THE
SPECTROGRAPHIC DETERMINATION
OF
TRACE ELEMENTS
IN
CITRUS LEAVES

- by -

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A thesis submitted in part-fulfilment of the requirements for the degree of Master of Science of Rhodes University.

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CONTENTS.

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INTRODUCTION.

With the rapidly growing knowledge on trace elements and their influence on plant nutrition the need for accurate and rapid methods for their determination arose. The essential plant nutrients are usually divided into two groups. The major or macronutrient elements, necessary in comparatively large amounts, and the trace or micronutrient elements. In the case of plants, the first group includes Sulphur, Phosphorus, Potassium, Magnesium, Calcium and Nitrogen. The essential trace elements are Iron, Manganese, Boron, Copper, Zinc, Molybdenum and Chlorine. Cobalt is essential for animal nutrition as a constituent of Vitamin B_{12}, but its essentiality for plants has not yet been proved. The latter group consists of metals which are catalysts in enzyme reactions and whose presence in the plant in minute amount determines whether the plant will be able to complete the vegetative or reproductive stage of its life cycle. Molybdenum may be quoted as an example of an essential trace element÷ It is generally recognised to be the catalyst responsible for the fixation of Nitrogen.

While the major elements in plants and soils may to-day be analysed with sufficient precision and speed, the accurate estimation of trace elements still requires complicated and time consuming analytical procedures. Polarographic and, especially, colorimetric methods have been developed which are very sensitive and yield results of high precision. However, they suffer from the disadvantage that each element has to be analysed for separately.

Spectrochemical analysis, on the other hand, offers the advantage of speed and high sensitivity. The pre-

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cision obtainable by this method may, however, not be very high, particularly, when a direct current is utilised for the excitation of the sample. In the analysis for minute quantities of metals chemical pre-treatment of the plant material is usually necessary, thus considerably increasing the length of the analytical procedure. However, on the basis of time consumed per element, spectrochemical analysis is relatively fast, since several trace metals may be determined simultaneously and a permanent record of the analysis is available in the form of photographic films or plates.

As mentioned above the accuracy of spectrographic analysis leaves something to be desired. This work was undertaken in order to investigate a few of the likely sources of error to which spectrochemical analysis may be susceptible with special regard to the determination of some minor and trace elements in citrus leaves. Factors influencing the accuracy of spectrographic analysis may be grouped under six main headings:-

1. Composition and Form of Sample.

The influence of the major constituent of a sample on the emission of minor constituents, i.e. the matrix effect. Analysis of a powder or a solution.

2. Introduction of the Sample to Discharge.

Very small amounts of sample are consumed in spectrographic analysis, introducing sampling errors. The influence of the dimensions and shape of the sample bearing electrode must be considered.

3. Excitation.

The manner in which the sample is excited is regarded as the most potent cause of inaccuracies. A distinction must be made between continuous (arc) and discontinuous (spark) discharges. The former, producing

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the highest sensitivity, may, however, be very unstable. Factors such as wandering of the discharge over the surface of the electrodes, ambient conditions, and the formation of stable compounds at the electrode can influence its behaviour considerably.

4. Interaction between Source and Electrodes.

Complex phenomena, such as selective distillation, Chemical reactions at high temperatures and the distribution of energy in the discharge may under some circumstances greatly affect accuracy.

5. Spectrograph.

The intensity of spectral background is dependent on the width of the spectrograph slit, which should therefore be maintained constant. A change in the focus of an instrument, e.g. by temperature changes or displacement of a lens or recording mechanism may cause a sharp line to become diffuse, modifying its apparent intensity relative to a naturally diffuse line. Schöntag (1) stated that the intensity ratio of two lines can vary by as much as 10% for a change of room temperature of 4°C.

6. Recording and Measurement of Spectra.

The characteristics of photographic emulsions, including such factors as calibration, contrast, grain, uniformity and processing methods must be carefully controlled and standardised. In the microphotometric evaluation of recorded spectra constancy of the light source, stability of galvanometer, relation between photometer slit width and width of spectrum lines and the effect of stray light all merit attention.

While, of course, a thorough investigation into the sources of error mentioned above would have been impossible, due to lack of facilities and time,

/ attention was

attention was paid to a few causes of inaccuracies connected with general techniques in spectrographic analysis. The main emphasis was placed on the development of a relatively rapid, reproducible and accurate method for the spectrochemical determination of the following trace elements in citrus leaves÷ Cobalt, Copper, Iron, Manganese, Molybdenum and Zinc. Of these elements Copper, Iron and Manganese may generally be determined directly on the ashed plant material and several successful methods have been developed in this respect, e.g. by Strasheim and Keddy (2), Pienaar (3) and Farmer (4). To this list may be added Zinc, since Strasheim and Eve (5) showed that the most sensitive Zinc line at 2138.6 A in the far ultra-violet may be utilised when special equipment and photographic materials are used. However, the quantitative estimation of Cobalt, Molybdenum and Zinc generally requires a preliminary chemical concentration technique.

A difficulty associated with the simultaneous spectrographic analysis of minor elements, such as Iron, Manganese and particularly Copper, and trace elements such as Cobalt, Molybdenum and Zinc is the following÷ The preliminary chemical treatment usually increases the concentration of the former group of elements to such an extent that 1.) their analytical lines become too dense to be measured successfully and 2.) the danger of coincidences of some of their spectral lines with analytical lines of other trace elements exists. The first case is particularly true in the case of Copper, which has a very simple spectrum containing two strong lines at 3274 and 3247 A respectively, which enable Copper to be determined directly on the plant ash from a concentration of appr. 1 p.p.m. Other Copper lines only become visible at very much higher concentrations. The second instance is true for Iron and Manganese, both possessing line-rich spectra. It was found, however, that at the concentration levels occuring in citrus leaves, no interferences were noted. In spite of self-reversal of the Cu 3274 A line Copper could be determined with a fair degree of accuracy up to approx. 10 p.p.m.

A survey of the literature revealed that a fairly rapid and simple enrichment of the trace and minor elements Cobalt, Copper, Iron, Manganese, Molybdenum and Zinc could be achieved by employing the method originally proposed by Stetter and Exler (6), who used Sodium pyrrolidine dithiocarbamate to precipitate the trace elements and separated the precipitated organic complexes from the macro-nutrients in plants by chloroform extractions. This method was made the basis of the spectrochemical analytical procedure in the work reported here.

The work, as presented in this thesis, is divided into four main parts÷

Part I gives a description of apparatus and techniques used.

Part II deals with an investigation into a few sources of error associated with spectrographic analysis in general.

In Part III the chemical concentration of trace elements in plants and their spectrographic determination are detailed.

Part IV contains a preliminary investigation into the possibilities of exciting a sample in atmospheres different from air.

PART I.

DESCRIPTION OF EQUIPMENT AND TECHNIQUES.

1. THE SPECTROGRAPH.

All the work described in this thesis was carried out using a Large Hilger Quartz Spectrograph, model E 472, (fig. 1.) fitted with a 35 mm film adaptor E 622, (fig. 2.) and plate holder. Plates were only employed in studies of rates of volatilisation of the elements from the electrode under different conditions, all other exposures being recorded on 35 mm film. Figure 3 shows the optical arrangement of the spectrograph. An image of the light source A is produced on the collimator lens L_4 of the spectrograph by means of the Zeiss Intermediate Image lens system as described by Nordmeyer (7). The light source A is focussed on the diaphragm D by a short-focus lens L_1 (Hilger F 958). Immediately behind the diaphragm a sphero-cylindrical long-focus condensor L_2 (Hilger F 997) produces an image of lens L_1 on the slit S of the spectrograph. The third lens L_3 is combined with a six-step sector (Hilger H 698) and placed immediately in front of the slit, it produces an image of the diaphragm opening on the collimator lens L_4 of the spectrograph. This system has the following advantages÷

1.) The spectrograph slit is evenly illuminated along its entire length, allowing the use of a step-sector.
2.) An enlargement of the light source, which is necessary in order that its image should fill the aperture of the collimator lens L_4 of the spectrograph is achieved in two stages. In this way convenient distances between lenses are obtained and the hot light source, i.e. the arc, is far removed

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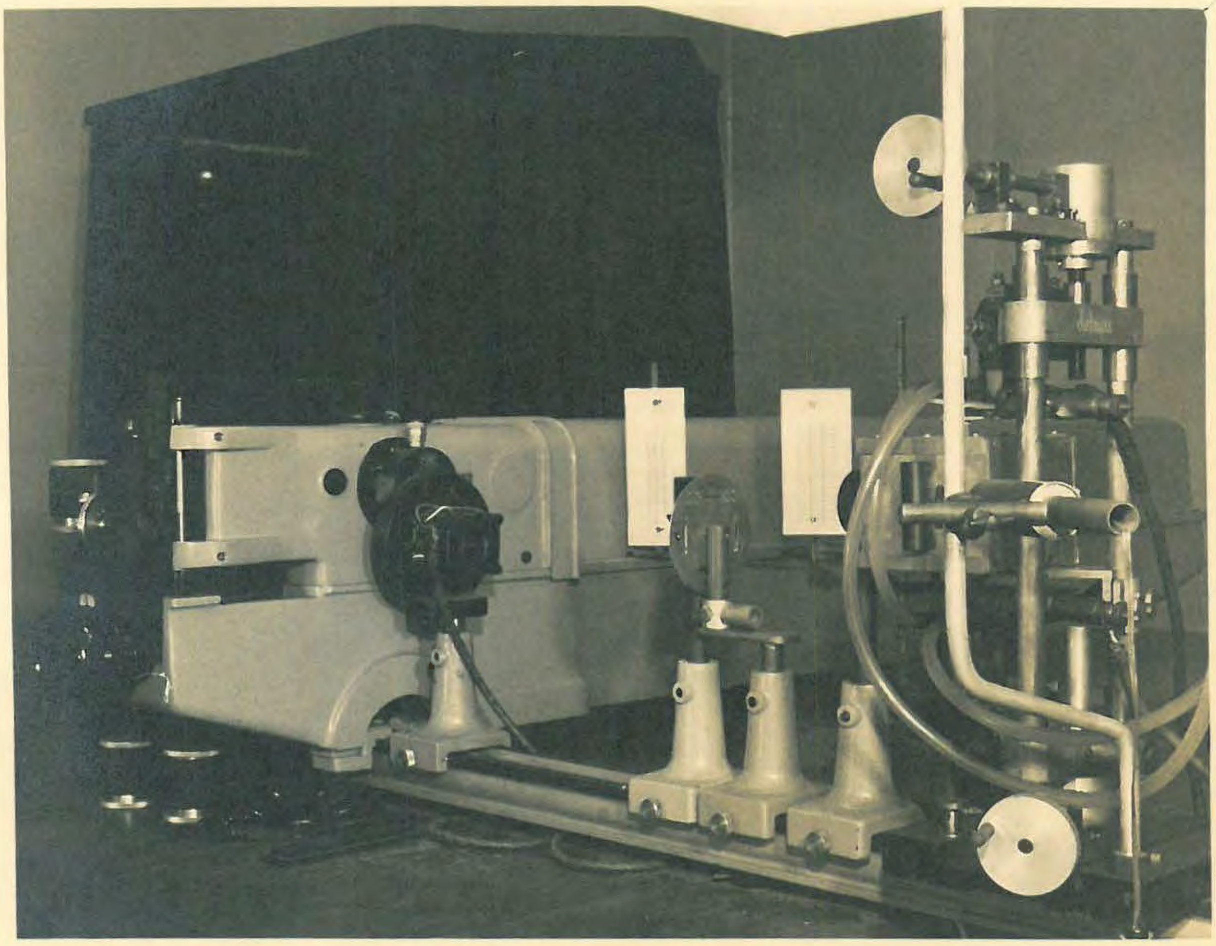

FIGURE 1.

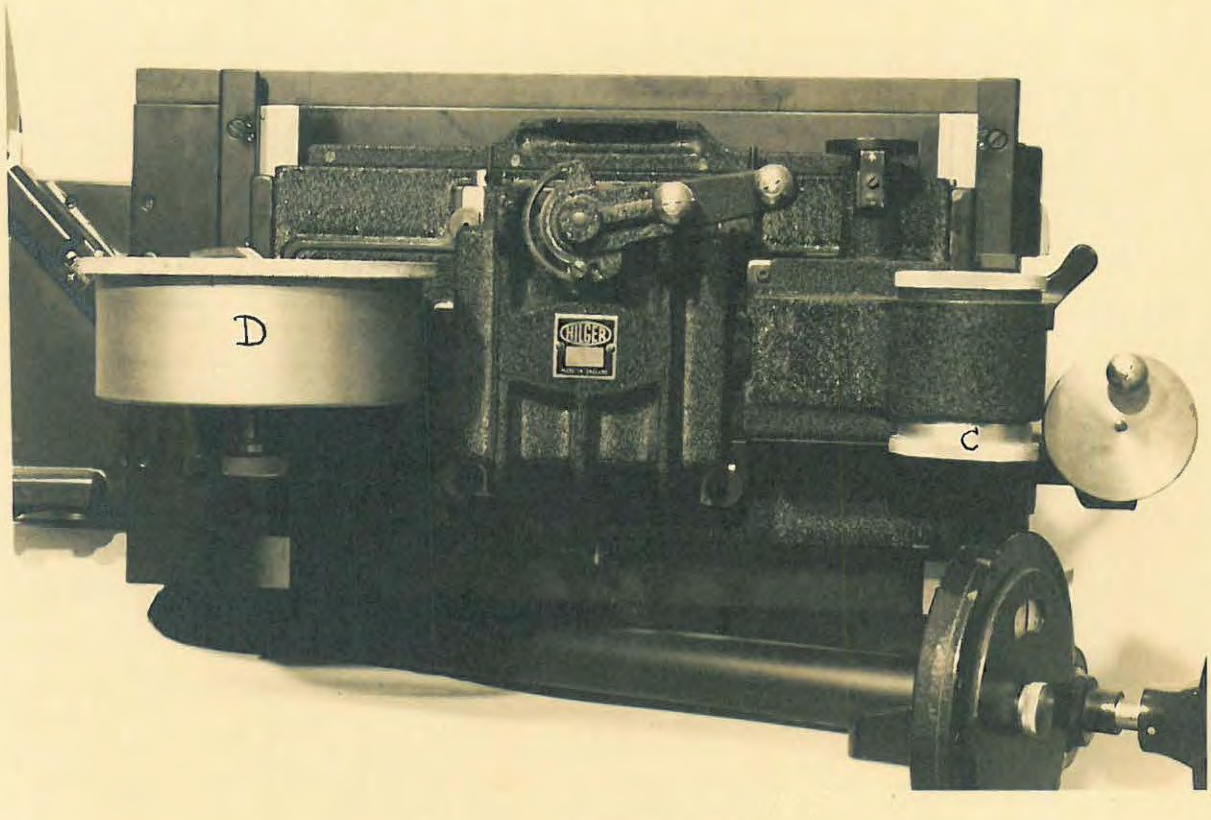

FIGURE 2.

SPECTROGRAPH OPTICS

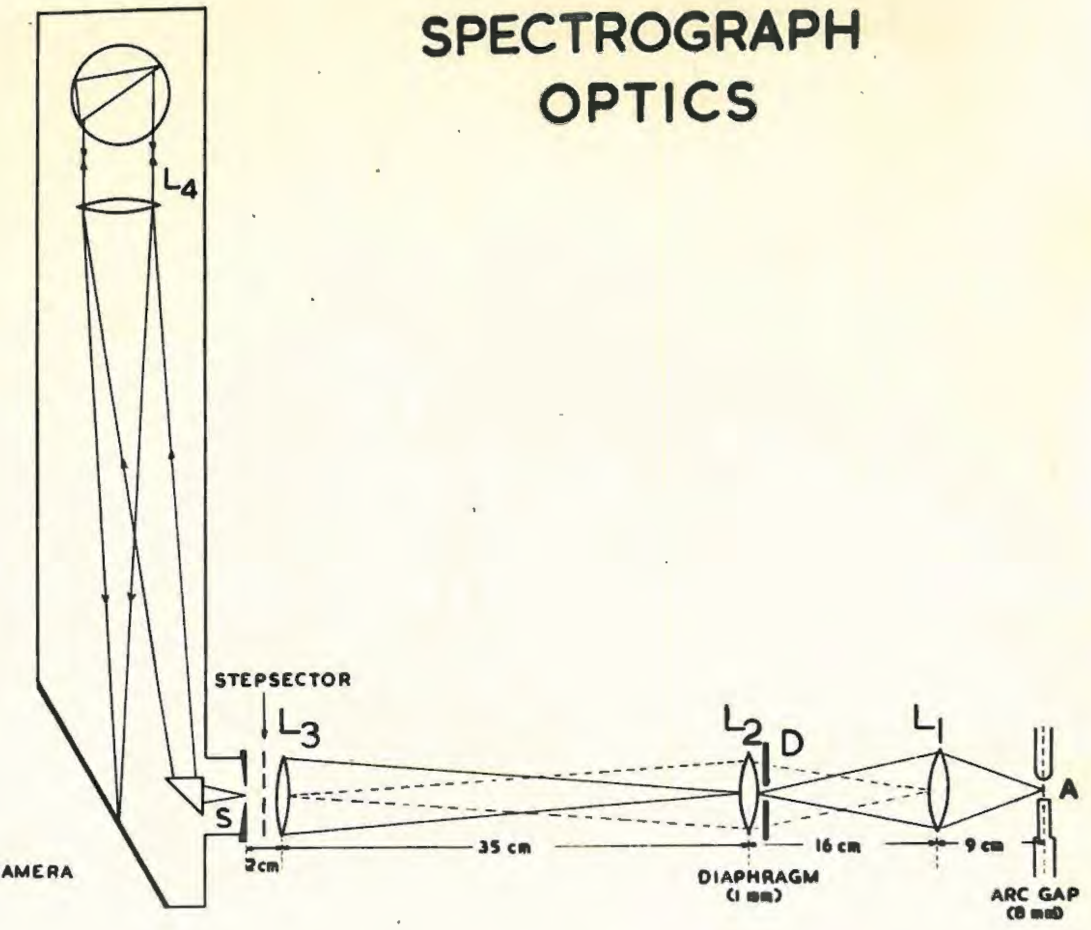

FIGURE 3.

