manganese and molybdenum. Of these the former was extracted from tartrate, but it was reported that tartrate interfered with the extraction of molybdenum.

Since the destruction of the tartrate in the sample solution after dithizone extraction, as required by the method of Gorbach and Pohl,^{is tedious} it was decided to make an investigation of the extraction of titanium, vanadium and molybdenum from tartrate solution. As recommended by Gentry and Sherrington, a 1% solution of oxine was used.

Experimental

(i) Apparatus

Hilger Large Spectrograph and ancillary equipment described fully in Chapter III.

(ii) Solutions

Solutions of each metal were prepared from A.R. potassium titanium oxalate, "Specpure" vanadium oxide (V_2O_5) and specpure molybdenum oxide (MoO_3), the oxides being dissolved in sodium hydroxide solution which was subsequently acidified with HCl. These solutions were diluted to give a standard solution of the required concentration.

(iii) Procedure

5 ml. of a solution containing 10 p.p.m. Mo, 10 p.p.m. V and 60 p.p.m. Ti were pipetted into a separatory funnel. 10 ml. of 8.2% tartaric acid solution were added from a burette and sufficient ammonia (and NaOH for higher pH values) to bring the final pH to the desired value. The solution was then made up to 100 ml. by the addition of double distilled water. 10 ml. of 1.0% chloroformic oxine solution were then added and the funnel shaken vigorously for three minutes. After the phases had been allowed to separate the chloroform layer was run into a 50 ml. Phillips beaker, traces remaining in the funnel being washed down with small volumes of pure chloroform. The chloroform extract was evaporated with 100 mg graphite containing an internal standard and after drying and ashing at 450° , the metal content was determined spectrographically. The pH of the aqueous solution was determined with the help of a pH meter immediately after the extraction.

Results

The results of this investigation are shown graphically in Figure 5, together with the curve published by Gentry and Sherrington for the extraction of manganese.

It can be seen that, whereas Ti and V appear to be satisfactorily extracted by oxine in the presence of tartrate, the extraction of Mo is too much affected to allow of the extraction of the metal in the presence of tartrate.

2. The Separation of Iron from Titanium, Vanadium and Molybdenum by Oxine Extraction.

In the investigation described in the previous section (II.B.1) it was shown that tartrate appreciably suppressed the extraction of molybdenum by oxine. It was to be expected that citrate, which from the investigation of dithizone extraction apparently has a stronger complexing action, would suppress the extraction of molybdenum (and possibly vanadium) more completely, thus allowing the separation of iron from these metals. It was therefore decided to investigate oxine extraction in the presence of citrate.

Experimental

(i) Extraction of Titanium, Vanadium and Molybdenum.

20 ml. of 40% ammonium citrate solution were run into a separatory funnel and sufficient redistilled 5N HCl was added to give the desired pH. 5 ml. of a solution containing 10 p.p.m. of Ti, V and Mo and sufficient water to make the total volume 100 ml. were added. Next the solution was extracted twice by shaking for two minutes with 10 ml. of 1% oxine and the extracts run into a beaker. The contents of the beaker were then evaporated onto graphite containing the internal standards and their metal content determined spectrographically. The pH of the solution was measured with a pH meter immediately after the extraction had been completed.

This was repeated at different pH values in the range 1 - 5.

(ii) Extraction of Iron

For the investigation of the extraction of iron, 10 ml. of a 20 p.p.m. iron solution prepared from A.R. ferric chloride, were used for the extraction. The

Oxine Extraction of Ti, V, Mo and Mn from Tartrate Solution.

(Mn according to Gentry and Sherrington³⁸)

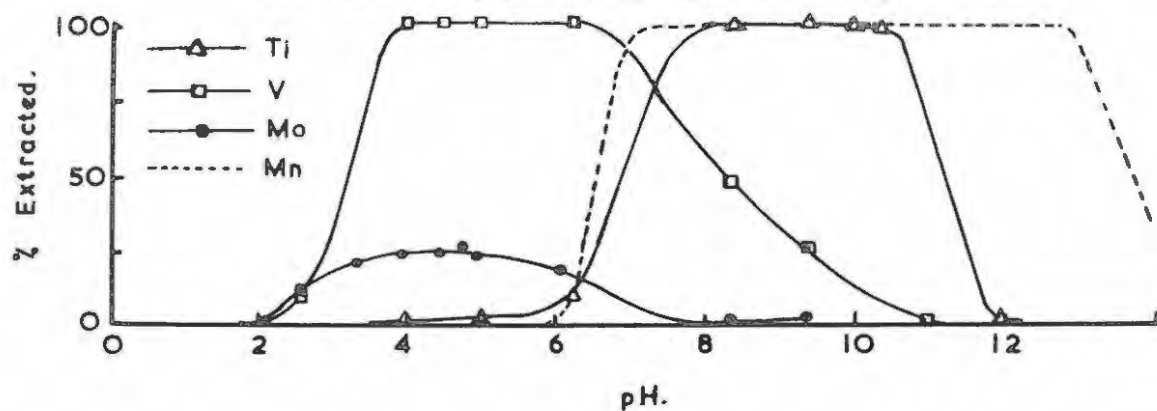


Figure 5.

Oxine Extraction of Fe, Ti, V, Mo.

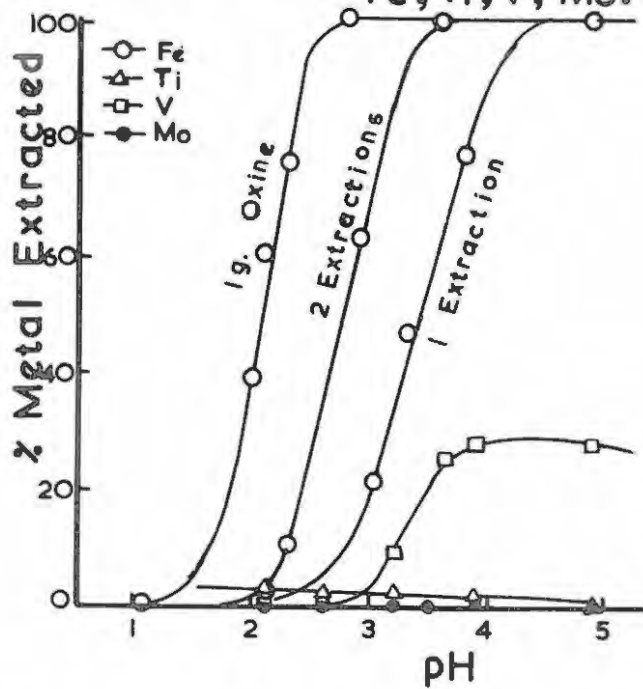


Figure 6.

procedure was similar to that followed for Ti, V and Mo except that, instead of evaporating the extract solution, this was run into a 50 ml. volumetric flask and diluted to that volume with pure chloroform. The iron content of the extract was determined spectrophotometrically by measuring the optical density at 470 m μ . In addition the extraction conditions for the iron were varied by making single as well as double extractions and extracting with 10 ml. of chloroform after the addition of 1 gram of oxine to the solution.

Results

From the results shown graphically in Figure 6, it was evident that ferric iron could readily be separated from molybdenum and titanium by oxine extraction in the presence of 8% citrate. The possibility of separating iron quantitatively from vanadium was, however, in some doubt, especially as it was apparent from the results of the various extraction methods for iron that appreciable quantities of the oxine were transferred to the aqueous phase and that, in consequence, the extraction equilibrium varied from one extraction to the next.

Oxine Extraction Equilibria - Variation of Distribution Coefficient with pH.

In order to assess the effect of this transfer, the following experiment was carried out.

Repeated extractions with 10 ml. of 1% oxine were made, using a solution containing ammonium citrate and hydrochloric acid only, and a 5 ml. portion of each extract was made up to 50 ml. with pure chloroform. The oxine content of the extract was then determined spectrophotometrically by measuring the optical density of this solution at 350 m μ and comparison with standards. This was done for three different pH values and the distribution coefficient for the system, oxine/chloroform/aqueous citrate solution, was calculated for each pH. The results are shown in Table VI. Graphs of the distribution coefficient K_D against pH and $\log K_D$ against pH were plotted and are shown in Figure 7. The results show a sharp increase in the solubility of oxine in the aqueous phase as the pH decreases. In spite of the very different experimental conditions, the results are of the same general form as those reported by Moeller and Pundsach⁽³⁶⁾, though the distribution was found to favour the aqueous phase more strongly, possibly due to the presence of citrate.

Influence of pH on Distribution Coeff.

k: Oxine/ CHCl_3 /Citrate Soln.

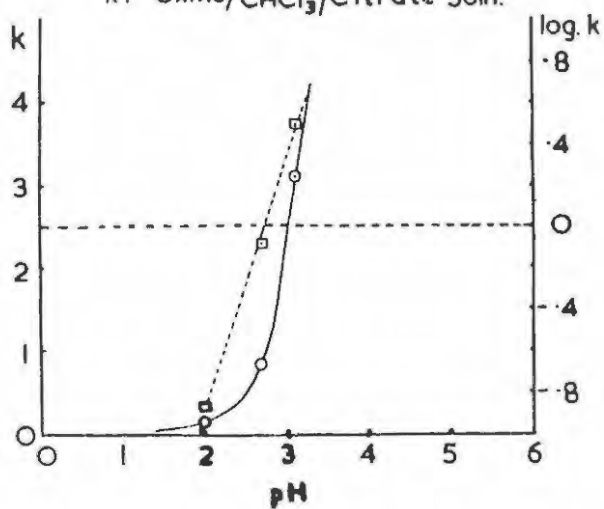


Figure 7.

Distribution of Oxine Between Aqueous and Organic Phase for Repeated Extraction at pH 3.

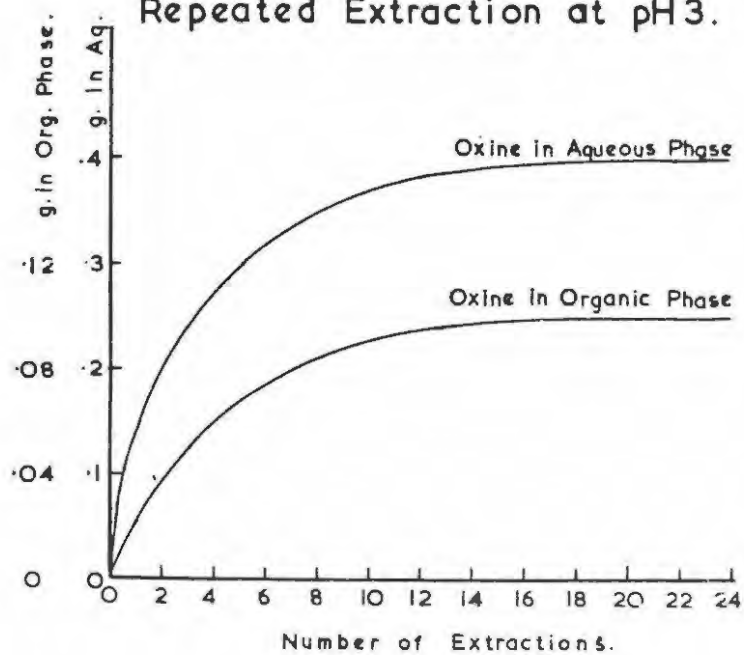


Figure 8.

TABLE VI

Variation of Distribution Coefficient of Oxine
with pH

pH	Extraction.	D	Oxine Concn. %	W _o Wt in CHCl ₃ g.	W _x Wt in H ₂ O g.	K_D $= \frac{W_o \times 100}{10 W_x}$
2.00	1	.048	0.0011	.0011	.0989	0.111
"	2	.090	0.0023	.0023	.1966	0.122
"	3	.145	0.0040	.0040	.2926	0.137
"	4	.225	0.0063	.0063	.3863	0.162
"	5	.263	0.0074	.0074	.4789	0.154
2.68	1	.270	0.0076	.0076	.0924	0.822
"	2	.510	0.0147	.0147	.1777	0.828
"	3	.720	0.0209	.0209	.2568	0.814
"	4	.940	0.0281	.0281	.3287	0.854
3.10	1	.800	0.0234	.0234	.0766	3.05
"	2	1.25	0.0420	.0420	.1346	3.12
"	3	1.42	0.0563	.0563	.1783	3.14

pH	K_D (mean)	log. K.
2.00	0.137	-0.863
2.68	0.829	-0.081
3.10	3.10	0.491

It was evident that a large number of extractions with 10 ml. of 1% oxine would be necessary before constant equilibrium conditions were approached. Figure 8, based on the distribution coefficient, read from Figure 7, shows how the distribution of oxine between aqueous and chloroform phase would vary with the number of extractions.

From Figure 6 the separation of iron from vanadium at pH3 appeared to be a distinct possibility and, from the following theoretical considerations, it appeared that it would be possible to remove the iron from the sample solution, while retaining 90% of the vanadium, by oxine extraction at this pH.

The extraction proceeds by the following steps:-

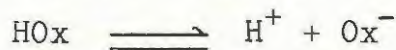
- (1) Oxine is transferred to the aqueous phase, the extent of this process being governed

by the distribution coefficient for oxine,

$$\frac{[\text{HOx}]_o}{[\text{HOx}]_w} = K_D \quad \text{-----} \quad (2.6)$$

where HOx represents unionised oxine and subscripts "o" and "w" label the concentrations in the organic and aqueous phase respectively.

(2) The oxine in the aqueous phase ionises



In acid solution we also get



We have thus:

$$\frac{[\text{H}^+][\text{Ox}^-]}{[\text{HOx}]_w} = K_I'$$

and
$$\frac{[\text{H}^+][\text{HOx}]}{[\text{HOx.H}^+]} = K_I''$$

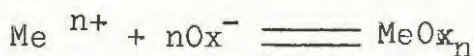
whence⁽³⁹⁾
$$[\text{Ox}^-] = \frac{\Sigma[\text{HOx}]_w}{1 + \frac{[\text{H}^+]}{K_I K_I'} + \frac{[\text{H}^+]}{K_I}} \quad \text{-----} \quad (2.7)$$

where $\Sigma[\text{HOx}]_w$ is the total concentration of oxine in the aqueous phase in all its forms.

At any chosen pH (2.7) becomes:

$$[\text{Ox}^-] = \frac{\Sigma[\text{HOx}]_w}{\text{constant}} = \frac{\Sigma[\text{HOx}]_w}{K_I} \quad \text{-----} \quad (2.8)$$

(3) The oxinate is formed by association of metal and oxinate ions:



for which we have:

$$\frac{[\text{MeOx}_n]_w}{[\text{Me}^{n+}][\text{Ox}^-]^n} = K_A \quad \text{-----} \quad (2.9)$$

(4) The metal oxinate is then transferred to the chloroform phase, a process governed by the distribution coefficient for the complex:

i.e.
$$\frac{[\text{MeOx}_n]_o}{[\text{MeOx}_n]_w} = K_p \quad \text{-----} \quad (2.10)$$

From (2.9) and (2.10) we get:

$$\frac{[\text{MeOx}_n]_o}{[\text{Me}^{n+}][\text{Ox}^-]^n} = K_p \cdot K_A \quad \text{-----} \quad (2.11)$$

and from (2.8) and (2.11) we get:

$$\frac{[\text{MeOx}_n]_o}{[\text{Me}^{n+}] [\text{HOx}]_w^n} = \frac{K_p K_A}{K_I} = \text{constant} = K \text{ ---- (2.12)}$$

The total metal concentration in the aqueous phase is taken as $[\text{Me}^{n+}]$. This simple treatment neglects the formation of intermediate oxinates and therefore cannot be expected to lead to accurate results. In addition, for simplicity, concentration is used instead of activity.

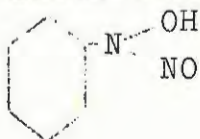
Calculation on this basis for the extraction of iron (FeOx_3) at pH 3 ($[\text{MeOx}_n]_o$ being obtained from Figure 6) gave $K=1.31 \times 10^9$ for the single extraction and $K=1.73 \times 10^9$ on the basis of the double extraction. K for vanadium which is reported to form an oxine complex of formula $\text{VO}(\text{OH})\text{Ox}_2$ ⁽³⁹⁾ was found to be 1.96×10^4 , calculated on the same basis from the value of 2.5% extraction read from the vanadium extraction curve (Figure 6).

If the sample solution were to contain 1000 μg of iron and 10 μg of vanadium, then the loss of oxine due to metal complex formation can be regarded as negligible and, on the basis of the above theory, assuming K for iron to be 1.5×10^9 , if three extractions were made with 10 ml. of 1% oxine, 93.1% of the iron would be removed and 5.9% of the vanadium, while four extractions would remove 99.1% of the iron and 10.5% of the vanadium. This result was borne out by later experiments. (III.A.3b).

C. CUPFERRON

(AMMONIUM-N-NITROSOPHENYL-HYDROXYLAMINE)

The Extraction of Titanium, Vanadium and Molybdenum



An examination of the literature led to the selection of cupferron as the reagent most likely to give quantitative extraction of titanium, vanadium and molybdenum from citrate solution. This reagent forms chloroform soluble chelate complexes in mineral acid solution with a large number of elements, but becomes more selective as the pH is reduced.

The method used was based on that described by Piper and Beckwith⁽⁴⁰⁾ for the separation of molybdenum. These authors indicated that molybdenum was extracted quantitatively

from 1.3% citrate solution at pH values below 1.0. Meunier⁽⁴¹⁾ has reported that titanium, vanadium and molybdenum are precipitated by cupferron from solutions more acid than pH 1.8, but no detailed study of the chloroform extraction of the cupferron complexes of these metals has been reported. It was therefore desirable to ascertain the optimum pH for their extraction.

Experimental

The metal solutions were prepared for extraction exactly as described in the previous section. Thus each solution of total volume 100 ml. contained 50 µg of each of the three metals, titanium, vanadium and molybdenum, 8% ammonium citrate and sufficient hydrochloric acid to give the desired pH. To each solution was added 1 ml. of a freshly prepared and filtered 6% aqueous solution of cupferron. The cupferrates were then extracted by agitating the solution for two minutes with 10 ml. of pure chloroform. The amount of each metal in the extract was determined spectrographically.

Results

The results of this investigation are presented graphically in Figure 9. As reported by Piper and Beckwith, the efficiency of extraction of molybdenum was found to increase with reduction of pH below 1. The efficiency of extraction of titanium was found to increase less rapidly with decrease in pH, while the extraction of vanadium fell off below pH 2. On the basis of the results it was decided that titanium, vanadium and molybdenum should be removed from the sample solution by a double extraction at about pH 2 and a further double extraction at pH 0.5.

D EXTRACTION METHOD

In a routine analysis method in which a number of solvent extractions have to be carried out, it is of advantage if mechanical agitation can be used, so that batches of six or more samples may be dealt with simultaneously. Mechanical shakers have advantages for certain extractions, but few produce sufficiently intimate a mixing of the solvent phases to allow of a rapid extraction. From a practical point of view a mechanical shaker is inconvenient because it necessitates repeated transfer of the funnel from stand to shaking machine.

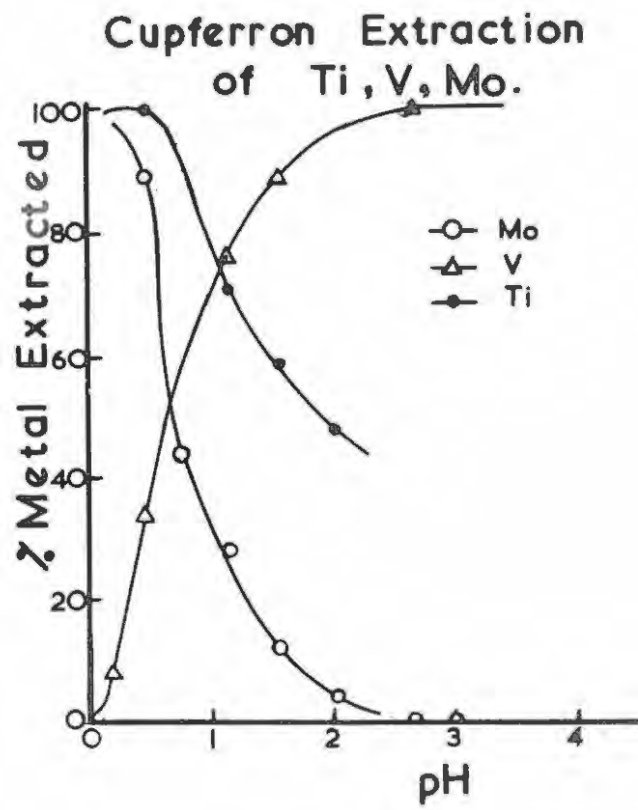


Figure 9.

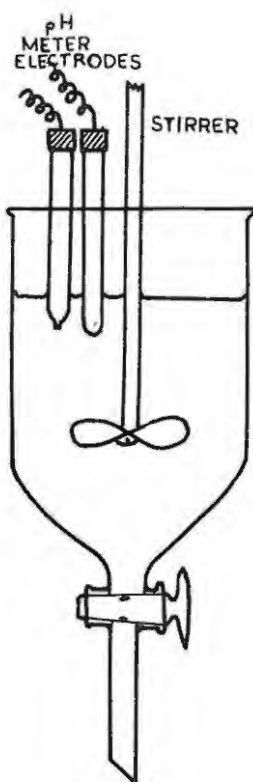


Figure 10.
Extraction
Apparatus

In addition, when a series of extractions have to be made from the same solution, numerous additions and accurate pH adjustments have to be made.

For these reasons it was decided to investigate the possibility of using open-topped separatory funnels with mechanical stirrers to produce the mixing of the solvents. This system was found to produce perfectly satisfactory extraction of the elements, while allowing easy access to the solution of pH meter electrodes and an apparatus was constructed* consisting of a bank of six electrically driven glass stirrers, for each of which there was a clamp to hold the separatory funnel. The extraction apparatus is shown in Figure 10.

* designed by the author and constructed in C.S.I.R. Workshop.

CHAPTER III

THE CHEMICAL CONCENTRATION AND SPECTROGRAPHIC
DETERMINATION OF Zn, Co, Ni, Pb, Ti, V and Mo
IN PLANT MATERIAL

A. PREPARATION OF SAMPLE FOR SPECTROGRAPHIC ANALYSIS -
DEVELOPMENT OF METHOD

1. Preparation of Sample Solution

The first step in the chemical preparation of the sample for analysis is to bring the trace elements into solution. Before this can be done organic matter has to be destroyed. This can be accomplished either by dry ashing in a muffle furnace, or by wet oxidation with nitric and perchloric acid.

The dry ashing method has the advantage of simplicity and no reagents which might contain trace impurities need to be added. Critics of the method⁽⁴²⁾ complain that it is lengthy, that some elements can be lost by volatilisation and that others are lost by adsorption on the walls of the vessel and on the silica of the sample itself.

Wet oxidation methods have the advantage of speed where small samples are used, while the danger of losses of metals due to adsorption and volatilisation is much reduced. On the other hand, the purification of reagents, particularly perchloric acid, presents a problem when large samples have to be used. It is for this reason that Mitchell⁽⁴³⁾ prefers the dry ashing method.

It has been found during the work described in this thesis that wet oxidation methods were very suitable for small samples of up to two grams, but that the dry ashing method was more convenient when bigger samples (5g - 20g) had to be used. A dry ashing method was therefore used for the preparation of all samples in the work described in this chapter.

It has been found that, if the ashing is carried out at temperatures above 450°C, there is a danger of losing zinc from the sample by volatilisation, but at lower temperatures no losses occur⁽⁷⁾. Care was therefore taken to insure that the muffle furnace temperature never exceeded 450°C during the ashing of the samples.

In the preliminary experiments the method of preparation of the sample solution from the ash was that originally described by Mitchell⁽³⁾. In this method the

ashed sample is fused with 4 grams of sodium carbonate and the melt taken up in hydrochloric acid. This solution is then evaporated to dryness, taken up in 1:2 hydrochloric acid and filtered to remove silica.

Mitchell⁽⁴³⁾ has since advocated a modified form of the above procedure in which the ashed sample is extracted with hydrochloric acid, the residue filtered off, reashed, fused with 1 gram of sodium carbonate in platinum and dissolved in hydrochloric acid, any silica remaining being filtered off. This modified procedure was adopted for later work and was used in the tests of the analysis method finally adopted.

Alternative methods of treating the ashed sample were compared with Mitchell's original method in the Onderstepoort laboratory⁽⁴⁴⁾. These included the method described by Piper⁽⁴⁵⁾ in which the ash is initially extracted with hydrochloric acid and the residue treated with hydrofluoric and sulphuric acids, and methods involving the treatment of the ash with perchloric and hydrofluoric acid, and perchloric, hydrofluoric and sulphuric acid. No significant difference was found on analysing sixteen sample solutions, four solutions being prepared by each of the above methods, for cobalt, molybdenum and zinc.

2. Study and Modification of the Chemical Concentration Method of Gorbach and Pohl.

The method for the concentration of trace elements which was proposed by Gorbach and Pohl⁽⁸⁾ has been briefly described in Chapter 1 of this thesis. Although it is long and somewhat complicated, its use for the analysis of plant material was shown by van Rooyen⁽¹⁰⁾ to be feasible and it was therefore proposed initially to make a study of this method, modifying it where improvement could be made. Only the elements zinc, cobalt, nickel, lead, titanium, vanadium, molybdenum, manganese and copper were considered.

The method is described in some detail below and outlined in the form of a "flow diagram" in Figure 11.

(i) Benzoate Precipitation

To the hydrochloric acid solution of the sample prepared by acid extraction of the ash, add 10 ml. of 10% ammonium acetate and sufficient 10% ammonium benzoate solution (20 ml are equivalent to 65 mg Al and 125 mg. Fe.) Adjust the pH to 3.8 - 4.0 with 5N NH₄OH (dropwise - spotting on indicator paper). Boil for two minutes and filter hot, washing with water containing 1% ammonium

benzoate and acetic acid.

(ii) Separation of Iron

Dissolve the benzoate precipitate in a little warm hydrochloric acid and transfer the solution to a 100 ml. separatory funnel, washing with hydrochloric acid, finally adding sufficient concentrated hydrochloric acid to make the solution 6.5 N with respect to HCl (8N if isopropyl ether is to be used). Extract twice with 25 ml. of ether. Evaporate the aqueous phase after ether extraction on a water bath and transfer the residue, with double distilled water, to the filtrate from the benzoate precipitation.

(iii) Dithizone Extraction

To the above mentioned aqueous sample solution from which iron has been removed, add 10 - 20 ml. of 10% ammonium tartrate and 5N ammonia dropwise, until a pH of 7 is reached. (Bromothymol blue or indicator paper). Extract with an 0.01% (weight to volume) chloroform solution of dithizone repeatedly until the organic phase remains green. Increase the pH to 9 by adding more ammonia and extract again with dithizone. Finally acidify the solution and remove the excess dithizone by extraction with pure chloroform. Retain the united extract for Spectrographic analysis.

(iv) Destruction of Tartrate

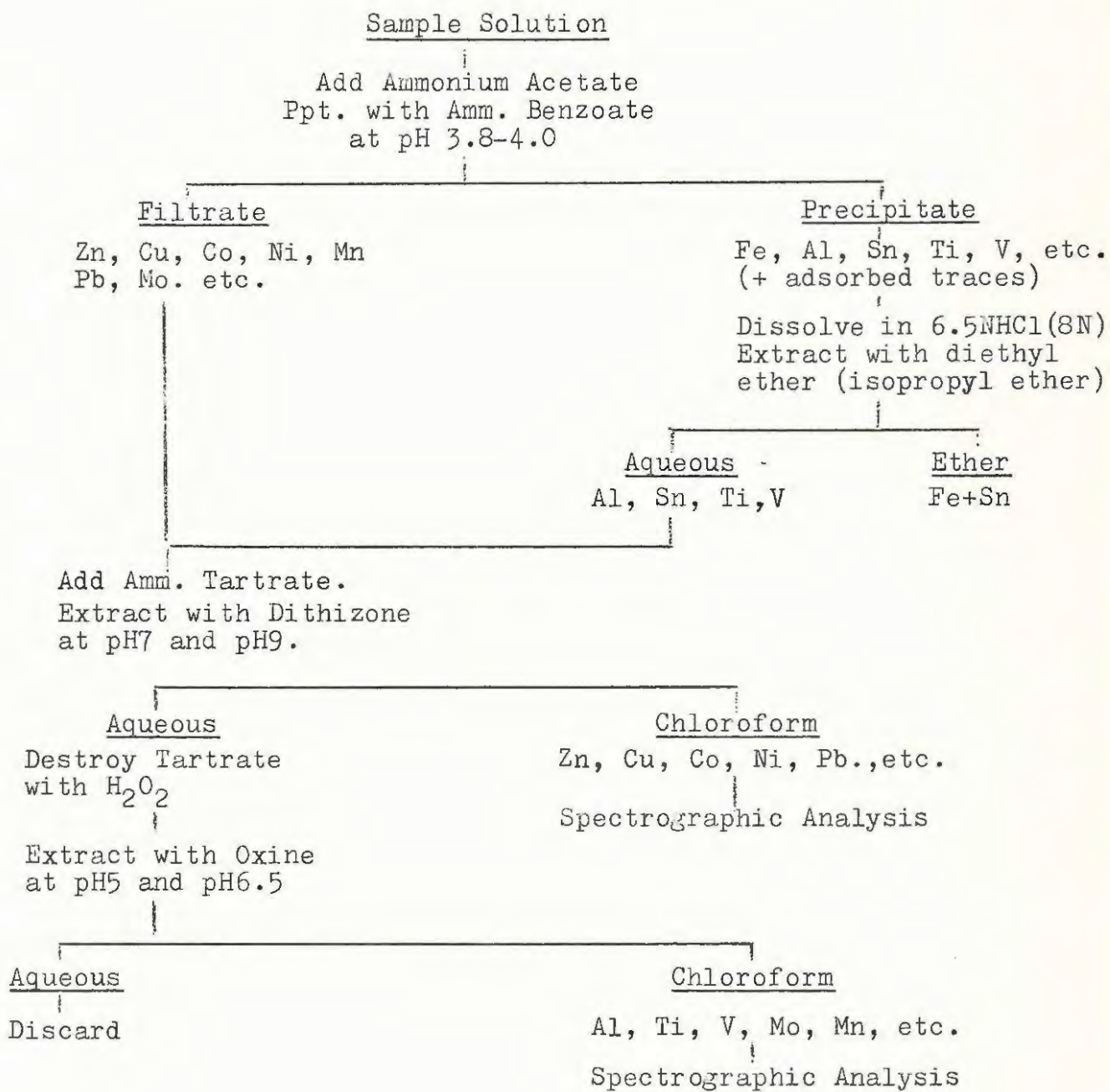
Transfer the aqueous solution after dithizone extraction to a beaker. Add cupric ions (3 ml. of 0.1% cupric chloride) as catalyst and destroy the tartrate by repeated oxidation with 30% hydrogen peroxide. Boil for fifteen minutes and return to the separatory funnel.

(v) Oxine Extraction

Adjust the pH of the solution to 5 (indicator paper) with 5N ammonia and extract with oxine (0.1% solution in chloroform) until the extract remains colourless. Repeat at pH 6.5. Retain the united oxine extract for spectrographic analysis.

FIGURE 11

GRAPHICAL OUTLINE OF THE CONCENTRATION
METHOD OF GORBACH AND POHL



(a) Modification of the Dithizone Extraction Procedure.

One of the main criticisms of the method of Gorbach and Pohl is its length and the number of steps involved. A short cut which immediately suggested itself was to carry out the dithizone extraction at one optimum pH instead of repeated extraction at pH7 and 9. To test this possibility the investigation of the extraction of dithizonates from tartrate solution, described in Chapter II, was carried out. As a result of this work, the method was modified to include dithizone extraction at pH 9.5 only.

(b) Removal of Iron by Direct Ether Extraction.

One of the more serious criticisms of the method of Gorbach and Pohl has to do with the benzoate precipitation and removal of iron.

The benzoate precipitate of Fe, Al, Sn, Ti, V, etc., was found to settle as a bulky gelatinous mass which was not easily separated even when a filter-stick was used, as proposed by Gorbach and Pohl. Precipitation, as a method for the separation of unwanted elements, is normally to be shunned in trace analysis and undoubtedly a large proportion of the wanted trace elements are adsorbed on the precipitate. According to the description of the method, these are recovered after the ether extraction of the iron, but any molybdenum adsorbed on the precipitate is liable to be quantitatively extracted with the iron, while traces of zinc and copper would probably be lost too. A trace of vanadium is also lost during the separation of iron.

It appeared that the method could be considerably shortened if, instead of precipitating with benzoate, iron was removed directly by ether extraction from the sample solution as done by Perrin⁽⁴⁶⁾ and the metals coextracted (particularly Mo) recovered by use of some other suitable reagent.

A solution of a tobacco leaf sample was therefore prepared in concentrated hydrochloric acid (8 N) and extracted repeatedly with 20 ml. volumes of isopropyl ether (Isopropyl ether has been shown by Dodson, Forney and Swift⁽⁴⁷⁾ to be better than diethyl ether for the extraction of iron). The ether extract was evaporated onto graphite and qualitatively analysed by a spectrographic method.

Apart from a large amount of iron, the ether

extract was found to contain titanium, aluminium and a trace of vanadium. No molybdenum was found, but it was uncertain whether this was due to its not being extracted, or to its extremely low concentration in the sample. The presence of titanium and aluminium was unexpected, as these are not extracted by diethyl ether, and their presence must be attributed either to the difference in the extracting reagent or to entrainment of some of the aqueous solution with the ether.

Generally the method was found to be unsatisfactory. A relatively large volume (50 ml.) of concentrated (11 N) hydrochloric acid had to be used to dissolve the sample, which would mean correspondingly large amounts of ammonia to neutralise it before further extractions could be carried out. In addition, the ether extraction was found to be time-consuming and inefficient due to the tendency to form an emulsion. This line of research was therefore dropped.