ELECTRODES

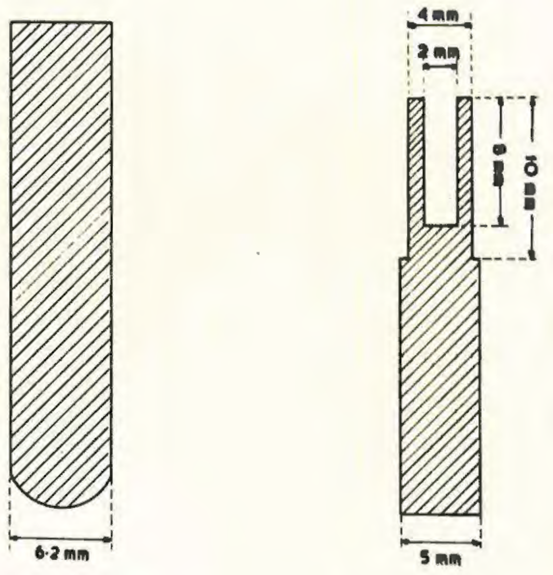

FIGURE 4.

from the delicate spectrograph slit.

3.) The aperture inside the spectrograph has been optically displaced to the diaphragm outside, where the correct positioning of the light source may be checked and unwanted regions of it may be eliminated (e.g. the electrode tips).

The adjustable slit was calibrated using a travelling microscope and interpolating to 2μ, which is the automatic stop provided by the manufacturers in order to prevent damaging the delicate jaws of the slit.

The calibration curve of the slit is shown in figure 5. A slit width of 15μ (Scale reading÷ 9) was chosen for all the work undertaken. A special Hartmann diaphragm was designed and constructed which enabled seven spectra to be recorded on one length of film without racking the film adaptor. This diaphragm, shown in figure 6, was made from a strip of brass and fitted immediately in front of the spectrograph slit. Appropriate size openings were also cut into this strip to allow only two and four steps respectively of the stepsector to be used. By means of this arrangement four two-stepped spectra or two four-stepped spectra could be recorded on one length of 35 mm film.

The diaphragm D consts of a circular aluminium disc into which slots of 1, 2, 3 and 5 mm were cut, the optimum exposure conditions can thus be chosen by revolving the correct opening into position in front of lens L_2.

The water-cooled universal arc-spark stand was designed by Strasheim (8) and constructed in the workshop of Rhodes University.

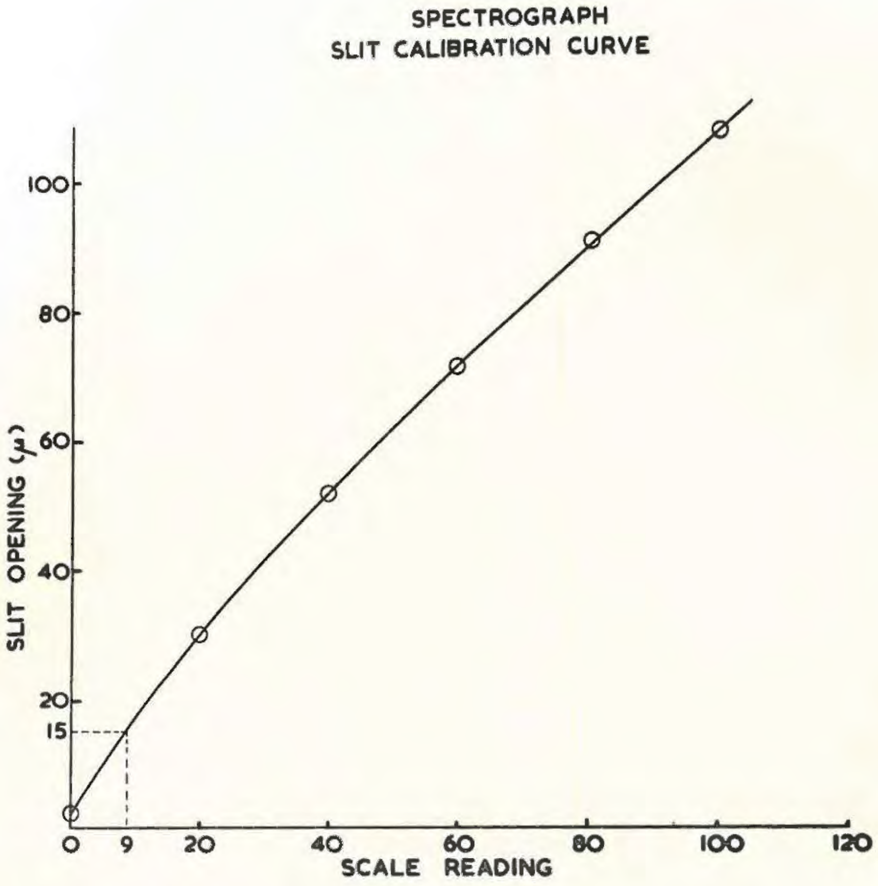

FIGURE 5.

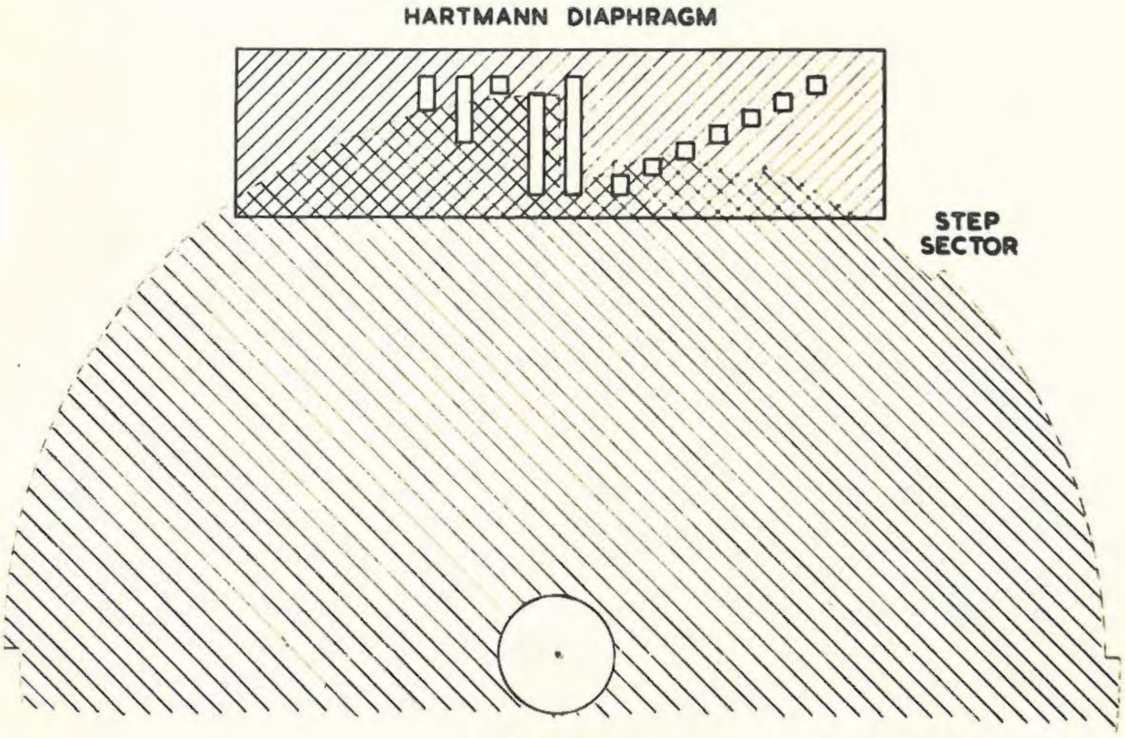

FIGURE 6.

1.1. Focussing of the Spectrograph.

This was carried out in two different ways. Firstly, a strong torch was placed at the position normally occupied by the film adaptor and a beam of light allowed to fall onto the collimator lens L_4. A piece of cardboard, into which irregular, saw-toothed openings had been cut, was placed in front of the lens. The spectrograph slit was opened to approx. 50μ and the wavelength drum was set on the visible region of the spectrum. The combination step-sector and lens F 1025 (L_3) was placed at a distance of 2 cm from the spectrograph slit. Since the slitcover containing the Hartmann diaphragm, was still in position, the distance piece provided on the stepsector had to be removed. Lens L_2 was placed at the position on the optical bench where a sharp image of the mask was formed. Lens L_1 and the arc stand A were then arranged in such a way that the irregular openings of the mask formed a sharp image on two graphite rods, one at the top and the other in the bottom clamp of the arc stand, and touching each other. In this way the optical path of the light from the focal plane at the film holder to the centre of the light source, was found. In the second method, used in conjunction with the first, light from a low amperage iron globule arc was focussed onto the diaphragm D by means of lens L_1. Using a large slit width, an enlarged image of the diaphragm opening was produced on the collimator lens L_4. This second method was used after focussing the spectrograph as described above and it was found that hardly any change in the position of the lenses was necessary. It was also found unnecessary to correct the lens positions for work in the ultra-violet region of the spectrum, since the visible region was used for focussing the instrument. Even illumination of the slit was tested by carrying out densitometric measurements of several lines of an iron globule arc spectrum, using the full length of the slit.

In order to be able to adjust the electrode gap accurately an image of the electrodes was produced on a scale graduated in millimeter separation by means of a lamp and lens system. For most of the work a separation of 8 mm was adhered to.

2. THE EXCITATION SOURCE.

A direct current arc was used in order to excite the sample in the electrode. The direct current, at 220 V, was obtained from a Standard Telephone & Cables, Ltd. Selenium rectifier of 30 amps maximum output. The current strength was regulated by means of a heavy duty resistance box. A d.c. arc has the advantage of very high sensitivity for all the elements. Its main drawback is its poor reproducibility due to wandering of the arc and selective volatilisation of the elements. However, by keeping all conditions of arcing absolutely constant (e.g. the filling of the electrodes, accurate control of the arc gap throughout the arcing period, etc.) the precision of d.c. arc analysis may to some extent be increased.

The resistance box and rectifier were calibrated by shortcircuiting the electrodes and measuring the current by means of a hot-wire ammeter.

Towards the end of 1961 a thyratron regulated constant current rectifier GTT 530 made by Präzisionsmessgeräte RSV, Hechendorf, West-Germany, of 30 amps maximum output at 300 V was acquired. Current control

/ was effected

was effected by means of six thyratrons, each carrying a current of 5 amps so that the output of the unit could be regulated in 6 steps of 5 amps each. In order to obtain intermediate current strengths, the resistance box was connected in series. This unit was also calibrated using a hot-wire ammeter.

Both source units produced a distinct, high-pitched hum in the arc of approx. 600 c.p.s. and when the selenium rectifier was tested by means of an oscillosope a 10% ripple in the rectification was recorded. When used in conjunction with the stepsector in order to calibrate the film, no stroboscopic effects were, however, observed.

3. THE ELECTRODE CUTTER.

The sample bearing electrodes were formed from National Carbon 3/16 inch graphite rods using a modified A.R.L. electrode cutter described by Strasheim (8). These electrodes had the following dimensions÷ An 8 mm deep cavity of 2 mm internal diameter was drilled into the graphite rod, while at the same time the three rapidly revolving cutting knives shaped the outer diameter down to 4 mm. These dimensions have been found suitable for work with Lithium carbonate buffered samples, producing a fairly even consumption of material and burning of electrode walls during the arcing period.

The counter electrode consisted of a 6.2 mm carbon rod, shaped hemispherically at one end using the same electrode cutter with different cutting knives. Unpurified carbon counter electrodes were found to contain traces of Copper and Iron which were effectively removed by burning the shaped rods in a 20 amp. arc for 15 seconds. The dimensions of sample and counter electrodes are shown in figure 4. The purification of sample bearing electrodes is discussed in Part II, section 3.

4. THE DEVELOPING EQUIPMENT.

The developing equipment consisted of a thermostatically controlled A.R.L. film developing machine, model 2300 with 3 separate trays for developer, stop-bath and fixer and designed to accomodate approx. 50 cm length of 35 mm film. Agitation of the film during development was provided by a slow rocking motion of all three trays. Work during the hot summer period necessitated the construction of a copper cooling spiral which was immersed in an insulated mixture of ice and water. The spiral was connected to the developing machine. The development of the film was carried out as follows÷ The length of film was stretched out across a special film holder which keeps it flat and immersed into the developing solution. The interval timer, previously set to the required time, is started. The film holder is transferred to the stop-bath tray when the alarm of the timer is heard. After approx. 15 seconds the film is placed into the fixer tray and the light is switched on after a further delay of approx. 10 seconds. Since a rapid working fixer (Amfix) is used, this time is sufficient for the film to be cleared. After 1½ minutes immersion in Amfix the film strip, still on its holder, is washed for 30 minutes in an A.R.L. film washer, model 2327. After a final rinse with distilled water it is dried on an A.R.L. infrared film drier, model 2352.

The emulsion used for all recordings of spectra was Kodak Spectrum Analysis No. 1, which has a high contrast and extremely fine grain. Its main disadvantage

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is the rapid change in contrast with wavelength in the region 3000 to 4000 A, which necessitates separate calibration for virtually each element and internal standard line.

After preliminary experiments, detailed in Part II, section 1, using Kodak D 19b developer it was decided to use Kodak D 153 high contrast developer, diluted 1÷1 with water. This developer is stored in two parts of which equal volumes are mixed immediately before use and diluted with the same volume of water.

The formula of D 153 is as follows÷

Soution A.

Hydroquinone	125 g.
Potassium metabisulphite	125 g.
Potassium Bromide	125 g.
Water to make	5000 ml

Solution B.

Potassium Hydroxide	250 g.
Water to make	5000 ml

Use solution A ÷ solution B ÷ water = 1 ÷ 1 ÷ 2
Development time÷ 4 min. at 21.5°C (70°F).

The developing solution was discarded after use. This method had the advantage that freshly mixed developer was used for each development, and hence, by careful control of the temperature, reproducible conditions were assured.

Kodak S.A. No. 1 plates, used in the study of the volatilisation of the elements (Part III, section 9) were developed in plastic trays, using the slow movement of a soft brush across the emulsion to provide agitation.

By trial and error the optimum time of development for Kodak S.A. No. 1 emulsions in Kodak D 153 was found to be 4 minutes at 21.5°C.

/ The reproducibility

The reproducibility of the slope of calibration curves obtained under these conditions is described in Part II, section 1.

4.1. 35 mm Spectrographic Film.

The advantages of using 35 mm film instead of plates to photograph spectra are manifold. The film is usually supplied in 100 foot rolls, so that between 115 and 120 sets of seven-stepped spectra or between 230 and 240 sets of four-stepped spectra may be recorded on one roll. This obviates the necessity to carry out frequent calibrations, which are so important when working with different batches of spectrographic plates of the same emulsion. It is generally agreed that a 35 mm celluloid film base may be coated more uniformly with an emulsion than is possible for thin glass plates, so that variations in the emulsion, such as thicknes and quality, are less liable to occur in films. The property of flexibility is a major attractionof films because all spectroscopists have suffered the exasperation of broken plates and the need to keep all pieces together somehow for densitometry and filing. Film is much easier to store in the refrigerator and to file after processing, as it only has about a fifth of the bulk and weight of glass. One should also mention the convenience of having any length of film readily available in the film storage drum (D in figure 2.), of which one or more exposed lengths may be wound into a small cassette (C in figure 2.) and cut off, ready for processing. Each plate has to be placed into an appropriate plate holder individually; this operation has to be carried out in absolute darkness, while film can be transported into position with the lights of the spectrographic laboratory switched on. It is often necessary to record only a few exposures and evaluate these before deciding on a modification or change in methods or conditions of an analysis. This is more economical when 35 mm film is used, instead of developing one plate containing two to three exposures, with three quarters of its useful surface wasted, film allows this operation to be carried out with the minimum amount of waste.

However, more often than not, a series of exposures, which can be accomodated on one plate, has to be recorded and evaluated. If film is used, this would involve at least two separate developments of two lengths of film each, since the useful space of one length of film is 15 × 250 mm^2 as compared to 70 × 250 mm^2 for a 4" by 10" spectrographic plate. Film is more susceptible to scratches and marks, particularly during transport into the focal plane of the film adaptor and when cutting off lengths for development. It cannot be used when studying certain phenomena, such as the rates of volatilisation of elements. In section 5 the difficulty of keeping a strip of 35 mm film flat in the densitometer for photometric evaluation will be mentioned. Plates possess greater dimensional stability, which may be useful for very accurate wavelength measurements but is not essential from the analytical point of view.

From the above discussion it can be seen that the advantages of using film to record spectra outweigh those of plates. However, both are essential in their respective fields of application.

5. THE DENSITOMETER.

A Steinheil Spectrum projector-densitometer, made by Optische Werke C.A. Steinheil Söhne, GmBH, Munich West-Germany and fully described by Rollwagen (9), was used for measuring optical densities of analysis and internal standard lines. The optical arrangement is shown schematically in figure 7.