(c) Oxine Extraction of Titanium, Vanadium, Molybdenum and Manganese.

Another rather tedious step in the original procedure is the destruction of tartrate before the oxine extraction. It is stated by Gorbach and Pohl that tartrate hinders the formation of oxinates of some of the metals, while Gentry and Sherrington⁽³⁸⁾ report that tartrate interferes with the extraction of molybdenum. As the extent of the interference was not known, it was decided to investigate the extraction of titanium, vanadium and molybdenum from tartrate solution. 1% oxine, as recommended by Gentry and Sherrington, was used.

From the results of this investigation, reported in Chapter II, it was apparent that while Ti, V and Mn could be quantitatively extracted in the presence of tartrate, molybdenum could not.

(d) An Initial Dithizone Extraction - The Use of Ammonium Citrate.

Since dithizone does not react with Ferric iron and almost all of the iron in the sample could be expected to be in the ferric state, dithizone extraction of zinc, copper, cobalt, nickel and lead was a logical first step in the concentration procedure and would avoid any losses of these elements during the separation of iron.

Preliminary experiments showed that 1% ammonium

tartrate in the solution, as used previously, was insufficient to prevent the precipitation of iron above pH 8 and that even in the presence of 6% of this complexing agent, precipitation took place at pH 8.5. Citrate was found to be more efficient in retarding the precipitation and it was found that, with 8% ammonium citrate in the solution, most sample solutions showed no sign of precipitation up to pH 9.5. With some samples, a light precipitate began to settle out slowly at this pH, but this did not appear to interfere with the extraction.

As a result of these observations, the dithizone extraction of Zn, Co, Ni, Pb and Cu from 8% citrate solutions was investigated, as reported in Chapter II.

(e) Separation of Iron by Oxine Extraction.

It was apparent from the observations reported above and the higher pH values necessary for the dithizone extraction of some metals in its presence, that citrate had a stronger complexing effect than tartrate. The possibility was therefore considered that 8% citrate would inhibit the oxine extraction of molybdenum and vanadium sufficiently to allow of a quantitative separation of iron from these metals.

As a preliminary test of this possibility, a sample solution prepared from 10 gm. of tobacco, to which was added 20 ml. of 40% ammonium citrate, was extracted first with 0.02% chloroformic dithizone at pH 9, and then with 1% oxine at pH 2.2, 3.2, 4.7 and 7.8. A qualitative spectrographic analysis was carried out on each of the extracts. The results of this analysis are shown in Table VII.

TABLE VII
TRIAL EXTRACTION OF SAMPLE SOLUTION

Extractant	Dithizone	Oxine			
	pH 9.5	2.2	3.2	4.7	7.8
Zn	Present	-	-	-	-
Ni	Strong	-	Trace*	Trace*	Trace*
Co	Strong	-	-	-	-
Pb	Strong	-	-	-	-
Ti	-	-	-	Strong	-
V	-	-	-	Weak	-
Mo	-	-	-	Weak	-
Mn	-	Sl.Trace	Trace	-	Strong
Fe	-	Trace	v.Strong	Trace	-

*Probably due to incomplete extraction by dithizone, as this sample contained a very high concentration of nickel.

The conclusions drawn from the results of this experiment were:-

- (i) Iron could be separated from titanium, vanadium and molybdenum by oxine extraction from citrate solution at about pH 3.
- (ii) Manganese is not extracted by dithizone from strong citrate solution at pH 9.5.

This experiment led to the full investigation of the possibility of separating iron by oxine extraction, which has already been described.

(f) Cupferron Extraction of Titanium, Vanadium and Molybdenum.

As has already been described, the answer to the problem of extracting molybdenum and vanadium from citrate solution was found in cupferron. The extraction of molybdenum from citrate solutions with this reagent has been described by Piper and Beckwith⁽⁴⁰⁾ and, after an examination of the pH extraction relationships for all three metals, the method was extended to include titanium and vanadium.

3. A Proposed New Chemical Concentration Scheme.

(a) Outline of Analysis Scheme

On the basis of the knowledge gained of the reagents, a new concentration scheme was proposed. This consisted of dithizone extraction at pH 9.5 to separate the dithizonates of zinc, cobalt, nickel and lead (and copper), followed by oxine extraction at pH 3 to remove iron and cupferron extraction at pH 2 and pH 0.5 to separate titanium, vanadium and molybdenum.

As copper and manganese can both be satisfactorily determined directly on the sample ash, their inclusion in the concentration scheme was relatively unimportant, but nevertheless some attention was given to the possibility of determining the concentrations of these elements as part of the general scheme.

As shown in Figure 4, copper can be extracted with dithizone together with the other metal dithizonates at pH 9.5. Manganese, according to the test extraction of a sample solution described above, is not extracted by dithizone from citrate solution at pH 9.5, but can

be extracted with oxine at pH 8. If concentrated to the same extent as the other elements, however, both of these elements would be present at inconveniently high concentration levels in the final extract. In the case of copper, the lines at 3247A and 3274A would both be too dense to allow of their measurement with those of the other elements and there is no suitable weaker spectrum line. One of the weaker lines of the manganese spectrum could be used for the determination of this metal, but the sample spectrum would be unnecessarily complicated by its presence in high concentration.

The alternative is to extract these metals separately, copper being separated by an initial extraction with dithizone at pH 3, and manganese by oxine extraction at pH 8. A suitable aliquot of each of their extracts could then be added to the combined extracts of the other metals, or alternatively, their concentrations in the extracts could be determined by some other convenient method such as flame photometry. Both of these techniques were tried with some success in the case of manganese and quite satisfactory results were obtained in the flame photometric analysis for copper as part of the scheme.

The proposed new scheme is outlined graphically below. (Figure 12)

(b) Experimental Trial of the Concentration Scheme.

As a preliminary test of the concentration scheme, a sample of lucerne was analysed for zinc, nickel, cobalt, lead, titanium, vanadium, molybdenum and manganese. (The flame photometric determination of copper was introduced after this initial test).

Procedure

The sample solution was prepared by ashing the sample at 450°C, sodium carbonate fusion and solution of the melt in hydrochloric acid. (i.e. according to the method originally described by Mitchell⁽³⁾). 20 ml. of 40% ammonium citrate were then added and the pH adjusted to 9.5 by the addition of redistilled 1:1 ammonium hydroxide solution. The following extractions were then made, further pH adjustments being made by the addition of ammonia or hydrochloric acid solution as required.

Flow-sheet Diagram of the Concentration Method

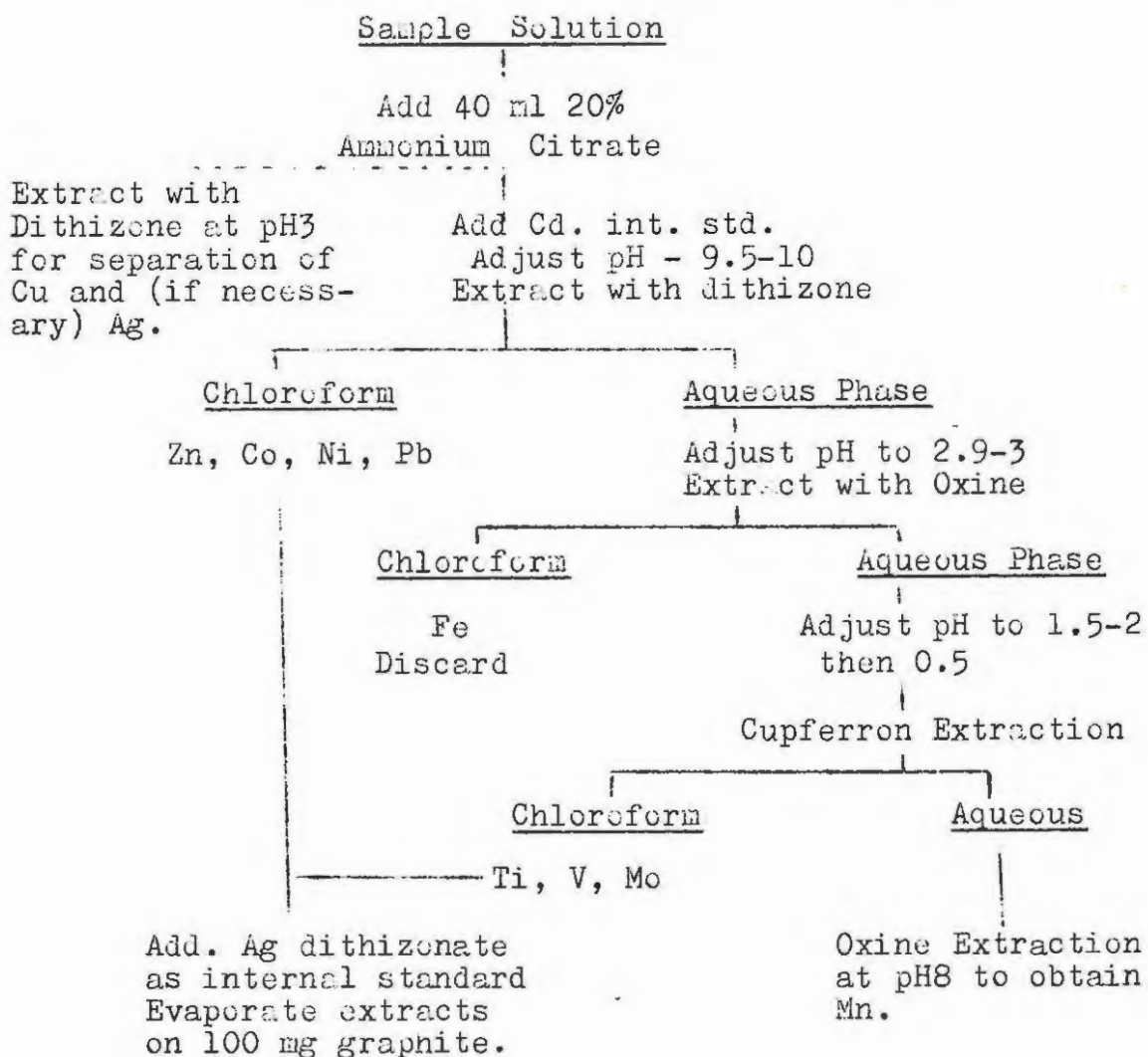


FIGURE 12

- (i) Dithizone at pH 9.5.
- (ii) Oxine at pH 3.
- (iii) Cupferron at pH 2 and 0.5.
- (iv) Oxine at pH 8.

Two or three extractions were made with each reagent at the corresponding pH and the extracts were retained in separate beakers.

Extract (iv) which contained the manganese was made up to 50 ml. with pure chloroform and 10 ml. aliquots taken for the spectrographic and flame photometric determination. The first aliquot of (iv) and each of the other extracts was then evaporated on to graphite containing internal standards and analysed spectrographically.

For the flame photometric determination of manganese, the 10 ml. aliquot was evaporated on a water bath and the residue digested with nitric and perchloric

acid, after which it was taken up in dilute hydrochloric acid and made up to 10 ml. This solution was atomised in the oxy-hydrogen burner of the Beckman flame attachment to the D.U. Spectrophotometer, and readings taken at 403.3 μ .

Results

The results, shown in Table VIII, are compared with the results obtained, using the modified Stetter method⁽⁵⁾⁽⁷⁾ (Analysis carried out by R.M. Fourie, Research Officer, C.S.I.R.).

It was apparent from the results that most of the separations were completely satisfactory.

TABLE VIII

Trial Analysis Using Proposed Concentration Scheme

Method	Extract	Element Concns. Found (p.p.m.)								
		Zn	Co	Ni	Pb	Ti	V	Mo	*Mn	**Mn
Trial of New Concn. Scheme	(i) Dz pH9.5	14	0.32	1.70	0.64	-	-	-	-	-
	(ii) Oxine pH3	-	-	-	-	-	0.07	0.05	-	-
	(iii) Cup- ferron pH2, 0.5	-	-	-	-	27	0.65	1.78	-	-
	(iv) Oxine pH8	-	-	-	-	-	-	-	33	34
Stetter	-	11	0.32	1.0	0.7	-	0.5	1.4	30	-

* Arc

** Flame

The proportion of vanadium lost in the oxine extraction of iron is approximately what was predicted from the study of this extraction. However, the apparent concentration may well have been enhanced by the presence of iron in this extract in the manner reported by Strasheim, Eve and Fourie⁽⁷⁾. The recovery of this metal was regarded as being satisfactory for nutritional studies.

The proportion of molybdenum lost in this extract (also probably enhanced by the presence of iron) was negligible for all practical purposes.

(c) Analysis of Four Standard Samples Using the New Concentration Scheme.

As a further test of the proposed scheme, four standard samples were analysed. [These samples, of which a number were retained in the laboratory, had been analysed previously by various methods, both spectrographic and chemical, in the C.S.I.R. and in other laboratories⁽¹⁾⁽⁴⁾]. In the analysis of these samples, the flame photometric determination of copper and manganese was included.

Procedure

The procedure followed was similar to that described above, except that the copper was extracted from solution at pH 3 and the whole of this extract, after evaporation and digestion with nitric and perchloric acid, was taken up in dilute hydrochloric acid for the flame photometric analysis, measurements being made at 324.8 m μ . The iron extract was not analysed and the dithizone extract, the cupferron extract and an aliquot of the manganese extract were combined for the spectrographic analysis.

Results

The results of these analyses are shown in Table IX. The figures obtained are compared with the means of results of previous analyses. These means can be somewhat misleading, but the actual figures obtained in previous analyses are given for comparison in the Appendix to this thesis (Page 103).

While these results demonstrated that the concentration method was workable, they still left much to be desired, particularly in the case of zinc, lead and titanium. This was attributed to the spectrographic analysis itself rather than to the concentration method. At this stage the spectrographic method used by van Rooyen⁽¹⁰⁾ was being used, with Cr and Bi (for Pb) as internal standards, but

full attention was given to the spectrographic steps in the procedure after this.

TABLE IX
Analysis of Four Standard Samples

Sample	*	Element Concentration (p.p.m.)									
		Zn	Co	Ni	Pb	Ti	V	Mo	Mn (Arc)	Mn (Fl)	Cu
4A Peach Leaves	1	13	0.15	2.2	2.7	16	.18	.06	49	45	7.9
	2	24	0.1	2.6	2.4	10	.24	.07	47		6.0
4B Bean Leaves	1	60	0.26	4.7	4.4	61	.53	2.3	48	38	11.5
	2	58	0.24	5.7	2.9	37	.71	2.3	38		12.1
4C Beet Leaves	1	13.5	0.14	4.0	1.9	47	.50	1.39	160	120	9.1
	2	22	0.25	2.5	2.1	21	.49	1.23	152		12.0
4E Plum Leaves	1	23	.48	1.3	2.5	13	.21	.05	150	100	5.3
	2	55	.64	1.4	2.4	8.5	.23	-	125		6.5

* 1 = Result of described analysis.

2 = Mean of Previous Results. (See Appendix p.103)

(d) The Direct Flame Photometric Determination of Copper and Manganese in the Organic Extract Solution.

The flame photometric determination of copper and manganese as part of the analysis scheme has been shown, from the results reported above, to be a practical possibility. In a number of recent

publications, Dean and others⁽⁴⁸⁾⁽⁴⁹⁾⁽⁵⁰⁾⁽⁵¹⁾ have recommended the direct atomisation of organic solutions of metal complexes in flame photometric analysis. As it appeared that this approach could easily be applied to the proposed analysis scheme and would possibly save time by eliminating the necessity for the destruction of the complexes, it was decided to test the method for chloroform solutions of copper dithizonate and manganese oxinate.

A preliminary experiment was carried out to determine the comparative sensitivities with chloroform solutions of the organo-metal complex and with aqueous solutions of the metals. It was found that a chloroform copper dithizonate solution gave 3.1 times the scale deflection given by an aqueous solution containing the same concentration of copper, while the sensitivity with a chloroform solution of manganese oxinate, was 5.3 times that with an aqueous solution.

Standard curves were then drawn up in the following way:

Measured volumes of standard solutions of the metals to be determined were run into separatory funnels, citrate added, the pH adjusted (To 3 in the case of copper, and 8 in the case of manganese) and the metal ions extracted with the appropriate complexing reagent (Dithizone in the case of copper and oxine in the case of manganese). The organic extracts were then made up to 50 ml. with pure chloroform and portions of them atomised into the flame directly, the photometer readings being recorded in each case. Photometer readings were made at 403.3 m μ for manganese and 324.8 m μ for copper.

The standard curves obtained are shown in Figure 13.

After this, five of the "standard" plant samples were analysed, using extracts obtained while working through the whole concentration scheme. The results, which are shown in Table X, were not very satisfactory though the practicability of the method has been proved. Difficulty was experienced from time to time, especially in the case of the manganese, due to clogging of the burner, and where

Standard Curves for the Flame Photometric Determination of Mn and Cu in their Chloroform Extracts

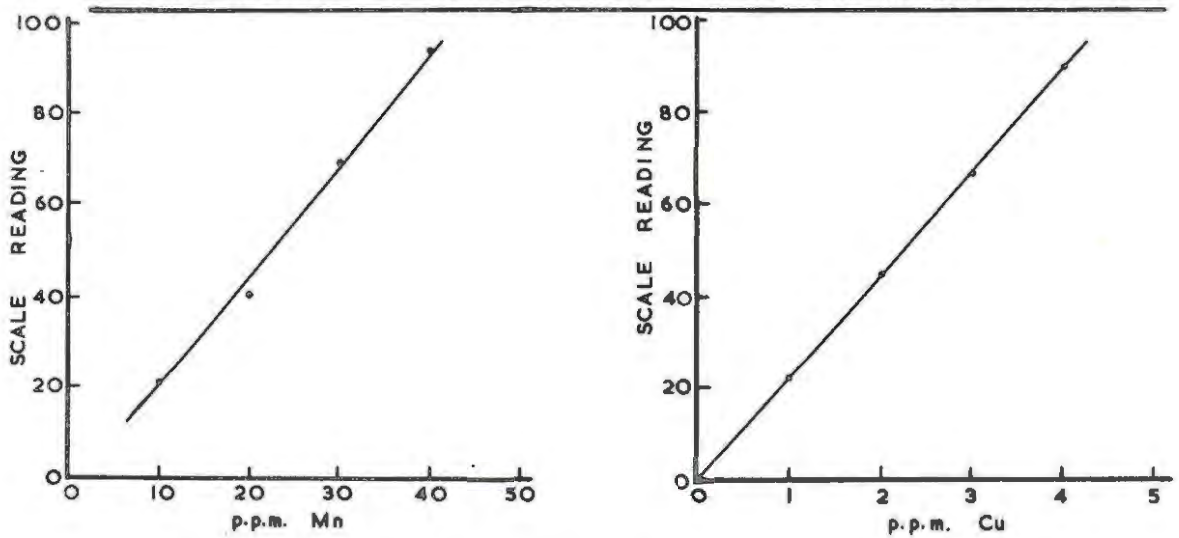
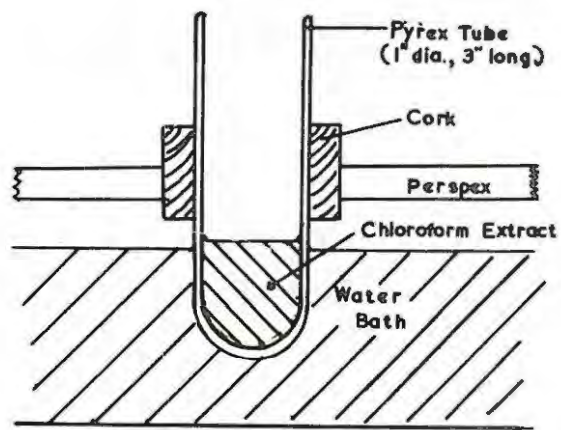


Figure 13.



Evaporation of Chloroform Extracts.

Figure 14.

the results are in poor agreement it can be attributed to erratic behaviour of the burner.

No further attention was given to the flame photometric determination of manganese and copper. There is no doubt that these elements could be determined satisfactorily along the lines suggested, but as these elements can both be determined satisfactorily directly on the ash, there seemed to be little point in pursuing this line of investigation any further.

TABLE X

Flame Photometric Determination of Copper and Manganese in Five Plant Samples Using their Organic-Chloroform Extracts Directly.

Sample	Cu(flame) ppm	Cu(std.)* ppm	Mn(Flame) ppm	Mn(std.)* ppm
4a 1.	6.2	6.7	26.4	51
4a 2.	6.4		35.5	
4b 1.	6.4	12.0	21	42
4b 2.	6.6		42	
4c 1.	9.0	10.9	100	166
4c 2.	-		-	
4d 1.	7.8	11.4	-	95
4d 2.	8.1		111	
4e 1.	5.4	5.4	146	136
4e 2.	4.6		-	

* = Mean of results obtained by other methods at Onderstepoort and in N.P.R.L.

4. Evaporation of the Chloroform Extracts on to Graphite.

The evaporation of the chloroform extracts on to graphite was found to present some difficulty. Under ordinary circumstances the extract tends to "creep" up the sides of the vessel from which the chloroform is being evaporated. This can be overcome by allowing some of the chloroform being distilled off to reflux down the sides of the vessel. Wark⁽²⁾ accomplished this by playing a jet

of air on the outside of the 1 ml. beaker in which the final evaporation was carried out. This method was found to be somewhat tedious as the extracts had to be first evaporated to a small volume and then transferred to 1 ml. beaker, while the last stages of the evaporation required the constant attention of the analyst.

In the preliminary experiments the combined extracts were evaporated slowly in 50 ml. Phillips beakers and the graphite added when only about 0.5 ml. of chloroform was left. This method was found to be fairly satisfactory as the tall sided beakers did cause the chloroform to reflux to some extent, but in many cases some of the extract was left on the walls of the beaker.

Experiments were carried out with a number of other methods and finally the following method, which was found to be very satisfactory and well suited to a routine analysis method, was adopted.

The apparatus used consisted of one inch diameter, thick-walled pyrex test-tubes cut to a length of three inches. These were mounted in a glass-trough water bath as shown in Figure 14. The chloroform extracts (first the dithizone extract and then the cupferron extract) were added bit by bit to the tubes and the graphite (100 mg.) added when there were only a few drops of chloroform left. During the last stages of the evaporation the tube was raised slightly from time to time to keep the liquid level inside it slightly above that of the water outside it. If at any stage the extract appeared to be adhering to the walls of the tube, all that was necessary was to remove the tube from the water bath and hold it in the air or blow on it gently. The graphite plus extract was dried first in the water bath, then in an oven at 100°C and finally placed in a muffle furnace at 450°C for about ten minutes to destroy the metal complexes.

In the initial experiments the internal standard elements in the form of oxide or salts were mixed with the graphite, but in later work, to reduce errors due to the chloroform extracts not all being taken up by the graphite, the internal standards were included as dithizonates in the chloroform extract.

B. SPECTROGRAPHIC PROCEDURE
DEVELOPMENT OF METHOD

1. The Spectrograph

Some of the preliminary experiments were carried out using a Jarrel Ash Co. Wadsworth grating spectrograph, but most of the work was done using an old model of the Hilger Large Quartz Spectrograph. This instrument differs from the newer models in that prism to plate distance, prism angle and plate angle are adjusted separately to predetermined settings, instead of being controlled by a single "wave length drum". The principle of the instrument is, however, the same.

A diagram of the optical arrangement is shown in Figure 15. Three lenses were used in the external optical system. The first produced an enlarged image of the source "s" at the diaphragm, "d" which had the effect of eliminating the light from the electrode tips. The second lens acted as a condenser and was placed so as to produce an image of the first lens on the third. The third lens, immediately in front of the spectrograph slit, focussed an image of the diaphragm gap and hence of the source itself, on collimator lens, "4" of the spectrograph. The first lens was protected from sputtering of the source by having a plain quartz window immediately in front of it. All the lens positions were calculated for a wavelength of 3500A (a convenient intermediate wavelength for general work in the near ultraviolet).

2. Excitation Source Conditions

It is normal in d.c. arc work to add to the sample some alkali salt to act as buffering agent and to reduce the emission of cyanogen bands. Lithium carbonate was used for this purpose. The behaviour of the elements in the arc is influenced by the proportion of buffer in the sample, the type of arc used, the electrodes and the current. It is therefore important that these factors be considered before adopting a particular method, and the most suitable conditions for the analysis selected.

During the preliminary investigation of the chemical preparation of the sample the spectrographic analyses were carried out according to the method used by van Rooyen⁽¹⁰⁾. The chloroform extracts were evaporated

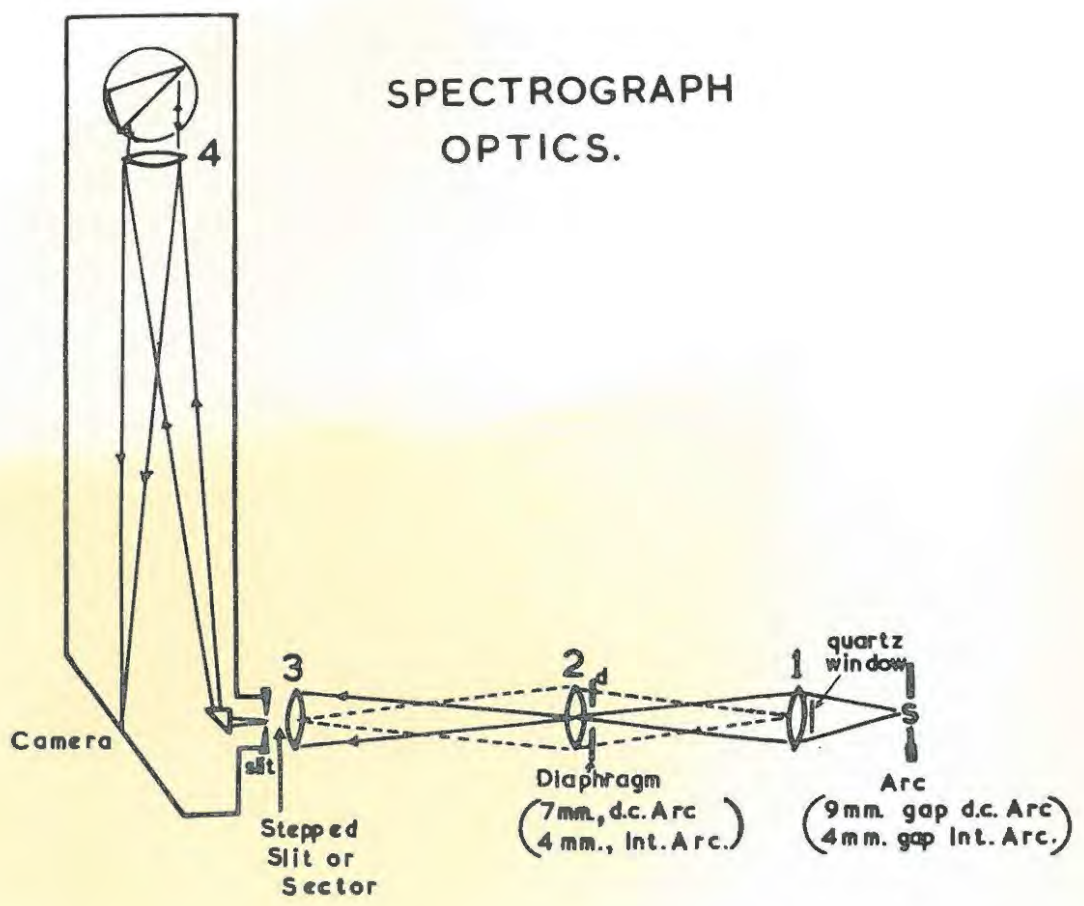


Figure 15.

ELECTRODES.

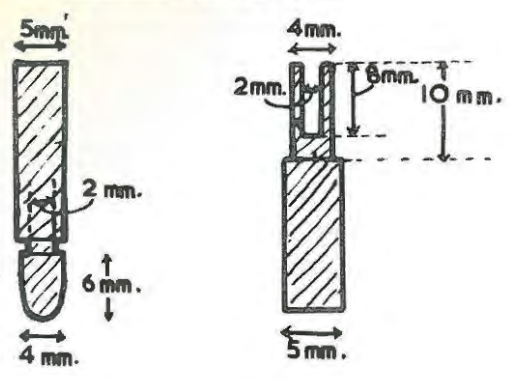


Figure 16.

on to 100 mg. of graphite containing the internal standard elements. After ashing the graphite-extract mixture, it was carefully mixed with 33 mg. of lithium carbonate, loaded into the sample electrode which was burned as anode in a 10 amp d.c. arc.

The electrodes used consisted of a graphite sample electrode with an 8 mm. deep sample cavity of internal diameter 2 mm. and external diameter 4 mm, and a carbon counter-electrode with a rounded and undercut tip. The form of the electrodes is shown in Figure 16. This combination is one of those recommended by Strasheim and Keddy⁽¹⁾.

After the completion of the investigation of the sample preparation method, attention was given to the selection of the most suitable source conditions. Under optimum source conditions the rate of volatilisation and emission of each element would be constant for the whole period of exposure. When this condition is attained, variations in exposure time and differences between analysis element and internal standard have little influence on the results. The source conditions listed in Table XI were therefore tested by plotting, for each element, curves showing the variation of intensity of spectrum line emission with time during the arcing of the sample.

Experimental*

(i) Source Units

For the work with the d.c. arc a constant current, d.c. arc source unit, designed and built in the C.S.I.R. laboratories by van der Walt⁽⁵²⁾, was used.

The interrupted arc source consisted of an a.c. arc source unit, used previously for other analyses, which was modified by the introduction of selenium rectifiers as shown in the circuit diagram (Figure 17a). The arc is ignited once every cycle by a high voltage, low energy spark. The phase of this spark is controlled by a rotary gap in the spark circuit, driven by a synchronous motor. The rotary control gap was set with the help of an oscilloscope so that the discharge took place at the peak potential (see Figure 17b).

*(This investigation was carried out with the assistance of Miss E.C. Maskew, Scientific Assistant, who worked under the direction of the Author).

TABLE XI

Source Conditions Tested

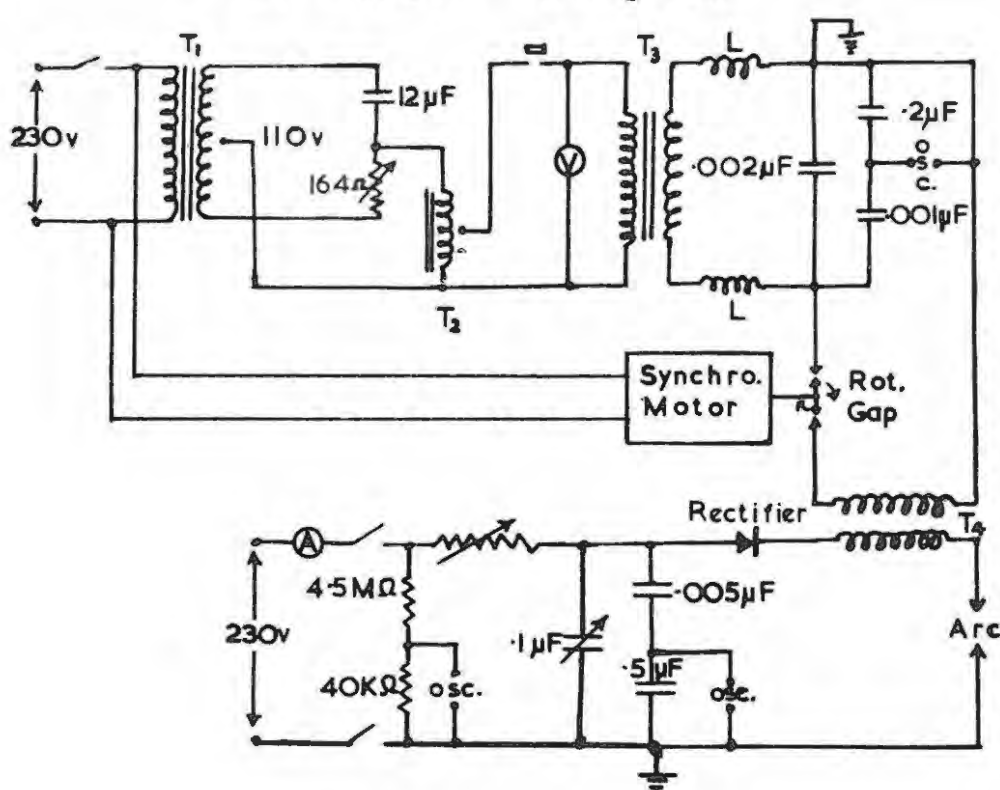
Source type	Con- dition	Sample Elec- trode *	Graphite Buffer Mixture	Current	Elec- trode Gap
<u>Constant</u>	(i)	1	25% Li_2CO_3	10 amp.	9mm.
<u>Current</u>	(ii)	1	42% Li_2CO_3	10 amp.	9mm.
<u>d.c. arc</u>	(iii)	1	25% Li_2CO_3	5 amp.	9mm.
<u>d.c. arc with Stallwood air jet</u>	(iv)	1	25% Li_2CO_3	10 amp.	9mm.
	(v)	1	42% Li_2CO_3	5 amp.	9mm.
<u>Interrupted d.c. arc</u>	(vi)	1	42% Li_2CO_3	3 amp.	4mm.
	(vii)	1	42% Li_2CO_3	5 amp.	4mm.
	(viii)	1	25% Li_2CO_3	4 amp.	4mm.
	(ix)	2	42% Li_2CO_3	3 amp.	4mm.

- * Sample electrodes
1. Cavity depth 8mm, internal diameter 2mm, external diameter 4mm.
 2. Cavity depth 1mm, internal diameter 4.5mm, external diameter 6.4mm.

Figure 17.

INTERRUPTED ARC SOURCE

(a) Circuit Diagram



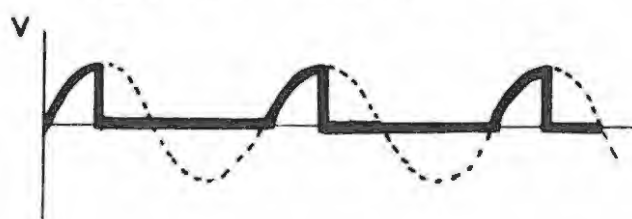
T₁ = 100 watt, 110 volt transformer.

T₂ = Hilger F282 A transformer 150v - 8000v

T₃ = Hilger high voltage transformer F282 B.

T₄ = Tesla transformer.

(b) Oscilloscope Trace for Correct Igniter Phase.



(ii) Procedure

To obtain the time-intensity curves, standard mixtures containing all the elements of interest were arced under each of the selected conditions. The spectral emissions during suitable fractions of the total arcing time were recorded consecutively in different positions on the photographic plate, the spectrograph shutter being closed for five seconds each time while the plate was moved to a new position. The densities of the recorded spectrum lines were measured and the corresponding relative intensity values (relative to 70% transmission) calculated. These values were then plotted as the mean relative intensity for each fraction of the arcing period.

Results

The results of this investigation are shown in Figures 18-26.

Condition (i) (Figure 18) 10 amps d.c. arc, 25% lithium carbonate in buffer mixture, 9 mm. arc gap.

This was the source condition used in the preliminary work. It can be seen from the time-intensity curves that the volatile elements such as Pb and Zn tend to be volatilised during the early part of the burn under these conditions and that the involatile elements such as Ti, Mo and V, after being fairly strongly excited initially, show low line intensity in the middle of the arcing period and emit again very strongly during the latter part of it.

Condition (ii) (Figure 19). 10 amps d.c. arc, 42% lithium carbonate, 9 mm. arc gap.

This condition was investigated in the hope that the extra lithium carbonate would have a greater buffering effect on arc temperature and hence produce greater constancy of volatilisation. The curves show, however, that only a slight improvement over condition (i) was obtained as far as constancy of volatilisation was concerned, while the general intensity level was reduced (hence the longer exposure periods).

Condition (iii) (Figure 20). 5 amps d.c. arc, 25% lithium carbonate.

It was to be expected that a lower arc current would mean lower arc temperatures and hence more constant volatilisation, particularly for the more volatile elements. This is born out by the curves obtained for this source

TIME - INTENSITY CURVES.

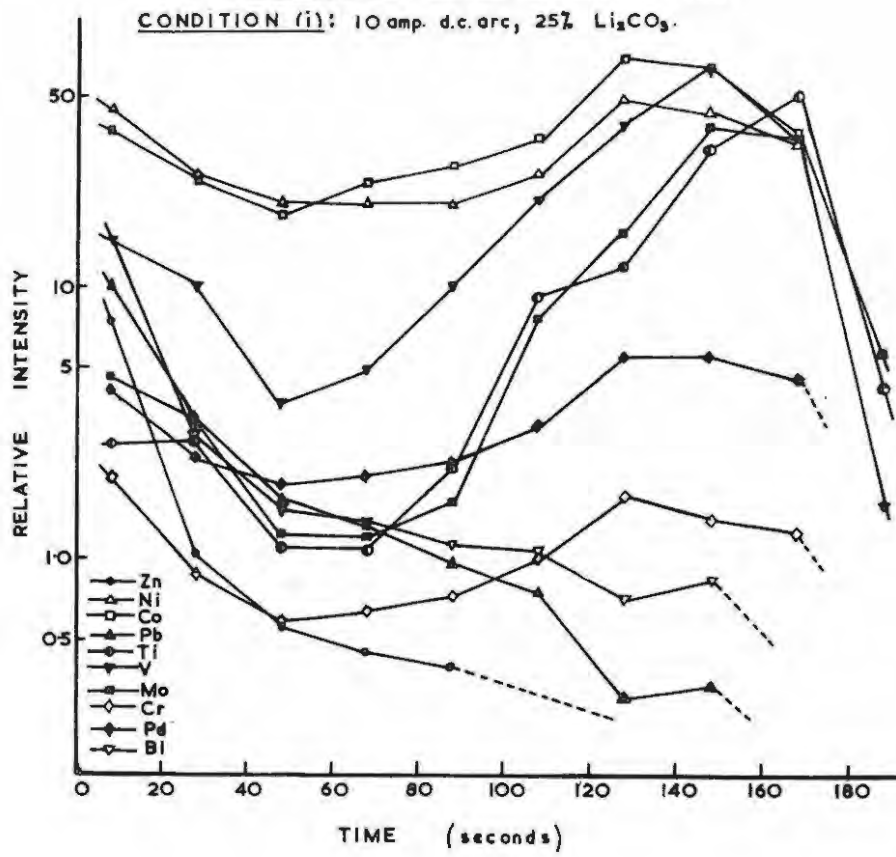


Figure 18.

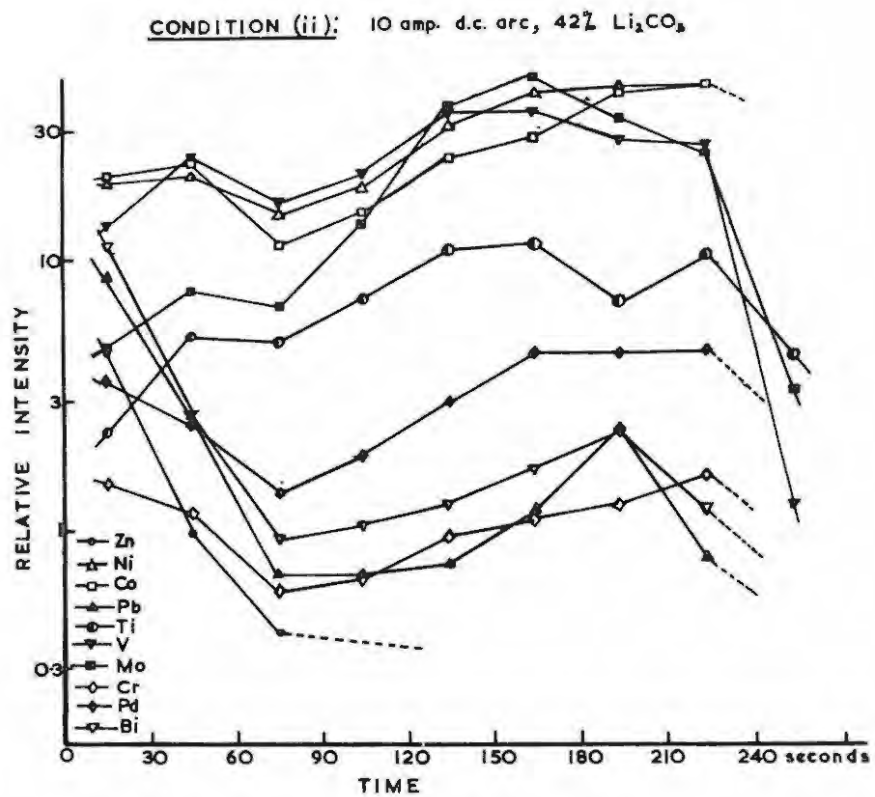


Figure 19.

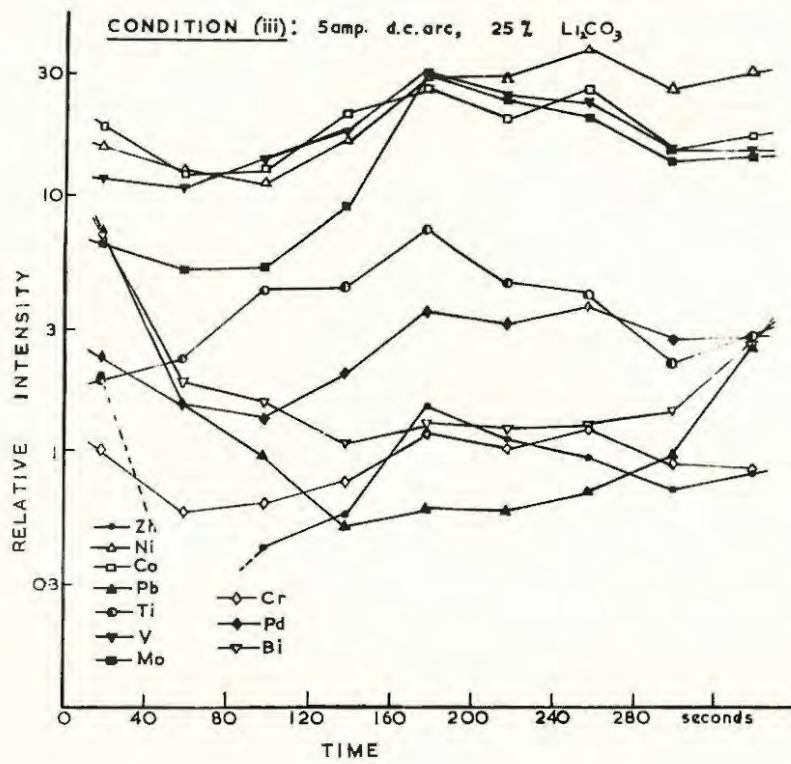


Figure 20.

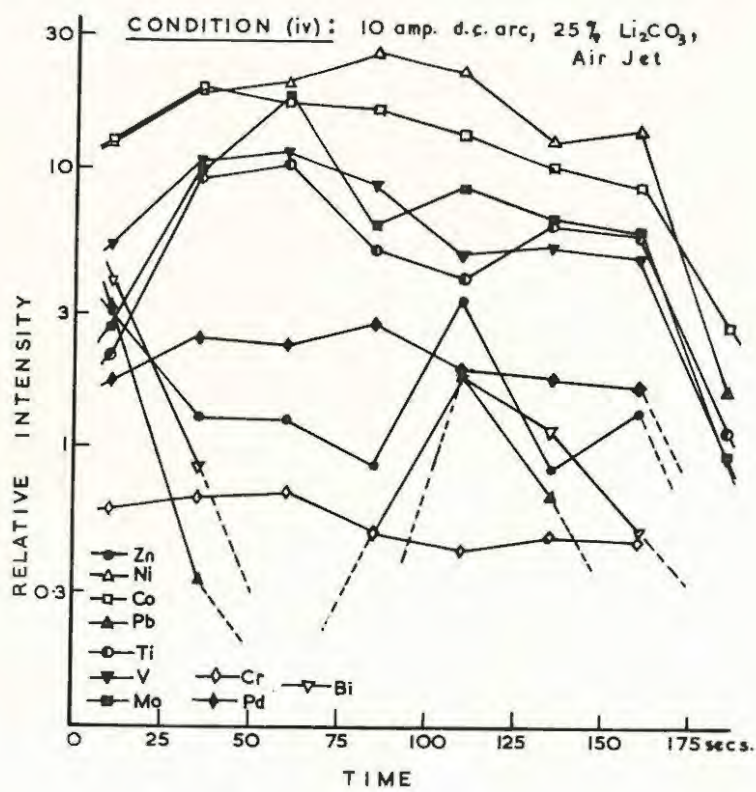


Figure 21.

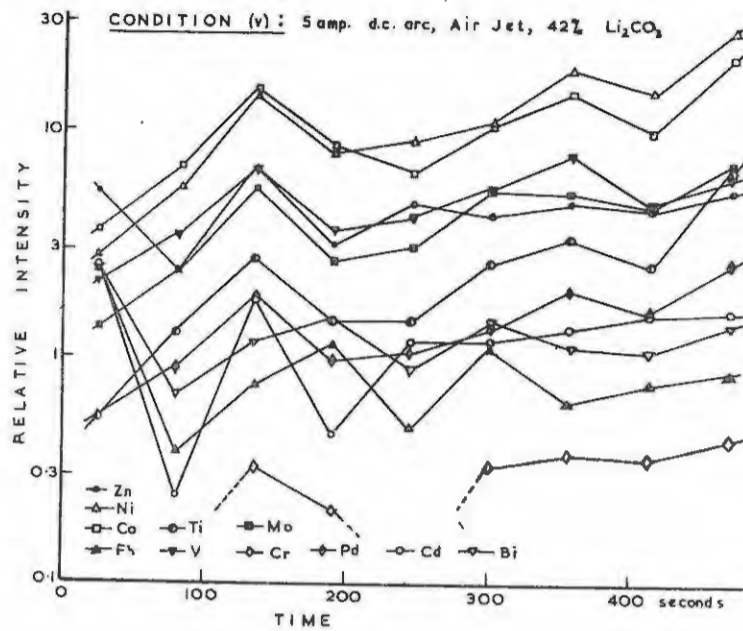


Figure 22.

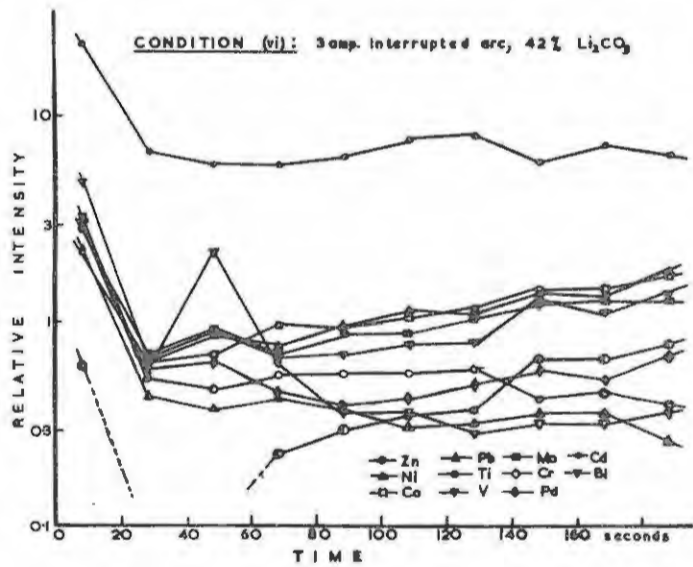


Figure 23.

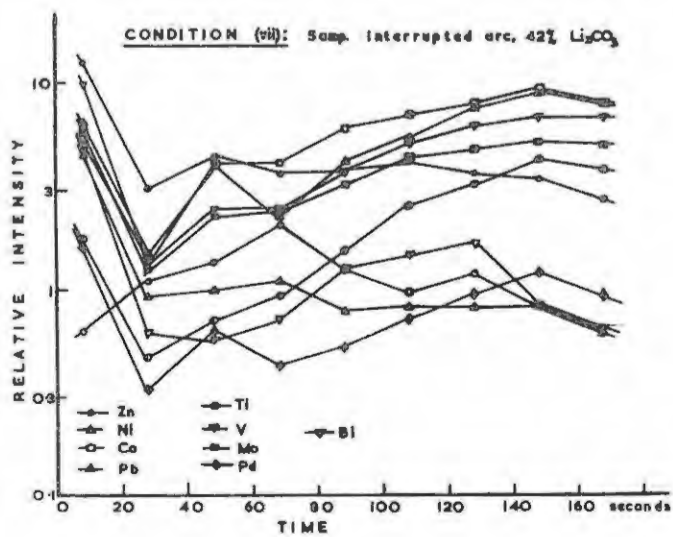


Figure 24.

condition, but the volatilisation rate or rate of emission was low, so that exposure times of over five minutes would be required. In addition a tendency remains for the less volatile elements to emit more strongly towards the end of the arcing period.

Condition (iv) (Figure 21). 10 amp. d.c. arc with Stallwood air jet. 25% lithium carbonate, 9 mm. arc gap.

For this condition an air jet of the type designed by Stallwood⁽⁵³⁾ was used. This consists of a circular metal jet which directs air upwards around the lower electrode, which has the effect of reducing wandering of the arc, and also cools the sample electrode, to some extent. A jet with an 8 mm. diameter aperture was used, through which compressed air was blown at a rate of 8 litres per minute.

This system was found to produce a more constant volatilisation of most of the elements, but the volatile elements, Zn and Pb behaved rather erratically as shown by the time-intensity curves.

Condition (v) (Figure 22). 5 amp d.c. arc with Stallwood air jet, 42% lithium carbonate.

The combined dampening effect of reducing the current and increasing the lithium carbonate concentration, together with the effect of the Stallwood air jet, produced a fair constancy of volatilisation and emission with all the elements, and though it would mean rather long exposure times, (7 or 8 minutes) this condition would undoubtedly give good results and would be recommended where only a d.c. source unit is available.

Condition (vi) (Figure 23). Interrupted d.c. arc, 3 amps. (R.M.S.), 42% lithium carbonate, 4 mm. arc gap. (The interrupted arc does not readily strike across a wide gap).

The interrupted or intermittent arc has the advantage of producing a relatively high energy of excitation without excessive heating of the electrodes. It is therefore particularly suited to the determination of elements with low boiling point and high excitation energy such as zinc.

The constancy of emission with this source was found to be remarkably good, but the rate of emission was inconveniently low.

Condition (vii) (Figure 24). Interrupted d.c. arc, 5 amps. (R.M.S.), 42% lithium carbonate, 4 mm. arc gap.

Increasing the current to 5 amps markedly increased the emission rate, but under these conditions differences in the volatility of the elements were once more strongly evident.

Condition (viii) (Figure 25). Interrupted d.c. arc, 4 amps (R.M.S.) 25% lithium carbonate, 4 mm. arc gap.

Reducing the current to 4 amps and the lithium carbonate concentration to 25% produced a source condition, which gave very nearly constant volatilisation of all the elements concerned and a satisfactory high rate of emission.

Condition (ix) (Figure 26). Interrupted d.c. arc, 3 amps (R.M.S.) 42% lithium carbonate, 4 mm. arc gap. Broad shallow sample electrode.

As not all of the sample in the 8 mm. cavity electrode was consumed under conditions (vi), (vii) and (viii), it was thought that an effectively higher sensitivity might be attained by using a broad shallow sample electrode (4.5 mm. diameter, 1 mm. deep). However, it was found that this was not the case and the emission became far more erratic due to the wandering of the arc.

Conclusion

On the basis of these results, condition (viii) was selected for the analysis.

3. Internal Standards

The selection of internal standard elements, when a number of elements in the sample are to be determined together, normally presents some difficulty. Ahrens⁽⁵⁴⁾ has listed several factors to be considered in the choice of an internal standard. These are as follows:-

Major Factors:

- (i) If the internal standard is to be added, it should be an element which has a negligibly small concentration in the analysis specimens.
- (ii) The rates at which internal standard and analysis element volatilise should be very similar.
- (iii) Internal standard and analysis lines should have similar excitation potentials.
- (iv) The internal standard line should be free from self-absorption.

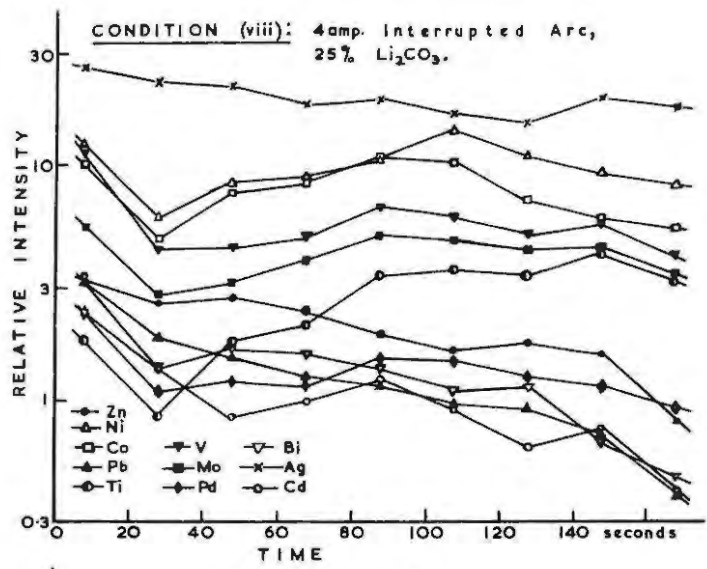


Figure 25a.

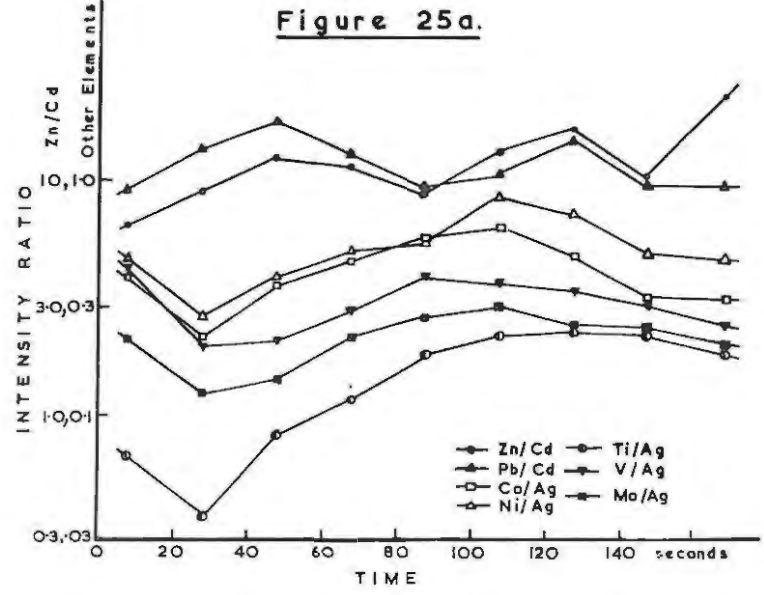


Figure 25b.

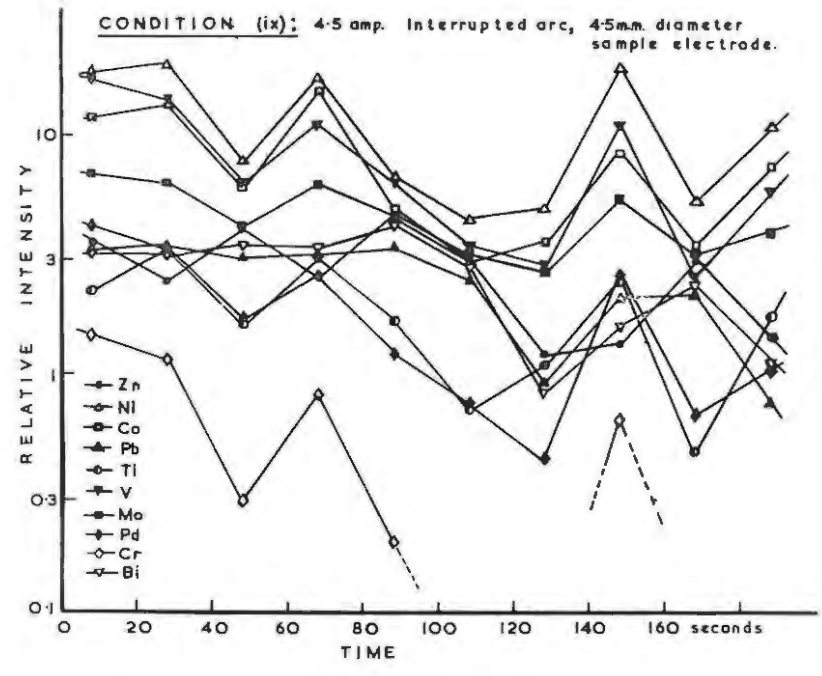


Figure 26.

- (v) Analysis and internal standard lines should have roughly the same wavelength, so as to reduce errors due to the photographic measurement of radiant energy.
- (vi) If the internal standard is added, it should be in a very high state of purity with respect to the elements sought.

Factors usually of less importance:

- (vii) The element chosen as internal standard should, if possible, be applicable to a relatively wide compositional variation, and consequently the presence of so-called "extraneous elements" should not influence the intensity ratio of the analysis pair of lines.
- (viii) The ionisation potential of the internal standard element should be similar to that of the analysis element.
- (ix) The atomic weight of the internal standard element should not differ much from the atomic weight of the analysis element if either is light.

To these conditions should be added the condition that the internal standard element should not have spectrum lines too close to the analysis element lines which are to be measured and, from this point of view, elements with simple spectra are to be preferred.

To fulfil all of these conditions for the seven elements sought would mean, if it were possible, the introduction of a large number of internal standards, which would mean a considerable amount of extra work during standard and sample preparation, in measuring the spectrum line densities and in the calculation of results. A compromise has therefore to be made.

Of the above factors (vi) and (vii) could be disregarded from the point of view of the type of analysis considered. Specpure chemicals could normally be used and since the trace elements are separated from all major constituents of the sample, the matrix effect drops away.

Information regarding some of the other factors is listed in Table XII for the analysis elements and for Cr, Cd, Pd, Ag and Bi, elements which were considered as internal standards. Of the internal standard elements considered, Ag has been used previously by Smit and Smit⁽⁵⁵⁾ in the spectrographic analysis of plants for Co, Mo and Cu; Cd has been used by a number of authors⁽⁶⁾⁽⁴³⁾ as internal standard for zinc and Bi has been shown to be a very satisfactory internal standard for lead.

Cr was used as an internal standard in some of the preliminary work but proved to be rather unsatisfactory. Some experimental work was carried out, using Pd as internal standard and this element proved to be quite satisfactory, but had the serious disadvantage that it interfered to some extent with the Co line at 3405.1 particularly when the latter element was present in very low concentration.

TABLE XII
Some Physical Properties of the Analysis Elements
and Possible Internal Standards

Element	Wave Length λ	Excit. Pot. eV (.)	Ion Pot. eV	Atomic Wt.	M.Pt. °C	B. Pt. °C
<u>Analysis Elements</u>						
Zn	3345.0 A	7.78	9.39	65.38	419	907
Co	3405.1 A	4.07	7.88	58.94	1480	3000
Ni	3414.8 A	3.65	7.63	58.69	1453	2732
Pb	2833.1 A	4.4	7.38	207.21	327	1744
Ti	3242.0 A	3.83	6.84	47.90	1800	3000
V	3185.4 A	3.96	6.74	50.95	1710	3000
Mo	3170.3 A	3.91	7.38	95.95	2622	4804
<u>Internal Stds.</u>						
Pd	3242.7 A	4.64	8.33	106.7	1553	2200
	3404.6 A	4.46				
Cr	3014.9 A		6.74	52.01	1615	2200
Bi	3067.7 A	4.02	8.0	209.0	271	1470
Cd	3261.1 A	3.80	8.99	112.41	321	765
Ag	3280.7 A	3.66	7.57	107.88	960	2212
	3382.9 A	3.78				

An idea of the volatilisation rates of these elements can be obtained from the time-intensity curves (Figures 18-26). It can be inferred from these curves that the volatilisation rates of Pb, Zn, Bi and Cd are similar under the source conditions tested. The volatilisation characteristics of cobalt, nickel, palladium and to some extent vanadium, seem to be intermediate between those of the volatile elements and the highly involatile elements, molybdenum and titanium, which show a tendency to be volatilised more strongly towards the end of the burn with all the source conditions tested. From Figure 25b it would appear that silver is also somewhat intermediate in character.

Cadmium and silver were eventually selected as

internal standards, the former for zinc and lead and the latter for the other elements. The main reason for the selection of these two elements was the fact that they both form chloroform soluble dithizonates and can therefore be present in the chloroform extract of the trace elements before it is evaporated. Cadmium was added to the sample solution before extraction and silver was added to the extract in the form of an aliquot of a standard silver dithizonate solution.

Silver has the advantage, as internal standard, of having only two strong spectrum lines in the spectral region used and therefore is unlikely to interfere with any of the analysis lines. As can be seen from Table XII, the excitation potential of each of the useful silver lines is sufficiently similar to those of the analysis elements to make it a suitable internal standard.

Silver does have the disadvantage as an internal standard that its concentration in biological materials may at times be high enough to interfere with the determination. Initially the co-extraction of silver with the other dithizonates was not expected in view of the high concentrations of chloride and ammonia present. The investigation of silver dithizonate extraction described in Chapter II showed, however, that some co-extraction could be expected. The concentration of silver in most plant materials is extremely low (.05 p.p.m.) and it was not found necessary to remove it from any of the standard samples analysed. Where the removal of silver is thought to be necessary, however, this could be easily accomplished by an initial dithizone extraction at pH3 in the presence or absence of citrate, a separation which would be made in any case if copper were to be determined as part of the analysis scheme.

The spectrum lines selected for the analysis are listed in Table XIII below. These are largely the same as those listed by Mitchell⁽⁴³⁾, but wavelengths much above 3400 were avoided (Co 3405 and not 3453) as the background due to cyanogen bands was found to increase rapidly with wavelength above this.

TABLE XIII

Spectrum Lines Used.

Element		Internal Std.	
Zn	3345.0 A	Cd	3261.1 A
Co	3405.1 A	Ag	3280.7 A
Ni	3414.8 A	Ag	3280.7 A
Pb	2833.1 A	Cd	3261.1 A
Ti	3242.0 A	Ag	3280.7 A
V	3185.4 A	Ag	3280.7 A
Mo	3170.3 A	Ag	3280.7 A

Figure 26 shows the variation with arcing time of the intensity ratios of the element-internal standard pairs. As can be seen from these curves the intensity ratios are reasonably constant for most of the elements.

4. Preparation of Standards

In the preliminary work, standards were mixed from spectrographically pure graphite and "specpure" oxides of the metals concerned. Suitable dilutions of the oxides in graphite were first prepared. Mixing was carried out in an agate mortar with a little pure methanol which was afterwards evaporated by placing the mortar in a drying oven. From these mixtures a top standard composed of graphite, oxides of the analysis elements and the internal standard elements, was prepared. This was diluted in the ratio 3.16:10 and 1:10 with a graphite dilution mixture containing the required concentrations of internal standards. The second of the diluted mixtures was then rediluted in the same ratios and the dilution process repeated three times to give a suitable range of standards. Lithium carbonate was finally mixed in the proportion 1:3 with all the standards.

As it was felt that it could not be insured that the elements in the final concentrated sample extract would be in the same form as in the standards mixed in the above manner, this method was not used in the preparation of standards for the analysis method as finally proposed. Instead standards were prepared by extracting a synthetic sample solution, prepared from measured volumes of standard metal solutions, with dithizone and cupferron and treating the extract in the same way as in the actual sample analysis.

C. RECORDING AND INTERPRETATION OF SPECTRA

1. Photographic Plates

Ilford ordinary plates were used throughout this part of the work, the main reason for the choice being their high sensitivity. After exposure the plates were developed for $6\frac{1}{2}$ minutes in 1:1 I.D.13 at 18°C. Development was stopped in a stop bath of 5% acetic acid and the plates fixed in Kodak "Xray" fixer. An A.R.L. developing machine was used for the processing. The plates were washed first in running tap water, then with distilled water and dried on an A.R.L. plate dryer.

2. Microphotometry

Two microphotometers were used for line density valuation in the analyses reported here. These were an Optica Milano double projector-microphotometer, which had been modified to some extent in the C.S.I.R. laboratories to reduce stray light errors, and an instrument of similar type, but improved design, which had been designed and built in the C.S.I.R.

3. Emulsion Calibration and the Calculation of Results

Quantitative spectrography depends on the assumption that there is a simple relationship between the intensity of radiant emission at a particular wave length and the concentration in the excitation source of the atoms which can produce it. In the ideal hypothetical case, when a state of thermal equilibrium exists in the source at a particular temperature, the number of atoms in an excited state, and hence the number of emitting atoms, will be proportional to the number of atoms in the ground state and very nearly proportional to the total number of atoms present.

Hence we can write

$$I = K.C \quad \text{-----} \quad 3.1$$

where I = intensity of emitted radiation.

C = concentration of atoms in the source.

K = proportionality constant.

Taking logs, we may write

$$\log I = \log K + \log C \quad \text{-----} \quad 3.2$$

In other words plotting log I against log C should give a straight line of 45° slope. This is found to be approximately true in practice but a truer expression is found to be

$$I = KC^n \text{ ----- } 3.3$$

$$\text{or } \log I = \log K + n \log C \text{ ----- } 3.4$$

where n is normally equal to, or slightly less than unity.

To minimise errors due to source condition variations, it is normal to determine the ratio of the intensity of the element line to that of a spectrum line of an added internal standard.

We then have

$$\log \frac{I_E}{I_S} = \log \frac{K_E}{K_S} + n \log C_E - n' \log C_S$$

$$\text{i.e. } n \log C_E = \log \frac{I_E}{I_S} + n' \log C_S - \log \frac{K_E}{K_S} \text{ ----- } 3.5$$

where subscripts E and S refer to analysis element and internal standard respectively

Since $n' \log C_S$ should be a constant 3.5 becomes

$$\log C_E = \frac{1}{n} \log \frac{I_E}{I_S} + K' \text{ ----- } 3.6$$

Thus standard calibration curves are normally obtained by plotting \log intensity ratio against \log concentration.

Since concentration can be fairly simply related to spectrum line intensity, it is important to be able to relate the observed blackening of the photographic emulsion to the line intensity. Before discussing the various methods available, however, it is as well to define some of the terms used.

It is usual to measure either the transmission, T, the fraction of light falling on the plate which is transmitted, the percentage transmission, T%, which is the same fraction expressed as a percentage, or the density which is $D = \log \frac{1}{T}$. Exposure is defined as being the product of the intensity of the light falling on the plate and the time for which it does so. i.e. $E = It$.

(a) Emulsion Calibration

(i) Emulsion Calibration Functions

One of the earliest means of relating exposure to the density of the photographic image was discovered by Hurter and Driffield⁽⁵⁶⁾ in 1890 who plotted Density against \log Exposure and obtained a curve having the form shown in Figure 27. This curve, known as a H and D curve, shows a straight line relationship between density and \log exposure over a fairly wide range of density.