By means of a selector slit a narrow beam of light from a 6 volt, 50 watt bulb was focussed onto the film or plate and a twenty times magnified image of the slit projected onto the photocell slit. The light source was stabilised by means of a model 1001 Sorenson A.C. voltage regulator. The response of the photocell was measured by means of a sensitive mirror galvanometer and a 500 mm scale graduated linearly from 0 to 50 (0 to 100% transmission) and logarithmically from ∞ to 0 in densities. The galvanometer was placed approx. 1.5 m away on a concrete stand.

A special film holder (fig. 8.) was constructed in the Rhodes University workshop in order to adapt the densitometer for use with 35 mm film. This adaptor fits snugly into the position normally reserved for 4" by 10" spectrographic plates. Preliminary experiments showed that by keeping the film strip flat between two clear glass plates a relatively large amount of the available light was absorbed, thus necessitating the use of a wider photocell slit opening.

Although bulbs varying in output from 35 to 70 watt were used in order to increase the illumination, the minimum slit width of the photocell which gave maximum deflection on a clear strip of film was 0.375 mm. For the most accurate measurement of densities of spectral

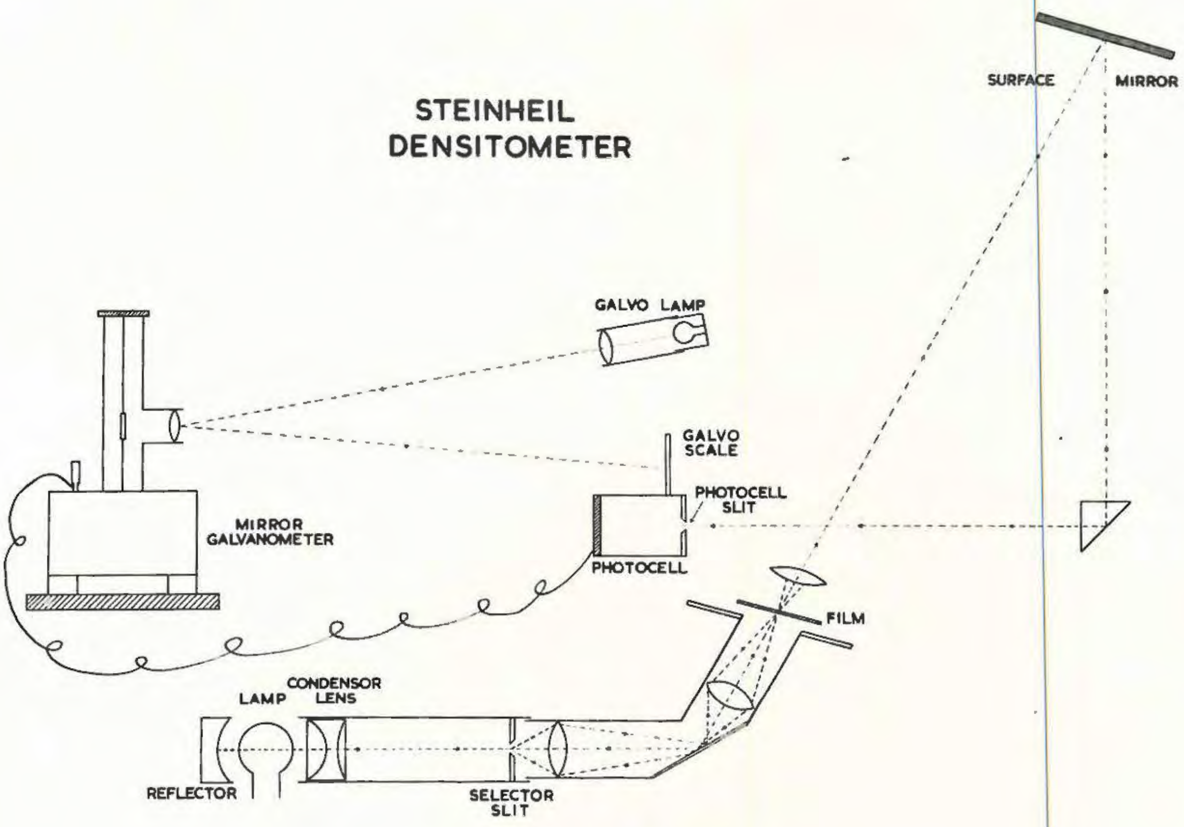

STEINHEIL DENSITOMETER

FIGURE 7.

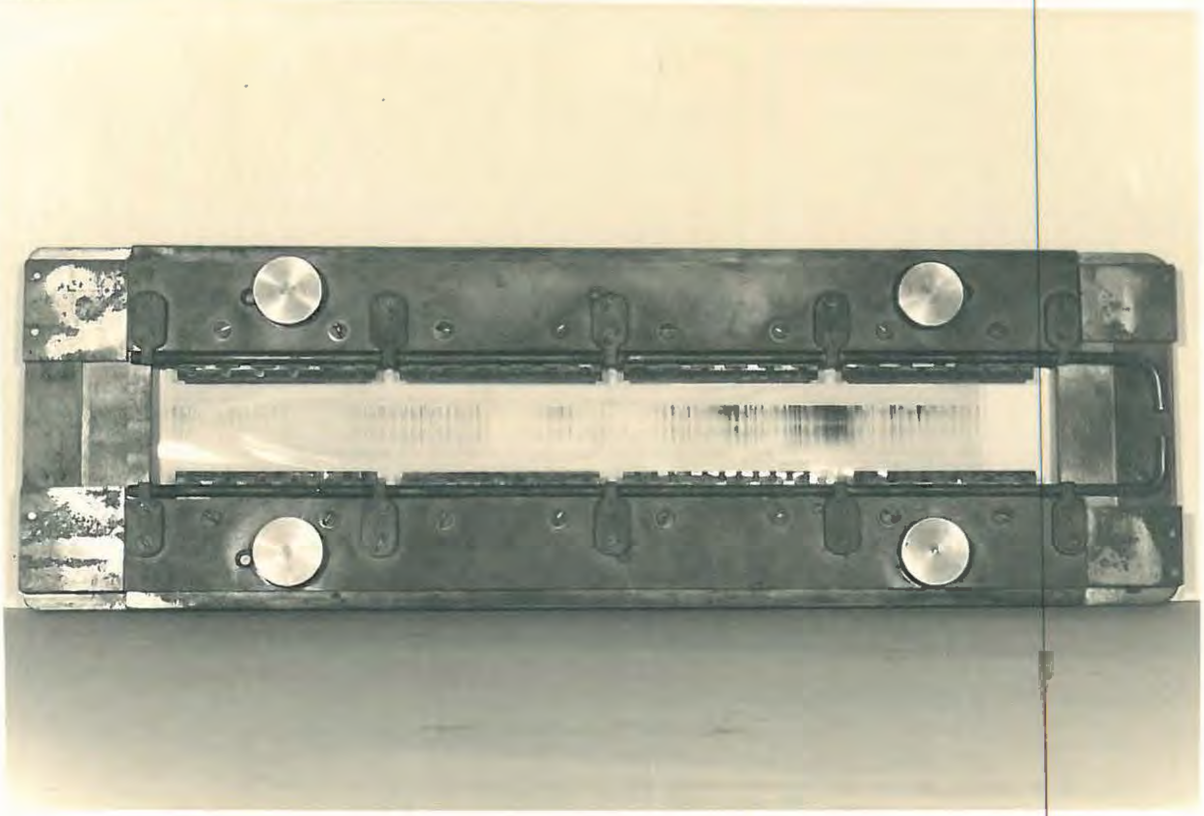


FIGURE 8.

lines the theoretical maximum width of the slit should not exceed two thirds of the width of the magnified image of the spectrograph slit. Since the magnification of the Steinheil densitometer amounted to approx. 20 × and the spectrograph slit was adjusted to 0.015 mm (15 μ) most precise readings should only be obtainable on using a photocell slit of less than 2/3 × 0.015 × 20 = = 0.20 mm width. Unfortunately this condition could not be adhered to, and resulted in relatively larger errors in the measurement of dense lines, and the apparent lowering of contrast of an emulsion by light scattering. A solution to this problem would be the replacement of the weak projection lens (Orthostigmat f4.5/70 mm) by a stronger lens. Such a lens was not available.

Since the film could not be placed absolutely flat in the focal plane by means of the film holder considerable delay in the evaluation of spectra was caused by the constant need for focussing the image of the lines at each region of the spectrum. The procedure adopted in order to obtain reproducibility in the densitometric evaluation was the following÷

The image of a line of medium density (0.5 to 0.7), usually one of the many lines of the iron spectrum, near the analytical line to be measured, was slowly moved across the photocell slit by means of the micrometer screw. The image was focussed by means of the projection lens until minimum deflection was obtained. The deflections produced by the analytical and internal standard lines were then measured and this procedure repeated for each region of the spectrum.

For the qualitative identification of spectra a simple wavelength atlas was compiled. Using two openings of a Hartmann diaphragm the spectra of Raies Ultimes

/powder,

powder, containing 50 elements, and an iron globule arc, respectively, were recorded over a wavelength range from 4600 to 2730 A. These spectra, magnified twenty times, were projected onto the working desk of the Steinheil projector-densitometer. All iron lines and the more persistent lines of the elements contained in R.U. powder were charted on foolscap sizes of paper, using the wavelength atlas given by Brode (10).

6. THE CALCULATING BOARD.

A modified A.R.L. calculating board was used in order to evaluate densitometric measurements. In its original version the board was provided with a horizontal sliding scale graduated logarithmically from 1 to 100 (Intensity scale) and a vertical sliding scale on which the percentage transmission was directly converted into density (Density scale). The modification adopted consisted of the replacement of the vertical scale by one on which the densitometer readings from 0 to 50 are directly converted into Seidel function values. An identical horizontal sliding scale was constructed for drawing preliminary calibration curves. The evaluation of spectra will be dealt with in section 7.

7. FILM CALIBRATION.

In order to obtain a relationship between the intensity of the light emitted by atoms and ions excited in an electrical discharge and the degree of blackening produced on a photographic emulsion by this radiation, calibration curves have to be plotted.

As mentioned earlier the spectrographic emulsion Kodak S.A. No. 1 requires a separate calibration for short regions of the spectrum due to rapid changes in contrast of the emulsion with wavelength, especially above 3200 - 3300 A.

In principle the preliminary curve method of emulsion calibration originally described by Churchill (11) was adopted. Using Seidel functions this method was refined by Schmidt (12) and is fully described by Feldmann (13).

The density of a spectral line is given by÷

$$D = \log \frac{\theta_o}{\theta}$$

where θ_0 is the galvo deflection for the clear film, and

θ the reading for the spectral line.

The transformed density or Seidel function Δ is defined by the equation÷

$$\Delta = \log \left(\frac{\theta_o}{\theta} - 1 \right)$$

The great advantage of using Seidel functions instead of ordinary density values in plotting calibration curves is that approximately straight lines are obtained. (fig. 9) Whereas the density vs. logarithmic intensity curve (also called "H & D" curve) exhibits a marked toe at low and high intensity values, the use of transformed density or Seidel functions produces a curve which is straight over a much wider range, especially at low intensity values. In most cases, however, a slight curvature in the opposite direction to the "H & D" curve is produced by the transformation. This led Kaiser (14) to suggest a modification, combining density and Seidel values in such a way that a straight line relationship between density and logarithmic intensity is obtained.

Tables have been published which enable the direct transformation of percentage transmission obtained from microphotometer readings to the corresponding Seidel function values (15). In the earlier part of this work,

/when the Seidel

when the Seidel scale modification to the A.R.L. calculating board had not been made yet, these tables were used.

In order to obtain the final emulsion calibration curve for a particular region of the spectrum a preliminary curve has to be drawn first.

7.1. The Preliminary Curve.

Some means of recording a spectrum of a line-rich element, such as iron, with exactly known intensity relationships between exposures have to be employed in order to draw the preliminary curve. This is most expediently accomplished by the use of a step sector or step filter of known transmittance values for the different steps. Relative intensities of 1, ½, 1/4, 1/8, 1/16, 1/32 and 1/64th of the light emitted from a Pfund type iron globule arc, run at 7 amps may be obtained by placing a sevenstep sector in front of the spectrograph slit. The plotting of the preliminary curve, however, only requires the recording of two steps, usually 100% and 50% of the total intensity to be made. This was achieved by inserting a diaphragm in front of the spectrograph slit which allowed only light from the first two steps to pass the slit (fig. 6). Four such two-stepped spectra can be recorded on one length of 35 mm film.

The deflection readings for the two steps of each iron line in the region of the spectrum to be calibrated were taken with the densitometer adjusted to give a full scale deflection of 50 cm for zero density (clear film) and zero deflection for infinite density (photocell shutter closed).

The preliminary curve was plotted on the calculating board with the vertical and horizontal Seidel scales as

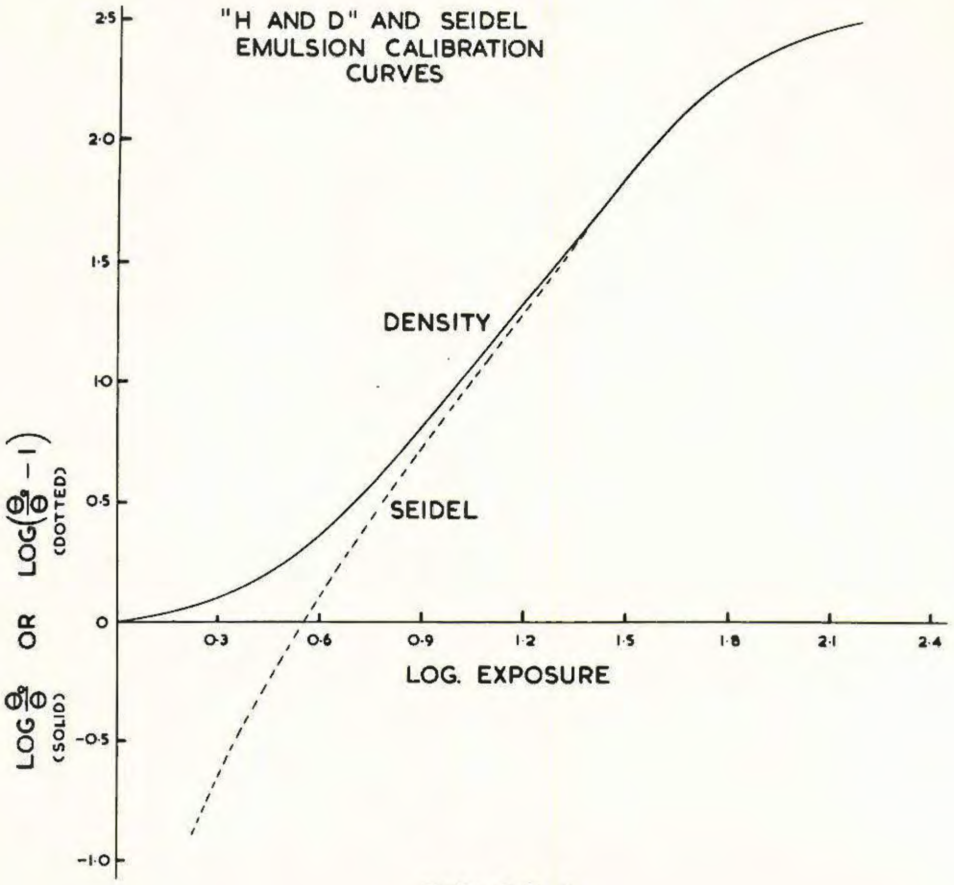


FIGURE 9.

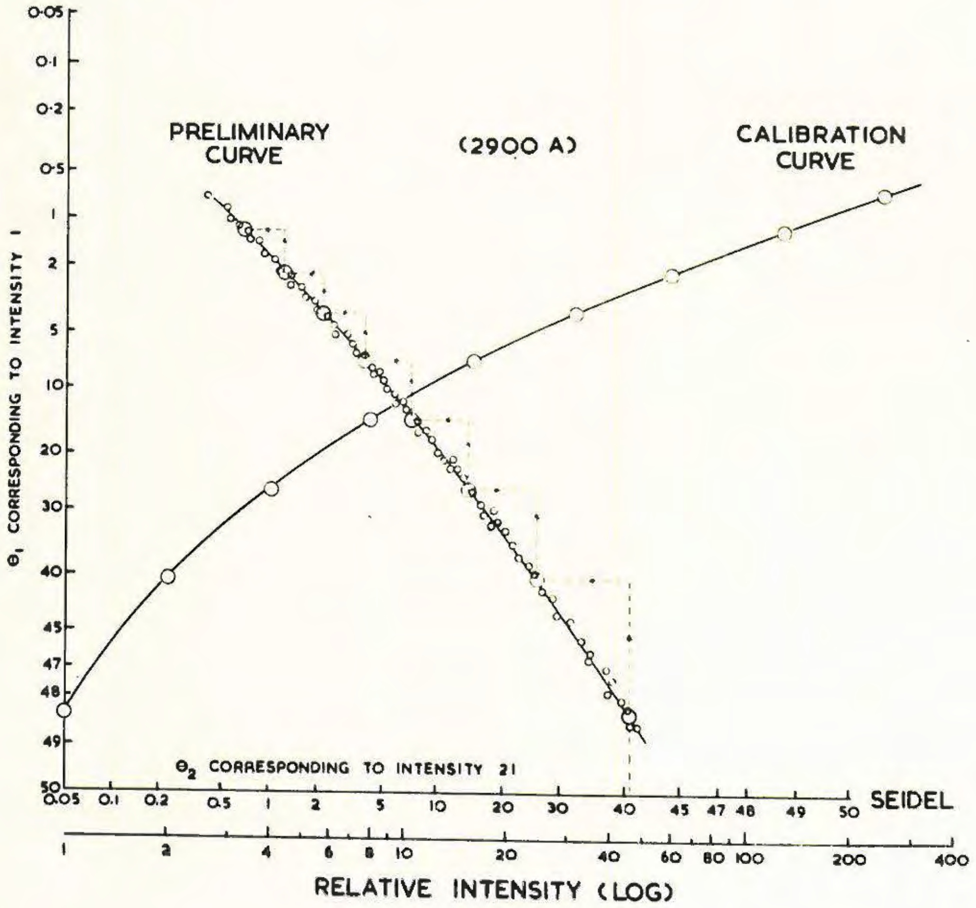


FIGURE 10.

ordinate and abscissa respectively. The Θ value of the first, most dense, step of a line (Θ_2) was plotted as a function of the second, less dense step (Θ_1) of the same line. Through the points thus obtained for all the iron lines in a particular wavelength range the best fitting curve was drawn. This gave the preliminary curve for the region of the spectrum chosen. In order to obtain a large number of values of Θ_1 and Θ_2, generally four exposures of 2, 5, 10 and 20 seconds of a 7 ampere iron globule arc were made. The preliminary curve allows the determination of the Θ value for an intensity 2I from any Θ value given by an intensity I.