A simple variation of the density - log exposure plot, due to Seidel, was reported by Kaiser⁽⁵⁷⁾. Instead of $D = \log \frac{1}{T}$, $S = \log(\frac{1}{T} - 1)$ is plotted against log exposure and a curve is obtained (Figure 27) which is straight over a much wider exposure range, the theory being that both $\log E$ and $\log(\frac{1}{T} - 1)$ approach $-\infty$ as exposure is reduced, while $\log \frac{1}{T}$ approaches zero. It thus only "corrects" (i.e. straightens) the lower part of the curve.

In actual fact the Seidel system over "corrects" the lower part of the curve and a slight curve in the opposite direction is obtained. For this reason Kaiser⁽⁵⁷⁾⁽⁵⁸⁾⁽⁵⁹⁾ introduced a further variation in which a function of T, which is a combination of the Seidel and H and D functions,

i.e. $f(T) = k \log(\frac{1}{T} - 1) + (1 - k) \text{Log} \frac{1}{T}$ ----- 3.7
is plotted against log E. "k" which is known as the "transformation constant" is effectively the fraction of the Seidel function which must be introduced with the H and D function to give a linear plot and can be quite simply calculated from the slope, at high and low density, of both the H and D curve and the Seidel curve. It is found that a plot of this function against log exposure gives points which very nearly lie on a straight line over a wide range of exposures. In Figure 28 H-D, Seidel and Kaiser-type functions have been plotted against log exposure for the Cd 3261 A line photographed on an Ilford Ordinary plate.

A general function which, it is claimed, will give a straight-line emulsion calibration for most photographic emulsions has been proposed by Hughes and Murphy⁽⁸²⁾. This is

$$f(T) = \log \left(\frac{T}{1-T} \right) - 0.4e^T \text{ ----- } 3.8$$

A number of other functions have been proposed for the preparation of emulsion calibration curves, but only the H and D, Seidel and Kaiser functions are in general use.

(ii) Practical Methods of Obtaining Emulsion Calibration Curves.

Both a step sector and a step filter were used at different times during the work described here to give spectrum-line steps of known exposure ratio for the calibration of the emulsion. The step sector method has the advantage of simplicity and constancy of the step

"H and D" and Seidel Emulsion Calibration Curves.

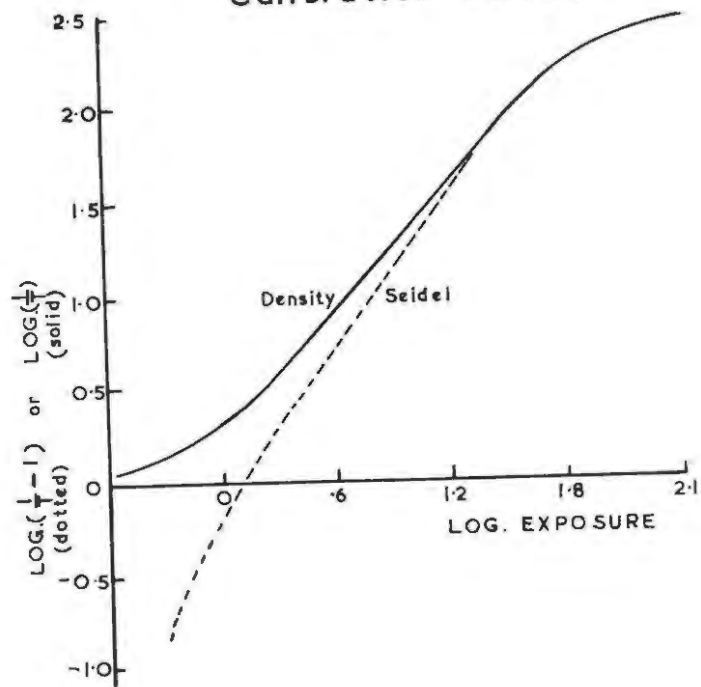


Figure 27.

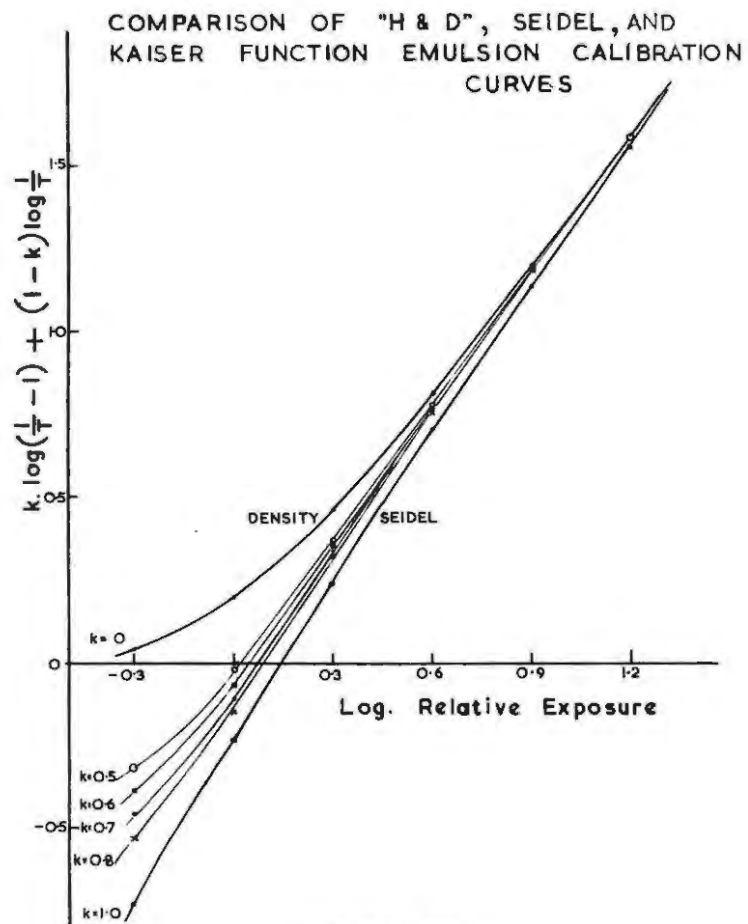


FIGURE 28

THE SLIDE RULE.

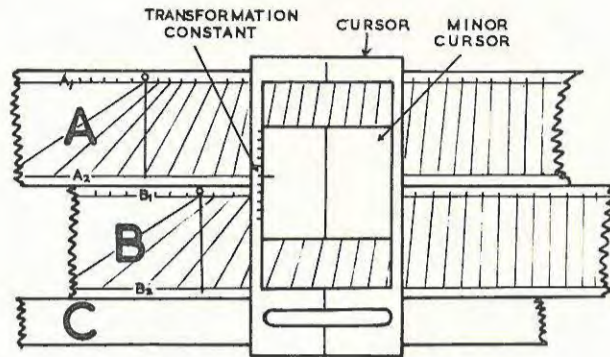


FIGURE 29.

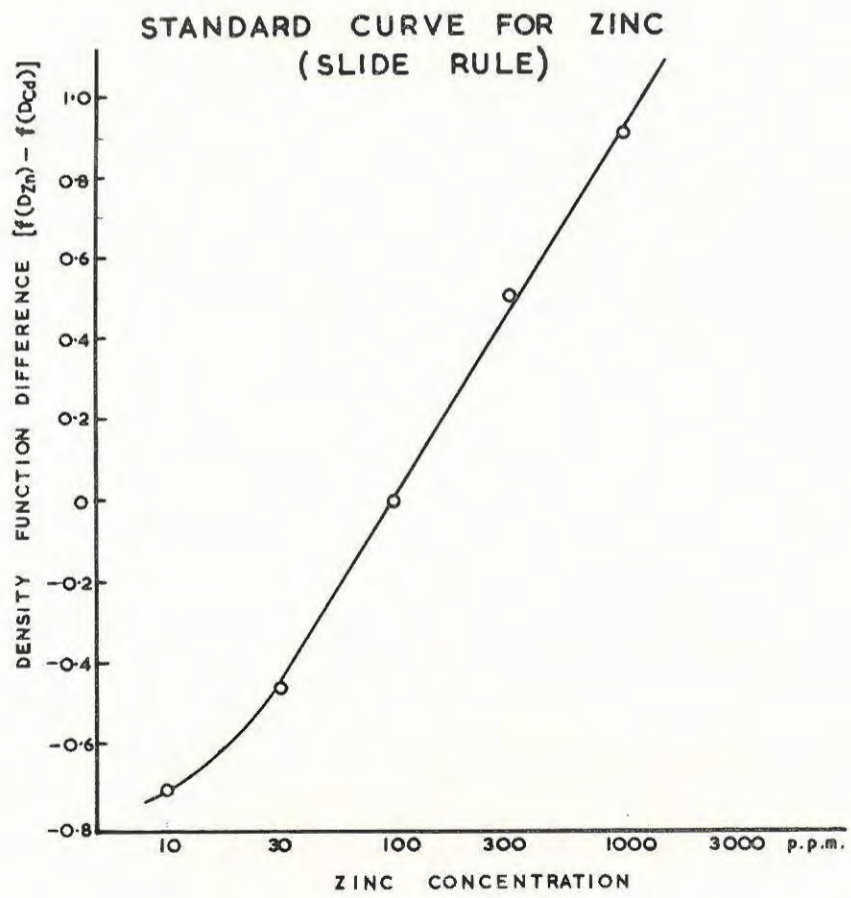


FIGURE 30.

ratios over the whole wavelength range. In spite of the findings of Schmidt, Manders and van Wijk⁽⁶⁰⁾, however, the step sector cannot be used with impunity with an intermittent source and, therefore, in the work with the interrupted arc a neutral step filter was used. This was nominally a 100% : 33% : 10% filter, but spectrophotometer measurements showed that the transmission of each step varied with wavelength as shown in Table XIV. In the calculation, however, it was assumed that the nominal filter calibration was correct for the first two steps which were used for the preparation of the emulsion calibration curve, according to the preliminary curve method described below.

TABLE XIV
Variation of Step Filter Transmission
With Wavelength.

λ	T ₁	T ₂	T ₃
3,800 A	100%	29.9%	8.8%
3,400 A	100%	31.6%	9.1%
3,100 A	100%	32.9%	9.3%
2,800 A	100%	33.9%	9.5%

(b) Calculation of Intensity Ratios.

The calculation of intensity ratios from the measured densities or transmittancies of the spectrum lines is often one of the more tedious steps in spectrographic analysis, as is evidenced by the large number of publications which have appeared from time to time describing graphical, mechanical and electrical methods for shortening the calculation. [See examples given by Churchill⁽⁶¹⁾, Hughes and Murphy⁽⁸²⁾, Frederickson⁽⁶²⁾ and Epstein⁽⁶³⁾]. Two important methods in general use are the Blackening Curve Separation Method and the Preliminary Curve Method.

(i) The Blackening Curve Separation Method.

The blackening curve separation method of calculating intensity ratios has been described by Mitchell⁽¹⁵⁾. In this method curves are plotted of density against log relative exposure (H and D curve) for the steps of each spectrum line. The horizontal distance (i.e. parallel to log relative exposure axis) between the curve for the analysis element and for the internal

standard line gives the log intensity ratio of the two lines. The density at which the separation is measured is chosen as a convenient value which falls on the straight-line portion of the H and D curve (0.4).

There are theoretical and practical advantages to obtaining the intensity ratio at a particular plate density, but there are serious disadvantages to this method. Since each emulsion calibration curve has to be obtained from readings made on a single spectrum line, it is necessary, when this method of calculation is used, to use a step sector with a large number of steps. (Mitchell⁽⁴³⁾ used a 6-step sector). This means that, unless extremely short steps are used, fewer spectrograms can be recorded on each plate than with other methods. Replacing the step sector with a step filter would further complicate the method, which is therefore not well suited for use in combination with an intermittent light source. Even with the large number of steps used, it cannot be insured, particularly with low element concentrations, that points on either side of the separation density will be obtained, and if all the points lie off the straight-line portion of the curve, the calculation becomes extremely inaccurate. The final result is very much dependent on individual density measurements even under ideal conditions.

This method has been improved by the use of Seidel density scales to give a longer straight line characteristic, as suggested by Black⁽⁶⁵⁾, and a slide rule based on the principle of this method, has recently been described⁽⁶⁶⁾.

(ii) The Preliminary Curve Method.

In the Preliminary Curve Method, which is described by Churchill⁽⁶¹⁾, a single emulsion calibration curve is prepared for internal standard and element line for a number of spectrograms. A preliminary curve for which the density or transmission function for one step is plotted against that for the next step, effectively averages the relationship between the densities of pairs of steps with the same exposure ratio. The final accurate emulsion calibration curve, giving the relationship between density or transmission and log relative exposure, is prepared from the preliminary curve. Then if the log relative exposures are read from the curve

for corresponding steps of element and internal standard lines, their difference gives the log exposure ratio, and provided there is no reciprocity failure, this is equal to the log intensity ratio.

This method has the advantage over the blackening curve separation method that less plotting has to be done and since an intensity ratio is obtained for each step of each spectrum, individual microphotometer readings are less important.

The preliminary curve method was used for the calculation of most of the results reported in this thesis. An A.R.L. calculating board, fitted with Seidel transmission scales, was used and the preliminary curve was obtained by plotting the transmission values of the less dense step of a pair, as abscissa, against that of the denser step as ordinate, for element and internal standard lines of all the spectrograms on a plate. In order to obtain the final emulsion calibration curve it was assumed that a transmission of 70% (density = 0.155) corresponded to unit relative exposure which gave the first point. For spectra photographed with the 1:2 step-ratio sector, further points were obtained from the preliminary curve for relative exposures of $\frac{1}{2}$, 2, 4, 8, 16, etc., and these points were plotted opposite corresponding values on a movable horizontal logarithmic scale. Intensity ratios were obtained as described above, except that 1.0 of the movable horizontal scale was placed opposite the internal standard transmission on the curve and log intensity ratio read directly, opposite the transmission of the element line.

(iii) Background Correction.

Nachtrieb⁽⁶⁷⁾ has stated that background densities below 0.1 can normally be neglected. In trace analysis, a background density as high as this would probably influence some results appreciably, but, in the analyses described here, conditions were so chosen that the background was always very much below this level (normally $<.04$). The necessity for the extra calculation work involved in making background corrections was thus avoided. Although no background corrections were made, the discussion of calculation methods would not be complete without some brief mention of the principal methods used, especially as the question of background

correction affects the general usefulness of the slide rule which will be described in the next section.

Two valid methods of background correction appear to be in common use. The first, described by Nachtrieb⁽⁶⁷⁾, consists of obtaining relative intensities from densities by means of the emulsion calibration curve, and subtracting background relative intensity from the relative intensity of line plus background. This method is sound but could prove tedious.

If log intensity ratios of line plus background to background, and background to background are first calculated, we have

$$\log \frac{L_E + B_E}{B_E}, \quad \log \frac{L_S + B_S}{B_S} \quad \text{and} \quad \log \frac{B_E}{B_S}$$

where L_E = element line intensity.

B_E = element line background intensity.

L_S = internal standard intensity.

B_S = internal standard background intensity.

$$\text{Then } \log \frac{L_E}{L_S} = \log \left(\frac{L_E + B_E}{B_E} - 1 \right) - \log \left(\frac{L_S + B_S}{B_S} - 1 \right) + \log \frac{B_E}{B_S} \quad \text{----} \quad 3.9$$

When element and internal standard line background intensities can be taken as equal, the above equation

becomes

$$\log \frac{L_E}{L_S} = \log \left(\frac{L_E + B}{B} - 1 \right) - \log \left(\frac{L_S + B}{B} - 1 \right) \quad \text{-----} \quad 3.10$$

The values of $\log \left(\frac{L+B}{B} - 1 \right)$ can be obtained from $\log \left(\frac{L+B}{B} \right)$ by using tables of subtractive logarithms⁽⁶⁸⁾.

This is the basis of the method for background correction described by Mitchell, Scott and Farmer⁽⁶⁹⁾ and is particularly suited to use with the blackening curve separation method.

(c) A New Slide Rule for the Calculation of Spectrochemical Results.

The preliminary curve method was found to give satisfactory results, but the calculation of intensity ratios remained one of the more tedious steps in the analysis, and some thought was therefore given to possible methods of shortening it. As a result, a special slide rule was designed which has proved perfectly satisfactory in preliminary tests.

The principle of this slide rule is the application of the Kaiser function in order to obtain a linear relationship between spectrogram line density and relative exposure. Discussion with associates who had used the Respektra Calculating Board⁽⁷⁰⁾, confirmed that calibration curves, obtained according to the Kaiser method, were, for all practical purposes, straight lines and that they remained virtually constant for a particular type of plate.

(i) Description of the Slide Rule

The slide rule, with which the tests described here were made, was designed by the author and made in the C.S.I.R. Workshop. It consists of three parts, as indicated in Figure 29. A and B have on them exactly similar scales which consist of a linear density scale at the top and a Seidel density scale at the bottom, corresponding values being joined by straight lines. C is merely a blank white surface. The cursor consists of two parts, the main cursor which slides from left to right, and a minor cursor which slides up and down in the main cursor. By means of the minor cursor, the scales A and B can be read, at top and bottom of the minor cursor, at the same distance between the normal density and Seidel scales. The position of the minor cursor is determined by the transformation constant and the cursor is marked accordingly for values of k between 0 and 1. The cursor has an aperture cut in it over C so that pencil marks can be made on C opposite the cursor line.

(ii) Methods of Use

The Transformation Constant

The first step in the calculation is the determination of the transformation constant k in the Kaiser function.

$$f(T) = k \log \left(\frac{1}{T} - 1 \right) + (1-k) \log \left(\frac{1}{T} \right) \text{-----} 3.7$$

It can readily be shown⁽⁶⁷⁾ that

$$k = \frac{(\Delta H - \Delta H')}{(\Delta H - \Delta H') - (\Delta S - \Delta S')} \text{-----} 3.10$$

where ΔH and ΔS are the differences in density function and Seidel function (i.e. ordinate distance) for one pair of exposures and $\Delta H'$ and $\Delta S'$ are the corresponding

THE SLIDE RULE.

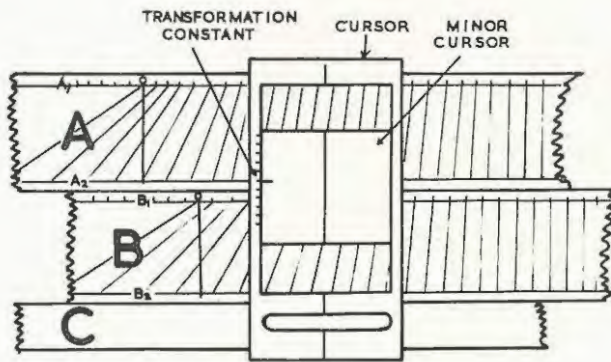


FIGURE 29.

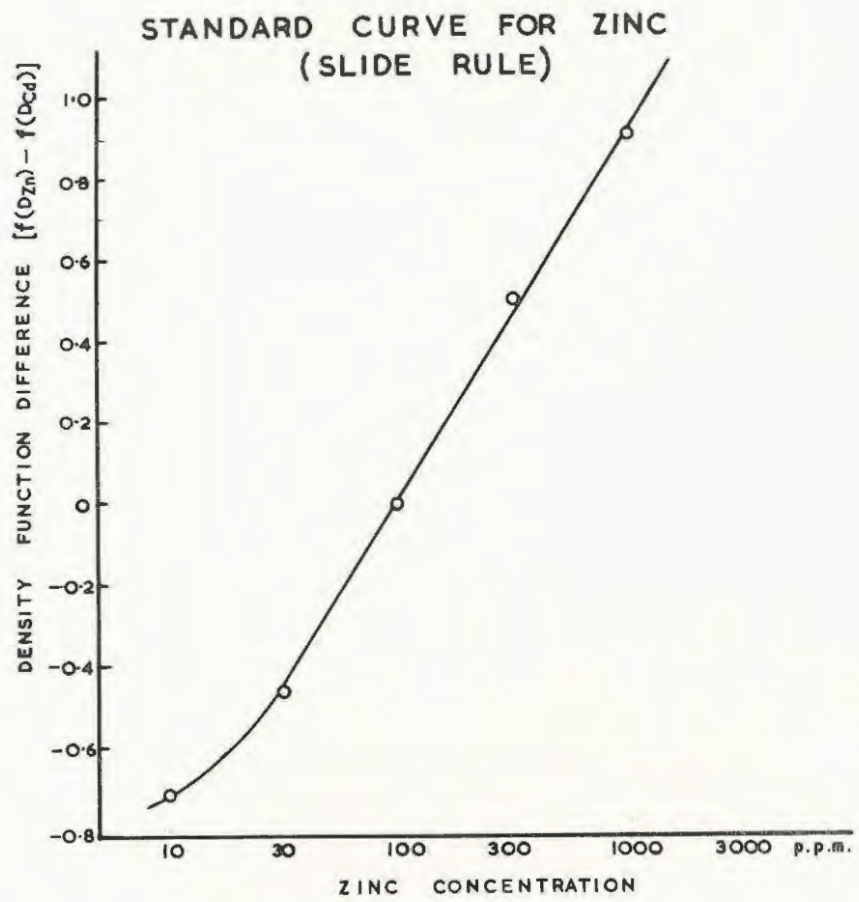


FIGURE 30.

differences for another pair of exposures of equal ratio to one another. ΔH and ΔS are thus expressions of the slope of the "H and D" and Seidel curves, and introduction of the correct value of k should have the effect of making the slope of the emulsion calibration constant.

To find $(\Delta H - \Delta H')$ the density of one step of a pair on scale A_1 is placed opposite the density of the other step on scale B_1 . The position of the zero of scale B is then marked with a pencil on C. This is repeated with a second pair of steps and the difference between the new zero position of scale B and the previous position (marked on C) is read off on scale B_1 .

To find $(\Delta S - \Delta S')$ a similar procedure is followed, but the density values on scales A_2 and B_2 are placed opposite and the zero differences are read from scale B_1 as before. Having calculated $(\Delta H - \Delta H')$ and $(\Delta H - \Delta H') - (\Delta S - \Delta S')$, k may be found by division with an ordinary slide rule.

This may be repeated once or twice to obtain a mean value for k .

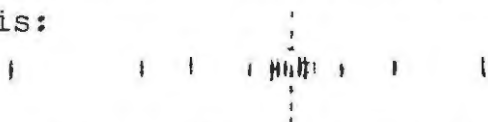
An alternative "trial and error" method for k was found to be both simpler and quicker. In this method an arbitrary value of k is selected. The minor cursor is then set for this transformation constant. The densities of two steps of a spectrum line near the lower end of the density scale are then placed opposite one another on those positions of the A and B scales which fall under the extremities of the cursor line on the minor cursor. The zero difference between scales A_1 and B_1 is then read off on scale A_1 . This is repeated for a step pair near the higher end of the density scale. If the correct transformation constant has been chosen, the zero difference on scale A_1 should be the same as the previous setting. If it is greater, a greater transformation constant is indicated and vice versa.

As it is found in practice that k varies with density, the step pairs at upper and lower ends of the scale should have approximately the same densities for each calculation of k and the lower step pairs should correspond, more or less, to the lowest density readings to be used in the calculation. It is found that single figure accuracy is quite sufficient in the determination of k .

After the determination of k the rest of the calculation can be carried out by either of two methods. The first of these corresponds in principle to the preliminary curve method and has only slightly fewer steps. The second method is much shorter.

Method 1.

The first step in this method is to determine the slope of the emulsion calibration. If the minor cursor has been set to the correct value of the transformation constant, the linear distance between density settings should be constant. In other words, for equal exposure steps, apart from minor errors in photometer readings, the zero differences read from scale A_1 should be constant when the densities of successive steps are placed opposite on the selected density function scale (at top and bottom of the minor cursor). These minor errors in photometer readings are eliminated by averaging. For each rule setting a pencil mark is made on C opposite the zero of scale B_1 . This gives a series of pencil marks which look like this:



The mean, as indicated by the dotted line can readily be judged by eye and with the cursor set opposite this mean position the corresponding mean value read off scale A_1 . To get the slope of the calibration curve this value is divided by the log of the step exposure ratio (.301 for a normal step sector).

This averaging procedure, to find the slope of the emulsion calibration curve, corresponds to the plotting of the preliminary curve in the Churchill method.

To calculate the intensity ratio, the k function (the Kaiser function with the selected value of k) density difference is found by placing the density values for element and internal standard lines opposite one another at top and bottom of the minor cursor and the zero difference read from scale A_1 . This value is divided by the slope of the characteristic curve, using an ordinary slide rule, to give the log of the intensity ratio.

This result may be used directly to give the concentration if the standards have been treated in the

Slide Rule Theory

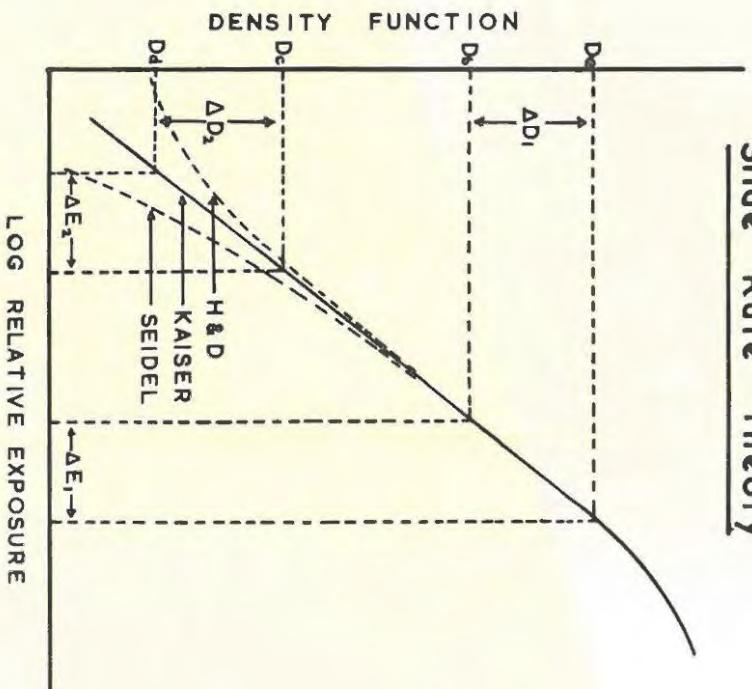


Figure 31.

same way and linear x log graph paper used to plot log intensity ratio against concentration.

Method 2.

It is found in routine analysis work that the transformation constant and the slope of the characteristic curve do not vary appreciably between plates of the same emulsion type and batch (This variation is even less in the case of film) provided development conditions are carefully controlled. It would, therefore, using Method 1, only occasionally be necessary to recalculate these values. The basis of the second method is that, if the transformation constant and characteristic curve slope do not vary, they need not be taken into account. The density values for element and internal standard line are simply placed opposite one another at top and bottom of the minor cursor and the linear zero difference read on scale A_1 . Concentrations are then read from a standard calibration curve which is prepared by plotting density function separations, obtained as above, against concentration on linear x log graph paper. A standard curve for zinc obtained in this way is shown in Figure 30.

(iii) Theory of the Use of the Slide Rule

It is found that, if the optimum transformation constant is used, the plot of density (on the Kaiser function scale) against log relative exposure is, for all practical purposes, a straight line over a wide density range ($D=0.05$ — $D=1.4$). (See Figure 28).

If D_a and D_b are the density readings for one pair of spectrum steps at a high relative exposure, D_c and D_d are the density readings for another pair of steps at low relative exposure, and δE_1 and δE_2 are the corresponding differences in log relative exposure (see Figure 31) then, if the step ratios are the same in each case, $\delta E_1 = \delta E_2$ and if, the correct transformation constant has been selected, $\delta D_1 = \delta D_2$.

This gives the "trial and error" method of finding k.

The formula for the first method for the determination of k,

$$k = \frac{(\Delta H - \Delta H')}{(\Delta H - H') - (\Delta S - \Delta S')}$$

is given by Anderson⁽⁷⁰⁾.

The slope of the emulsion characteristic is merely the average δD divided by δE .

Method 1 consists of finding δD for element and internal standard lines and dividing by the slope to get the corresponding δE .

$$\begin{aligned} \text{i.e. } \delta E &= \log \text{ exposure 1} - \log \text{ exposure 2.} \\ &= (\log \text{ intensity 1} \times \text{time}) - (\log \text{ intensity 2} \times \text{time}) \\ &= \log \frac{\text{intensity 1}}{\text{intensity 2}} \end{aligned}$$

(since time of exposure is the same for element and internal standard).

In Method 2 it is simply assumed that δD is proportional to δE .

(iv) Background Correction Using the Slide Rule

Either of the two basic methods for background correction already described could be used in conjunction with the slide rule.

The first method would involve obtaining the relative intensities equivalent to the measured densities. To do this the density value (of line or background) should be placed opposite the top of the line on the minor cursor and the corresponding value read from Scale A_1 . Division of this reading by the characteristic slope and looking up the antilog of the result, gives a relative intensity value. Relative intensity of background can then be subtracted from relative intensity of line plus background.

For the application of the second background correction method, line plus background to background, intensity ratios can be calculated as described under Slide Rule Method 1. Then, since the relationship between density and Seidel density scales is the same as between logarithm and subtractive logarithm, if the cursor is set to $\log \frac{L+B}{B}$ on A_2 the corresponding value read from A_1 will be $\log \frac{L}{B}$. Division would then give $\log \frac{L_E}{L_S}$.

Where the slope of the emulsion calibration does not deviate very much from unity (as is often the case), a shorter background correction could probably be made along the lines of the Slide Rule Method 2 and the second background correction method without incurring appreciable errors.

(v) Results Obtained Using the Slide Rule

In order to test the slide rule, a number of analysis results which had been previously obtained using the preliminary curve method of Churchill, were recalculated using both the first method and the second method described above. In Table XV a series of analysis results for lead in plant samples, calculated by both slide rule methods, are compared with the results obtained by the Churchill method.

TABLE XV

Comparison of Results Calculated
by the Churchill Method and with
the Slide Rule

Sample	A Churchill Method (p.p.m. Pb)	B Slide Rule 1 (p.p.m. Pb)	C Slide Rule 2 (p.p.m. Pb)
1	1.70	1.72	1.76
2	3.13	3.14	3.40
3	3.25	3.17	3.46
4	0.93	0.96	0.91
5	3.50	3.55	3.65
6	2.18	2.22	2.38
7	2.40	2.45	2.65
8	2.15	2.13	2.29
9	2.20	2.18	2.35

The difference between the results obtained by the Churchill method and by Method 1 with the slide rule are insignificant. The results obtained by Method 2 are significantly different, but it must be emphasised that the same standard curve was used in A and B, but a new standard curve had to be drawn up to get the results reported under C. The differences shown by C therefore incorporate possible differences in the standard calibration curve.

In order to get an idea of the comparative accuracy of the second slide rule method and the Churchill method, some analysis results were calculated with the slide rule and three times using the Churchill method. These results are shown in Table XVI. The slide rule calculations were carried out by the author,

while the three sets of calculations by the Churchill method were carried out by an assistant. For the first two sets of figures obtained by the Churchill method, the same standard curves were used, but a period of about six months elapsed between the two sets of calculations. For the third set of figures obtained by the Churchill method, standards were recalculated and new standard calibration curves drawn up. These results suggest that the error involved in using the slide rule by the second method is no greater than the error involved normally in graphical methods of calculation.

In the results reported at the end of this chapter, which were obtained in the tests of the accuracy of the proposed analysis method, figures obtained for zinc, molybdenum and cobalt are compared with figures obtained by the second slide rule method. These show no significant difference.

TABLE XVI
Further Comparison of Calculation Methods

Sample	Slide Rule (Method 2) (p.p.m. Zn)	Churchill 1 (p.p.m. Zn)	Churchill 2 (p.p.m. Zn)	Churchill 3 (new std. curve). (p.p.m. Zn)
A	14.6	13.8	14.3	14.2
	14.4	13.6	14.7	14.5
	14.2	13.7	14.7	14.6
B	13.4	12.5	13.6	13.4
	12.8	13.0	13.8	13.5
	13.2	11.3	13.4	13.3
C	32.4	31.7	33.2	34.2
	35.6	36.0	37.2	38.2
	30.1	29.8	31.4	32.3
D	41.8	40.9	42.2	41.6
	46.4	45.1	48.0	46.6
	43.0	42.2	44.2	43.6
E	25.0	25.5	25.6	25.8
	23.6	23.2	23.2	23.6
	30.1	31.3	33.2	33.8
F	13.6	13.0	13.4	13.9
	13.2	13.2	13.7	14.0
	12.8	13.1	13.1	13.6
G	36.4	36.0	38.6	37.4
	38.6	38.0	40.2	39.9
	40.8	40.8	43.4	43.1

D. THE PROPOSED ANALYSIS METHOD

The findings made in the preliminary investigations described above were finally consolidated in the proposed analysis method described below and test analyses were made to assess the precision and accuracy of analysis.

1. Experimental*

(a) Apparatus

The apparatus used has been fully described in preceding sections.

(b) Reagents

All the reagents used were the purest available. Acids and ammonia were redistilled in glass. Distilled water was further purified by passage through an ion exchange column. (Amberlite M.B.3)

All aqueous solutions were stored in polythene bottles.

Dithizone

0.02% (weight to volume) prepared by dissolving Eastman Kodak Dithizone in redistilled chloroform.

Oxine

1% Analytical Grade 8-hydroxyquinoline dissolved in redistilled chloroform.

Cupferron

A 6% aqueous solution was prepared fresh for each set of analyses by weighing the appropriate quantity in to a beaker, adding the required volume of water and filtering off the insoluble portion.

Ammonium Citrate Solution

400 gms of ammonium citrate were dissolved in 1 litre of water, ammonia added to give pH=9 and the solution extracted with dithizone. Finally water was added to make 2 litres.

Standard Solutions

Zinc. 100 ppm. Prepared by dissolving "Specpure" zinc oxide in a little dilute HCl and diluting.

Cobalt. 200 ppm. Prepared from analytical grade cobalt chloride.

* During the experimental work to test the precision and accuracy of the proposed method, the author was assisted with parts of the analyses by Miss E.C. Maskew, Scientific Assistant.

<u>Nickel.</u>	1000 ppm.	Prepared from analytical grade nickel chloride.
<u>Lead.</u>	100 ppm.	Prepared from "Specpure" lead nitrate.
<u>Titanium.</u>	100 ppm.	Prepared from analytical grade potassium titanium oxalate.
<u>Vanadium.</u>	500 ppm.	Prepared by dissolving "Specpure" vanadium oxide in dilute sodium hydroxide, the solution being made slightly acid by addition of dilute hydrochloric acid.
<u>Molybdenum.</u>	100 ppm.	MoO ₃ dissolved in dilute sodium hydroxide and solution made slightly acid with dilute HCl.

Portions of these solutions were mixed and suitably diluted just before running a series of standards to give a solution containing 10 ppm. Zn, 0.2 ppm. Co, 2 ppm. Ni, 1 ppm. Pb, 0.5 ppm. Mo, 10 ppm. Ti, 0.5 ppm. V. Suitable volumes of the latter solution were then treated in the same way as the sample solution.

Internal Standards Solutions.

Cadmium. 20 ppm. prepared by diluting a 500 ppm. solution prepared from "Specpure" cadmium sulphate.

Silver. 1 ppm. in Chloroform:

A 20 ppm. solution was prepared from "Specpure" silver nitrate. 5 ml. of this solution was diluted, acidified slightly with sulphuric acid and extracted with dithizone. The extract was diluted to 100 ml.

(c) Procedure

(i) Sample Solution

Weigh 5 grams of dried and powdered plant material into a silica basin and ash in a muffle furnace at 450°C overnight. After allowing the ashed sample to cool, add a little water and 5 ml. of redistilled 1:1 hydrochloric acid and cover with a watch glass. When the initial reaction has subsided, wash down the watch glass and add more hydrochloric acid, drop by drop, until there is no further effervescence. Filter off the residue and ash the filter paper and residue in a platinum crucible. Fuse this ash with 1 gram of sodium carbonate and, after cooling the fusion mixture, dissolve it in 1:1 redistilled hydrochloric acid.

Filter the resulting solution to remove silica, the residue being washed with hot water containing a little hydrochloric acid, and add it to the first filtrate.

(ii) Dithizone Extraction

Transfer the solution to a 250 ml. open-topped separatory funnel and add 40 ml. of 20% ammonium citrate and 5 ml. of standard cadmium solution. Adjust the pH to about 9.5 by the addition of ammonia (redistilled 1:1). Add 20 ml. of 0.02% dithizone solution and agitate the mixture with the mechanical stirrer for two minutes. Run the extract into a beaker and re-extract the solution with 5 ml. of dithizone and finally with 5 ml. of pure chloroform. If the latter extract is pure green, the extraction can be regarded as complete. Otherwise repeat the extraction with 10 ml. of the dithizone solution.

(iii) Oxine Extraction (Removal of Iron)

Next adjust the pH of the solution to 2.9-3.0 by the addition of 5N hydrochloric acid and extract with 20 ml. portions of the oxine solution. The extraction should be repeated (normally three or four times) until the chloroform phase is only slightly coloured. Discard this extract.

(iv) Cupferron Extraction

Reduce the pH of the solution further to about 2 by the addition of hydrochloric acid and add 2 ml. of freshly prepared 6% cupferron solution. Then extract twice with 10 ml. volumes of pure chloroform. Repeat this extraction after reducing the pH to about 0.5.

(v) Preparation of the Extracts for Arcing.

Add a 5 ml. aliquot of the standard silver dithizonate solution to the dithizone extract. Evaporate the dithizone and cupferron extracts in turn on to 100 mg. of graphite by the method described. After drying and ashing the graphite-concentrate mixture, add 33 mg. of lithium carbonate and mix thoroughly. Load portions of the mixture into 2 sample electrodes. [Using the "Elpac" electrode packing machine⁽¹³⁾]

(vi) Spectrographic Step.

Burn each electrode as anode in an interrupted d.c. arc at 4 amps, allowing a "pre-burn" of 30 seconds and an exposure of two minutes. Photograph the spectrum on an Ilford Ordinary plate, duplicate spectra

being recorded for each sample.

(vii) Standard Calibration Curves.

Standard calibration curves were prepared by working through the above procedure, using measured volumes of the standard solution instead of the sample. Standard curves obtained by this method are shown in Figure 32.

2. Analysis Results

(a) Precision of the Method.

In order to insure that the coefficients of variation obtained were a reflection of the precision of the analysis method alone and unaffected by inhomogeneity of the sample material, the reproducibility test was carried out by repeated analysis of aliquots of a sample solution rather than a number of samples. A solution of 100 grams of plant material was prepared and sixteen aliquots, each equivalent to 5 grams of sample, were analysed. The results are shown in Table XVII.

TABLE XVII

RESULTS OF ANALYSIS OF SIXTEEN ALIQUOTS
OF A PLANT SAMPLE SOLUTION

(Each Aliquot Equivalent to 5 gms. of sample)

	Zn	Co	Ni	Pb	Ti	V	Mo
Mean Result (p.p.m.)	30.4	0.65	2.08	2.2	11.0	0.32	0.97
Std. Dev. (p.p.m.)	1.1	0.09	.13	0.5	0.13	0.04	0.10
Coeff. of Var.	3.6%	14.5%	6.4%	22%*	11.6%	11%	11%

* The last 4 aliquots seem to have become slightly contaminated.

- std deviation for Pb on first 12 analyses = 0.3 p.p.m. or 16.4%.

(b) Comparison of Analysis Results with those obtained by other Methods.

In order to get an idea of the accuracy of the method, eight standard samples which had been analysed previously by other methods were re-analysed and the results compared. These results are shown in Table XVIII.

STANDARD CALIBRATION CURVES

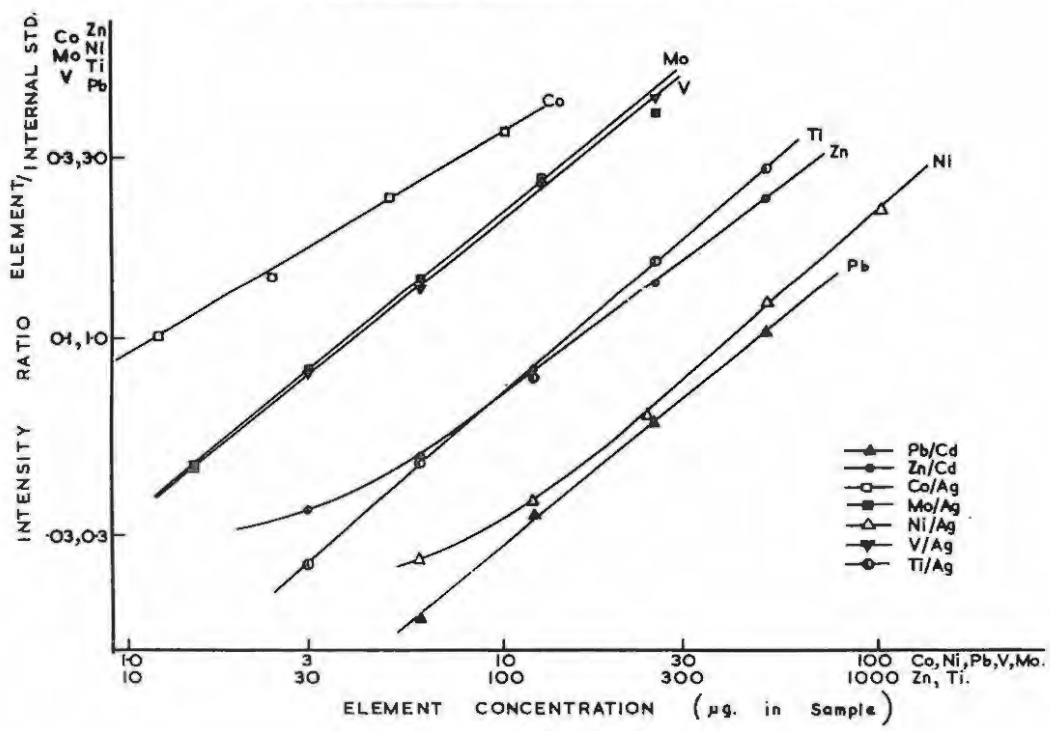


FIGURE 32.

TABLE XVIII

Comparison of Analysis Results

Sample		Element Concentrations (p.p.m.)						
		Zn	Co	Ni	Pb	Ti	V	Mo
4A Peach Leaves	1*	21	0.10	4.3	2.9	7.8	0.26	<0.2
	2**	24	0.1	2.6	2.4	10	0.24	0.07
4B Bean Leaves	1	50	0.18	7.3	4.6	28.9	0.47	1.81
	2	58	0.24	5.7	2.9	37	0.71	2.3
4C Beet Leaves	1	25	0.27	2.8	2.3	19.2	0.40	0.74
	2	22	0.25	2.5	2.1	21	0.49	1.23
4D Gem Leaves	1	55	0.50	3.2	2.3	27.9	0.40	2.10
	2	48	0.31	3.3	4.0	41	0.92	3.98
4E Plum Leaves	1	49	0.46	1.4	3.5	6.8	0.29	<0.2
	2	55	0.64	1.4	2.4	8.5	0.23	-
4G Bush	1	30	0.73	2.2	1.9	11.6	0.39	<0.2
	2	30	0.41	4.4	1.2	27	0.70	
4H Tobacco	1	34	0.84	8.7	1.7	18.3	0.57	<0.2
	2	34	0.92	8.2	1.1	17	0.39	
4I Tobacco	1	50	1.95	15.2	0.9	80.8	2.18	<0.2
	2	54	2.16	13.3	1.5	74	2.38	

* 1. Results obtained by the method described.

** 2. Average value for results obtained by various other methods (See Appendix p.103).

(c) Discussion of Results

The precision of the method, as indicated by the coefficients of variation, is very good in the case of zinc and quite adequate for nutritional studies in the case of the other elements, with the possible exception of lead. It is evident from previously reported results that analysis for the latter element presents peculiar difficulties, possibly due to reagent contamination.

According to the criteria of accuracy suggested by Ahrens⁽⁷¹⁾ for rock and soil analysis, the precision of the zinc determination may be classed as very good,

that for nickel as good and that for the other elements as fair to good. Except in the case of zinc the precision compares rather unfavourably with that reported by Mitchell⁽⁴³⁾. However, the figures reported by him have apparently been calculated for repeated arcing of the same concentrate and do not represent the overall precision of the analysis method. The actual element concentration in the arc material is also considerably higher than that obtained here.

Due to poor agreement, in most cases, between the previous analyses of the "standard" samples, it is difficult to draw definite conclusions as to the absolute accuracy of the method. In most cases, however, the figures obtained show the same trends as the means of previous results and in a few cases the agreement is very good. In general it can be concluded that the accuracy compares well with that of other methods.

The sensitivity in the case of molybdenum is not as high as might be desired. This difficulty could undoubtedly be overcome by using a ten gram sample instead of five grams (as was done in the preliminary tests), or by evaporating the sample on to 50 mg. of graphite instead of 100 mg.

Conclusions.

The concentration method described is both rapid and suitable for routine use. With the extraction apparatus used it was found possible to work through the concentration procedure, for six samples together, in about two and a half hours. The precision and accuracy of the method should prove adequate for most trace element nutritional studies.

(d) Slide Rule Calculated Results for Zn, Co, and Mo.

In order to obtain a further indication of the reliability of results calculated with the help of the proposed slide rule the figures for zinc, cobalt and molybdenum, obtained in the analysis of eight of the standard samples, were recalculated using the second slide rule method. The results obtained are compared in Table XIX with figures taken from Table XVIII (Calculated

by the Churchill Method).

TABLE XIX
Comparison of Results Calculated With
Slide Rule.

Sample	Zn (p.p.m.)		Co (p.p.m.)		Mo (p.p.m.)	
	Slide Rule	Churchill	Slide Rule	Churchill	Slide Rule	Churchill
4A	20	21	0.08	0.10	-	-
4B	51	50	0.13	0.18	1.54	1.81
4C	25	25	0.24	0.27	0.64	0.74
4D	56	55	0.42	0.50	1.72	2.10
4E	48	49	0.43	0.46	-	-
4G	29	30	0.62	0.73	-	-
4H	33	34	0.81	0.84	-	-
4I	50	50	1.99	1.95	-	-

While the figures obtained are not identical with those obtained previously, the differences are certainly much smaller than the expected analysis error and there are indications that they are no greater than the error normally incurred in the graphical calculation.

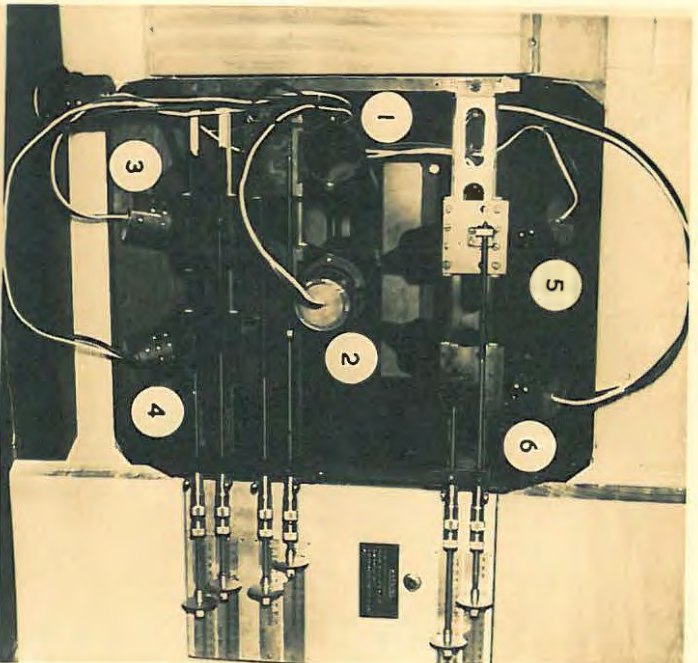


Figure 33.
Photomultiplier and Exit Slit
Assembly.

CHAPTER IV

DIRECT READING SPECTROMETRIC ANALYSIS OF
PLANT MATERIAL

In the introductory chapter, the advantages of direct reading spectrometric methods have been briefly discussed. These advantages over the more conventional photographic methods of emission spectrochemical analysis are generally recognised and the scope of direct reading spectrometry is being rapidly widened to include samples of greater variety and complexity. Direct reading methods for the determination of trace metals in plant material are therefore highly desirable.

For reasons already mentioned, if direct reading methods are to be used to the best advantage, each step in the analysis procedure must be carefully considered. Since the instrument used was of a medium dispersion type with relatively wide entrance and exit slits, the development of a suitable excitation source which would produce simple spectra free from excessive background, received particular attention.

The limitations on the proximity of the exit slits would have made it difficult to analyse for a large group of elements and it was decided initially to develop a method for zinc, lead and copper only. The experimental work described in this chapter, although it only deals with the determination of this small group of elements, has established the applicability of direct reading spectrometry to the analysis of plant material.

A. APPARATUS

The spectrometric equipment used consists of a Hilger Medium Quartz Spectrograph with a direct reading attachment built in the laboratory. The photomultiplier unit, which is mounted in place of the plate holder of the spectrograph, consists of six movable E.M.I. head-on-type photomultiplier tubes mounted in the manner shown in Figure 33. The light from the spectrum line to be measured passes through a slit attached to each photomultiplier assembly and is reflected by a small mirror into the photomultiplier itself. The position of each tube can be accurately adjusted by means of the micrometer screws shown in the photograph. The mounting is so arranged that the slit of either tube 3 or 4 can

be placed as close as is desired to that of tube 5 or 6, or on the same line if necessary. The same conditions apply as regards the setting of either tube 5 or 6 with relation to the positions of tubes 3 or 4. The nearest possible approach of exit slits in the same bank is, however, about 10 mm. Tubes 1 and 2 are intended for use in the weak far-ultraviolet region and their slits are designed and set to include the whole spectrum image.

During the exposure the current produced by the photomultipliers is used to charge condensers. An electronic measuring system consisting of a modified Naish-Ramsden⁽⁷²⁾ circuit with a servo mechanism, is used to indicate the charge on the condensers after the exposure has been made.

The spectrograph is inclosed in a wooden case lined with slag wool and the whole apparatus is housed in a temperature and humidity controlled room. These precautions are taken to avoid positional changes in the spectrum due to temperature variation, and variation in electronic measuring circuit due to humidity.

Slit Widths: Entrance slit, 0.04 mm.; Exit 0.06 mm.

External Optics: The external optical arrangement was similar to that used with the Large Hilger Spectrograph, an image of the source being focussed first on a diaphragm and then on the collimator of the spectrograph.

Source Units: A "Multisource" unit, built in the laboratory, was used in some of the preliminary work and for providing the spark source which was used in setting the positions of the exit slits.

Auxiliary Spectrograph: A Fuess medium dispersion spectrograph was used from time to time during the preliminary development work.

B. DEVELOPMENT OF METHOD

1. Excitation Source Conditions

For direct reading spectrometric analysis it is desirable to have an excitation source for which the emission of the various elements is constant, or nearly constant, for the whole period of the exposure. The reason for this is that exposures have to be made for constant periods in order that correction can be made for the electronic background noise. In addition, since relatively large entrance and exit slits are used,

it is essential to have a source which will provide a simple spectrum, free from excessive background.

Spark sources are usually suitable for direct reading work, but they do not normally give sufficiently high sensitivity for trace element analysis. As the rotating platform electrode with an interrupted d.c. arc had been used successfully in the laboratory for the analysis of mineral oils, it was decided to try this type of source for the analysis of the dithizone extract of a solution of plant material. Portions of such extracts were evaporated on to rotating platform electrodes and subjected to an interrupted d.c. arc of 5 amps (R.M.S.). The spectra, photographed with a medium dispersion spectrograph, indicated that strong spectrum lines could be obtained for zinc, copper and lead for concentrations equivalent to less than a gram of plant material.

A number of further experiments were carried out with this type of source, but the evaporation of the extracts on to the electrode proved to be a somewhat tedious step in the analysis and attention was therefore given to the possibility of using a source into which the extract solution could be introduced directly.

The first method tried consisted of spraying the extract solution into an A.C. arc between two horizontal graphite electrodes. A Beckman atomiser-burner was used to spray the solution between the electrodes, compressed air at 10 lbs/sq. inch pressure being connected to the oxygen inlet. This form of source was tried in various ways with different positions of electrodes and atomiser, but no satisfactory results were obtained. Strong band spectra were produced and the Zn and Pb lines were only weakly emitted. Aluminium electrodes were tried in an attempt to reduce the background, but this was only partially successful. No improvement in sensitivity was observed when the horizontal arc was used in conjunction with an oxy-acetylene flame.

A type of source commonly used for spark analysis of solutions takes the form of a rotating disc electrode which dips into the sample solution. This method was tried with the interrupted arc. An aqueous standard

solution was used at first, but this was found to give strong OH bands in the spectrum. A chloroform solution of metal dithizonates was found to give satisfactory excitation, spectrum lines of Zn, Cu, Cd and Pb being clearly visible in the spectrogram, while the background was considerably less than with the aqueous solution. Only a short exposure was possible, however, due to the fact that the heat produced caused the chloroform to boil.

To overcome this difficulty it was decided to try spraying the sample solution on to the disc with a Beckman atomiser as shown in Figure 34. This proved to be satisfactory as far as the production of Zn, Cu, Cd and Pb lines was concerned, but the background produced was rather strong. Use of an aluminium counter electrode with the disc as cathode was found to reduce the background a little. Next a pure aluminium disc was prepared and used in place of the graphite disc. This system reduced the CN band background to a very low level, but the background due to the aluminium itself became inconveniently strong.

Finally discs prepared from a mixture of sodium carbonate and "S.P.1" graphite were tried. These proved to be very suitable. The element lines were produced with sufficient strength, while the background was held to a minimum. Tests showed that a 4:1 mixture of graphite and sodium carbonate was most suitable. The discs were prepared by compressing 4 grams of the graphite-sodium carbonate mixture in a specially made mould in an A.R.L. briquetting press. The mould was so constructed as to produce a one inch diameter disc with a hole in its centre so that it would fit on to the spindle above the atomiser.

For the analysis the disc was placed on the spindle which was connected by a flexible drive (a piece of pressure tubing) to a variable-speed electric motor.

It was found that 10 lbs/sq. inch was the most suitable pressure for the atomiser spray, both higher and lower pressures being found to give weaker spectrum lines.

Experiment showed that the sample sprayed on to

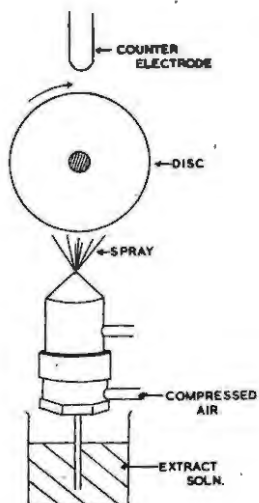


FIGURE 34
ATOMISER - ROTATING DISC
SOURCE

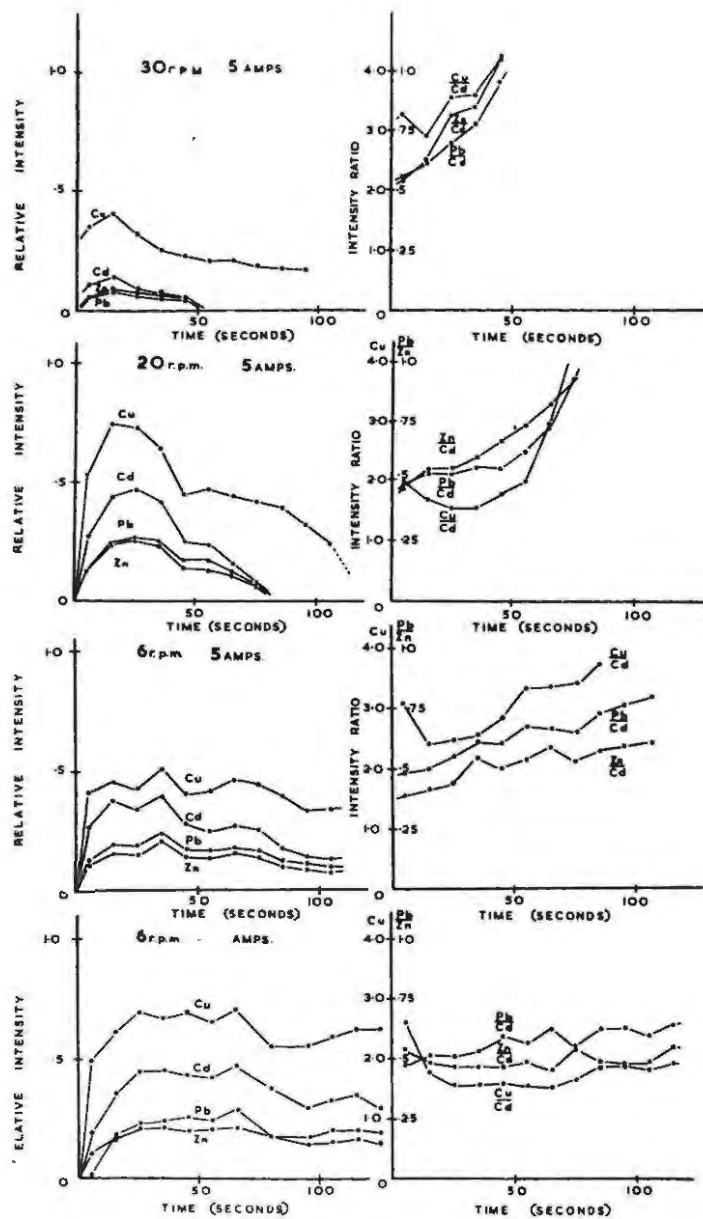


FIGURE 35
EFFECT OF CURRENT AND ROTATION
SPEED ON "VAPORISATION" CURVES

the disc was not immediately burned away, so that a new disc was necessary for each sample, but it was evident that a certain amount of control over the vaporisation rates could be exercised by varying the disc rotation speed. The results of an experiment to determine the effect of disc rotation speed are shown in Figure 35. To obtain these curves, chloroform solutions of the dithizonates of zinc, copper, lead and cadmium were sprayed on to the disc. The plate holder on the Fuess spectrograph, which was used for this work, was moved on every ten seconds of the exposure time. The relative intensity of each line was then determined and plotted against exposure time. A unidirectional interrupted arc was used with the disc as anode, the current being as indicated.

It was apparent that the volatilisation and excitation of the elements became more constant as the rotation speed and current were reduced. The rotation speed was not reduced below 6 revolutions per minute, however, as it was felt that there would not be an effective random sampling of the disc surface at lower speeds. As can be seen, the time-emission curves are virtually parallel with the time axis for the condition 3.5 amps and 6 revolutions per minute, while the intensity ratios are also nearly constant for these conditions. On the basis of these curves it was decided to expose from the 20th to the 60th second of the arc.

The excitation source finally used thus consisted of an interrupted d.c. arc struck at mains frequency (50 cycles per second) between a graphite counter electrode (cathode) and a rotating graphite-sodium carbonate disc (anode), on to which the chloroform solution of the extracted metal dithizonates was sprayed with a flame photometer atomiser. It was found necessary to control air pressure and rate of revolution of the disc very carefully for reproducible results.

2. Chemical Preparation of the Sample.

(a) Preparation of Sample Solution

As the excitation source developed was sensitive enough to allow the use of less than a gram of sample, it was convenient to use a wet-oxidation method for the

preparation of the sample solution. The possible danger of the loss of zinc when the sample is dry-ashed was thus avoided, while the method is much quicker. The procedure followed was that described by Verdier, Steyn and Eve⁽⁷³⁾.

(b) Dithizone Extraction of Zinc, Lead, Copper and Cadmium.

Since only 1 g. of sample was used, it was not necessary to have such a high concentration of citrate as formerly. The extract of the metals in question from a 1% ammonium citrate solution was therefore investigated. On the basis of the results of this investigation, which are reported in Chapter I, a pH of 8.5-9 was selected for the extraction which was made with 0.02% Dithizone in the apparatus previously described.

3. Internal Standard

Cadmium was selected as internal standard element, as it does not occur in appreciable concentrations in plant material and can readily be extracted as a dithizone complex together with zinc, copper and lead, thus providing a standardisation of the chemical extraction step as well as the spectrometric part of the procedure. It has in addition been shown to be a most suitable internal standard for zinc.

4. Spectrum Lines Used.

Since, with the instrument used, no more than two slits can be placed nearer than about 10 mm. apart, the choice of spectrum lines, even for this small group of elements, was severely limited. The following spectrum lines were used in the analysis:

- Zn 2138.6 Å, photomultiplier (1)
- Pb 2833.1 Å, photomultiplier (3)
- Cu 3247.5 Å, photomultiplier (4)
- Cd 3261.1 Å, photomultiplier (5)

In positioning the exit slits use was made of the fact that, for each of the arc spectrum lines used, there was a corresponding strong line in the spark spectrum of the element. A Feussner-type spark, struck between electrodes of the metal concerned, was used to provide a constant reference spectrum while the exit-slit-

photomultiplier assemblies were moved into position. Each exit slit was first set to approximately the correct position to receive the light from the selected spectrum line. Then, with the spark source in operation, the exit-slit-photo multiplier assembly was moved slowly across the spectrum line with the corresponding micrometer screw. Meanwhile the ratio of the moving photomultiplier tube signal to that of one of the other stationary tubes was plotted on an automatic recorder and the exit slit was finally set on the position corresponding to the maximum of the recorded curve. (commonly known as the spectrum-line profile).

5. Self Absorption of the Zinc Line

From the standard curves shown in Figure 36, it is evident that the zinc line at 2138.6 A shows marked self absorption at the higher concentrations. This means that the zinc concentration must be kept low and the conditions must be kept as constant as possible if this line is to be used. The line at 3345 A would probably have proved more satisfactory had it been possible to position the exit slits for this purpose.

C. THE ANALYSIS METHOD

1. Method

(a) Solutions and Reagents

Most of the solutions used were prepared and purified in the manner described in the previous chapter.

Standard solutions of zinc, copper, lead and cadmium were prepared from spectrographically pure salts of these metals.

5% Ammonium Citrate Solution: This was prepared by dissolving analytical grade ammonium citrate (dibasic) in half the required quantity of water. Ammonia solution was then added to bring the pH to about 9 and the solution extracted repeatedly with small volumes of dithizone solution until these remained green. The solution was then made up to the required volume.

Redistilled nitric acid and analytical grade perchloric acid were used for the wet-oxidation of the sample.

(b) Procedure

Weigh between 0.5 and 1 gram of finely ground dry plant material into a wide-necked 150 ml. conical flask. Add a mixture containing 10 ml. of redistilled nitric acid plus about 2 ml. of perchloric acid and,

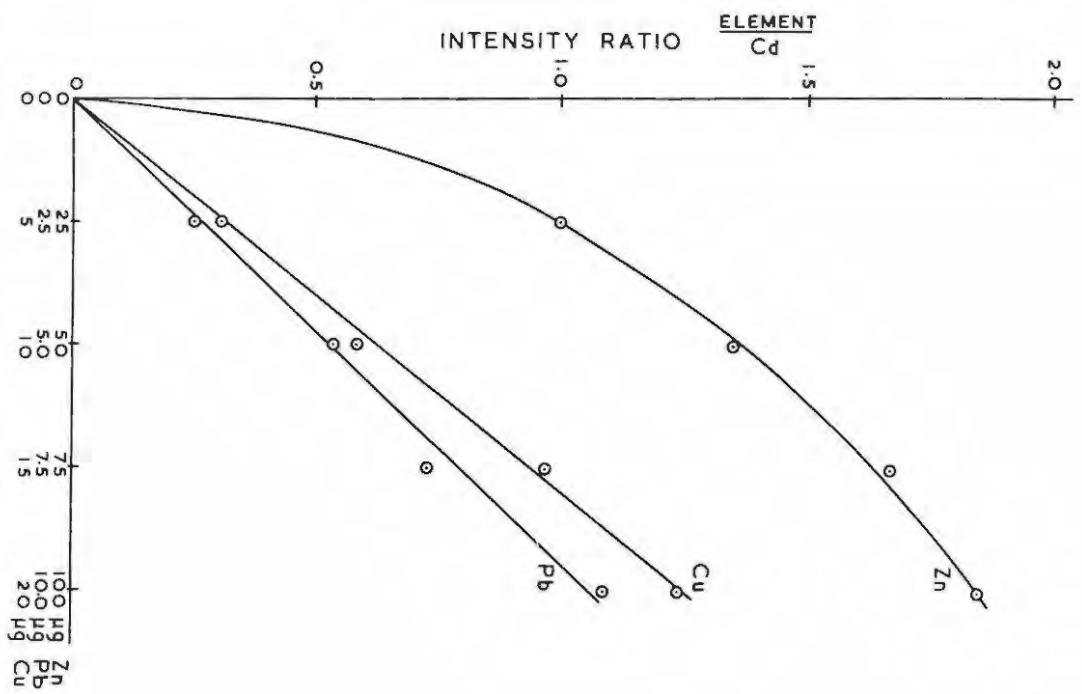


Figure 36.

after placing a reflux cover on the flask, warm the contents gently on a sand bath. After the initial vigorous reaction has subsided boil the mixture gently until a clear solution is obtained. Finally remove the cover and evaporate the contents of the flask to about 0.5 ml. Take up the residue in dilute HCl and filter through a Whatman No.40 filter paper to remove silica.

Bring the pH of the sample solution to 8.5-9 with redistilled ammonia solution after the addition of 40 ml. of 5% ammonium citrate solution and 5 ml. of standard 10 pp. cadmium solution and transfer it to a separatory funnel. Next agitate the solution with 15 ml. of an 0.02% chloroform solution of dithizone for two minutes and separate the chloroform phase. Repeat the extraction after adding 5 ml. of dithizone solution and 5 ml. of pure chloroform. Evaporate the extract partially on a moderate-temperature sand bath, transfer to a 25 ml. volumetric flask and make up to volume with pure chloroform.

Portions of this solution should now be poured into 5 ml. beakers to be sprayed on to the rotating disc for the exposure, two exposures being made for each sample and the mean intensity ratio taken.

Calculate the intensity ratio of element line to internal standard line and read the concentration from a standard graph.

The procedure followed for the standards is exactly the same, except that, in place of the sample solution, suitable volumes of a standard solution containing 5 ppm. zinc, 1 ppm. copper and 0.5 ppm. lead are used. (One or two of the standards should be re-arc'd every day so that correction can be made for changes in the instrument).

2. Analysis Results

(a) Reproducibility Test

To test the reproducibility of the method 10 aliquots of a solution prepared from 20 grams of plant material were analysed. The results of these analyses are shown in Table XX. The standard deviations were calculated for each element and found to be 5.1% in the

case of zinc, 7.3% for copper and 6.1% for lead.

TABLE XX

Results of Analysis of Aliquots of Sample Solution

Aliquot.	Element Concentrations (ppm)		
	Zn.	Cu.	Pb.
1	33.3	8.9	12.2
2	32.4	7.6	10.9
3	33.3	9.3	12.9
4	31.5	8.0	12.9
5	32.0	8.9	12.8
6	34.6	9.7	12.0
7	32.8	8.9	12.5
8	30.0	7.6	12.8
9	29.5	8.4	12.0
10	34.1	9.2	11.0
Mean	32.35	8.65	12.20
Standard Deviation (ppm)	1.66	0.63	0.75
% Standard Deviation	5.1	7.3	6.1

(b) Comparison of Analysis Results

As a further test of the method, six "standard" plant samples which had been analysed by other methods, were analysed using the proposed method and the results are given in Table XXI.