7.2. The Calibration Curve.

The calibration curve was plotted as follows÷ The highest Θ value ever to be expected in the densitometric evaluation of spectra was assumed to be 48.5, corresponding to 97% transmittance. To this value a relative intensity I of 1 is assigned. The Θ value for the intensity 2I is now read off from the preliminary curve, and the relative intensity **of** 2 assigned to it. Knowing the latter Θ value, the Θ value for an intensity 4I can now be read from the preliminary curve. In this manner the Θ - values for intensities I, 2I, 4I, 8I etc. or relative intensities 1, 2, 4, 8, ... etc. are obtained which are plotted as ordinates on the vertical Seidel scale against 1, 2, 4, 8 etc. on the horizontal logarithmic scale. In this way the final calibration curve is drawn, which is usually a straight line with a slight curvature in the region of high Θ values.

Figure 10 shows a typical set of preliminary and calibration curves obtained.

Recently Strasheim (16) suggested that instead of

/ plotting each value

plotting each value of the Seidel functions Δ_1 and Δ_2 corresponding to relative intensities 1 and 2 respectively of a series of lines in an iron spectrum, the preliminary curve should be drawn through approx. eight points which are the mean of a range of values. The reproducibility of the contrast factor of Kodak S.A. No. 1 emulsion and the development using this method is discussed in Part II, section 1.

PART II.

INVESTIGATION OF SOME SPECTROGRAPHIC ERRORS.

1. REPRODUCIBILITY OF DEVELOPMENT.

Under the description of the development equipment it was mentioned that after some consideration the X-ray film developer Kodak D 19b was replaced by the high-contrast, two-solution developer Kodak D 153 (also known under the trade name Ilford ID 13) in order to develop Kodak S.A. No. 1 emulsions. This is the developer used in the National Physical Laboratories of the Council for Scientific and Industrial Research for the development of Ilford spectrographic emulsions, such as Ilford Thin Film Half Tone and Ilford Ordinary. Experiments were therefore carried out to find the suitability of D 153 for the development of S.A. No. 1 films.

Three series of two stepped iron spectra were recorded as described in Part I, section 7 and developed for 3, 4 and 5 min. respectively at 21.5°C in Kodak D 153. The contrast factor gamma of S.A. No. 1 film for the regions 4300 A and 3000 A obtainable under these conditions of development was calculated in each case, using the method of preliminary curves.

TABLE 1.

Contrast Factor Gamma.

Development Time	4300 A	3000 A
3 min	1.72	0.78
4 min	1.84	1.02
5 min	1.87	1.09

It will be seen from the above table that a longer development than 4 min only increases the contrast to a slight extent. It did, however, increase the background considerably.

A comparison was made on the density of background in the neighbourhood of the Cu 3274.51 A line obtained when 100, 10, 1, and 0 p.p.m. respectively of Copper in a 1÷1 mixture of Lithium carbonate and National Carbon special spectroscopic graphite powder, grade SP 2, hereafter referred to as SP 2 graphite, were arced at 10 amp for 1 minute. These spectra were recorded twice and developed in÷

a.) Kodak D 153 for 4 minutes at 21.5°C

b.) Kodak D 19b for 5 minutes at 21.5°C.

The mean background densities obtained were÷

a.) 0.013, and b.) 0.032.

It was therefore decided to standardise procedures by developing S.A. No. 1 emulsions in Kodak D 153, diluted 1÷1 with water for 4 minutes at 21.5°C.

In order to determine the reproducibility of development and hence, of the contrast factor gamma obtainable, the following series of experiments was carried out÷

Fourteen strips of film, each containing four two-stepped spectra of an iron globule arc, run at 7 amp (exposures÷ 2, 5, 10 and 20 seconds, respectively) were developed separately. The percentage transmission of the two steps of all iron lines in the region of 3000 A was measured with the microphotometer and converted to Seidel functions by using tables (15).
To establish the preliminary curve, the Seidel value of the "weak" step of a given line (Δ_w) was plotted as ordinate against the corresponding value for the

/ "strong" step

"strong" step (Δ_s) of the same line as abscissa. From this preliminary curve the calibration curve was plotted as described in Part I, section 7. This procedure was repeated for each strip of film and the gamma values calculated using the slopes of the calibration curves between log. relative intensities of 0.9 and 1.8 resp. This work was carried out before the Seidel scales were fitted to the A.R.L. calculating board.

A comparison was made between the reproducibility of gamma obtainable when mean Seidel function values were plotted as described by Strasheim (16) on the one hand and when all values were used for plotting preliminary curves. No significant difference was obtained in the preliminary curves drawn by the two methods. Table 2 shows an example of the calculations involved in plotting a preliminary and calibration curve, while figures 12a and 12b represent a plot of one of these curves.

/ TABLE 2.

TABLE 2.

Seidel Functions corresponding to Intensities I and 2I resp.

Interval

	1.90 - 1.60		1.60 - 1.30		1.30 - 0.90	
	Δ_s	Δ_w	Δ_s	Δ_w	Δ_s	Δ_w
	1.86	1.58	1.37	1.05	1.32	0.902
	1.77	1.48	1.34	1.02	1.22	0.881
	1.89	1.62	1.39	1.09	1.19	0.847
	1.81	1.53	1.42	1.10	1.05	0.683
	1.79	1.50	1.46	1.12	1.08	0.733
	1.77	1.44			0.91	0.555
	1.72	1.40			0.91	0.546
	1.62	1.30			0.91	0.545
	1.65	1.32			1.23	0.881
	1.63	1.28			1.26	0.921
Mean÷	1.75	1.445	1.40	1.075	1.10	0.750

	0.900 - 0.500		0.500 - 0.200		0.200 - 0.00	
	Δ_s	Δ_w	Δ_s	Δ_w	Δ_s	Δ_w
	0.716	0.349	0.447	0.027	0.153	-0.335
	0.751	0.378	0.385	-0.049	0.037	-0.477
	0.887	0.527	0.430	-0.006	0.077	-0.420
	0.853	0.477	0.252	-0.191		
	0.713	0.332	0.278	-0.181		
	0.881	0.505	0.236	-0.233		
	0.695	0.295	0.320	-0.132		
	0.725	0.320	0.263	-0.181		
	0.695	0.260				
	0.540	0.132				
	0.659	0.257				
Mean÷	0.738	0.348	0.326	-0.118	0.089	-0.411

	0.000 - -0.200		-0.200 - -0.600		-0.600 - -1.000	
	Δ_s	Δ_w	Δ_s	Δ_w	Δ_s	Δ_w
	-0.054	-0.540	-0.154	-0.744	-0.612	-1.284
	-0.086	-0.694	-0.281	-0.870		
	-0.016	-0.545	-0.577	-1.210		
			-0.527	-1.177		
			-0.345	-1.016		
			-0.416	-1.063		
			-0.572	-1.210		
			-0.457	-1.117		
			-0.212	-0.807		
			-0.413	-1.103		
			-0.345	-0.962		
Mean÷	-0.052	-0.593	-0.391	-1.025	-0.612	-1.284

In Table 3 the results for the contrast factor of Kodak S.A. No. 1 film at 3000 A obtained by the two different methods and their reproducibility are compared.

/ TABLE 3.

TABLE 3.

Contrast Factor Gamma for the Region 3000 A.

	Plotting of	
	Mean Values	All Values
	1.038	1.038
	1.033	1.022
	1.027	1.027
	1.038	1.038
	1.020	1.020
	1.010	1.010
	1.016	1.032
	0.984	0.984
	0.987	0.998
	1.029	1.029
	0.997	0.997
	1.014	1.026
	1.019	1.019
	1.018	1.018
Mean÷	1.016	1.018
Standard Deviation÷	0.0169	0.0160
Coefficient of Variation÷	1.67%	1.56%*

*) The Standard Deviation of the Mean is given by÷

$$s = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$$

Where x = Individual measurement,

$\bar{x}$ = Mean of n measurements,

n = Number of measurements.

The Coefficient of Variation is given by÷

$$C.V. = \frac{100 \times s}{\bar{x}}$$

The standard deviation was calculated on 13 degrees of freedom.

There is a slight improvement in the reproducibility of the contrast of an emulsion if the preliminary curve is drawn as the best fitting curve through all the points of Seidel functions corresponding to intensities 2I and I respectively. This is due

/ to the neglect

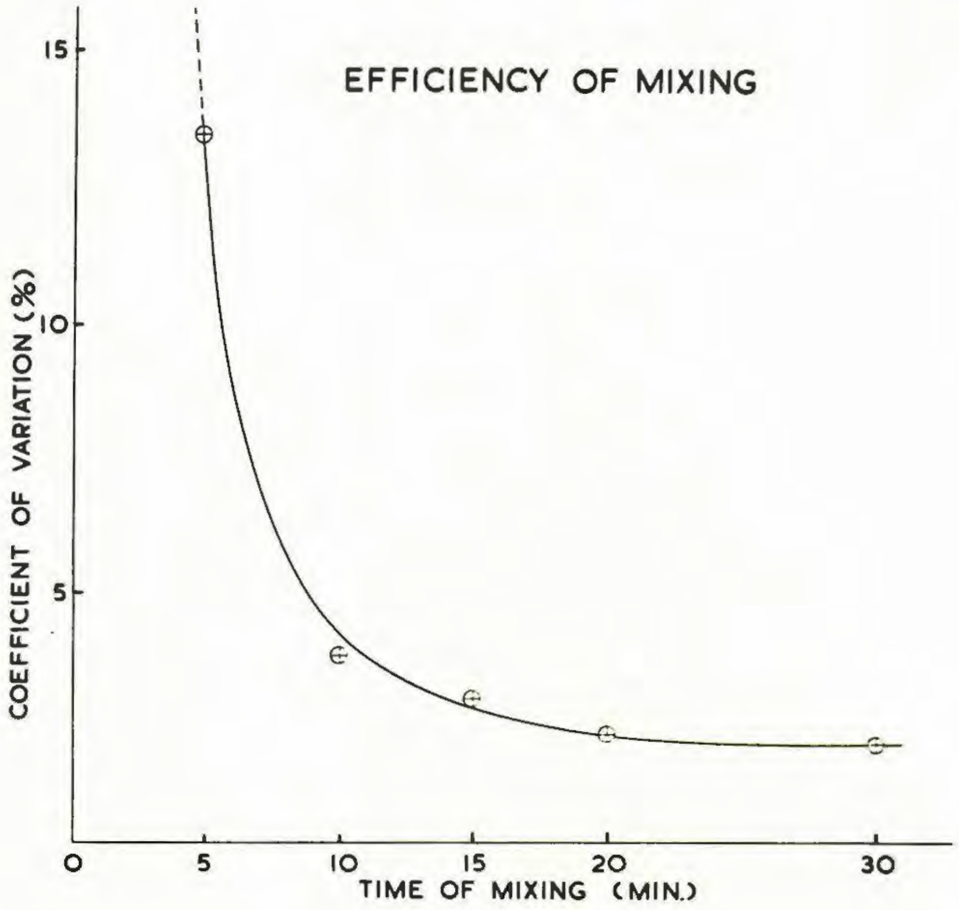


FIGURE 11.

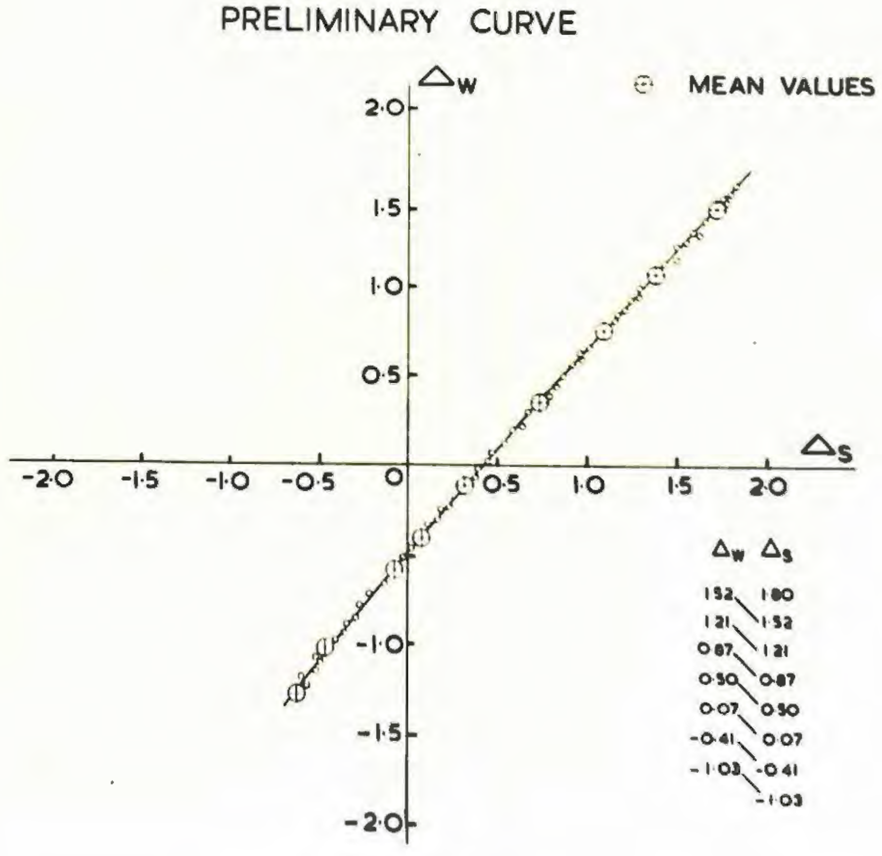


FIGURE 12a.

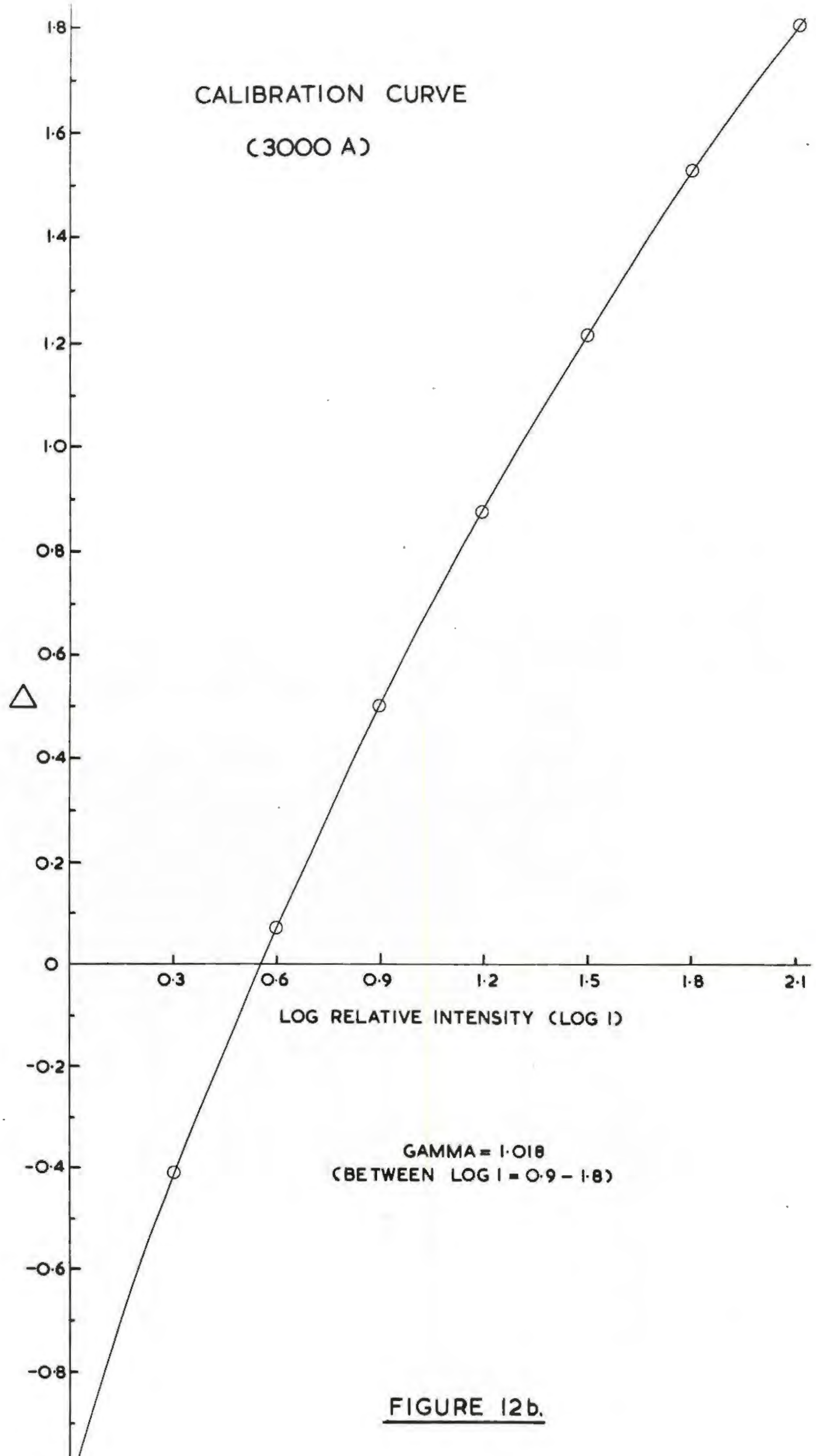

CALIBRATION CURVE
(3000 A)
1·8
1·6
1·4
1·2
1·0
0·8
0·6
△
0·4
0·2
0
-0·2
-0·4
-0·6
-0·8
-1·0
0·3
0·6
0·9
1·2
1·5
1·8
2·1
LOG RELATIVE INTENSITY (LOG I)
GAMMA = 1·018
(BETWEEN LOG I = 0·9 – 1·8)

FIGURE 12b.

to the neglect of widely scattered points in this method as compared with the case when the mean of a range of Seidel functions is plotted. However, the difference is insignificant. Strasheim (16), recording nine four-stepped iron spectra on a single plate, obtained a coefficient of variation of 1.15% for the reproducibility of gamma values. The considerably higher value of the standard deviation obtained above may be explained by the fact that each set of four spectra used for the determination of gamma was developed separately. It was found that a very critical control of the temperature of the developing solution used is necessary in order to achieve reproducibility of contrast factors.