In the case of lead, it was found necessary to make a blank correction for contamination which took place during the wet oxidation step (about 1 ug.).

TABLE XXI

Comparison of Analysis Results

Sample	Analysis	Element Concentration (p.p.m.)		
		Zn	Pb	Cu
4A Peach Leaves	1	24	2.3	8.5
	2	20	1.0	9.1
	Mean	22	(1.7)	8.8
	Std.*	24	2.4	6.0
4B Bean Leaves	1	69	5.3	12.5
	2	59	4.0	12.8
	Mean	64	4.7	12.7
	Std.	58	2.9	12.1
4C Beet Leaves	1	21	1.0	16.0
	2	34	2.1	10.2
	Mean	27	(1.6)	13.1
	Std.	22	2.1	12.0
4D Gem Leaves	1	53	1.5	12.4
	2	56	2.1	11.7
	Mean	55	1.8	12.1
	Std.	48	4.0	11.9
4E Plum Leaves	1	47	2.0	8.5
	2	53	2.5	10.3
	Mean	50	2.3	9.4
	Std.	55	2.4	6.5
4G Bush	1	26	0.7	12.4
	2	30	2.0	11.7
	Mean	28	(1.4)	12.0
	Std.	30	1.2	-

* Std. = Mean of results of other methods.
(see Appendix p.103).

(c) Discussion of Results

The results of ^{the} reproducibility test indicate that the precision of/method is good for all three metals. It should be noted, however, that the lead concentration in the solution used for this set of analyses ~~was~~ unusually high. The precision of the zinc analysis would undoubtedly have been better had a non-reversal line been used for the determination of this element.

In the comparison of the analysis results with those obtained by other methods, (see also Appendix p.103) the figures for copper show fairly good agreement with one another and with previous analyses. The zinc results are not always in good agreement with one another, but in general agree fairly well with the results obtained by other methods. The lead results are not in good agreement but this is to be expected with the blank correction generally of a similar order to the analysis result (1 μ g). A comparison of the figures for lead given in the Appendix (p.103), shows that analysis for this element is generally troublesome, probably due to some form of reagent contamination.

In general it can be concluded that the proposed method gives results at least as reliable as those obtained by any of the other methods with which comparison has been made and that precision is good, while the speed of analysis is much greater than can be attained by most other methods. With an instrument of greater dispersion more elements could undoubtedly be determined at the same time and the zinc results improved by using another more suitable spectrum line. The technique for the introduction of the sample extract into the source could probably be used with organic extracts of other elements.

CHAPTER V

THE SPECTROGRAPHIC DETERMINATION OF ZINC
USING THE 2138.6 A SPECTRUM LINE*

Zinc is generally recognised as one of the more important trace elements for plant nutrition and symptoms of deficiency of this element in various types of crops are commonly observed in South Africa. Rapid, reliable methods for the determination of this element are therefore especially desirable.

In most of the commonly used methods for the spectrographic determination of zinc in plant material it is necessary to carry out a preliminary chemical separation and concentration of this element. An alternative is to use the very sensitive spectrum line at 2138.6 A, when the metal can be determined directly in the ashed sample without further chemical treatment. Methods of this type were described by a number of authors⁽⁷⁴⁾⁽⁷⁵⁾⁽⁷⁶⁾⁽⁷⁷⁾ more than a decade ago. Since then spectrochemical analytical technique has improved considerably and new types of photographic plates for the far ultraviolet have become available. A critical re-evaluation of the possibilities of the spectrographic method for the determination of zinc in plant materials using the line at 2138 A thus seemed worthwhile.

Rogers and Gall⁽⁷⁵⁾ found, with the procedure which they describe, the mean of 5 arcings being taken for each result, that the probable error varied from 3% to 17% and they attribute the errors to one or more of five possible causes, which they listed as follows:-

- (1) Contamination from the internal standard or from the graphite electrodes;
- (2) Non-uniform distribution of the internal standard throughout the sample;
- (3) Variation of exposure conditions (wandering of the arc, change of line voltage, etc.);
- (4) Influence of varying major constituents of the ash on the volatility and "excitability" of the zinc atoms;
- (5) Photometric errors.

* A large proportion of the practical work described in this chapter was done by scientific assistants, Miss G. Behrman (Dec. 1957 to Feb. 1958) and Miss E.C. Maskeu (Feb. 1958 to June 1959) under direction of the author.

O'Connor⁽⁷⁶⁾ used the 2138.6 Å line in the analysis of fertilizers for zinc. He carried out eight replicate analyses to test the precision of the method and the standard deviation calculated from his results is 11.7%

Eastmond and Williams⁽⁷⁷⁾ found that self absorption caused errors, especially at higher concentrations and they recommended the use of line width measurements as a measure of zinc concentration. The standard deviation of their method was given as 10-14%.

In the investigation described it was found possible to reduce the errors due to the various causes listed above. To accomplish this each part of the analysis procedure was carefully considered.

A. DEVELOPMENT OF METHOD

1. Spectrography in the 2100Å Region

Spectrography in the far ultraviolet region of the spectrum presents peculiar difficulties. Below 2500Å the absorption of light by quartz optics becomes appreciable and ordinary photographic emulsions tend to become insensitive due to the absorption of the light by the gelatin. The initial task, therefore, was to establish that the 2138.6Å Zn line could be photographed with sufficient sensitivity for the analysis.

(a) Optics

The spectrograph used for this project was the same as that described in Chapter III. As this is a very old instrument it was felt to be unlikely that the silvering on the back of the prism would be suitable for use in the spectral region required. The prism was therefore removed from the instrument, cleaned and the back surface aluminised in the Optics laboratory of the C.S.I.R. After this, as the instrument had not been used in this low wavelength region before, it was necessary to focus it for the 2100Å - 2200Å region. Focus plates were therefore taken with varying prism position and plate angle. Ilford thin-film half-tone plates were used for this work and a copper globule arc provided the spectrum source. (An iron globule is normally used for the focussing of a spectrograph, but copper gives a more suitable spectrum in this

wavelength region).

The external optical system used initially was similar to that used for the analyses described in Chapter III (Figure 15). The focal lengths of the lenses were calculated for a wavelength of 2,200A and their position adjusted accordingly. As the sensitivity appeared to be rather low, however, the transmittancies of these lenses were measured with the aid of the Beckman D.U. spectrophotometer at a number of wavelengths when it was found that appreciable amounts of light were being lost by absorption (The results of these measurements are shown in Table XXIII). The original external optical system was therefore replaced by a simpler single lens system in which an image of the source is focussed on the collimator by a lens placed in front of the slit (as shown in Figure 37). This was done at first using lens 3 of the original system, the relative positions of lens and source being adjusted so that image of the central 4 m.m. of the arc column would fill the collimator aperture. Since, under this system, a certain amount of light from electrode tips does pass through the slit, non-reflective black paper was placed around the collimator in order to reduce background due to stray reflections. This change in the external optical system was found to bring about a marked improvement in sensitivity and a further improvement was obtained by using a "suprasil" lens instead of the quartz lens. ("Suprasil" is claimed by the manufacturers to exhibit negligible absorption down to 1800A).

TABLE XXIII

Light Transmitted by External Optics at
Various Wavelengths

Wavelength	Percentage Transmission		
	Lens 1	Lens 2	Lens 3
3500A	100%	100%	95%
3000A	100%	100%	94%
2560A	100%	98%	93%
2140A	70%	86%	90%
2050A	62%	77%	88%

The transmittancy of the quartz window in front of lens 1 was found to drop from 95% at 3500 to 84% at 2140.

50.5% of light transmitted by external optics at 3500A is transmitted at 2140A.

Optical Arrangement for Zn Analysis
Using the 2138.6A Spectrum Line

(Idealised Diagram)

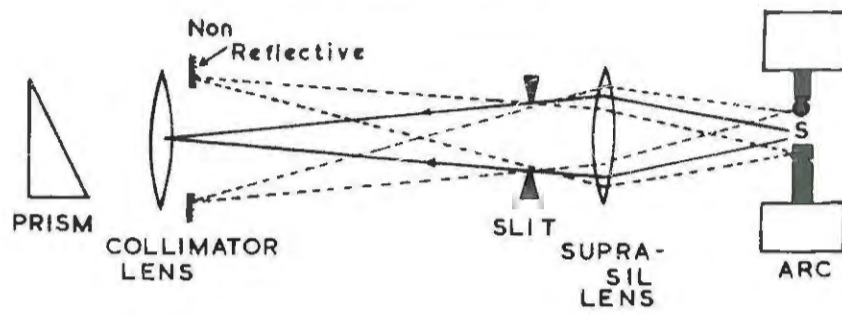


Figure 37.

Sample Electrode

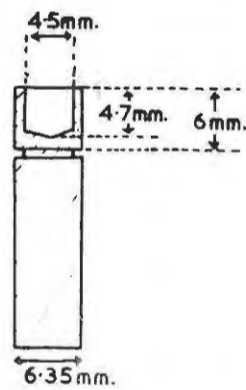


Figure 38.

(b) Photographic Plates

Of the photographic plates commonly used in the laboratory, Ilford thin-film half-tone plates were found to give the best results but these were insufficiently sensitive and various methods for sensitising plates for use in the far ultra-violet were therefore investigated.

Preparation of plates for use in this spectral region can be done by either of two methods: the plate may be coated with a substance which fluoresces in the visible region of the spectrum under the action of ultraviolet light, or the amount of gelatin in the emulsion can be reduced to a minimum.

Sensitization by coating the plates with a thin layer of mineral oil was first tried as proposed by Harrison⁽⁷⁸⁾. Tests were made with two light lubricating oils and with paraffin. The method consisted of submerging the plate in the oil and leaving it to drain for about ten minutes in the darkroom before exposure. After exposure it was necessary to remove the oil so that it would not interfere with the developing. This was done by immersing the plate in acetone. This technique was found to be somewhat tedious and the results obtained were not appreciably better than those with untreated plates.

Coating with kodak ultraviolet sensitizing solution was tried next. This gave only a slightly improved sensitivity and was found to produce patchy spectrograms which would be unsuitable for quantitative work.

The method described by Allen and Franklin⁽⁷⁹⁾ was next considered. Two of the fluorescent compounds which they examined were tested, namely amino G acid (2-naphthylamine -6:8-disulphonic acid) and Tobias Acid (2-naphthylamine-1-sulphonic acid). The latter was found to give some improvement with Ilford thin-film half-tone plates and quite a marked increase in sensitivity with Ilford Ordinary plates. These sensitizing compounds have the advantage of being water soluble and are thus easily removed before developing.

A very thin emulsion can be produced by the method of Duclaux and Jeantet⁽⁸⁰⁾ in which most of the

gelatin is removed by immersing the plate in concentrated sulphuric acid. This method was tried but was found to be most inconvenient. Thin emulsion plates for use in the ultraviolet region are, however, available commercially and some of these were obtained. Ilford "Q" plates, which were the type used, are produced in three different speed grades, "Q1", "Q2" and "Q3". Of these the "Q1" plates were found to be most suitable, the faster "Q2" and "Q3" plates having too large a grain size for accurate density measurements. For economy these plates were cut into 2 in. x 4 in. pieces and placed in the plate holder so as to record the zinc spectrum line near the centre.

2. Source Conditions

(a) Source Unit

D.C. arc excitation was used for all the work described in this chapter. The electrodes were arced at 10 amps, current being drawn from a constant current arc source. The use of this source unit, to which reference has already been made in Chapter III⁽⁵²⁾, should have countered to a large degree the third source of error listed by Rogers and Gall.

(b) Electrodes

The deep, narrow cavity sample electrodes which were used in the method described in Chapter III were found to be unsuitable for the zinc analysis as the background emission from them was found to be high, and insufficient sample was consumed during their arcing. A wide, shallow cavity electrode, for which cutting tools were available in the laboratory, was therefore tried. This was found to produce much improved results and undercutting the cavity was found to improve the results even further.

In the analysis, therefore, the anode consisted of a 6.4 mm. ($\frac{1}{4}$ ") diameter graphite rod having a 4.7 mm. deep 4.5 mm. diameter sample cavity. This electrode was undercut to a depth of 1 mm. at a distance of 6 mm. from the top (see Figure 38). An undercut carbon counter electrode, as described in Chapter III⁽¹⁾ served as the cathode.

The arc gap in all the analyses reported here was 9 mm., the central 4mm. of the arc column being

photographed.

(c) Buffer

In order to eliminate the fourth source of error listed by Rogers and Gall, namely the influence of varying major constituents of the sample, it was decided to mix only a small proportion of the ashed sample with a dilution mixture containing the internal standard and buffer. In order to ascertain the best analysis conditions, experimental arcings were made with a number of different buffer mixtures containing suitable concentrations of zinc oxide.

- (i) 10% CaCO_3 in graphite.
- (ii) 20% CaCO_3 in graphite.
- (iii) 35% CaCO_3 in graphite.
- (iv) 50% CaCO_3 in graphite.
- (v) 75% CaCO_3 in graphite.
- (vi) 90% CaCO_3 in graphite.
- (vii) 33% Na_2CO_3 in graphite.
- (viii) 33% Al_2CO_3 in graphite.
- (ix) 33% K_2CO_3 in graphite.
- (x) 33% Ag powder in graphite.
- (xi) 33% Ag Powder + 33% CaCO_3 in graphite.
- (xii) 33% MgCO_3 in graphite.
- (xiii) 33% K_2SO_4 in graphite.
- (xiv) 10% K_2SO_4 in graphite.

It was found that the alkali carbonates and potassium sulphate reduced the background emission most effectively, but at the same time were found to reduce the intensity of the zinc line. 10% K_2SO_4 was found to be better than 33% K_2SO_4 in this respect and was used in some of the preliminary analyses. Calcium carbonate was found to produce the highest line to background ratio and of the concentrations tried, 50% was found to be the best. With this buffer, however, the background remained rather higher than that observed with the alkali salts.

(d) Reversal of the Zinc Line - The Stallwood Air Jet

In the preliminary analyses carried out using a 10% K_2SO_4 buffer, the results were found to be in poor agreement with those obtained by other methods and the errors were attributed to poor buffering action and consequent major-element-variation effects. Somewhat improved results were obtained with 50% CaCO_3 , but the standard curve was unusually flat and curved until nearly horizontal at the high concentrations, indicating

marked self absorption of the zinc line.

Attempts were made to combat self absorption in a number of ways. First a buffer mixture containing both calcium carbonate and potassium sulphate was used, the potassium sulphate being added to reduce and control the vaporisation rate of the zinc more strongly. Vaporisation curves recorded under the arc conditions finally used, confirmed that this buffer mixture was very suitable and considerably better than the calcium carbonate alone (see Figure 39).

As a further means of reducing the amount of zinc vapour in the arc at any time, sample electrodes with a deep narrow cavity (2 mm. diameter x 8 mm. deep) were tried. This did reduce self absorption to some extent, but the line intensity was found to be inconveniently low even when two electrodes were burned at each plate position.

It was eventually found possible to reduce self absorption considerably by blowing an air stream around the sample electrode. This had the effect of removing some of the absorbing vapour from the outer portion of the arc column. An 8 mm. wide air jet of the type described by Stallwood⁽⁵³⁾ was used. This was set approximately 6 mm. below the top of the electrode and compressed air was caused to flow through it at a rate of 8 litres per minute.

The reduction of self absorption by the use of narrow electrodes and the air jet is illustrated in Figure 40 which shows standard curves produced under three different conditions, 4.5 mm. electrodes without air jet, 2 mm. electrodes and 4.5 mm. electrodes with air jet.

The arc conditions finally selected therefore consisted of burning the 4.5 mm. electrodes with the air jet and using a buffer dilution mixture containing 40% calcium carbonate and 10% potassium sulphate with which the sample was mixed in the proportion 1:9. Time-intensity curves were recorded according to the method described in Chapter III for both the above buffer mixture and the 55% calcium carbonate mixture. These curves, shown in Figure 39, demonstrate the

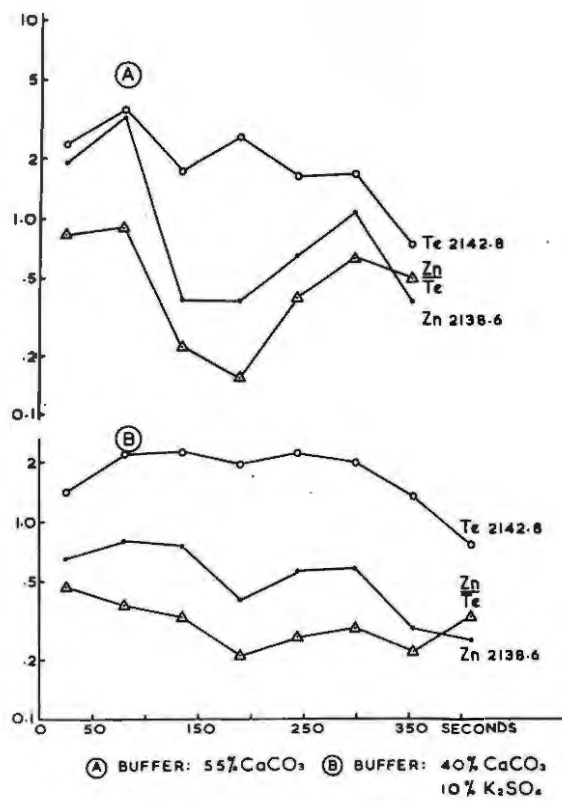


Figure 39.
Time - Intensity Curves.

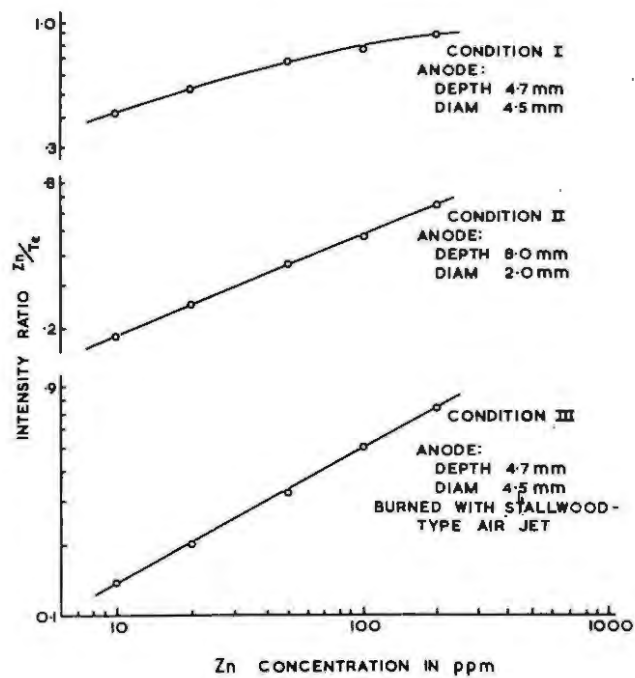


Figure 40.
Standard Curves Obtained under Various Source Conditions.

suitability of the buffer mixture finally used.

3. Interpretation of Spectra

(a) Internal Standard

Tellurium was used as internal standard by Rogers⁽⁷⁵⁾ and was found to be perfectly satisfactory in the work described here. It has a strong spectrum line close to the zinc line (Te 2142.8A) and is unlikely to occur in appreciable quantities in plant material. The boiling point of tellurium (1390°C) is somewhat higher than that of zinc (907°C), but its other physical properties are suitable (Excitation potential: Zn 2138.6, 5.8 eV; Te 2142.8, 5.8 eV. Ionisation potential: Zn 9.392 eV, Te 9.007 eV). In addition, as can be seen from Figure 39, the vaporisation rates of the two elements in the arc are fairly similar.

(b) Plate Calibration

The spectrum lines photographed were found to be of uneven density along their length, probably due to some optical imperfection of the spectrograph becoming more marked at the low wavelength used. For this reason an ordinary step sector could not be used to obtain a reliable plate calibration for the sample spectrum. The possibility of using the two-line method was considered, as it appeared that the 2147A Te line was fairly strongly emitted from the source, but the intensity ratio of these two Te lines was found to vary considerably. Eventually a two-step sector was made so that spectrum lines could be photographed in two long steps on each of which a number of measurements could be made. In order to obtain a wide range of densities, two copper globule arc spectra were photographed on each plate with different exposure times. The preliminary curve method was then used to obtain the emulsion calibration curve. The step sector was not used when photographing the sample spectra. Instead five microphotometer readings were made for each line and the mean used in the calculation of intensity ratios. Increasing the spectrograph slit width from 0.015 mm. to 0.030 mm. was found to increase line density a little and to facilitate the density measurement.

(c) Calculation

Intensity ratios were obtained graphically by the preliminary curve method and concentrations read from a standard calibration curve.

(d) Standards

Standards were prepared from specpure zinc oxide and S.P.2 graphite, lower concentrations being obtained from an original top standard by diluting with graphite. Suitable quantities of the standards were mixed in the ratio 1:9 with the $\text{CaCO}_3/\text{K}_2\text{SO}_4$ /Graphite dilution mixture and arced in the same way as the samples.

B. THE ANALYSIS METHOD

1. Procedure

Weigh about one gram of dry powdered plant material into a clean platinum crucible and ash overnight at a temperature of 450°C. Weigh the crucible again after the ashing to determine the "ash factor" (ratio of weight of ash to weight of dry plant material). Mix the ash well and dilute a suitable portion 1:9 with the buffer-dilution mixture. Load three sample electrodes with the diluted sample and burn these in the d.c. arc with air jet. Make the exposure for 5³/₄ minutes from the striking of the arc.

2. Analysis Results

(a) Precision of the Analysis Method

In order to test the precision of the method 10g. of plant material were ashed and, after mixing with buffer, loaded into 30 electrodes which were then burned according to the method described. The results of this series of analyses are given in Table XXIV.

The standard deviation calculated from these results is 5.48 ppm. which is equivalent to a coefficient of variation of 9.3% on individual arcings. On this basis the coefficient of variation on the means of three arcings for each sample should be 5.4% which is quite adequate for plant nutritional studies.

TABLE XXIV

Results of Repeated Arcing of Plant Ash

Arcing No.	Zn p.p.m.	Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.
1	67.3	11	54.4	21	52.3
2	68.1	12	51.8	22	57.3
3	63.3	13	54.0	23	60.5
4	66.4	14	54.7	24	69.6
5	54.5	15	51.1	25	58.9
6	53.1	16	58.5	26	63.3
7	55.5	17	54.5	27	54.5
8	68.1	18	59.8	28	63.7
9	57.8	19	58.5	29	60.8
10	65.1	20	62.4	30	54.5

Mean = 59.1 p.p.m.

Std. Deviation = 5.48 p.p.m.

% Std. Deviation= 9.3%

(b) Comparison of Analysis Results with those Obtained by Other Methods

In order to assess the accuracy of the method nine of the "standard" samples were analysed as described. The results obtained are compared in Table XXV with the results obtained by other analysis methods.

It is difficult to obtain any definite idea of the accuracy of the analysis method from the figures compared in the table, but for the first few samples, for which previous results show fair agreement, the accuracy seems good with perhaps a slight tendency for the results to be low. In general the results show the same trends and it can be assumed that the analysis method described here will give as reliable results as any of the other methods with which comparison is made.

TABLE XXV

Comparison of Analysis Results with those of
Other Methods

Sample	Zn Concentration p.p.m.								
	4A	4B	4C	4D	4E	4G	4H	4J	4K
Analysis	19.2	60.5	17.1	59.4	37.9	21.3	26.9	75.1	40.6
Results	19.4	53.2	17.1	65.5	36.4	22.5	26.9	69.1	45.5
	19.4	57.8	18.7	59.4	40.4	23.3	23.2	72.4	47.0
Mean	19.3	57.2	17.6	61.4	38.2	22.4	25.7	72.2	44.4
Results* 1	28	60	22	59	64	25	28	48	27
Obtain- 2	22	60	18	39	93	28	28	76	28
ed by 3	21	60	30	60	52	26	50	45	40
Other 4	26	56	23	38	30	41	43	60	18
Methods 5	(10)	54	19	48	35		21	42	
Mean	24	58	22	48	55	30	34	54	28

* See full list of analysis results and laboratories in Appendix (p.103)

3. Conclusion

The method described is simple and, apart from the time required for the ashing, relatively fast. It should be particularly suited for use in a laboratory where other elements are determined spectrographically after ashing the sample. From the point of view of accuracy and precision the method compares well with other spectrochemical methods.

C. DIRECT READING SPECTROMETRIC DETERMINATION
OF ZINC IN PLANT ASH

In the method described above one of the main sources of error lay in the evaluation of the zinc concentrations from measurements made on the photographic plate. As was to be expected at such a low wavelength, the slope of the emulsion characteristic, or gamma was very low which meant a relatively small variation in density with line intensity. In addition there were indications that the emulsion properties were not as constant from plate to plate, or even from one part of a plate to another, with the Q plates as for more common photographic emulsions. It was therefore felt that

some advantage might be gained by transferring the method to a direct reading spectrometer. It was hoped to be able to do this with a minimum of change in the procedure. Some modifications had to be made, however, and an account of these and the results obtained is given below

1. Adapting the Method to Direct Reading Spectrometry

(a) Internal Standard Line

The limitations of the instrument with regard to dispersion and the relative positioning of the exit slits made it impossible to use the 2142.8A Te line as internal standard. The 2383.3A Te line was therefore used.

(b) Setting the Exit Slit Positions

The exit slit positions were set using the same technique as described in the previous chapter, namely by adjusting the slit to the position which gave a maximum signal with a source consisting of a spark between electrodes of the metals concerned. A Te electrode was prepared by forming a briquette of pure Te powder in the A.R.L. press.

(c) Exposure Time

Since the sensitivity attainable with the photomultipliers of the direct reading instrument was much higher than could be achieved photographically, it was only necessary to expose for a fraction of the time used previously. It was decided to make a relatively short exposure a minute after striking the arc, thus allowing time for the arc to achieve some stability, and terminating the exposure before the background emission, which normally became more pronounced towards the end of the arcing time, had increased sufficiently to interfere with the analysis.

Two different methods were tried with exposure times of 30 seconds and 60 seconds respectively. Results obtained by both methods are reported below.

(d) Standards

Standards were prepared in exactly the same way as for the photographic procedure. A number of extra electrodes were packed with the top standard and these were used for preliminary readings while the sensitivity

settings on the instrument were adjusted. The standard curves obtained by each of the two methods are shown in Figure 41.

2. Analysis Results

(a) First Method (Preburn 60 seconds, exposure 30 seconds)

The precision of this method was tested by carrying out repeated analyses on the ash of "standard" sample 4B. The results are shown in Table XXVI.

TABLE XXVI

Reproducibility Test on First Method

Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.
1	44.4	10	38.1	19	54.4
2	49.5	11	46.8	20	44.4
3	38.2	12	48.8	21	44.9
4	54.5	13	54.9	22	47.7
5	43.2	14	44.7	23	45.7
6	48.6	15	48.8	24	50.2
7	37.8	16	46.7	25	59.8
8	50.6	17	32.2	26	45.9
9	43.8	18	50.5		

Mean 46.8 p.p.m.
 Standard Deviation = 5.90 p.p.m.
 Coefficient of Variation = 12.6%

The precision indicated by the results of these analyses compares rather unfavourably with that of the photographic method. It is also noteworthy that the mean result is significantly different from that obtained for this sample by the photographic method. The second method in which a longer exposure is made, was therefore tried in the hope that by using a longer exposure the influence of random fluctuations of the arc would be reduced.

(b) Second Method (Preburn 60 seconds, exposure 60 seconds)

Eight electrodes were burnt as a preliminary test of the precision of this method. The coefficient of variation for these analyses was found to be 4.8%, which indicated that better reproducibility might be expected.

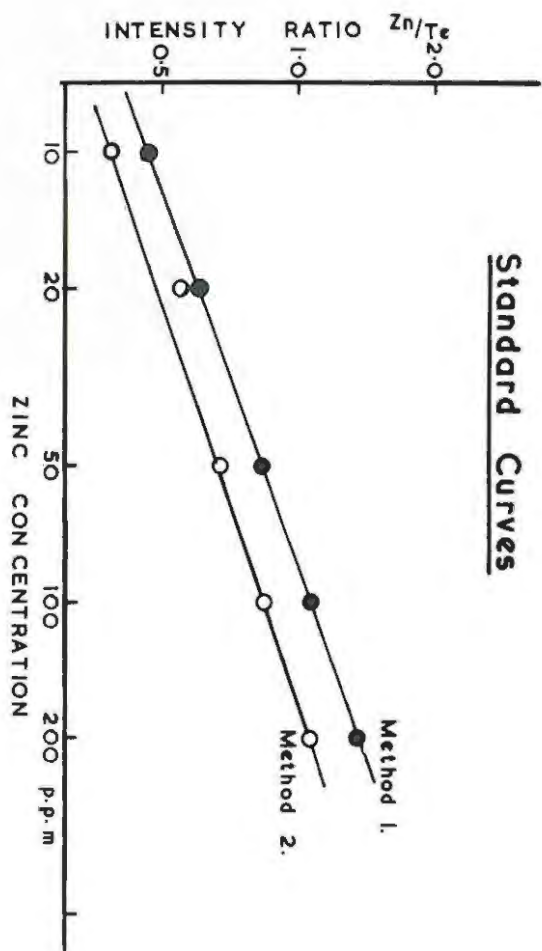


Figure 41.

Seven of the "standard" samples were thereupon analysed using the method. It was apparent, however, from these results, shown in Table XXVI, that the precision and accuracy was much less than that of the photographic method and a further test of the reproducibility was made by the analysis of seventeen portions of the ash of 4B. These analysis results, which are shown in Table XXVII, gave a coefficient of variation of 14.8% in complete disagreement with the figure calculated for the initial eight analyses.

TABLE XXVII

Results of Analysis of Standard Samples

Sample	4A	4B*	4C	4D	4E	4G	4H
Analysis	11.7		24.4	31.4	51.5	30.5	34.0
Results	19.5		11.7	37.7	59.5	35.8	34.6
	13.9		19.1	42.6	35.3	26.9	26.9
Mean	15.0	52.9	18.4	37.2	48.8	31.1	31.8
Mean Result by Photo- graphic Method	19.3	57.2	17.6	61.4	38.2	22.4	25.7

* See Table XXVIII for individual analysis results.

TABLE XXVIII

Reproducibility Test on Second Method

Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.
1	48.3	7	52.2	13	53.6
2	48.9	8	47.6	14	62.2
3	46.3	9	47.6	15	71.4
4	53.2	10	43.7	16	48.9
5	49.3	11	43.7	17	57.5
6	58.2	12	66.1		

Mean = 52.9 p.p.m.

Standard deviation = 7.82 p.p.m.

Percentage standard deviation = 14.8%

As a final test the spectrometer settings, including exit slit positions, were checked thoroughly and twenty nine analyses carried out on another sample of plant ash. The results of these analyses are shown in Table XXIX. The precision attained in these analyses appears from the results to be even worse than indicated by the previous test.

TABLE XXIX
Final Reproducibility Test

Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.	Arcing No	Zn p.p.m.
1	31.1	11	37.7	21	36.4
2	29.8	12	46.4	22	36.1
3	37.3	13	35.7	23	30.7
4	35.4	14	32.4	24	31.7
5	50.3	15	35.7	25	33.7
6	40.3	16	28.6	26	31.1
7	41.0	17	33.7	27	28.8
8	37.7	18	49.9	28	29.8
9	36.4	19	32.3	29	27.5
10	32.0	20	33.1		

Mean = 35.2 p.p.m.

Standard deviation = 5.84 p.p.m.

Percentage standard deviation = 16.6%

3. Conclusion

It was apparent at this stage that the method was not usable in its present form in conjunction with a direct reading spectrometer. Major errors were apparently introduced due to variation in background, wandering of the arc and inconstancy of emission and it was plain that a further full scale investigation of each step in the procedure would be necessary before satisfactory results could be obtained. Therefore, in view of the success which had been achieved with other methods, no further work was done on this project.

CHAPTER VI

CONCLUSION.

In the work that has been described in the last four chapters, many of the problems involved in the spectrochemical determination of trace elements in plant material have been studied from different points of view and, while it cannot be claimed that this thesis represents a final answer to all the difficulties of the analyst engaged in this type of work, some very worthwhile advances have been made, some new ground has been broken and a fresh approach has been made to some old problems. At all times during the progress of the work described, the needs of an agricultural research laboratory, where a large number of analyses have to be carried out, have been kept in mind and simple rapid techniques have been favoured.

The study which has been made of the properties of the three organic reagents used has shown how the selectivity of reagents of this type can effectively be increased by the use of suitable concentrations of other complexing agents and control of pH, to suit the needs of a particular analysis. The main purpose of a large part of this study was the rapid and effective separation of iron from the other elements, but the knowledge gained could be used in the preparation of alternative analysis schemes, particularly those which involve smaller groups of the elements studied.

In the development of the spectrographic method described in Chapter III important observations have been made on the effect of variation in source parameters. The problem of evaporating a chloroform extract on to graphite has been tackled in a simple but effective manner and the technique proposed should prove useful to any analyst contemplating using a spectrographic method in conjunction with solvent extraction.

The calculation of results has long proved one of the more tedious steps in spectrographic analysis. Further work must be done with the proposed slide rule before its general applicability can be claimed, but it has been shown to be perfectly satisfactory for use in conjunction with the proposed spectrographic method and

its use promises a considerable shortening of the work involved in spectrographic analysis.