2. THE EFFICIENCY OF MIXING.

In spectrographic d.c. arc analysis the sample is commonly mixed with a suitable diluent or buffer before being filled into the electrode. This mixing is quite often carried out with the aid of a small agate mortar and pestle. In order to find the optimum time for which the sample should be ground in order to become homogeneous the following series of experiments was carried out÷

1.50 g. of specpure Lithium carbonate were carefully ground with 1.50 g. of SP 2 graphite for 15 min in a small agate mortar. 0.9 ml of a 1% iron solution and 0.6 ml of a 0.05% cobalt solution were added to the well-mixed buffer and left overnight in an oven set at 110°C. The sample was then carefully ground for exactly 5 minutes and placed into 14 electrodes. These were arced at 10 amp for 2 minutes, using a specially small diaphragm of approx. 1 mm^2 opening in front of lens F 997 in order to avoid overexposure. The procedure

/ was repeated

was repeated after grinding the remaining sample for further periods of 5, 10, 15 and 25 minutes resp.

The percentage transmission of the Co 3453.51 A and Fe 3443.88 A lines was measured and converted to Seidel function values using tables (15) as discussed in section 1. From a calibration curve the logarithmic intensities of the lines were read off and the ratio Intensity Fe 3443.88 A / Intensity Co 3453.51 A obtained by subtraction. The intensity ratio was obtained from logarithmic tables. This work was carried out before Seidel scales were fitted to the calculating board. The results are recorded in tables 4 and 5.

TABLE 4.

Intensity Ratios after 5 Minutes Mixing.

Fe 3443.88 A			Co 3453.51 A				
%T	Δ	Log I_1	%T	Δ	Log I_2	Log $\frac{I_1}{I_2}$	$\frac{I_1}{I_2}$
27.3	.425	.737	67.9	-.325	.352	.385	2.427
22.1	.547	.808	59.0	-.158	.434	.378	2.366
16.4	.707	.903	67.6	-.319	.355	.548	3.532
18.4	.647	.868	53.8	-.066	.480	.388	2.443
20.7	.583	.831	57.2	-.126	.448	.383	2.415
20.1	.599	.840	55.4	-.094	.463	.377	2.382
19.0	.630	.875	54.0	-.070	.475	.400	2.512
27.8	.414	.732	64.8	-.265	.382	.350	2.239
25.4	.468	.762	63.4	-.239	.395	.367	2.328
33.3	.302	.672	72.1	-.412	.308	.364	2.312
25.2	.473	.765	65.0	-.269	.375	.390	2.455
21.3	.568	.822	55.5	-.096	.465	.357	2.275
26.6	.441	.746	64.2	-.254	.387	.359	2.286
33.6	.296	.667	70.1	-.370	.330	.337	2.173

Mean÷ 2.439
Standard Deviation÷ 0.3283
Coefficient of Variation÷ 13.46%

The standard deviation was calculated on 13 degrees of freedom in each case except for the last series, in which only sufficient sample to fill 12 electrodes was available. In the following table (table 5) only the final intensity ratios obtained will be given.

/ TABLE 5.

TABLE 5.

Intensity Ratios of Fe 3443.88 A / Co 3453.51 A.

Time of Mixing

	10 min.	15 min.	20 min.	30 min.
	2.296	2.259	2.234	2.415
	2.193	2.173	2.193	2.366
	2.323	2.339	2.254	2.421
	2.371	2.265	2.138	2.404
	2.317	2.296	2.228	2.382
	2.443	2.460	2.239	2.339
	2.399	2.410	2.259	2.432
	2.259	2.291	2.254	2.449
	2.382	2.328	2.265	2.438
	2.432	2.328	2.213	2.466
	2.228	2.312	2.244	2.421
	2.350	2.328	2.203	2.495
	2.427	2.301	2.286	
	2.432	2.382	2.291	
Mean÷	2.347	2.319	2.236	2.419
Standard Deviation÷	0.0806	0.0692	0.0400	0.0429
Coefficient of Variation÷	3.43%	2.99%	1.79%	1.77%

From tables 4 and 5 it is evident that longer mixing than 20 minutes does not increase the homogeneity of the sample. Figure 11 shows the dependence of the coefficient of variation on the time of mixing.

3. PURIFICATION OF ELECTRODES.

The unpurified sample electrodes, shaped from 3/16" National Carbon graphite rods (described in Part I, section 3) were examined qualitatively by use of the cathode layer technique (17) and the following elements were found to be present÷ Calcium, Magnesium, Silicon, Iron, Copper, Boron, Aluminium, Vanadium and Titanium. For this purpose the light from the arc (10 amps short-circuited) was focussed directly onto the spectrograph slit by means of the lens F 958. The sample electrode was empty and made the cathode.

/ Since Iron and

Since Iron and Copper were amongst the elements whose concentration in plant ash was to be determined, their removal from the electrode material was essential.

Strong heating of the graphite rods in an oxy-acetylene flame for approx. 30 seconds resulted in almost complete removal of Copper and Iron. However, because of the rather thin diameter of the graphite rods used, considerable amounts of material were burnt away on igniting to incandescence and this method was therefore abandoned.

Extraction of impurity elements by means of different acids at elevated temperatures was therefore considered. Cholak and Story (18) recommended heating of the shaped electrodes in a mixture of equal parts of redistilled Hydrochloric acid and Nitric acid at 70°C over a period of 48 hours, during which time the bath was changed four to five times. This treatment was followed by a corresponding period of immersion in four to 5 changes of triple distilled water, after which the electrodes were heated at from 900 to 1000°C in a muffle furnace. Cholak and Story claim that all impurities except Boron and Silicon and traces of Magnesium and Vanadium were practically removed. Staud and Ruehle (19) heated the cut electrodes with an oxy-gas flame in a silica dish and refluxed with 1÷1 Sulphuric acid for 24 hours. The electrodes were washed by decantation and boiled with two to four changes of water after which the heat treatment was repeated. The following elements were reported to be removed÷ Aluminium, Calcium, Copper, Iron, Magnesium, Manganese, Silver, Sodium, Titanium and Vanadium, while the Boron and Silicon concentrations in the graphite electrodes remained unchanged. Vanselow and Liebig (20) purified graphite electrodes by

/ extraction with

extraction with 20% HCl for three to four days followed by a similar extraction with concentrated Nitric acid using an all-Pyrex glass soxhlet apparatus. The electrodes were freed from acid by extraction with several changes of water. They claimed that only Silicon and Titanium were occasionally found in the purified electrodes.

An attempt was made to assess the degree of purity obtainable from various acid treatments by using the intensity of the Ca I 4226.76 A line as a measure of the purification.

For this purpose the purified electrodes were filled with a 1÷1 mixture of Lithium carbonate and SP 2 graphite. Due to the low ionisation potential of an alkali matrix a considerable enhancement of the low temperature Ca 4226 A line (Excitation Potential÷ 2.92 v) is obtained. Very small traces of Calcium impurities in the graphite may thus be determined. A qualitative examination for contaminating elements remaining behind after different methods of extraction, was carried out, using spectra recorded by burning the electrodes filled with a 1÷1 mixture of specpure Lithium carbonate÷ SP 2 graphite in a 10 amp d.c. arc for 1 min. These spectra were recorded under conditions identical to those subsequently adopted for the quantitative determination of trace elements.

In addition to the unpurified electrodes (Type A) electrodes purified by the methods described below, were examined÷

<u>Type B</u>÷ Electrodes were extracted with 20% HCl for 3 days, followed by extraction for a similar period with concentrated HNO_3 in an all-Pyrex

/ glass soxhlet apparatus.

glass soxhlet apparatus. This was followed by heating the electrodes for approximately 5 min. each in 12 changes of distilled and deionised water (hereafter referred to as "pure" water) in a pyrex beaker. They were dried by igniting them over a Meker type burner in a silica crucible for approx. 1 hour.

Type C÷ Electrodes were extracted with 1÷1 Sulphuric acid in an all-Pyrex glass soxhlet apparatus for 3 days and finally washed free of acid as under B.

Type D÷ Electrodes were refluxed with 1÷1 Sulphuric acid in an all-Pyrex glass apparatus for 1 day, the acid was changed three times. They were freed of acid as under B.

Type E÷ Similar to D, but refluxing continued for 3 days.

Type F÷ Combination of treatments B and C.

Type G÷ Combination of treatments B and E.

Fourteen exposures of each type of purified electrodes were recorded and the percentage transmission of the Ca 4226 line measured in each spectrum and converted to Seidel function values, using tables (15). The corresponding relative intensities were interpolated from a calibration curve. The mean of the relative intensities of one series was taken as a measure of the purity attainable under the conditions of purification. The results are recorded in table 6.

/ TABLE 6.

TABLE 6.

Comparison of the Purity of Electrodes.

(Relative Intensities of the Ca 4226 A Line)

	Method of Purification						
	A	B	C	D	E	F	G
	8.12	5.20	5.95	5.28	5.10	4.20	4.95
	7.30	4.93	6.05	5.28	5.48	4.85	4.50
	7.78	5.23	5.82	5.30	5.13	4.85	4.48
	7.60	5.05	6.15	5.70	4.68	4.98	4.25
	8.40	5.27	6.20	5.55	5.48	4.95	4.63
	8.05	5.40	5.62	5.55	5.20	4.23	4.55
	7.95	4.60	5.87	5.42	5.48	4.48	4.55
	8.05	5.40	6.15	5.42	4.80	4.58	5.00
	8.55	5.05	5.87	5.23	4.97	4.82	4.78
	7.47	5.08	6.30	5.65	5.42	4.43	4.50
	8.22	5.25	5.55	5.30	5.13	4.72	4.90
	7.72	5.53	6.55	5.42	5.35	4.90	4.53
	7.47	5.38	6.40	5.42	5.00	4.55	4.90
	7.10	5.10	6.05	4.78	4.88	4.85	5.13
Mean÷	7.84	5.18	6.10	5.38	5.15	4.66	4.69
Standard * Deviation÷	0.422	0.269	0.283	0.225	0.266	0.264	0.252
Coeff. of Variation÷	5.38%	5.20%	4.63%	4.17%	5.16%	5.67%	5.38%

*) The standard deviation was calculated on 13 degrees of freedom in each case.

It will be evident from the results in table 6, that complete removal of calcium from the electrode by different acid treatments was virtually impossible, only a fractional reduction in the concentration of the Calcium being produced. A qualitative examination of the spectra of the electrodes revealed that the following impurities could not be removed completely by any of the extraction processes tested÷

Calcium (Ca 4226.73 A), Aluminium (Al 3961.53 A), Silicon (Si 2881.59 A) and Magnesium (Mg 2852.13 A). Iron and Copper, as judged from the absence of the lines Fe 3020.64 A and Cu 3247.54 A were effectively removed by extraction processes B, F and G, while Sulphuric acid treatment appeared to leave traces of these elements behind, probably due to impurities in the Analytical Reagent quality acid itself. Because of the wavelength range chosen (4600 - 2740 A) no qualitative examination for Boron (B 2497.7 A) was undertaken.

All graphite electrodes were thenceforth purified according to method B (20).

/ PART III

P A R T III.

DEVELOPMENT OF THE SPECTROGRAPHIC ANALYTICAL METHOD.

1. SAMPLE PREPARATION.

In order to determine minute concentrations of elements in any sample it is often necessary to convert the sample into a form which will eliminate the bulk of the material, and hence raise the relative concentration of trace and minor elements to a higher value. In the case of plant leaves this concentration technique is achieved in two stages, the first of which removes the organic constituents amounting to between 80 and 95% of the bulk of the material, leaving behind the inorganic constituents which are mainly chemical compounds of Calcium, Silicon, Magnesium, Sodium, Aluminium and Potassium. This concentration step is usually sufficient for the spectrographic determination of minor elements, such as Iron and Manganese. However, the quantitative estimation of trace element concentrations usually requires a second stage in the concentration procedure which will remove the main bulk of the alkalis and alkaline earths in addition to Silica. The second stage will be discussed in section 2.

The plant sample is collected, washed with tap water containing 0.1% Teepol and finally three times in distilled water to remove any residual detergent. This washing process is essential for the removal of any traces of chemicals with which the leaves may have been sprayed before being collected. The plant sample is dried in a forced draught oven at 65°C for two days, the dried leaves crushed by hand and ground to a fine powder in an agate ball mill.

1.1. Ashing of the Sample.

The elimination of carbonaceous matter from plant leaves is effected by either dry ashing or wet ashing of the dried plant leaves. The latter method consists of treatment of the dried plant sample with combinations of acids, such as Aqua regia, Sulphuric, Nitric and Perchloric acids, Sulphuric and Nitric acids, or Nitric and Perchloric acids; the various combinations are discussed by Smith (21). The most commonly employed wet ashing procedure is that using Nitric and Perchloric acids for the destruction of organic matter (22). Its disadvantage, as far as spectrographic analysis is concerned, is the limitation in the size of the sample which may be conveniently ashed, namely approx. 3 g., although samples of up to 5 g. have been used (23, 24). However, since larger samples necessitate the use of larger amounts of Perchloric acid, which is very difficult to purify, contamination of the sample with some of the trace elements to be analysed for is a serious consideration against this method.

The advantages of the dry-ashing method are its simplicity and convenience in handling samples of between 10 to 20 g., amounts of sample required for the second stage of the enrichment procedure. Important disadvantages to be considered are possible loss of ash-forming constituents by volatilisation and by mechanical entrainment in the walls of the vessel used for ashing. However, by careful control of the ashing temperature, these losses may be minimised to a large extent and even eliminated.

Dry-ashing of 10 g. samples of dried and crushed plant leaves was adopted as the first stage of the concentration procedure. The ashing was carried out in a muffle furnace by slowly raising the temperature to 450°C and stirring the sample contained in a silica basin from time to time during the early stages of burning off the carbonaceous matter, in order to avoid local overheating. A slow draught of air was allowed to pass through the muffle furnace during this stage. Ignition was completed by leaving the sample overnight in a thermostatically regulated muffle furnace at 450°C. The necessity for strict control of the ignition temperature at 450°C was confirmed by the following series of experiments÷ Two 10 g. samples of dried plant leaves were ignited at controlled temperatures of 450°C and 550°C respectively and analysed as described later. Ignition at 550°C resulted in a loss of approx. 40% of the Zinc as compared to that obtained by ignition at the lower temperature.

1.2. Solution of Plant Ash.

As mentioned earlier, dry-ashing is in many cases sufficient for the spectrographic determination of some minor elements in plants (25, 26, 20, 3, 4). In order to determine traces of elements spectrographically, reducing the bulk of material by ashing is not sufficient to enrich the concentration of many elements. The second stage of the concentration procedure generally requires that a solution of the plant ash be made. As the name implies, wet ashing methods obviate the necessity for an extra step in order to obtain the plant solution.

Solution of the ash is achieved by either of two main methods. Mitchell's (27) Sodium carbonate fusion procedure and Piper's (28) Hydrochloric-Hydrofluoric acid treatment.

In the former method the ash obtained from the ignition of approx. 10 g. of dried plant leaves is thoroughly mixed with 2 g. of specially purified anhydrous Sodium carbonate and fused in a platinum crucible. The melt is taken up in 50 ml redistilled constant boiling point Hydrochloric acid (approx. 6N) and evaporated to dryness on a steambath to dehydrate the silica. The latter is filtered off on Whatman No. 40 filter paper after addition of 50 ml approx. 6N HCl to the dried residue. The filtrate is made up to 100 ml in a volumetric flask.

A modification of the above procedure proposed by Mitchell (29) consists of first extracting the plant ash with Hydrochloric acid and filtering off the residue. The residue is re-ashed and fused with 1 g. anhydrous Sodium carbonate in a platinum crucible, dissolved in Hydrochloric acid and filtered to remove any silica.

In the earlier part of the work described Mitchell's original method was used in order to obtain a plant solution.

For this purpose Sodium carbonate used in the fusion was purified as follows÷

200 g. of Analytical Reagent quality Sodium carbonate were dessoved in 1 litre of hot "pure" water and filtered through Whatman No. 40 filter paper, using a Buchner funnel. The solution was extracted three times with 50 ml 0.02% Dithizone in chloroform and finally washed with 3 portions of 50 ml chloroform. $Na_2CO_2.10\ H_2O$ crystallised out on cooling in an ice-salt mixture and was filtered off in a Buchner funnel. Gradual heating

/ to 300°C

to 300°C in a muffle furnace finally produced anhydrous Sodium carbonate.

2 g. of one bath of the purified salt extracted and analysed spectrographically as described in later sections, was found to contain traces of Molybdenum, which, of course, cannot be removed by dithizone extraction. Using a different brand of anhydrous A.R. Sodium carbonate as starting material, satisfactory purification was obtained, obviating the necessity for blank corrections for Molybdenum.

It was found that plant solutions obtained in the manner described above retarded the development of colour in the spectrophotometric determination of iron as described in section 3. Also, because of the relatively high concentration of Sodium salts present in the solution, retardation in the separation of phases in the concentration procedure discussed in section 5.3 and sometimes the formation of an emulsion was experienced. For these reasons Piper's method of obtaining a plant solution was adopted. This method lends itself more easily to the treatment of a large number of samples simultaneously.

The ash obtained from the ignition of exactly 10 g. of dried plant leaves was transferred to a 100 ml Pyrex beaker, moistened with a few ml of "pure" water and 20 ml of approx. 6.5N (constant boiling point) redistilled Hydrochloric acid were slowly added down the side of the beaker. To prevent loss of material in the ensuing reaction of the acid with the carbonates formed during dry ashing the beaker was almost completely covered with a watchglass during the addition of the acid.

The resultant solution was evaporated to dryness on a hotplate adjusted to a sufficiently low setting to prevent boiling and spluttering. To the dry residue 10 ml 6.5N HCl and 10 ml "pure" water were added, the solution warmed on the hotplate until all soluble salts were dissolved and then filtered through a 9 cm Whatman No. 41 filter-paper into a 100 ml volumetric flask. The beaker was washed twice with 2 ml of warm 6.5 N HCl and 10 ml "pure" water. The filter paper and contents, consisting mainly of dehydrated silica and some incompletely ashed plant material, were transferred to a platinum crucible and carefully ignited over a Meker burner to destroy the filter paper and any carbon present in the ash. Ignition was continued until the residue was nearly white, and, after cooling, 3 - 4 drops of concentrated A.R. Sulphuric acid and approx. 3 ml of A.R. Hydrofluoric acid were added. The latter addition has to be carried out very carefully because of the violent reaction occurring between the acid and the silica. Elements occluded in or adsorbed onto the silica were recovered by evaporating off the Hydrofluoric acid on a hotplate in a fume cupboard until white fumes of Sulphuric acid were evolved. Actual boiling of the Hydrofluoric acid was avoided and care was taken not to remove the Sulphuric acid completely. After cooling, 2 ml 6.5N Hydrochloric acid and 2 ml "pure" water were added to the platinum crucible which was then gently heated on a hotplate and the contents were filtered through a 9 cm Whatman No. 41 filter paper into a 100 ml volumetric flask containing the filtrate previously obtained. This procedure was repeated twice, care being taken to avoid prolonged heating of the Hydrochloric acid in the crucible because of the slow attack of the acid on the material of the crucible.

Filtering was necessary in many cases since all carbonaceous matter could only be removed by igniting for a considerably long time.

The plant material was now in a convenient form for the second stage of the concentration procedure.

During the early part of the work described here the solution was diluted to 50 ml in volumetric flasks. This practice was discontinued for several reasons as mentioned in later sections.

2. THE CONCENTRATION STEP.

It was mentioned in section 1 that removal of the bulk of organic material from plant leaves by ashing is in most cases not sufficient to raise the relative concentration of some trace elements to above the limits of spectrographic sensitivity. Several methods for the enrichment of traces prior to spectrographic analysis have been proposed. Electrolysis was used extensively in the early days of spectrography (30, 31, 32) but is only rarely applied in modern work (33, 34). Ion exchange chromatography is gaining in importance, especially in the investigation of Rare Earths(35) and other geological specimens (36), but its application to biological materials is still limited (37).

Enrichment techniques of main interest, as far as the spectrographic analysis of trace elements in plants and soils is concerned, are carrier precipitation and solvent extraction. The pioneering work in the former field of application was carried out by Mitchell and Scott (38), who precipitated trace elements in the presence of Aluminium as carrier by the use of mixed organic reagents (8-Hydroxyquinoline, Tannic acid and Thionalide) at pH 5.2. The enduring success of this early method may be judged from the fact that it has been adopted, sometimes with slight modifications, as standard practice in many laboratories all over the world (39, 40, 41, 42). A criticism of Mitchell's concentration method is the amount of time spent on the preparation of the sample until it is in a form suitable for spectrographic analysis.

Solvent extraction has been applied with great success as a means of enriching traces of metals in agricultural samples for subsequent spectrochemical determination. In all solvent extraction procedures one or more organic complexing agents are added to the solution of the plant sample, buffered to an optimum pH value, and the metal complexes formed extracted with organic solvents immiscible with water, such as Chloroform or Carbontetrachloride. Wark (43) extracted Copper, Cobalt and Zinc with dithizone from a plant solution buffered at pH 8.3. However, in order to concentrate as many trace metals as possible, mixtures of organic reagents are quite often employed and successive extractions carried out at different pH values. Gorbach and Pohl (44, 45) succeeded in the quantitative enrichment of traces of Cadmium, Cobalt, Indium, Nickel, Lead, Thallium, Zinc, Bismuth, Gold, Copper, Mercury and Palladium by the use of dithizone extractions at pH 7 and 9 resp. followed by the recovery of Aluminium, Titanium, Vanadium, Gallium, Molybdenum, Cerium, Manganese, Antimony, Yttrium and Zirkonium on extracting with a 0.1% 8-Hydroxyquinoline solution in Chloroform at pH 5 and 6.5 resp. This concentration procedure was shortened and improved by Strasheim and Eve (46) using Dithizone, 8-Hydroxyquinoline and Cupferron and extracting at pH values of 9.5 - 10, 2.9 - 3.0, 1.5 - 2.0 and 0.5 resp.. Pohl (47) described a lengthy method by which twenty trace elements were enriched by extraction with a mixture of diethyldithiocarbamate, 8-Hydroxyquinoline and Dithizone at pH values of 3, 5, 7 and 9. Doll and Specker (48) investigated the possibility of extracting precipitated trace metal complexes using Pyrrolidine dithiocarbamate and Cupferron.

In all these methods stress was laid upon the elimination of Iron from the final extract, which lengthened the enrichment process considerably in may cases. The removal of Iron was achieved by Wark by carrying out Dithizone extraction at pH 8.3, while Gorbach and Pohl precipitated Iron along with a number of other elements with a buffered solution of Ammonium Benzoate followed by Oxine extraction at pH 2 and Ethyl ether extraction. Strasheim and Eve found that after a preliminary extraction with Dithizone at pH 9.5, Iron could be transferred to the organic phase on shaking with a 1% solution of Oxine in Chloroform at pH 2.9 - 3.0. Doll and Specker advocated the use of high concentrations of either Hydrochloric acid or Lithium chloride followed by extraction with iso-Butyl Methyl Ketone. However, quantitative recovery of some elements such as Molybdenum, Cobalt, Copper and Zinc was not achieved. Pohl described a procedure by means of which Iron together with several other elements was extracted into Ethyl Ether from a 6.5N Hydrochloric acid solution and reduced to Fe (II) with Sodium dithionate. After the addition of Ammonium thiocyanate, elements coextracted with Iron, e.g. Molybdenum, Gallium, Indium, Tin, Vanadium and Zinc may be recovered by a second ether extraction, which left the Iron in the aqueous phase.

The main criticism of enrichment methods employing mixed organic complexing reagents and extracting at different pH values and all methods of removing Iron is the amount of time spent in order to obtain the final extract in a convenient form for spectrographic analysis.

The partial or complete elimination of Iron from the final concentrate is, however, advisable if spectrographs of low or medium dispersion are used since the line-rich spectrum of iron may cause interfering coincidences.

It was shown by Strasheim, Eve and Fourie (49), that the Iron concentration in a sample excited by means of a d.c. arc has a profound influence on the analytical calibration curves of some elements, and that it should therefore be kept constant if a removal of Iron is not envisaged as part of the enrichment scheme.

Mitchell (50) concentrated Iron along with all other trace elements. Using Iron as internal standard for most of the precipitated traces necessitated the plotting of a series of working curves for each element, covering the usual concentration ranges, but making allowance for the Iron concentration (between 2 - 15%) in the final concentrate. This concentration was determined colorimetrically on 5 - 10 mg of the concentrate and the appropriate working curve referred to in order to determine the particular trace element concentration. This is the basis of the variable internal standard method of analysis.

Scharrer and Judel (24, 51) suggested a method obviating the necessity for different working curves for the same element. Also using iron as internal standard they determined its concentration in the plant solution colorimetrically on a small aliquot of the solution. An appropriate aliquot of a standard Iron solution was then added to the plant solution to give a predetermined constant amount of Iron in the sample. If the Iron concentration in the sample was higher than the predetermined value a correspondingly smaller aliquot was used for subsequent enrichment methods.

In order to avoid the necessity for several extractions to be carried out to concentrate the trace and minor elements Cobalt, Copper, Manganese, Molybdenum and Zinc prior to spectrographic analysis, the concentration method originally proposed by Stetter and Exler (6) was adopted in principle. Iron was concentrated with the other trace elements and used as internal standard.

Since Stetter and Exler's concentration procedure forms the basis of the method adopted for the enrichment of trace elements it will be described in more detail.

2.1. Stetter and Exler's Method.

The above authors proposed the use of Sodium pyrrolidine dithiocarbamate (or Na-t-carbate) for a rapid method of concentrating trace elements. The heavy metal traces were isolated from the main constituents of agricultural samples (alkalies, alkaline earths and Aluminium) by precipitation with Sodium pyrrolidine dithiocarbamate and subsequent chloroform extraction. Silica apparently interfered with the method and had to be removed. The precipitation of hydroxides during the enrichment procedure was prevented by the addition of Sulphosalicylic acid. This complexing agent appeared to be particularly suited for this purpose since it did not interfere with the precipitation of the carbamates. In addition the intense colouration of the Fe (III) - Sulphosalicylic acid complex served as a useful indicator for the quantitative precipitation and extraction of Iron and hence also of the other metal traces.

Stetter and Exler carried out the extraction as follows÷

1 g. of a plant or soil sample, from which silica has been removed with Perchloric and Hydrofluoric acids was taken up in a small amount of Hydrochloric acid and transferred to a 100 ml separating funnel. After addition of 20 ml 13% Sulphosalicylic acid the pH of the solution was adjusted to 4.8 with dilute Ammonium hydroxide and diluted to approx. 80 ml. With vigorous shaking of the contents of the separating funnel, 15 ml of a 5% Na-t-carbate solution were slowly added and the solution heated to 50 - 55°C, in order to coagulate the precipitate formed which collected at the surface. The solution was quickly cooled and the precipitate extracted three times with 10 ml of Chloroform each. Leaving the last Chloroform extract in the separating funnel, 1 ml 2N Hydrochloric acid was added. The pH of the solution, which increased as a result of the hydrolysis of the Na-t-carbate, thus again dropped to between 4.8 and 5.0. The precipitated Dithiocarbamic

/ acid, in addition to

acid, in addition to the remaining heavy metal carbamates were then extracted with the Chloroform remaining in the extraction funnel. The trace metals may be isolated from the combined Chloroform extracts in a form suitable for spectrographic analysis.

Stetter and Exler claimed quantitative enrichment of the following elements÷ Silver, Copper, Cadmium, Zinc, Gallium, Indium, Lead, Tin, Vanadium, Bismuth, Molybdenum, Manganese, Cobalt, Nickel, Iron and Palladium. All chemical manipulations were carried out in silica vessels and platinum crucibles to avoid contamination. The time required for one complete extraction was claimed to be 20 to 25 min.

Scharrer and Judel (24, 51) and Strasheim, Eve and Fourie (49) reported the successful application of this concentration method to the spectrographic determination of trace elements in agricultural samples. Pohl (52) used a related compound, Ammonium pyrrolidine dithiocarbamate in conjunction with Dithizone to effect similar enrichments. The former reagent obviated the pH adjustments necessary when the Sodium salt was employed in inadequately buffered solutions. In addition, the Ammonium salt was claimed to be more stable at lower pH-values than the commercially available Sodium pyrrolidine dithiocarbamate.

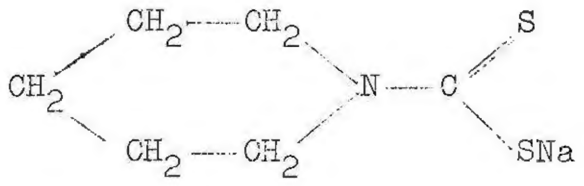
Scharrer and Judel's application of Stetter and Exler's concentration method will be described in more detail in section 2.3 since most of the work reported below is a development of their procedure.

2.2. The Organic Precipitant.

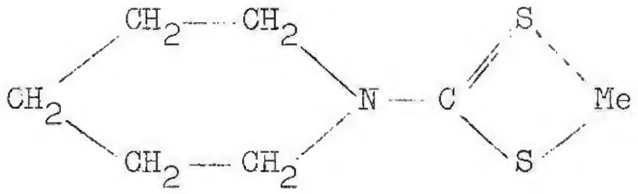
The structure of the organic precipitant,

/ Sodium pyrrolidine dithiocarbamate

Sodium pyrrolidine dithiocarbamate or Na-t-carbate is given below.



Under the correct conditions of pH, chelate type metal-organic complexes of the type



are formed, which are soluble in Chloroform. A somewhat better known disubstituted dithiocarbamate is Sodium diethyl dithiocarbamate, frequently used as a colorimetric reagent for Copper (53).

According to Gleu and Schwab (54) Na-t-carbate may be prepared from Pyrrolidine as follows÷

Eighteen ml of pure Pyrrolidine and 12 ml redistilled Carbon disulphide are added to 200 ml denatured Ethyl alcohol. After the addition of 30 ml 8N Sodium hydroxide the white substance Sodium pyrrolidine dithiocarbamate crystallises out on cooling. After allowing to stand for 2 hours the crystals are filtered off at the pump, washed with 1 ml each of Ethyl alcohol and Ethyl acetate. Dried between filter paper, the yield is approx. 32 g.

Both Sodium and Ammonium salts of Pyrrolidine dithiocarbamic acid have been successfully applied to concentration procedures in the field of analytical chemistry. Koch (55) used NH_4-t-carbate in conjunction with Dithizone to concentrate trace impurities in

/ Zirconium and its

Zirconium and its compounds prior to spectrographic analysis. Jones and Watkinson (56) carried out spectrophotometric determinations of Vanadium in plant material using NH_4-t-carbate. Yamamoto (57) applied an extraction method using Cupferron and Na-t-carbate to the simultaneous polarographic determination of Iron, Zinc and Manganese in biological materials.

Since an aqueous solution of Sodium pyrrolidine dithiocarbamate is slowly decomposed, a fresh solution should be made up whenever required.

2.3. Scharrer and Judel's Method.

After wet-ashing of 5 g. of biological material with 2 ml Sulphuric acid, 5 ml Perchloric acid and 40 ml Nitric acid the resultant plant solution was made up to 100 ml in a volumetric flask. An aliquot of from 1 to 10 ml, depending on the concentration of Iron was transferred to a 25 ml volumetric flask for the spectrophotometric determination of Iron using α-α'-Dipyridyl (58). Iron (40 mg. per sample) was used as the internal standard for the spectrographic determination of Cobalt, Copper, Manganese, Molybdenum, Nickel, Silver and Vanadium, while Cadmium (200 µg. per sample) served as internal standard for Zinc.

Depending on the result of the colorimetric Iron determination, a concentration of 40 mg. Iron in the plant solution was obtained either by the addition of an appropriate amount of a standard Iron solution or by taking a correspondingly smaller aliquot of the plant solution, as was described in section 2. In addition 200 µg. Cadmium as a solution of Cadmium acetate was added to each sample.

/ For the separation

For the separation and enrichment of trace elements the authors used a modification of Stetter and Exler's method. The plant solution, analysed for Iron and containing the correct amounts of this element and Cadmium, was transferred to a 250 ml beaker and 30 ml 12.5% Sulphosalicylic acid and 2 ml Sodium acetate - Acetic acid buffer (pH = 4.8) were added. Using a pH-meter, the pH of the solution was adjusted to 3.8 by addition of dilute Ammonium hydroxide. The solution was then heated to 68°C and transferred to a 250 ml separating funnel. Immediately, 15 ml of an aqueous 5% Sodium pyrrolidine dithiocarbamate solution was poured into the separating funnel, which was then shaken vigorously for 15 - 30 seconds and cooled under running tap water. The black carbaminate precipitate, the colour of which is due to the excess of the Iron carbaminate, coagulated on the surface, leaving the solution itself clear. The presence of appreciable amounts of Titanium resulted in a yellow coloration of the solution due to the Titanium-Sulphosalicylic acid complex. The appearance of turbidity indicated that insufficient Sulphosalicylic acid had been used. This could still be corrected by adding a small volume of the 12.5% reagent and heating. After cooling, the extraction of the precipitated heavy metal carbaminates was carried out by shaking for approx. 15 seconds with from 5 to 10 ml portions of re-distilled Chloroform until during one extraction the organic phase remained lightly yellow to colourless. This extract was not run off, but shaking of the separating funnel was repeated for 15 to 20 seconds after the addition of 1 ml of 1÷10 re-distilled Hydrochloric acid (approx. 0.6N). A white precipitate of free carbamic acid was formed when the acid was added.