The successful application of direct reading spectrometry to the analysis of plant material for zinc, lead and copper has further demonstrated the wide possibilities of this powerful technique. The direct introduction of trace element extract into the excitation source in combination with direct reading spectrometry is likely to provide an important challenge to other methods of trace analysis. For this reason solution methods of spectrochemical analysis undoubtedly warrant further attention.

Although a good case cannot normally be made for the use of a spectrographic method for the determination of a single element, the study of the determination of zinc using the 2138.6A spectrum line has been well worthwhile. The proposed method has the advantage of speed and simplicity and would probably prove most useful in laboratories where major constituents of plant ash are determined by similar techniques.

The subject of spectrochemical determination of trace elements in plants and related biological materials is one which is likely to occupy analytical research workers for many years to come. A large part of their research will undoubtedly have to do with direct reading spectrometric methods, and the use of X-ray fluorescence for this type of analysis is receiving increasing attention. The first paper dealing with the application of atomic absorption spectroscopy to chemical analysis in the laboratory⁽⁸¹⁾ appeared during the progress of the work described here and the phenomenal development of this technique during the past few years has opened up a whole new field to the spectro-analyst.

A P P E N D I X

RESULTS OF ANALYSIS OF STANDARD
SAMPLES BY OTHER METHODS

Sample	Method*	Element Concentrations (p.p.m.)								
		Zn	CO	Ni	Pb	Ti	V	Mo	Mn	Cu
4A	1	28	<0.3	2.4	1.7	11	0.20	<0.1	46	8.7
	2	22	<0.1	2.2	1.1	9	0.21	0.04		
	3	21	0.15	3.3	6.5	11-13	0.3	0.05	58	5.7
	4	26	0.1	2.8				0.1	12	4
	5	(10)	0.08	2.4	1.4		0.23	0.10	58	
	6								58	5.5
4B	1	60	<0.1	4.8	3.3	42	0.51	2.0	41	13.8
	2	60	0.16	6.4	1.9	32	0.78	1.7		
	3	60	0.36	6.6	3.0	40-82	1.0	1.7	53	12.9
	4	56	0.3	0.5				3.5	17	12
	5	54	0.28	5.0	3.9		0.56	2.5	37	
	6								42	9.6
4C	1	22	0.1 <0.3	2.2	1.3	22	0.40	0.85	162	13.9
	2	18	0.21	3.3	1.2	20	0.45	0.67		
	3	30	0.37	2.0	6.0	24-39	0.70	0.85	182	10.6
	4	23	0.1	2.5				2.7	69	15
	5	19	0.31	2.4	1.3		0.4	1.1	168	
	6								179	8.4
4D	1	59	<0.3	3.0	4.6	42	0.9	5.3	86	13.6
	2	39	0.15	3.3	3.8	40	1.0	3.9		
	3	60	0.33	4.0	5.5	45-74	1.1	3.3	109	10.5
	4	38	0.5	3.0				5.5	26	14
	5	48	0.25	3.1	2.0		0.7	5.3	84	
	6								107	9.5
4E	1	64	0.49	1.5	2.2	9.3	0.28	<0.1	129	6.8
	2	93	0.54	2.1	0.8	7.7	0.13	<0.05		
	3	52	0.76	1.8	5.5	9-13	0.3	0.05	141	5.8
	4	30	0.8	0.19				1.5	60	9
	5	35	0.61	1.33	3.0		0.2	0.03	145	
	6								150	4.5

Sample	Method*	Element Concentrations (p.p.m.)								
		Zn	Co	Ni	Pb	Ti	V	Mo	Mn	Cu
4G	1	25	0.37	2.5	1.7	23	0.72	0.1		
	2	28	0.35	4.5	0.7	31	0.68	0.03		
	3	26	0.63	4.1		22-30	0.7	8.0		
	4	41	0.30	6.5				1.5		
	5									
4H	1	28	0.88	9.9	0.6	16	0.25	0.1	68	8.8
	2	28	0.83	7.0	0.5	18	0.46	0.04		
	3	50	1.20	12.5	2.0	18-62		0.1	62	9.3
	4	43	0.8	2.1				0.2	33	7
	5	21	0.91	9.7	1.2		0.47	0.1	75	
	6								81	7.8
4I	1	48	1.74	11.6	1.3	83	1.53	0.3	72	17.0
	2	76	1.91	12.1	0.7	65	2.0	0.05		
	3	45	2.90	17.0	2.0	34-84	4.0	0.18	50	17
	4	60	2.2	13.3				0.5	45	22
	5	42	2.03	12.3	1.8		2.0	0.13	76	
	6								100	12.6
4K	1	27	0.66	2.5	1.6	51	1.2	0.3		
	2	28	0.64	3.5	1.0	43	1.5	0.18		
	3	40	1.1	3.8	2.0	47-66	2.2	0.40		
	4	18	0.9	3.8				1.0		

- * 1 = C.S.I.R. Anode (Strasheim + Camerer⁽⁴⁾), (Cu, Mn, Strasheim + Keddy⁽¹⁾).
- 2 = C.S.I.R. Cathode (Strasheim + Camerer).
- 3 = Macaulay Institute (Cathode layer method).
- 4 = Chemical Services, Dept. Agriculture. (Chemical method).
- 5 = C.S.I.R. (Strasheim, Eve and Fourie⁽⁷⁾).
- 6 = Onderstepoort Laboratory, Dept. Agriculture (Spectrographic).

Note: For Pb 1 and 2 are each the mean of two results.

REFERENCES

- (1) Strasheim, A. and Keddy, R.J. : J. S.African Chem. Inst., 11, 21, (1958).
- (2) Wark, W.J. : Anal. Chem., 26, 203, (1954).
- (3) Mitchell, R.L. and Scott, R.O. : J. Soc. Chem. Ind. 66, 330, (1947).
- (4) Strasheim, A. and Camerer, L. : J. S.African Chem. Inst., 8, 28, (1955).
- (5) Stetter, A. and Exler, H. : Naturwissenschaften, 42, 45, (1955).
- (6) Scharrer, K. and Judel, G.K. : Z. Anal. Chem., 156, 340, (1957).
- (7) Strasheim, A., Eve, D.J. and Fourie, R.M. : J. S.African Chem. Inst., 12, 75, (1959).
- (8) Gorbach, G. and Pohl, F. : Mikrochemie, 38, 258, 328, 335, (1951).
- (9) Doll, W. and Specker, H. : Z. Anal. Chem., 161, 354, (1958).
- (10) van Rooyen, E. : University of Pretoria M.Sc. Thesis, 1956.
- (11) Mannkopff, R. and Peters, C. : Z. Physik, 70, 444, (1931).
- (12) Ahrens, L.H. and Liebenberg, W.R. : Trans. Geol. Soc. S. Afr., 49, 133, (1946).
- (13) Strasheim, A. and Tappere, E.J. : Appl. Spectroscopy, 13, 12, (1959).
- (14) Churchill, J.R. : Ind. Eng. Chem. Anal. Ed., 16, 653, (1944).
- (15) Mitchell, R.L. : "The Spectrographic Analysis of Soils, Plants and Related Materials", Technical Communication No.44, Commonwealth Bureau of Soil Science, 1948, p.17.
- (16) Kaiser, H. : Spectrochim. Acta, 2, 1; 3, 278; 4, 351.
- (17) Truhaut, R. and Soulet, M. : Rep. 15me Congr. G.A.M.S., 201 (1952); Spectrochem. Abs. 1691, (1952-3).
- (18) Brooks, R.R., Ahrens, L.H. and Taylor, S.R. : Geochim. and Cosmochim. Acta, 18, 162, (1960).
- (19) Ahrens, L.H., Brooks, R.R. and Edge, R.E. : S.African Ind. Chemist, 14, 5, (1960) (Summary of paper read at XIV Convention S.A. Chem. Inst.).
- (20) Fischer, H. : Mikrochemie, 30, 38, (1942).
- (21) Irving, H. and Bell, C.F. : J. Chem. Soc., 4253, (1954).
- (22) Sandell, E.B. : "Colorimetric Detn. of Traces of Metals", 3rd ed., Interscience Publishers, N.Y., 1959, pp. 139-176.
- (23) Walkley, A. : Australian Chem. Inst. J. and Proc., 9, 29, (1942).
- (24) Hibbard, P.L. : Ind. Eng. Chem. Anal. Ed., 9, 127, (1937).
- (25) Cholak, J. Hubbard, D.M. and Burkey, R.E. : Ind. Eng. Chem. Anal. Ed. 15, 754, (1943).

- (26) Marston, H.R. and Dewey, D.W. : Australian J. Exptl. Biol. Med. Sci, 18, 343, (1940).
- (27) Geiger, R.W. and Sandell, E.B. : Anal. Chim. Acta., 8, 197, (1953).
- (28) Yasumutsu Uzumasa and Isao Mujashita : J. Chem. Soc. Japan, 1951, p.41.
- (29) Biefield, L.P. and Patrick, T.M. : Ind. Eng. Chem. Anal. Ed., 14, 275, (1942).
- (30) Sandell, E.B. : "Colorimetric Detn. of Traces of Metals", 3rd Ed., Intersci. Publ., N.Y., 1959, p.951.
- (31) Koltoff, I.M. and Sandell, E.B. : J. Amer. Chem. Soc., 63, 1906, (1941).
- (32) Sandell, E.B. : "Colorimetric Detn. of Traces of Metals", 3rd Ed., Intersci. Publ., N.Y. 1959, p.54.
- (33) Oosting, M. : Anal. Chim. Acta, 21, 301, (1959).
- (34) Irving, H. and Williams, R.J.P. : J. Chem. Soc., 1841, (1949).
- (35) Friedeberg, H. : Anal. Chem., 27, 305, (1955).
- (36) Moeller, T. and Pundsack : J. Amer. Chem. Soc., 75, 2258, (1953).
- (37) Moeller, T. : Ind. Eng. Chem. Anal. Ed., 15, 346, (1943).
- (38) Gentry, C.H.R. and Sherrington, L.G. : Analyst, 71, 432, (1946).
- (39) Sandell, E.B. : "Colorimetric Detn. of Traces of Metals", 3rd. Ed., Intersci. Publ., N.Y., 1959, pp.179-188.
- (40) Piper, C.S., and Beckwith, R.S. : J. Soc. Chem. Ind., 67, 374, (1948).
- (41) Meunier, P. : Bull. Soc. Chim. Biol., 17, 584, (1935).
- (42) Sandell, E.B. : "Color. Detn. of Traces of Metals", 3rd Ed., Intersci. Publ., N.Y. 1959, pp.21-23.
- (43) Mitchell, R.L. : "Trace Analysis," Yoe and Koch, Ed., John Wiley, (1957).
- (44) Dr. Radmacher, Dept. of Agriculture, Onderstepoort, (Private Communication).
- (45) Piper, C.S. : "Soil and Plant Analysis", Intersci. Publ., N.Y., 1947, p. 265.
- (46) Perrin, D.D. : N.Z. J. Sci. Tech. 28A, 183, (1946).
- (47) Dodson, R.W., Forney, G.J. and Swift, E.H. : J. Amer. Chem. Soc., 58, 2573, (1936).
- (48) Dean, J.A. and Lady, J.H. : Anal. Chem., 27, 1533, (1955).
- (49) Dean, J.A. and Lady, J.H. : Anal. Chem., 28, 1877, (1956).
- (50) Dean, J.A. and Cain, C. : Anal. Chem. 29, 530, (1957).
- (51) Bryan, H.A. and Dean, J.A. : Anal. Chem., 29, 1289, (1957).
- (52) van der Walt, N.T. : Rev. Sci. Instru., 27, 157, (1956).
- (53) Stallwood, B.J. : J. Opt. Soc. Amer., 44, 171, (1954).

- (54) Ahrens, L.H. : "Spectrochemical Analysis", Addison-Wesley Publ. Co., Cambridge, Mass., 1954, p.81.
- (55) Smit, J. and Smit, J.A. : Anal. Chim. Acta., 8, 274, (1953).
- (56) Hurter, F. and Driffield, V.C. : J. Soc. Chem. Ind., 9, 455, (1890).
- (57) Kaiser, H. : Spectrochim. Acta, 2, 1, (1941).
- (58) Kaiser, H. : Spectrochim. Acta, 3, 278, (1948).
- (59) Kaiser, H. : Spectrochim. Acta, 4, 351, (1951).
- (60) Schmidt, R., Manders, H. and van Wijk, G.J. : Rec. Trav. Chim. Pays-Bas. 67, 745, (1948).
- (61) Churchill, J.R. : Ind. Eng. Chem. Anal. Ed., 16, 653, (1944).
- (62) Frederickson, L.D. : Anal. Chem., 24, 2019, (1952).
- (63) Epstein, S. : Appl. Spectroscopy, 14, 7, (1960).
- (64) Mitchell, R.L. : "The Spectrographic Analysis of S Soils, Plants and Related Materials", Tech. Communication, No.44, Commonwealth Bureau of Soil Sci., 1948, p. 18.
- (65) Black, I.A. : Spectrochim. Acta, 4, 512, (1952).
- (66) Philcox, H.J. : Spectrochim. Acta., 10, 199, (1957).
- (67) Nachtrieb, N.H. : "Principles and Practice of Spectrochemical Analysis", McGraw Hill, 1950. p.136.
- (68) Mitchell, R.L. : "The Spectrographic Analysis of Soils, Plants and Related Materials", Tech. Communication, p. 174. No.44, Commonwealth Bureau of Soil Sci., (1948).
- (69) Mitchell, R.L., Scott, R.O. and Farmer, V.C. : Nature, 157, 193, (1946).
- (70) Anderson, J.W. : Appl. Spectroscopy, 10, 195, (1956).
- (71) Ahrens, L.H. : "Spectrochemical Analysis," Addison Wesley Publ. Co., Cambridge, Mass., 1954, p. 85.
- (72) Naish, J.M. and Ramsden, Spectrochim Acta, 5, 295, (1952).
- (73) Verdier, E.T., Steyn, W.J.A. and Eve, D.J. : Agr. Food Chem., 5, 354, (1957).
- (74) Rogers, L.H. : Ind. Eng. Chem. Anal. Ed., 7, 421, (1935).
- (75) Rogers, L.H. and Gall, O.E. : Ind. Eng. Chem. Anal. Ed., 9, 42, (1937).
- (76) O'Connor, R.T. : Ind. Eng. Chem. Anal. Ed., 13, 597, (1941).
- (77) Eastmond, E.J. and Williams, B.E. : J. Opt. Soc. Amer. 38, 666, (A.41)/801 (1948).
- (78) Harrison, G.R. : J. Opt. Soc. Amer. 11, 113, (1925).
- (79) Allen, A.J.A. and Franklin, R.G. : J. Opt. Soc. Amer. 22, 499, (1932).
- (80) Duclaux, J. and Jeantet, P. : J. Phys. et Radium, 2, 156, (1921).
- (81) Walsh, A. : Spectrochimica Acta, 7, 108, (1955).
- (82) Hughes, H.K. and Murphy, R.W. J. Opt. Soc. Amer., 39, 501, (1949).

THE SPECTROGRAPHIC DETERMINATION OF ZINC IN PLANT MATERIAL ASH USING THE 2138.6 Å SPECTRUM LINE

by

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OPSOMMING

'n Metode word beskryf vir die spektrografiese ontleding van plantmaterialas vir sink deur gebruik te maak van die spektrumlyn te 2138.6 Å. Ilford „Q1” ultravioletgevoelige plate is gebruik om die spektrum vas te lê. 'n Stallwood-tipe lugtregter is om die hoog gebruik om die moeilikheid van selfabsorpsie te oorkom. Vir dertig herontledings van een monster is 'n standaard afwyking van 9.3% bereken. 'n Reeks standaard monsters is volgens die metode ontleed en die resultate met die van ander metodes vergelyk.

SUMMARY

A method is described for the spectrographic analysis of plant material ash for zinc using the spectrum line at 2138.6 Å. Ilford "Q1" ultraviolet-sensitive plates were used for recording the spectrum. A Stallwood-type air jet was used round the arc to overcome the difficulty of self absorption. The standard deviation of the method was calculated to be 9.3% from 30 replicate analyses of one sample. A series of standard samples was analysed and the results are compared with those obtained by other methods.

INTRODUCTION

Zinc in plant materials can be determined spectrographically either directly on the plant ash^{1, 2, 3, 4}, or after carrying out a preliminary chemical separation and concentration of this element^{5, 6}.

The direct determination of zinc in the plant ash has certain advantages such as:

- (1) less manipulation of the sample compared to the concentration methods and thus the possibility of loss of zinc is diminished;
- (2) shorter lapse of time before results are available;
- (3) larger volume of samples, which is of value in many fruit culture problems where only zinc is of importance.

Most spectrographic methods based on analysis of the ash, without a preliminary chemical separation, were reported in the literature more than a decade ago. Since then the spectrochemical analytical technique has improved considerably and new types of photographic plates for the far ultraviolet have become available. A critical re-evaluation of the possibilities of the spectrographic method for the determination of zinc in plant materials using the line at 2138.6 Å thus seems long overdue.

Rogers and Gall² found that, with the mean of five arcings taken for each result, their probable error varied from 3% to 17% and they attributed the error to one or more of five possible causes, viz.:

- (1) contamination from the internal standard or from the graphite electrodes;
- (2) non-uniform distribution of the internal standard throughout the sample;
- (3) variation of exposure conditions (wandering of the arc, change of line voltage, etc.);

- (4) influence of varying major constituents of the ash on volatility and "excitability" of the zinc atoms;
- (5) photometric errors.

O'Connor³ used the 2138 Å line in his analysis of fertilisers for zinc. He carried out eight replicate analyses to test the precision of the method and the percentage standard deviation calculated from these results is 11.7%.

Eastmond and Williams⁴ found that self absorption caused errors, particularly at higher concentrations, and recommended the use of line-width measurements for determining the zinc content. The percentage standard deviation of their methods was given as 10-14%.

In the method described in this paper the precision has been improved by overcoming to some extent the errors caused by self absorption, variation of source conditions and variation of the major constituents.

EXPERIMENTAL

Spectrograph. Hilger Large Littrow with quartz optical system.

Source unit. Constant current source unit⁷.

External optics. As it was found that the quartz lenses normally used in the external optical system of the spectrograph absorbed a large proportion of the radiation between 2100 and 2200 Å, these were replaced by a single "suprasil" lens (high transmittancy down to 1800 Å) which focussed an image of the source on to the collimator of the spectrograph. This lens was placed so that an image of the central 4 mm of the arc column filled the 5 cm collimator aperture which was surrounded by non-reflective material.

Plates. Ordinary photographic emulsions are not very sensitive to radiation below 2500 Å. To overcome this difficulty experiments were carried out with various types of plates and a number of ultraviolet sensitizing agents. It was found that, while these materials gave increased sensitivity in the ultraviolet, they often gave rise to "patchy" spectrograms which were unsuitable for quantitative density measurements. Experiments with thin emulsion plates proved more successful and Ilford "Q1" plates were finally selected as being the most suitable. These were cut into pieces 2 inches by 4 inches, one being used for each exposure with its centre at about 2140 Å. The plates were developed at 18° C for 6½ minutes in 1:1 I.D. 13 and fixed in Kodak X-ray fixer. Development was stopped in a stop bath of 5% acetic acid.

Excitation conditions. By using a constant current source⁷ variations in emission due to current fluctuations were reduced to a minimum. Anode excitation was used and the arc burned at 10 amps.

To reduce background and increase sensitivity a relatively wide and shallow sample cavity in the anode was used. The sample electrode consisted of a 4.7 mm deep 4.5 mm diameter cavity cut into a 6.35 mm (¼ inch) diameter graphite rod. The electrode was undercut to a depth of 1 mm at a distance of 6 mm from the top.

An undercut carbon counter-electrode as described by Strasheim and Keddy⁸ served as cathode.

It was evident from the slope and curvature of the first standard curves obtained that self-absorption was affecting the results (Fig. 1). Some improvement was ob-

tained by using a deeper narrower sample electrode (8.0 mm deep, 2 mm diameter cavity) but the spectrum density was inconveniently low even when two electrodes were burned at each plate position. As shown in Fig. 1, however, the effect of self-absorption was greatly reduced by blowing an air stream upwards around the sample electrode. This had the effect of removing some of the absorbing vapour from the outer portion of the arc column. An 8 mm wide air jet of the type described by Stallwood⁹ was used. In addition to reducing the influence of self absorption this was found to give a much steadier arc. The excitation conditions finally selected are given in Table I.

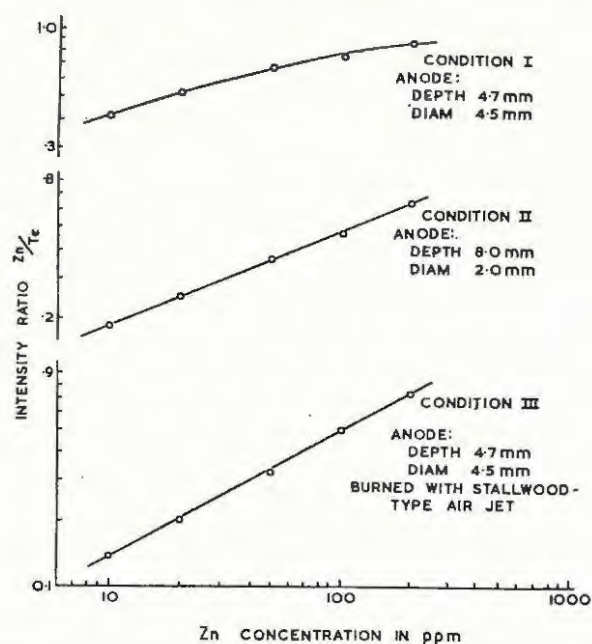


FIG. 1.—Standard calibration curves for zinc obtained under different excitation conditions

TABLE I

Standard Excitation Conditions

Light source	Positive direct current arc of 10 amp at 230 volts (constant current unit supplying the E.M.F.)
Upper electrode	Carbon electrode of 5 mm diameter with undercut rounded end.
Sample electrode	Graphite electrode of 6.35 mm diameter. The electrode craters were 4.7 mm deep and 4.5 mm in diameter. The electrodes were also undercut to a depth of 1 mm at a distance of 6 mm from the top.
Jet	A jet with an 8 mm orifice was placed 1 mm below the undercut of the sample-holding electrode.
Electrode gap	A gap of 8 mm was maintained throughout the exposure. The central 4 mm portion of this gap was photographed.

Buffer mixture. The mixture of potassium sulphate, calcium carbonate and graphite was selected after experiments had been carried out with a number of different mixtures. A 1:10 potassium sulphate, graphite mixture was found to give a good line to back-ground ratio, but was found to give inconsistent results. A mixture of 55% calcium carbonate in graphite was used with some success, but a mixture of 10% potassium sulphate and 40% calcium carbonate in graphite was found to give a more constant emission (Fig. 2). By diluting the sample 1:9 with this buffer mixture the influence of variation in the major constituents of the sample was greatly reduced.

Internal standard. Tellurium, previously used by Rogers¹, was selected as internal standard element. It has a strong spectrum line (2142.8 Å) close to the zinc line, is unlikely to occur in plant materials and it has suitable physical properties. (Excitation potential: Zn 2138.6 Å, 5.8 eV; Te 2142.8 Å, 5.8 eV. Ionisation potential: Zn 9.392 eV, Te 9.007 eV). In addition, as can be seen from Fig. 2, the vaporisation rates of tellurium and zinc are similar under the arc conditions. One per cent of tellurium oxide was added to the buffer mixture to give a suitable constant concentration of tellurium.

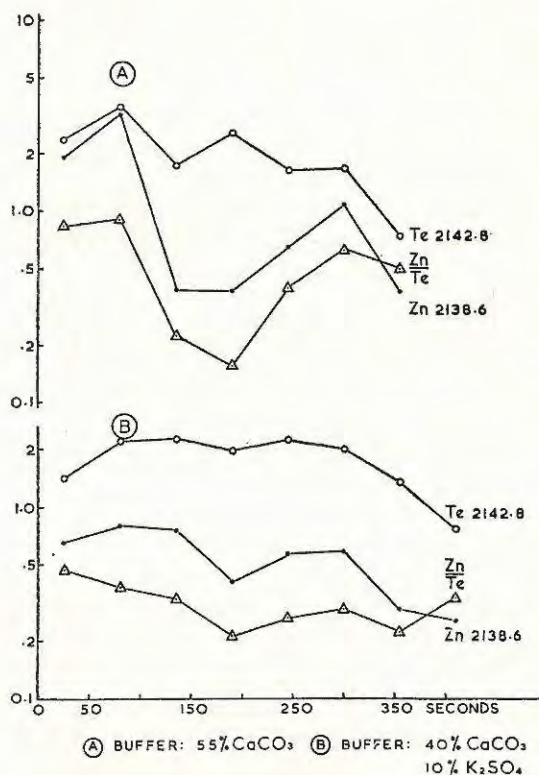


FIG. 2.—Time/intensity curves for:
 (1) Zn 2138.6 Å
 (2) Te 2142.8 Å
 (3) Zn 2138.6 Å/Te 2142.8 Å (intensity ratio)

Plate calibration and calculation of intensity ratios. The spectrum lines were observed to be unevenly illuminated. This was attributed to some optical imperfection of the spectrograph becoming more marked at low wavelengths. For this reason no step sector or filter was used while recording the sample spectra and the mean of five transmission readings was taken for each line. For the emulsion calibration a number of copper spectra, obtained by burning a globule arc for varying periods with a two-step sector, were used to give a wide range of densities for the emulsion calibration.

An A.R.L. calculating board fitted with seidel scales was used for the calculation of intensity ratios according to the preliminary curve method described by Churchill¹⁰.

Standards. Standards were prepared from "Specpure" zinc oxide and S.P.2 graphite to give concentrations ranging from 10 to 200 p.p.m. These standards were then mixed with the buffer-internal standard mixture in the same proportion as the samples.

Procedure. About one gram of plant material was weighed into a clean platinum crucible and ashed overnight at 450°C. The ash factor (ratio of ash: dry plant material) was determined by reweighing the crucible after ashing. The ash was well mixed and a suitable portion diluted 1:9 with the buffer. The diluted sample was then loaded into three electrodes and burned in the d.c. arc with air jet. The exposure was made for 5½ minutes from the striking of the arc. Two or three copper spectra, obtained from a globule arc burned for different periods with a two step rotating sector, were recorded on the same plate.

RESULTS

To tests the precision of the method 10 grams of plant material were ashed and the ash, after mixing with buffer, loaded into 30 electrodes which were then burned according to the method described. The results of this series of analyses are given in Table II.

Nine standard plant samples, which had been analysed a number of times previously by chemical and spectrographic methods by the Macaulay Institute for Soil Research, by the Division of Chemical Services of the South African Department of Agriculture and in the C.S.I.R., were analysed using the method described. The results are shown in Table III.

TABLE II
Results of repeated arcing of plant ash

Arcing No.	Zn p.p.m.	Arcing No.	Zn p.p.m.	Arcing No.	Zn p.p.m.
1	67.3	11	54.4	21	52.3
2	68.1	12	51.8	22	57.3
3	63.3	13	54.0	23	60.5
4	66.4	14	54.7	24	69.6
5	54.5	15	51.1	25	58.9
6	53.1	16	58.5	26	63.3
7	55.5	17	54.5	27	54.5
8	68.1	18	59.8	28	63.7
9	57.8	19	58.5	29	60.8
10	65.1	20	62.4	30	54.5

Mean=59.1 p.p.m.
Std. deviation=5.48 p.p.m.
% Std. deviation=9.3%.

TABLE III
Results of analysis of standard samples*

Sample	4a	4b	4c	4d	4e	4g	4h	4j	4k	
Analysis using present method	19.2	60.5	17.1	59.4	37.9	21.3	26.9	75.1	40.6	
	19.4	53.2	17.1	65.5	36.4	22.5	26.9	69.1	45.5	
	19.4	57.8	18.7	59.4	40.4	23.3	23.2	72.4	47.0	
Mean	19.3	57.2	17.6	61.4	38.2	22.4	25.7	72.2	44.4	
Results obtained by other methods	C.S.I.R. anode	28	60	22	59	64	25	28	48	27
	C.S.I.R. cathode	22	60	18	39	93	28	28	76	28
	Macaulay Inst.	21	60	30	60	52	26	50	45	40
	Chem. Services	26	56	23	38	30	41	43	60	18
Mean	24	59	23	49	60	30	37	57	28	

*For particulars of samples see Strasheim and Camerer¹¹.

CONCLUSIONS

The method described is simple and, apart from the time required for the ashing, relatively fast. It should be particularly suited for use in a laboratory where other elements are determined spectrographically after ashing the sample⁸.

From the standard deviation obtained for single exposures it can be expected that the error of an analysis in which the mean of three exposures is taken would be about 5.4%, which is quite good enough for trace element nutritional studies.

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REFERENCES

- ¹ Rogers, *Ind. Eng. Chem. Anal.*, 1935, 7, 421.
- ² Rogers and Gall, *ibid.*, 1937, 9, 42.
- ³ O'Connor, *ibid.*, 1941, 13, 597.
- ⁴ Eastmond and Williams, *J. Opt. Soc. Amer.*, 1948, 38, 666, (A.41)/801.
- ⁵ Mitchel and Scott, *J. Soc. Chem. Ind.*, 1947, 66, 330.
- ⁶ Scharrer and Judel, *Z. anal. Chem.*, 1957, 156, 340.
- ⁷ Van der Walt, *Rev. Sci. Instr.*, 1956, 27, 157.
- ⁸ Strasheim and Keddy, *J.S. African Chem. Inst.*, 1958, 11, 21.
- ⁹ Stallwood, *J. Opt. Soc. Amer.*, 1954, 44, 171.
- ¹⁰ Churchill, *Ind. Eng. Chem. Anal.*, 1944, 16, 653.
- ¹¹ Strasheim and Camerer, *J.S. African Chem. Inst.*, 1955, 8, 28.

THE INFLUENCE OF pH ON THE EXTRACTION OF ZINC, COBALT, NICKEL AND COPPER FROM TARTRATE SOLUTIONS USING DITHIZONE

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OPSOMMING

Die invloed van pH op ekstraksie, met chloroform-oplossings van ditisoon, van sink, kobalt, nikkell en koper, uit waterige tartraat oplossings, is ondersoek. Die eenvoudige verwantskap, voorgestel deur Koltoff en Sandell, is met sukses in die geval van nikkell en kobalt aangewend en die ewewigskonstant vir die ekstraksie van nikkell, kobalt en sink ditisoonate is bereken.

SUMMARY

The influence of pH on the extraction with chloroformic dithizone solutions of zinc, cobalt, nickel and copper from aqueous tartrate solutions, has been investigated. The simple expression proposed by Koltoff and Sandell has been shown to apply fairly well in the case of nickel and cobalt and equilibrium constants for the extraction of nickel, cobalt and zinc dithizonates have been calculated.

In recent years a number of publications have dealt with the application of dithizone extraction methods to the concentration of trace metals for spectrographic analysis. Dithizone extraction has also been applied extensively to the separation and concentration of metals for colorimetric and polarographic analysis. Except in the case of lead^{1,2} however, knowledge of the effect of pH on the efficiency of extraction is far from complete.

In the analysis of plant material it is common practice to add citrate or tartrate to the solution before it is neutralised in order to prevent the precipitation of metal hydroxides, especially iron and aluminium. Biefield and Partick¹ have shown that citrate solutions are unsuitable when lead is to be extracted and recommend the use of tartrate where a complexing agent is required. Unfortunately most of the work that has been carried out in connection with the influence of pH, deals with the extraction of metals from citrate solutions, and as the work of Biefield and Patrick shows, the nature of the complexing anions present can have a profound effect on the pH range of quantitative extraction. Furthermore, Yasumutsu Uzumasa and Isao Mujashito² have reported that tartrate and citrate interfere with the quantitative extraction of copper dithizonate by carbon tetrachloride.

A number of somewhat conflicting publications dealing with the extraction of zinc dithizonate have appeared^{4,5,6}, but in none of these is extraction from tartrate solution considered. Less work has been done on the extraction of cobalt. Marston and Dewey⁷ have investigated the extraction of this element by a large excess of dithizone from the citrate and acetate solutions. They found a remarkable difference between carbon tetrachloride and chloroform as extractants.

Apparently little work has been done on the extraction of nickel with dithizone and no similar investigation of the extraction of copper from tartrate solutions has been published.

Fischer⁸ has studied the pH ranges of stability of a number of metal dithizonates, but, as the results show, these have little relation to the pH ranges of complete extraction. Gorbach and Pohl⁹ have recommended repeated extraction with dithizone over the pH range 7-9 for the extraction from plant material solutions of the trace metals zinc, copper, cobalt, nickel and lead, prior to their spectrochemical determination. As this procedure was found to be too slow, this investigation was primarily undertaken to investigate the possibility of extracting the elements in question at one optimum pH.

EXPERIMENTAL

During the course of this investigation two commercial brands of dithizone were used. In the first series of experiments a 46 *per cent.* pure product was used. Solutions prepared from this product were used for the preparation of the first series of pH-extraction curves for the individual metals. (These represent the smallest excess of dithizone). The second commercial product, obtained later, was shown to be 96 *per cent.* pure and this was used in all subsequent investigations.

8.2 *per cent.* (0.546 M) Tartaric acid solution (10 ml.) (corresponding to 10 *per cent.* ammonium tartrate) was run into a 200 ml. separatory funnel. Sufficient N ammonia solution was added to give approximately the desired pH (previously determined). A 10 p.p.m. solution (10 ml.) of the metal was then pipetted into the funnel and the mixture diluted to 100 ml. Dithizone solution (20 ml.) was added and the contents of the funnel were shaken vigorously for two minutes. The chloroform layer was separated and the pH of the aqueous phase determined.