The combined Chloroform extracts were collected in a 100 ml porcelain dish and freed of the solvent by infrared radiation. The residue was ignited at 450° C in a muffle furnace overnight. The ashed Chloroform extract, consisting mainly of Ferric oxide, was finally used for the spectrographic determination of the co-extracted trace elements.

2.4. Purification of Reagents.

The high degree of purity of reagents required in trace analysis cannot be stressed enough. Especially when the spectrographic analysis is preceded by chemical manipulations, care must be taken not to introduce impurities of elements to be determined. The water used in all chemical operations was distilled and passed through a mixed Cation-Anion exchange resin column to remove the last traces of ions like Copper and Zinc, dissolved from the brass vessel in which the distilled water is collected. All vessels used in the chemical pre-treatment of the sample, such as beakers and separating funnels, were cleaned with hot Aqua Regia, followed by at least three to four rinsings with distilled and deionised, or "pure" water. Whenever possible, acids and alkalis were employed which could be conveniently purified by redistillation. All solutions were kept in polythene bottles, with the exception of Chloroform.

/ 2.4.1. Hydrochloric Acid.

2.4.1. Hydrochloric Acid.

Approximately 6.5N Hydrochloric acid was obtained by distillation of a 1÷1 solution of A.R. HCl and water from an all-Pyrex glass still. The first fraction of the distillate was rejected. The concentrated acid was used in the preparation of the plant solution (Section 1.2.) while 1÷10 HCl, employed in the solvent extraction, was obtained by diluting the 6.5N acid ten-fold with "pure" water.

2.4.2. Ammonium Hydroxide.

An approximately 8N solution of Ammonium hydroxide was produced by re-distilling A.R. concentrated Ammonia at as low a temperature as possible and absorbing the gas in "pure" water contained in a polythene bottle cooled in an ice-salt mixture. The high concentration was achieved by continuing the distillation for two days and replacing the cooling mixture from time to time. Part of this solution was diluted four-fold for more accurate adjustment of pH values.

2.4.3. Sulphosalicylic Acid.

Two hundred and fifty gram of A.R. quality Sulphosalicylic acid were dissolved in approx. 200 ml "pure" water and 8N re-distilled Ammonium hydroxide added until a pH of approx. 4.8 was measured on a pH-meter. Accurate adjustment of pH was carried out after cooling the solution. After diluting to approx. 800 ml with "pure" water, the Sulphosalicylic acid solution was warmed to 68°C, transferred to a clean 1 litre Pyrex separating funnel and 20 ml 5% Na-t-carbate solution added. The funnel was shaken vigorously for

/ approx. 30 seconds,

approx. 30 seconds, the contents cooled under running tap water, and extracted three times with 20 ml portions of re-distilled Chloroform. After leaving to stand overnight, the extraction was repeated with a further 20 ml of Chloroform. The solution was then transferred to a 1 litre beaker and heated to approx. 80°C in order to drive off any remaining Chloroform and also to decompose any un-used carbaminate reagent. The cold solution was finally stored in a ½ gallon polythene bottle.

2.4.4. Sodium Pyrrolidine Dithiocarbamate Solution.

Before every extraction a 2.5% aqueous solution of Sodium pyrrolidine dithiocarbamate was freshly prepared from the Analytical Reagent quality product available commercially. 10 ml of this solution was used for each precipitation and extraction. Since large Iron concentrations, as recommended by Scharrer and Judel, were avoided (see section 5.1.), 0.25 g. of the reagent was found to be sufficient to precipitate all heavy metals. Six extractions could conveniently be carried out simultaneously so that 1.5 g. of the reagent were dissolved in 60 ml "pure" water before each series of solvent extractions.

2.4.5. Sodium Acetate Buffer.

Seven parts of a 10% Sodium acetate solution, made up from A.R. quality reagent, were added to three parts of N Acetic acid, yielding a buffer solution of pH 4.88, which was checked and corrected to pH 4.8 using a pH-meter. The buffer was purified as described under section 2.4.3. and stored in a polythene bottle. Two ml were used for each determination.

/ 3. THE SPECTROPHOTOMETRIC DETERMINATION OF IRON.

3. THE SPECTROPHOTOMETRIC DETERMINATION OF IRON.

Since a constant amount of Iron was used as internal standard for the elements Cobalt, Copper, Molybdenum and Manganese, its concentration in the sample must be accurately known. For this purpose the standard method used in the Chemistry Department of Rhodes University for the colorimetric determination of Iron in plant and soil samples, was adopted. This method employs α-α'-Dipyridyl to form a stable coloured complex with ferrous ion showing maximum absorption at 522 mμ and is a modification of the one described by Moss and Melon (59). One ml of plant solution was pipetted into a 50 ml volumetric flask and then shaken to reduce the ferric ion after the addition of 5 ml M Sodium acetate and 1 ml 10% Hydroxylamine hydrochloride. The absorbance of the solution at 522 mμ was measured on a Unicam spectrophotometer one hour after the addition of 5 ml 0.1% α-α'-Dipyridyl in 0.2N Hydrochloric acid.

Since the concentration of Iron in citrus leaves varies from approx. 50 to 300 p.p.m. of oven-dry material, corresponding to 500 - 3000 μg. in a 10 g. sample of leaf poweder ashed and made up to 100 ml, a calibration curve for 0 to 50 μg. Fe was drawn up using matched 4 cm absorption cells. This calibration curve is reproduced in figure 13.

Using plant solutions obtained according to Mitchell's Sodium carbonate fusion method, it was found that the development of the colour was considerably retarded. No equilibrium conditions were attained even when the solutions were allowed to stand overnight. The use of a freshly prepared solution of α-α'-Dipyridyl improved matters somewhat, but the full development of colour still did not occur until after approx. 8 h. Aliquots taken from plant solutions prepared according to Piper's Hydrochloric acid extraction method led to the attainment of steady absorption readings after 30 minutes.

The reason for the slow development of colour in the case of plant solutions obtained by Mitchell's method could not be ascertained. pH measurements revealed that the acidity of the coloured solution was well within the limits recommended by Moss and Mellon.

A very convenient method of determining Iron would be a utilisation of the colour of the Iron-Sulphosalicylic acid complex produced when Sulphosalicylic acid is added to the plant solution prior to the extraction. Koulter-Andersson (50) described a procedure using Sulphosalicylic acid for the determination of Iron in soils. No experiments regarding the application of this method were, however, undertaken, because of possible interference of added reagents with the subsequent solvent extraction.

4. PREPARATION AND COMPOSITION OF PLANT STANDARDS.

Scharrer and Judel (51), in their proposed method of soil analysis, recommended the use of standard solutions of the trace elements covering the range of concentrations normally encountered. These solutions should simulate the composition of the soil extract as closely as possible, as far as the concentration of macro-elements is concerned, and are extracted in exactly the same way as the actual soil extract. For this purpose Scharrer and Judel prepared two solutions, A and B, both of which contained the average concentration of macro-elements found in a soil extract. The highest concentration of trace elements expected to be met with in soil analysis was added to solution B and this diluted in varying proportions with the blank A, thus yielding a graded series of soil standards.

The choice of graded standard solutions was adopted in the work described below. The use of standards similar in composition to the samples analysed and treated in identical fashion has the advantage that any errors arising out of the chemical treatment are virtually eliminated. For instance, mechanically mixed standards, containing trace elements in the matrix chosen for spectrographic analysis do not take account of possible incomplete extractions. On the other hand, if great care is not exercised, these solutions may introduce errors due to impurities contained in the blank A.

The average concentrations of macro-elements in dried citrus plant leaves was estimated from previous analyses carried out in the Chemistry Department, Rhodes University, amounting to÷

Calcium÷ 4.5%
Potassium÷ 0.5%
Magnesium÷ 0.5%
Aluminium÷ 0.03%
Phosphate÷ 0.2%

Two litres of a synthetic plant leaf solution containing only the macro-elements, hereunder referred to as "plant blank", were made up and purified as described below. The amounts of macro-elements contained in 10 ml of this plant blank were equivalent to the average amounts of Calcium, Potassium, Magnesium,

/ Aluminium and Phosphorus

Aluminium and Phosphorus in 10 g. of dried citrus leaves.

Two hundred and twenty five grams of A.R. Calcium carbonate (equivalent to 90 g. Ca) and 29.8 g. A.R. Magnesium carbonate (equivalent to 10 g. Mg) were weighed out into a two litre beaker and dissolved by the careful addition of 1÷1 Hydrochloric acid. When everything was dissolved, 19.1 g. A.R. Potassium chloride (equivalent to 10 g. K), 10 ml Sodium acetate buffer and 30 ml 12.5% Sulphosalicylic acid were added and the pH of the resultant solution adjusted to 4.8 by the addition of 8N and 2N Ammonium hydroxide. Dilution to more than 1 litre was necessary, since it was found that at lower dilutions some Calcium hydroxide started to precipitate out because of the relatively high pH of 4.8. The solution was heated to approx. 65°C and quickly transferred to two 1 litre separating funnels. After the addition of 10 ml freshly prepared 5% Na-t-carbate solution to each funnel they were shaken vigorously for approx. 30 seconds, cooled under running water and extracted four times with 10 ml portions of Chloroform. The pH of the solution was found to have increased to 5.3. After the addition of 2 ml 0.6N Hydrochloric acid the extraction was repeated with 10 ml Chloroform. Since the pH had only dropped to 5.0, two further extractions with 10 ml portions of Chloroform were carried out, in each case acidifying with a further 3 ml of Hydrochloric acid.

An amount of A.R. Disodium phosphate (31.2 g. of $Na_2HPO_4.12\ H_2O$) corresponding to 4 g. of PO_4^{3-}, was purified in a similar way and combined with the above solution after acidifying with approx. 20 ml 6N Hydro-

/ -chloric acid

chloric acid to prevent precipitation of Calcium phosphate. Any remaining Chloroform was evaporated off and any unused carbaminate reagent decomposed by heating the combined solution to approx. 80°C.

An amount of "specpure" Aluminium oxide (1.132 g. Al_2O_3), corresponding to 0.6 g. Aluminium was dissolved in 6N Hydrochloric acid and added to the purified plant blank. After cooling and diluting to two litres the solution was stored in a polythene bottle.

In addition to the plant blank, five more solutions were prepared, containing the trace elements in a graded series of concentrations. For this purpose Mitchell's suggestion was followed. Mitchell (61) proposed the use of a series of standards in which the ratio of concentrations of trace elements of one step to the next was $10 \div \sqrt{10}$. This assured an equal spacing of points plotted for analytical working curves for each element.

The following concentrations were chosen to cover the range normally encountered in plant analysis÷

Manganese and Zinc÷ 316 to 3.16 p.p.m.
Copper÷ 100 to 1 p.p.m.
Cobalt and Molybdenum÷ 3.16 to 0.0316 p.p.m.

In order to prepare the above plant standards, 100 ml of the following solutions were made up from "specpure" chemicals, by dissolving in 1÷1 Hydrochloric acid, except for Molybdenum trioxide, which was first dissolved in dilute Sodium hydroxide and then acidified with 1÷1 HCl.

1% Zinc solution÷ 1.2448 g. ZnO / 100 ml
1% Manganese solution÷ 1.3884 g. Mn_3O_4 / 100 ml
1% Copper solution÷ 1.2518 g. CuO / 100 ml
0.1% Cobalt solution÷ 0.1362 g. Co_3O_4 / 100 ml
0.1% Molybdenum solution÷ 0.1500 g. MoO_3 / 100 ml.

/ One hundred millilitres

One hundred millilitres of 0.01% solutions of Cobalt and Molybdenum were prepared by dilution of the respective 0.1% solutions.

Two hundred ml of the plant standard with the highest concentration (1) were obtained by pipetting the following volumes of standard solutions into a 200 ml volumetric flask and making up to the mark with the plant blank÷

Manganese and Zinc÷ 6.32 ml of 1% solution,
Copper÷ 2.00 ml of 1% solution,
Cobalt and Molybdenum÷ 6.32 ml of 0.01% solution.

In addition to the average concentration of macroelements, plant standard 1 contained per 10 ml÷ 3.16 mg. of Mn and Zn, 1.0 mg. Cu and 31.6 µg. of Co and Mo, corresponding to 316 p.p.m. of Mn and Zn, 100 p.p.m. Cu and 3.16 p.p.m. of Co and Mo in 10 g. of oven-dry plant leaves.

63.2 ml of plant standard 1 were buretted into a 200 ml volumetric flask and made up to the mark with plant blank solution, yielding plant standard 2. Similar dilutions were carried out in order to prepare plant standards 3, 4 and 5 resp.. The concentrations of trace elements in the different solutions are tabulated below.

TABLE 7.

Concentration of Trace Elements in p.p.m.

Plant Standard	1	2	3	4	5	0
Manganese	316	100	31.6	10.0	3.16	0
Zinc	316	100	31.6	10.0	3.16	0
Copper	100	31.6	10.0	3.16	1.00	0
Cobablt	3.16	1.00	0.316	0.100	0.0316	0
Molybdenum	3.16	1.00	0.316	0.100	0.0316	0

/ During a later stage

During a later stage of the work described here these solutions were diluted in the ratio of 5.6 ml plant standard to 4.4 ml plant blank (0) in order to obtain another series of intermediate concentrations tabulated below÷

TABLE 8.

Concentration of Trace Elements in p.p.m.

Plant Standard	1a	2a	3a	4a	5a
Manganese	177	56	17.7	5.6	1.77
Zinc	177	56	17.7	5.6	1.77
Copper	56	17.7	5.6	1.77	0.56
Cobalt	1.77	0.56	0.177	0.056	0.0177
Molybdenum	1.77	0.56	0.177	0.056	0.0177

The necessity for duplicating the average composition of plants in plant standards was checked by omitting the main constituents Calcium, Magnesium, Potassium, Aluminium and Phosphate from some of the above plant standards and plotting a series of working curves. Six modified synthetic plant solutions of 200 ml each were prepared by pipetting the appropriate volumes of 0.1% and 0.01% solutions of Copper, Manganese and Zinc and 0.001% and 0.0001% solutions of Cobalt and Molybdenum into 200 ml volumetric flasks and making up to the mark with "pure" water. 10 ml of each solution were therefore equivalent to a plant solution obtained from 10 g. oven-dry plant material containing the concentrations of trace elements listed below÷

TABLE 9.

Concentrations of Trace Elements in p.p.m.

Plant Standard	1b	2b	3b	4b	5b	6b
Manganese	100	56	31.6	17.7	10.0	5.6
Zinc	100	56	31.6	17.7	10.0	5.6
Copper	31.6	17.7	10.0	5.6	3.16	1.77
Cobalt	0.56	0.316	0.177	0.100	0.056	0.0316
Molybdenum	0.56	0.316	0.177	0.100	0.056	0.0316

Each modified plant standard was made up separately by pipetting the volumes of solutions tabulated below into 200 ml volumetric flasks and making up to the mark.

TABLE 10.

Volumes of Trace Element Solutions used for modified Plant Standards.

Std. No.	Mn and Zn	Cu	Mo and Co
1b	20 ml 0.1% soln	6.32 ml 0.1%	11.2 ml 0.001%
2b	11.2 " " "	3.54 " "	6.32 " "
3b	6.32 " " "	20.0 " 0.01%	3.54 " "
4b	3.54 " " "	11.2 " "	20.0 " 0.0001%
5b	20.0 " 0.01% "	6.32 " "	11.2 " "
6b	11.2 " " "	3.54 " "	6.32 " "

All plant standards were stored in polythene bottles. A comparison of the working curves obtained by using the synthetic plant solutions on the one hand and the modified standard solutions on the other will be given in section 10.

5. THE MODIFIED SOLVENT EXTRACTION METHOD.

5.1. Addition of Internal Standards.

Scharrer and Judel's (51) method of preparing the sample for solvent extraction was adopted in principle. However, several modifications were introduced and examined. The above authors extracted the trace elements Mn, Zn, V, Cu, Ni, Pb, Sn, B, Co and Mo together with a large excess of Iron, namely 40 mg. Fe per sample. Strasheim, Eve and Fourie (49) reported that there was no necessity for as much as 40 mg. of Iron to be present for satisfactory recovery of the

/ trace elements.

trace elements. Scharrer and Judel (24) observed that due to the high concentration of Iron in the extract a weak Iron line, not listed in any wavelength table, seriously interfered with the use of the Zn 3345.02 A line for the analysis of this element. In order to obtain a standard deviation of 5% in the quantitative spectrographic determination of Zinc under the influence of this disturbing Iron line, amounts exceeding 70 μg. of Zn had to be present in the electrode. Since concentrations exceeding 500 p.p.m. Iron (i.e. yielding more than 5 mg. Fe on extraction of a 10 g. plant sample) are unlikely to occur in citrus plants, it was decided to use this amount of Iron as carrier and internal standard. One litre of a standard 0.1% Iron solution was prepared by dissolving 1.4297 g. "specpure" Ferric oxide (Fe_2O_3) in approx. 20 ml of 1÷1 redistilled Hydrochloric acid and diluting to the mark with "pure" water. 5 ml of this solution was added to each 10 ml of plant standard before solvent extraction. The Iron concentration in a plant sample was determined colorimetrically as described in section 3 and a correspondingly smaller aliquot of the 0.1% Iron solution measured out by means of a graduated pipette. E.g. if the Iron determination yielded a result of 150 p.p.m. in 10 g. of oven-dry plant material, 3.5 ml standard Iron solution were added.