In the estimation of cobalt, nickel and copper the chloroform layer was evaporated and the metal complexes destroyed by evaporation with nitric acid (5 ml.) containing a few drops of perchloric acid. The residue was dissolved in water containing a few drops of N HCl and, after diluting to 10 ml., the metal content was determined flame photometrically using a Beckman D.U. spectrophotometer with photomultiplier and flame attachments.

In the case of zinc, the excess dithizone in the chloroform phase was removed by extraction with .04 *per cent.* sodium sulphide as described by Sandell¹⁰ and the zinc content determined photometrically after dilution to 50 ml.

Nickel. Because of the metals studied, least is known about the extraction of nickel, a more thorough study was made of the effect of varying excesses of dithizone in the extraction of this metal than the others.

The results are shown in Figure 1.

Curve 1 shows the effect of variation in pH on the extraction of nickel using 20 ml. of .0047 *per cent.* Dithizone (Dz), a 6.8 *per cent.* excess of the reagent.

Curve 2 shows the extraction with 20 ml. of .0096 *per cent.* Dz (120 *per cent.* excess).

Curve 3 represents the result obtained when extractions were made with 14 ml. of .0096 *per cent.* Dz to give a 54 *per cent.* excess.

Curve 4 shows the extraction with the same excess of reagent (54 *per cent.*), obtained by using 20 ml. of 0.007 *per cent.* Dz.

Cobalt. Figure 2 shows the results obtained for the extraction of cobalt. Extractions were made with two dithizone solutions, .0096 *per cent.* and .0047 *per cent.* giving excesses of reagent of 7.2 *per cent.* and 121 *per cent.* respectively.

Zinc and Copper. In Figure 3 the results obtained for the extraction of zinc and copper shown. In each case 20 ml. of .0047 *per cent.* Dz were used for the extraction.

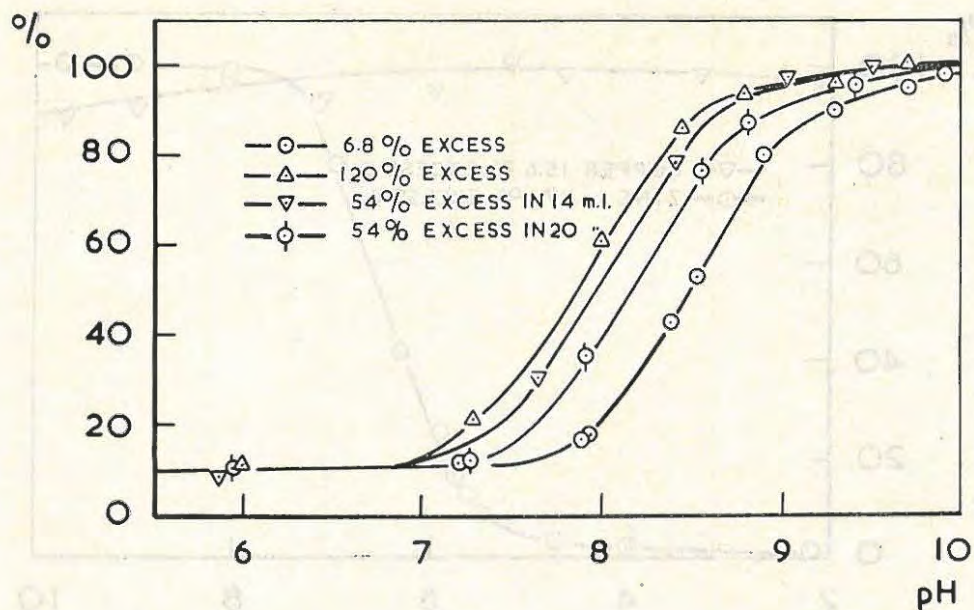


Fig. 1
pH—Extraction relationship for nickel.

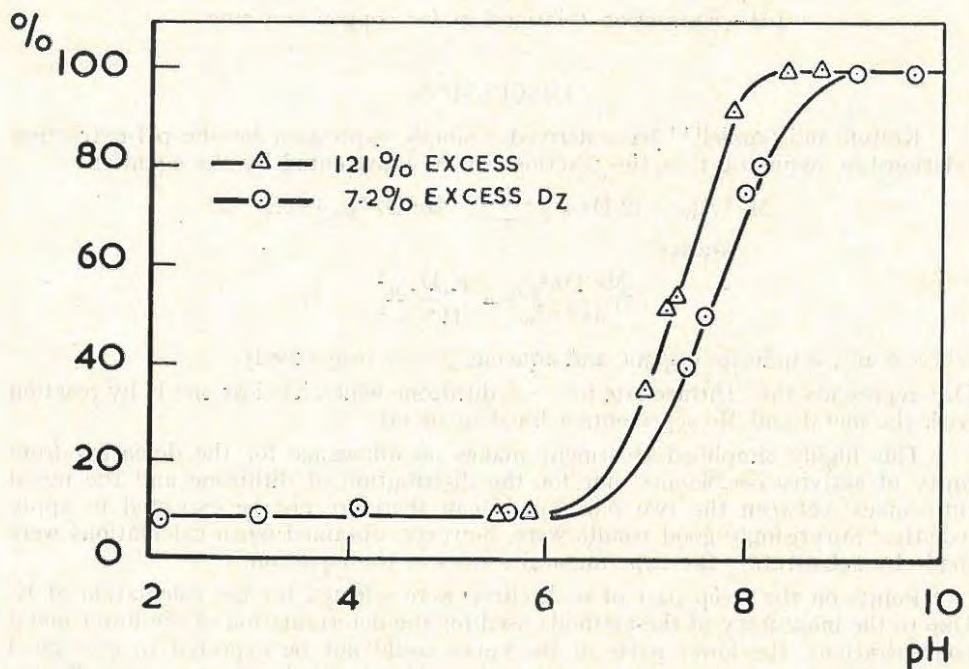


Fig. 2
pH—Extraction relationship for cobalt.

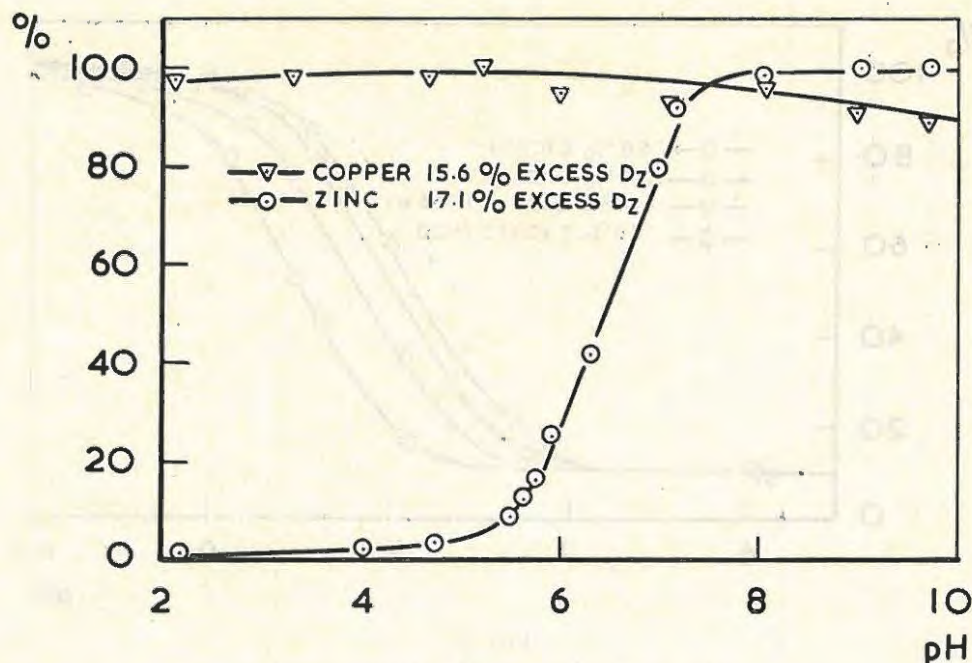
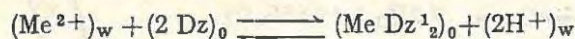


Fig. 3

pH—Extraction relationship for copper and zinc.

DISCUSSION

Koltoff and Sandell¹¹ have derived a simple expression for the pH-extraction relationship, assuming that the reaction can be represented by the equation:



whence

$$\frac{[\text{Me Dz}^1_2]_o}{[\text{Me}^{2+}]_w} = \frac{K[\text{Dz}]_o^2}{[\text{H}^+]_w^2}$$

where o and w indicate organic and aqueous phases respectively.

Dz¹ represents the "Dithizonate ion" i.e. dithizone which has lost one H by reaction with the metal and Me represents a bivalent metal.

This highly simplified treatment makes no allowance for the deviation from unity of activity coefficients, nor for the distribution of dithizone and the metal dithizonate between the two phases. It can therefore not be expected to apply exactly. Surprisingly good results were, however, obtained when calculations were made by substituting the experimental results in the equation.

Points on the steep part of each curve were selected for the calculation of K. Due to the inaccuracy of the methods used for the determination of the lower metal concentrations, the lower parts of the curve could not be expected to give good results. Where possible points were selected at pH values below 8, as in more alkaline solutions the position becomes somewhat complicated by the partial solution of the dithizone in the aqueous phase.¹

Calculated values of K for the extraction of nickel, cobalt and zinc, are shown in Tables I, III and V while Tables II and IV illustrate the use of the expression for predicting the effect of varying the Dz concentration.

TABLE I
Calculation of K for Extraction of Nickel.

Curve	pH	% Extracted	K
1	8.5	51.0	$.55 \times 10^{-8}$
1	8.0	20.0	$.57 \times 10^{-8}$
2	8.0	60.0	1.01×10^{-8}
3	8.0	52.2	1.27×10^{-8}
4	8.0	40.5	0.91×10^{-8}

As the steep portion of curve 1 lies in the pH region above 8, values for K from this curve were neglected.

Mean K for curves 2, 3, 4, $= 1.06 \times 10^{-8}$.

TABLE II
Calculation of points on the curves assuming that $K = 1.06 \times 10^{-8}$

Curve	% Extracted	Calculated pH	pH found
1	70	8.72	8.79
1	40	8.19	8.34
2	70	8.21	8.17
2	40	7.76	7.68
3	70	8.29	8.27
3	40	7.88	7.81
4	70	8.37	8.45
4	40	7.96	8.00

TABLE III
Calculation of K for the Extraction of Cobalt

Curve	pH	% Extracted	K
1	8.0	71.5	3.40×10^{-7}
1	7.5	44.5	3.56×10^{-7}
2	8.0	96.5	3.11×10^{-7}
2	7.5	90.6	3.01×10^{-7}
2	7.0	35.0	2.26×10^{-7}

Mean K, 3.07×10^{-7}

TABLE IV
Theoretical Calculation of Position of Curve 2 from Curve 1—Cobalt
K (from curve 1), 3.48×10^{-7}

% Extract	Calculated pH	pH Found
40	7.00	7.07
70	7.36	7.50

TABLE V
Calculation of K for the Extraction of Zinc

pH	% Extracted	K
7.0	79.5	5.38×10^{-5}
6.5	53.0	5.50×10^{-5}

Mean K, 5.4×10^{-5}

From these results it has been concluded that at pH 9.5, using only a small excess of reagent, over 90 *per cent.* extraction was obtained in each case and therefore zinc, nickel, cobalt, copper and, from the results reported by Biefield and Patrick¹, lead may be quantitatively extracted from tartrate solutions at this pH. These observations led to the selection of pH 9.5 for all dithizone extractions for spectrographic analysis.

Comparison of the results obtained for zinc with those of Walkley⁴ indicates that zinc may be extracted at a lower pH from tartrate than from citrate solution. This fact may prove valuable in the separation of zinc from other metals for colorimetric or polarographic analysis.

The results demonstrated in curves 3 and 4 of Fig. 1 for nickel indicate (with support from the theoretical calculation) that more efficient extraction may within certain limits be obtained by using the same weight of dithizone in a smaller volume of chloroform. These results also show the importance of stating all the experimental conditions when reporting work of this nature.

For nickel and cobalt, the expression derived by Koltoff and Sandell¹¹ may be used to predict the position of the pH extraction curves for any concentration of dithizone, provided K is known. Above pH 8, however, where dithizone becomes appreciably soluble in the aqueous phase, the relationship cannot be expected to apply.

The value of K as defined depends on the nature of the complexing anions in solution, as comparison with the observations of other workers shows. The possibility that the concentration of these anions may also affect the value of K should not be overlooked.

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REFERENCES

- ¹ Biefield and Patrick, *Ind. Eng. Chem., Anal. Ed.* 1942, **14**, 275.
- ² Clifford and Wichmann, *J. Assoc. Offic. Agric. Chemists*, 1936, **19**, 130.
- ³ Yasumitsu Uzumasa and Isao Mujashita, *J. Chem. Soc. Japan*, 1951, 41.
- ⁴ Walkley, *Australian Chem. Inst. J. and Proc.* 1942, **9**, 29.
- ⁵ Hibbard, *Ind. Eng. Chem. Anal. Ed.*, 1937, **9**, 127.
- ⁶ Cholak, Hubbard and Burkey, *Ind. Eng. Chem. Anal. Ed.*, 1943, **15**, 754.
- ⁷ Marston and Dewey, *Australian J. Exptl. Biol. Med. Sci.*, 1940, **18**, 343.
- ⁸ Fischer, *Microchemie*, 1942, **30**, 38.
- ⁹ Gorbach and Pohl, *Microchemie*, 1951, **38**, 258, 328, 335.
- ¹⁰ Sandell, "Colorimetric Detn. of Traces of Metals," 2nd Edn. Interscience Publishers Inc., New York, 1950, p. 623.
- ¹¹ Koltoff and Sandell, *J. Amer. Chem. Soc.*, 1941, **63**, 1906.

Direct Reading Spectrometric Determination of Zinc, Copper and Lead in Plant Material

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Abstract

A method is described for the direct reading spectrometric determination of zinc, copper and lead in plant material. The metals to be determined are separated by dithizone extraction from a solution of 1 gram of the plant material. The excitation source consists of an interrupted d.c. arc struck between a graphite counter electrode and a rotating graphite-sodium carbonate disc, on to which a chloroform solution of the metal dithizonates is sprayed. Spectrum line emission measurements are made by means of a medium dispersion quartz prism spectrograph with direct reading attachment. A series of plant material samples has been analysed by the method and the standard deviations of the results obtained from the mean of two arcings in each of ten replicate analyses were found to be 5.1% for zinc, 7.3% for copper and 6.1% for lead.

Introduction

The advantages of direct-reading methods over the more conventional photographic methods of emission spectrochemical analysis are generally recognised and their scope is being rapidly widened to include samples of greater variety and complexity. In this paper a method is described for the direct reading spectrometric determination of zinc, copper and lead in plant materials. Spectrographic analysis for trace elements, especially when the instrument used is of the medium dispersion type, normally requires that these elements be separated and concentrated chemically as a preliminary step in the procedure. To obtain the full advantage of the use of direct reading equipment, however, the time spent on preliminary steps must be reduced as far as possible and it is to this end that the method described in this paper was developed.

Apparatus*

The spectrometric equipment used consisted of a *Hilger Medium Quartz Spectrograph* with a direct reading attachment built in the laboratory. The photomultiplier unit, which is mounted in place of the plate holder of the spectrograph, consists of six movable *E.M.I.* head-on-type photomultiplier tubes mounted in the manner shown in Figure 1. The light from the spectrum line to be measured passes through a slit attached to each photomultiplier assembly and is reflected by a small mirror into the photomultiplier itself. The position of each tube can be accurately adjusted by the micrometer screws shown in the photograph which allow for both a coarse and a fine adjustment. The mounting is so arranged that the slit of either tube 3 or 4 can be placed as close as is desired to

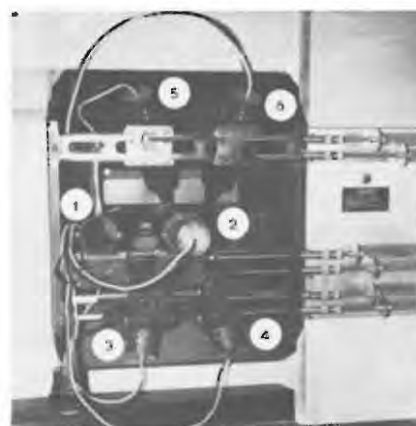


FIG. 1. ARRANGEMENT OF PHOTOMULTIPLIERS AND EXIT SLITS.

that of tube 5 or 6, or on the same line if necessary. The same conditions apply as regards the setting of either tube 5 or 6 with relation to the positions of tubes 3 or 4. The nearest possible approach of exit slits in the same bank is, however, about 10 mm. Tubes 1 and 2 are intended for use in the weak, far-ultraviolet region and their slits are designed and set to include the whole spectrum image.

During the exposure, the current produced by the photomultipliers is used to charge condensers. An electronic measuring system consisting of a modified Naish-Ramsden (1) circuit with a servomechanism, is used to indicate the charge on the condensers after the exposure has been made.

The spectrograph is enclosed in an insulating case and the whole apparatus is housed in a temperature and humidity controlled room. Slit widths: Entrance slit, 0.04 mm; exit slits, 0.06 mm.

Development of Method

Excitation Source

A number of experiments were carried out to find an excitation method for the chloroform extract solution which would provide a simple spectrum, free from excessive background. Used in conjunction with the rotating platform type of electrode, an interrupted arc was found

*Detailed drawings of the apparatus and electronic circuits will be supplied by the authors on request.

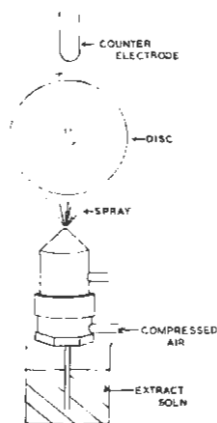


FIG. 2. THE ATOMISER-ROTATING DISC EMISSION SOURCE

to give strong spectrum lines of zinc, copper and lead for concentrations equivalent to less than a gram of plant material, but the transfer of the sample to the electrode was found to be tedious and time-intensity curves showed that all the zinc and lead were vaporised early in the arcing period.

Other methods were tried, but most proved to be insufficiently sensitive. It was found, however, when the interrupted arc was used in conjunction with a rotating disc electrode, that the sensitivity remained high while some measure of continuous sampling was obtained. It was not possible to keep the rotating disc in contact with the sample solution, as it was found that the heating of the electrode by the arc caused the solution to boil violently and the technique of spraying the solution on to the edge of the disc was therefore adopted. For this a *Beckman Flame Photometer* atomiser-burner was found to be satisfactory (See Figure 2.) A compressed air line was connected to the oxygen inlet tube while the fuel inlet was left open. The air pressure used was 10 lbs/square inch. This is the pressure of oxygen which was recommended by the manufacturers of the atomiser for flame photometry and was found to be the most suitable for this work.

Spectrograms obtained using pure graphite disc electrodes showed the presence of background which would be inconveniently high for direct reading work. It was found that this background was considerably reduced if the disc was made up of a mixture of graphite and sodium carbonate. Suitable discs were prepared by pressing 4 grams of a graphite (S.P.1): sodium carbonate mixture (4:1) in a special 1 inch diameter mould in an A.R.L. press.

For the analysis the disc was placed on a spindle connected to a variable-speed motor, as with the normal rotating disc-spark technique. Each disc was arced for 30 seconds before use to prepare the surface and free it from impurities picked up in handling. A new disc was used for each arcing.

Experiments carried out with varied rotation speeds and current strengths, seemed to indicate that a temperature equilibrium could be set up which gave a more or less constant vaporisation and excitation of the elements in question. This is illustrated by the time-intensity curves shown in Figure 3. As can be seen, the time-emission curves are virtually parallel with the time axis for the conditions 3.5 amps at 6 revolutions per minute, while the intensity ratios are also nearly constant for these conditions. On the basis of these curves it was decided to expose from the 20th to the 60th second of the arc.

The excitation source finally used thus consisted of an interrupted d.c. arc struck at mains frequency (50 cycles per second) between a graphite counter electrode (cathode) and a rotating graphite sodium carbonate disc (anode), on which the chloroform solution of the extracted metal dithizonates was sprayed with a flame photometer atomiser, as shown in Figure 2. It has been found necessary to control temperature, air pressure and rate of revolution of the disc very carefully for reproducible results.

Chemical Preparation of the Sample

As the excitation source developed was sensitive enough to allow the use of less than a gram of sample, it was convenient to use a wet ashing method for the preparation of the sample solution. The danger of the loss of volatile elements such as zinc, when the plant sample is dry-ashed, was thus avoided. The procedure followed was similar to that described by Verdier, Steyn and Eve (2).

Solvent extraction methods have shown themselves to be particularly suited for the rapid, efficient separation of trace metals from other constituents of the sample. The method used was similar to that described by Wark (3), but a smaller concentration of citrate was used, 40 ml of 5% am-

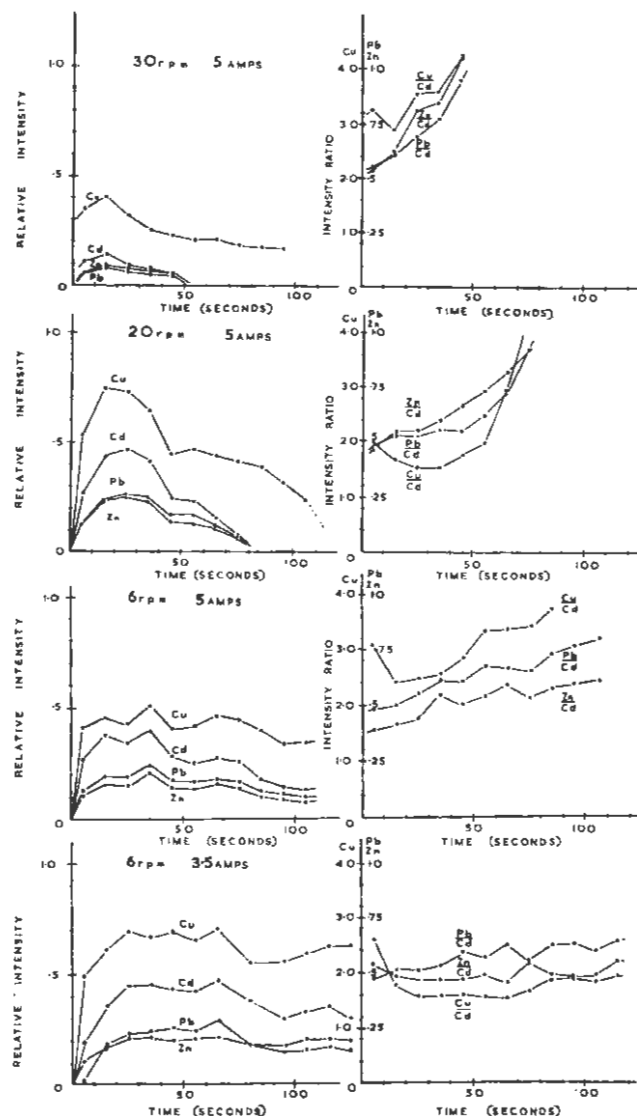


FIG. 3. TIME-INTENSITY CURVES FOR VARIOUS SOURCE CONDITIONS

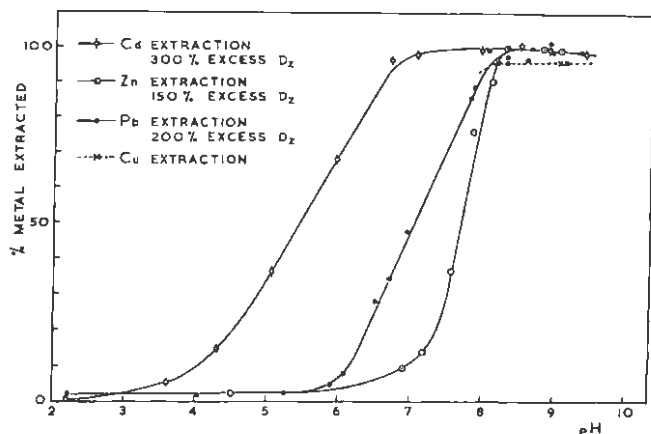


FIG. 4. INFLUENCE OF pH ON DITHIZONE EXTRACTION

monium citrate being found to be adequate for the much smaller samples taken. The influence of pH on the dithizone extraction of zinc, copper, lead and cadmium from citrate solutions of similar concentration to those obtained in the analysis, was investigated and the results are shown graphically in Figure 4. On this basis a pH of 8.5-9 was selected for the extraction. To insure a rapid and complete extraction, 0.02% dithizone (weight to volume in chloroform) was used for the extraction, which was carried out with the help of a mechanical-stirrer extraction apparatus designed for the simultaneous extraction of six sample solutions. (This apparatus is to be fully described in another publication (4)).

Internal Standard

Cadmium was selected as internal standard, as it does not occur in large concentrations in plant material and can readily be extracted as a dithizone complex together with zinc, copper and lead, thus providing a standardisation of the chemical extraction step as well as the spectrometric part of the procedure. It has, in addition, been found to be a suitable internal standard for zinc by a number of other analysts (5) (6).

Spectrum Lines Used

The following spectrum lines were used in the analysis, the cadmium being used as internal standard: Zn 2138.6A, Pb 2833.1A, Cu 3247.5A, Cd 3261.1A.

Self Absorption of the Zinc Line

From the standard curves shown in Figure 5, it is evident that the zinc line at 2138.6A shows marked self absorption at the higher concentrations. This means that the zinc concentration must be kept low and the conditions must be kept as constant as possible if this line is to be used. The line at 3345A would probably have proved quite satisfactory had it been possible to position the exit slits for this purpose, but this was made impossible by the relative proximity of the other lines used.

Chemicals

All the chemicals used were of the highest grade of purity available.

- (i) Distilled water was further purified by passage through an ion exchange resin which has been found particularly suitable for this purpose.
- (ii) Chloroform was twice distilled before use.
- (iii) Standard solutions of zinc, copper, lead and cadmium were prepared from spectrographically pure salts of these metals.

- (iv) 5% Ammonium Citrate Solution: This was prepared by dissolving analytical grade ammonium citrate (dibasic) in half the required quantity of water. Ammonia solution was then added to bring the pH to about 9 and the solution extracted repeatedly with small volumes of dithizone solution until these remained green. The solution was then made up to the required volume.

Method

Procedure

0.5 to 1 gram of finely ground dry plant material was wet-ashed. The filtered sample solution (20-30 ml) was brought to pH 8.5-9 with redistilled ammonia after the addition of 40 ml of 5% ammonium citrate solution and 5 ml of standard 10 ppm cadmium solution, and transferred to a separatory funnel. The solution was then agitated with 15 ml of a 0.02% chloroform solution of dithizone for two minutes and the chloroform phase separated. A further 5 ml of dithizone solution and 5 ml of pure chloroform were then added and the extraction repeated. Remaining traces of chloroform solution of dithizonates were washed down with pure chloroform. The combined extract was evaporated partially on a moderate temperature sand bath, transferred to a 25 ml volumetric flask and made up to volume with pure chloroform.

Portions of this extract solution were poured into 5 ml beakers to be sprayed on to the rotating disc for the exposure. Two exposures were made for each sample and the mean intensity ratio taken.

The ratio of the intensity of the element line to that of cadmium was calculated and the concentration read from graphs, drawn from standards on linear paper, of intensity ratios against concentration.

The procedure followed for the standards was exactly the same, except that in place of the sample solution, suitable volumes of a standard solution containing 5 ppm zinc, 1 ppm copper and 0.5 ppm lead were used. (One or two of the standards should be re-arc'd every day to allow for correction for change in the instrument).

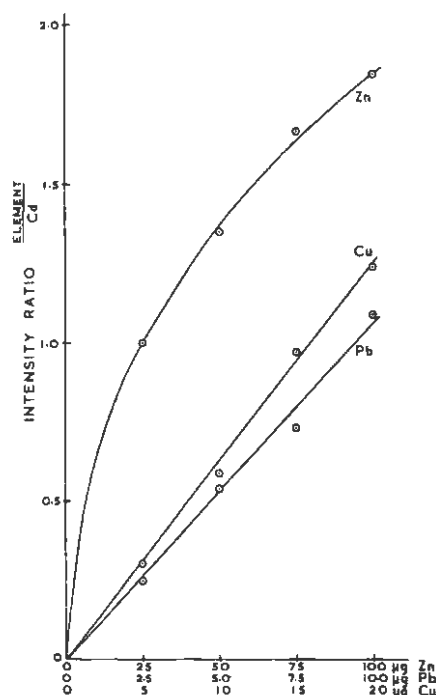


FIG. 5. STANDARD ANALYSIS CURVES

TABLE I. RESULTS OF ANALYSIS OF ALIQUOTS OF SAMPLE SOLUTION

Aliquot	Element Concentrations (ppm)		
	Zn	Cu	Pb
1	33.3	8.9	12.2
2	32.4	7.6	10.9
3	33.3	9.3	12.9
4	31.5	8.0	12.9
5	32.0	8.9	12.8
6	34.6	9.7	12.0
7	32.8	8.9	12.5
8	30.0	7.6	12.8
9	29.5	8.4	12.0
10	34.1	9.2	11.0
Mean	32.35	8.65	12.20
Std. Dev. (ppm)	1.7	0.6	0.7
Coefficient of Variation, %	5.1	7.3	6.1

Reproducibility

To test the reproducibility of the method 10 aliquots of a solution prepared from 20 grams of plant material were analysed. The results of these analyses are shown in Table I. The standard deviations were calculated for each element and found to be 5.1% in the case of zinc, 7.3% for copper and 6.1% for lead.

Analysis Results

As a further test of the method, six plant material samples, which have been repeatedly analysed previously by both chemical and spectrographic methods in this and other laboratories*, were analysed in duplicate using the proposed method, and the results are given in Table II.

In the case of lead it was found necessary to make a blank correction for contamination which took place during the wet-ashing step (about 1 µg).

From the results in Table II it can be seen that the method gives as reliable results as any of the previously used methods.

Conclusions

The method described is rapid and sufficiently accurate for trace element studies.

The excitation technique was developed primarily for use in conjunction with a direct reading instrument, but could no doubt be used to advantage with photographic instruments. It has a high sensitivity and allows control of the excitation conditions. (For the standards, about 2 ml of chloroform solution were required for each exposure and for the lowest standard, which was well above

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Onderstepoort Laboratory, Department of Agriculture, Union of South Africa.

TABLE II. ANALYSIS OF STANDARD PLANT SAMPLES (PPM)

Sample	Laboratories where Previous Analyses were carried out ¹	Results of Previous Analyses			Results of Direct-Reading Analyses described in this paper		
		Zn	Pb	Cu	Zn	Pb	Cu
4a Peach Leaves	N.P.R.L. (A)	28	1.7	8.7	—	—	—
	N.P.R.L. (C)	22	1.1, 1.1	—	—	—	—
	O.	—	—	5.5	23.6	2.3	8.5
	M.	21	6.5	5.7	19.8	1.0	9.1
4b Bean Leaves	C.S.	26	—	4	—	—	—
	N.P.R.L. (A)	60	3.9, 2.7	13.8	—	—	—
	N.P.R.L. (C)	60	2.2, 1.5	—	—	—	—
	O.	—	—	9.6	69.4	5.3	12.9
4c Beet Leaves	M.	60	3.0	12.9	59.3	4.0	12.8
	C.S.	56	—	12	—	—	—
	N.P.R.L. (A)	22	1.4, 1.2	13.9	—	—	—
	N.P.R.L. (C)	18	1.3, 1.1	—	—	—	—
4d Gem Squash Leaves	O.	—	—	8.4	20.6	1.0	16.0
	M.	30	6.0	10.6	33.9	2.1	10.2
	C.S.	23	—	15	—	—	—
	N.P.R.L. (A)	59	4.6	13.6	—	—	—
4e Plum Leaves	N.P.R.L. (C)	39	2.7, 4.8	—	—	—	—
	O.	—	—	9.5	53.2	1.5	12.4
	M.	60	5.5	10.5	56.2	2.1	11.7
	C.S.	38	—	14	—	—	—
4f Bush	N.P.R.L. (A)	64	1.4, 3.0	6.8	—	—	—
	N.P.R.L. (C)	93	0.5, 1.0	—	—	—	—
	O.	—	—	4.5	47.3	2.0	8.5
	M.	52	5.5	5.8	53.0	2.5	10.3
4g Bush	C.S.	30	—	9	—	—	—
	N.P.R.L. (A)	25	1.6, 1.7	—	—	—	—
	N.P.R.L. (C)	28	0.9, 0.5	—	—	—	—
	O.	—	—	—	26.4	0.7	12.4
4h Bush	M.	50	—	—	30.0	2.0	11.7
	C.S.	43	—	—	—	—	—

¹N.P.R.L. (A) = C.S.I.R. Anode Excitation Method
N.P.R.L. (C) = C.S.I.R. Cathode Excitation Method
O. = Onderstepoort (Spectrochemically)
C.S. = Div. Chemical Services (Chemically)
M. = Macaulay Institute (Spectrochemically)

the sensitivity limit of the method, the concentrations were 1 ppm or 2 µg of zinc, 0.1 ppm or 0.2 µg of lead and 0.2 ppm or 0.4 µg of copper).

Acknowledgements

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Literature Cited

- J. M. Naish and W. Ramsden, *SPECTROCHIM. ACTA* **5**, 295 (1952)
- E. T. Verdier, W. J. A. Steyn and D. J. Eve, *AGR. FOOD CHEM.* **5**, 354 (1957)
- W. J. Wark, *ANAL. CHEM.* **26**, 203 (1954)
- A. Strasheim and D. J. Eve, *J. S. AFRICAN CHEM. INST.* (*In press*)
- R. L. Mitchell and R. O. Scott, *APPLIED SPECTROSCOPY* **11**, 6, (1957)
- K. Scharrer and G. K. Judel, *Z. ANAL. CHEM.* **156**, 340 (1957)

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