An internal standard of 200 μg. Cadmium was recommended by Scharrer and Judel for the determination of Zinc. 100 ml of a 1% Cadmium solution were prepared by dissolving 2.2816 g. A.R. Cadmium Sulphate ($3\ CdSO_4.8\ H_2O$) in "pure" water. A 0.01% Cadmium solution was obtained by diluting 2 ml of this stock solution to 200 ml in a volumetric flask. Using 400, 200 and 100 μg. resp. of Cd in the electrode (corresponding to 4, 2 and 1 ml 0.01% Cadmium solution added before extraction) the optimum concentration for the excitation conditions chosen was established. The amount used in all subsequent work, namely 200 μg. Cd, was that recommended by the above authors.

The introduction of internal standards to the plant solution before extraction has the advantage that mechanical losses of the extract, due to bumping on evaporation, spilling, etc., reduce the amounts of analysis and internal standard elements proportionally, thus minimising errors due to imcomplete extraction and allied losses of material.

5.2. Preparation of Plant Solution for Solvent Extraction.

Standards and sample solutions were treated in exactly similar manner throughout. Ten millilitres of each plant standard (or 99 ml of plant solution, remaining after the Iron determination) were transferred to 250 ml Pyrex beakers. Five millilitres of 0.1% Iron solution (correspondingly less in the case of the sample solutions, see section 5.1.) plus 2 ml 0.01% Cadmium solution, 2 ml Sodium acetate buffer and 20 ml 12.5% Sulphosalicylic acid were pipetted into the beaker and the contents diluted to approx. 150 ml with "pure" water. By adding concentrated (8N) and dilute (2N) redistilled Ammonium hydroxide drop by drop the pH of the solution was adjusted to 4.8 (± 0.05 pH units) using a Beckmann, model H-2 pH meter. The correct pH could be judged fairly accurately from the colour of the Iron (III) - Sulphosalicylic acid complex, which changes rapidly from a deep purple at pH < 3 to light yellow at a pH of approx. 6. The pH adjustment was therefore changed somewhat by adding 8N Ammonium hydroxide drop by drop until a light purple to yellow colour was obtained, indicating a pH of between 5 and 6. Final adjustments to pH 4.8 were effected by the addition of 0.6N Hydrochloric acid. Contact of the sensitive glass and calomel electrodes of the pH meter with solutions of pH lower than 1 was thereby avoided.

It should be mentioned at this stage that it was found inadvisable to leave plant solutions or standards in contact with Sulphosalicylic acid for longer than 1 to 2 hours, since a slow decomposition of the acid appeared to take place, which was accelerated at pH 4.8. Colloidal Sulphur was deposited on the walls of the vessel and when attempting to extract the precipitated metal carbamates with 10 ml portions of Chloroform, a clear, colourless organic phase could never be obtained. The resultant combined extracts spluttered violently on evaporation.

5.3. The Solvent Extraction.

The standard or plant solution, after adjustment to pH 4.8, was washed into 250 ml separating funnels and heated to between 50 and 60°C over the open flame of a Meker burner, no thermometer being used. Immediately, 10 ml of freshly prepared 2.5% Sodium pyrrolidine dithiocarbamate were added and the separating funnel shaken vigorously for approx. 15 sec. It was found convenient to carry out six extractions simultaneously; while the first separating funnel was allowed to cool half-submerged in a basin filled with water and provided with an overflow, the second precipitation could be carried out. After cooling, the outside of the separating funnel was wiped dry with a clean towel, the outflow washed with "pure" water and the interior of the stem dried with a small roll made from filter paper.

The extraction was carried out by shaking vigorously with 10 ml re-distilled Chloroform for approx. 30 seconds, running off the extract after allowing approx. 1 minute for the phases to separate, and repeating the procedure with two portions of 5 ml Chloroform each. The last extract was in most cases light yellow to colourless. The pH of the aqueous phase was re-adjusted by the addition of 2 ml 0.6N Hydrochloric acid and a final extraction carried out with a further 5 ml portion of Chloroform.

In the earlier part of the work described here, plant solutions were made up to 50 ml in volumetric flasks, and, after preparation of the solution as described under section 5.2. in a 100 ml beaker, the solutions were extracted in 100 ml separating funnels. When the precipitated metal-organic complexes were shaken with 10 ml portions of Chloroform, the phases only separated with great difficulty after allowing to stand for excessively long periods of time. In approximately 50% of the separations attempted in 100 ml separating funnels, emulsions were produced. This difficulty was overcome to a certain extent by slowly pouring 10 ml volumes of Chloroform down the inside walls of the separating funnels. The mass of precipitated metal carbamates, floating on the surface, was dissolved in the organic phase. However, excessive-

/ -ly large volumes

ly large volumes of Chloroform had to be employed, sometimes up to 50 ml. When the final extraction was attempted by shaking with small volumes of Chloroform, emulsions were still obtained quite frequently. This difficulty was experienced when using either standards or plant solutions. Plant samples ashed and dissolved according to Mitchell's Sodium carbonate fusion method, were particularly prone to emulsion formation on extraction, probably due to the higher concentration of salts in the aqueous phase.

5.4. Completeness of Extraction.

The quantitative recovery of trace elements was investigated on the aqueous phases remaining after the metal carbamates were extracted.

According to Sandell (62) precipitation of Iron, Manganese and Molybdenum with 8-Hydroxyquinoline is quantitative at a pH of approximately 6.5, while Cadmium, Cobalt, Copper and Zinc may be precipitated with Dithizone in the presence of Ammonium citrate at pH 9.7 (46).

The following concentration scheme was therefore applied to the extracted plant standards 1, 3 and 5÷ The pH of the solution was adjusted to 6.5 with dilute Ammonium hydroxide, extracted with 10 ml of a 1% solution of 8-Hydroxyquinoline in Chloroform and washed with two portions of 10 ml pure Chloroform.

To a duplicate set of extracted standards 20 ml of purified 0.5M Ammonium citrate were added, the pH adjusted to 9.7 and the solutions extracted with 20 ml 0.02% Dithizone in Chloroform. The organic phase remained pure green.

/ The Chloroform extracts

The Chloroform extracts were combined and evaporated onto 40 mg. SP 2 graphite, ashed, mixed with 40 mg. Lithium carbonate and analysed spectrographically in duplicate.

At the same time 10 ml of the plant blank was extracted and analysed in a similar way.

It was found that Copper was not quantitatively recovered with Sodium pyrrolidine dithiocarbamate. Subtracting the reagent blank, approximately 10 and 2 μg. resp. of Copper were found in the extracted plant solutions 1 and 3. This corresponded to between 97 and 98% recovery of Copper. The analysis was semi-quantitative, comparing the intensities produced by standard mixtures of the oxides of the elements in a 1÷1 mixture of Lithium carbonate and SP 2 graphite.

Cobalt, Molybdenum and Zinc were extracted quantitatively whilst traces of Iron were found in all extracted plant standards. Approximately the same amount of Manganese remained behind in the most concentrated plant standard 1.

Fortunately the extraction errors appeared to be proportional to the concentration of Copper and could therefore be virtually eliminated by treating both standards and plant solutions in exactly similar fashion.

5.5. Evaporation and Ashing of the Extract.

Various experiments were carried out in order to determine the most suitable and convenient procedure for the evaporation of Chloroform extracts of the heavy metal carbamates. When the extracts were collected in 100 ml silica basins and the Chloroform

/ was evaporated

was evaporated by the use of an infrared lamp, as suggested by Scharrer and Judel (24), excessive "creeping" of the black extract up the sides of the vessels occurred. When attempting to carry out the evaporation in 50 ml pyrex beakers on a steam bath, the same difficulty was experienced. Wark (43) prevented creeping by directing a jet of air against the outside of a 1 ml beaker in which the final evaporation was carried out. Eve (63) proposed a relatively simple solution to this problem. The extract was evaporated in one inch diameter thick-walled Pyrex test-tubes cut to a length of three inches and mounted in a water-bath in such a way that during the final stages of the evaporation the liquid level inside the test-tubes was always slightly above that of the water level outside. Partial condensation of the evaporated Chloroform takes place on the long, relatively cooler walls of the test-tubes which dissolves any extract which may have crept up the sides. In order to achieve the confinement of the extract to as small a volume as possible, the test-tubes have to be raised out of the waterbath from time to time in order to keep the respective liquid levels in the same relative position to each other.

Eve's suggestion was followed and a simple water bath, consisting of a 3 litre Pyrex beaker heated on an electric hot-plate, was constructed. The top of the beaker was cut off and snugly fitted with a wooden lid into which six circular openings were drilled of diameter slightly larger than the outside diameter of a Pyrex boiling tube. These tubes were cut down to a length of approx. 3 inches and prevented from slipping through the opening by putting a tight-fitting rubber

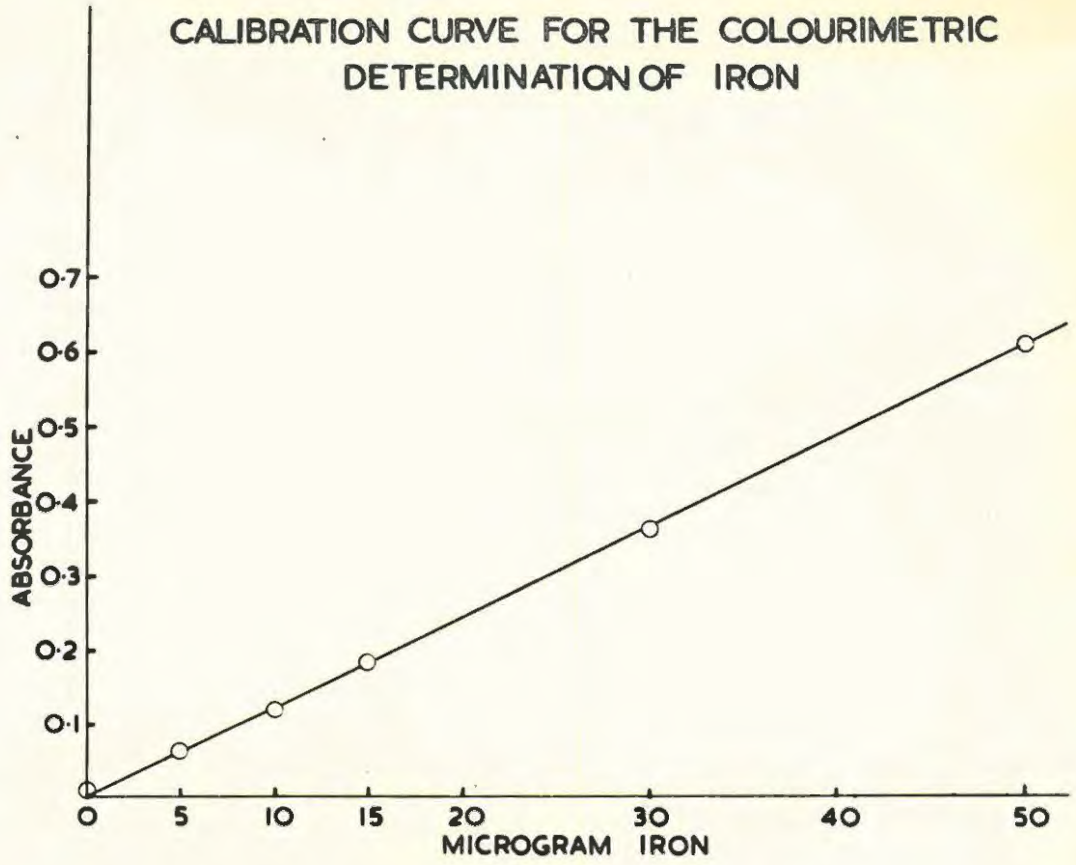

CALIBRATION CURVE FOR THE COLOURIMETRIC DETERMINATION OF IRON

FIGURE 13.

EVAPORATION OF CHLOROFORM EXTRACTS

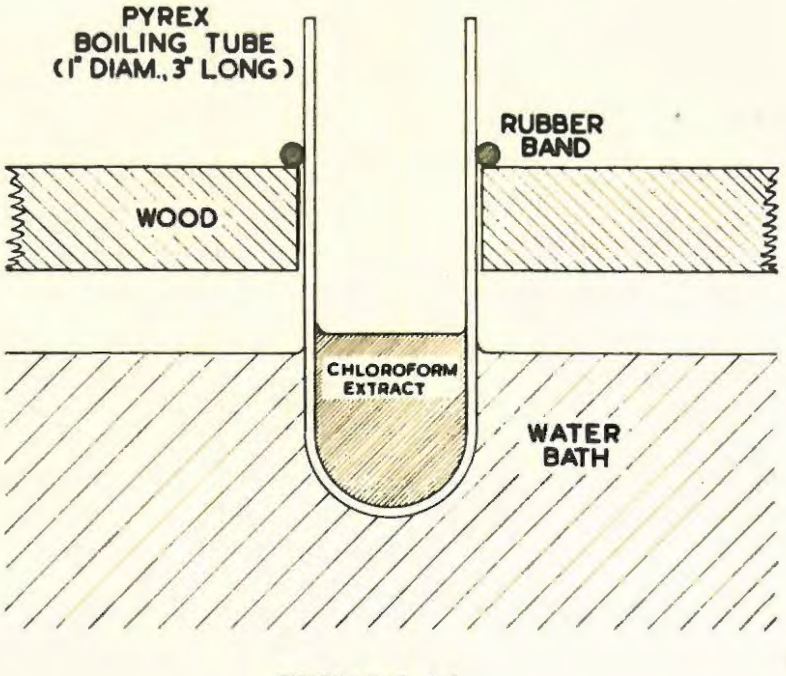


FIGURE 14.

FIGURE 15.

FIGURE 16.

band around the outside. The arrangement is shown diagrammatically in figure 14 while figure 15 shows the actual water bath used. The technique of evaporating extracts in these vessels proved to be very satisfactory. However, constant attention has to be paid to the position of the liquid level inside the test-tube with respect to the water level of the bath, particularly during the later stages of the evaporation. If, through neglect, the extract did creep up the side of the test-tube, the tube was dipped into a beaker of cold water, resulting in condensation of Chloroform on the cold walls of the vessel and solution of the extract.

Initially it was attempted to evaporate the extract onto 40 mg. SP 2 graphite. However, serious spluttering occurred in many cases during the last stages of the evaporation. Another reason for discontinuing this practice is discussed in section 8.

When extracting six samples simultaneously a considerable amount of time was saved by adopting the following procedure÷ The first 10 ml Chloroform extract was run into 3 inch test-tubes, supported in numbered 100 ml Pyrex beakers, and allowed to evaporate on the specially constructed waterbath while the second extraction was carried out. This was repeated for all subsequent extractions so that only the evaporation of the final small volume of Chloroform run off from the separating funnels (5 ml) had to be attended to with the precautions described above. When all the Chloroform had been removed, (judged by the smell of the vapours) the tubes were immersed as far as possible into the water bath for approx. 15 minutes to dry the extract. Drying was completed in an electric oven for approx. 1 hour at 110°C. For this purpose the test-tube was placed into an appropriately numbered 100 ml beaker, the rubber band slipped off and the opening of the test-tube loosely covered with a 20 ml beaker. The arrangement is shown in figure 16. After drying, the extract was converted to the oxides of the metals by placing in a muffle furnace and slowly raising the temperature to 450°C, leaving the residue in the muffle overnight. By taking all the precautions described above no sputtering was ever observed to occur on ignition to 450°C; the added precaution of leaving a 20 ml beaker over the mouth of the test tube while in the muffle furnace was therefore unnecessary. The practice was, however, adhered to in order to avoid possible Iron contamination from the open-element furnace used. The oxides formed could be easily removed from the test-tube by means of a spatula. Further treatment of the ignited extract will be described in section 7.

The test-tubes were thoroughly cleaned after use by boiling them in Aqua Regia for approx. 10 minutes, followed by a smililar heating in 30% Sodium hydroxide. After finally placing the tubes in boiling Aqua Regia for a few minutes they were rinsed three times with distilled water, twice with "pure" water and dried in an oven at 65°C.

Six samples can be concentrated comfortably by one operator in approximately 1½ hours, from the addition of internal standard solutions up to the evaporation of the final extract. The extraction itself, including precipitation of the trace metals, cooling and shaking with small volumes of Chloroform, does not take longer than approximately 45 minutes.

/ 6. CHOICE OF DILUENT OR MATRIX.

6. CHOICE OF DILUENT OR MATRIX.

In addition to effecting a high relative concentration of trace elements so that they can be determined by spectrographic methods, chemical enrichment techniques serve several useful purposes. The composition of the major constituents in ash obtained from different varieties of plants may differ considerably and if a direct spectrographic analysis is carried out on the ash it is the composition, or matrix, which determines the nature of the emission of radiation from minor constituents excited in a d.c. arc.(65) Chemical concentration methods, however, separate the trace elements from a variable base so that one set of spectrographic standards can be used for a large variety of materials. A constant matrix is obtained by adding a suitable buffer to the enriched elements and, finally, the effects of differences in the chemical composition of trace elements is eliminated since the final arcing mixture consists of all the elements in the same type of chemical combination.

The most important properties governing the choice of a good diluent or buffer are÷

1.) It must be free from the elements to be determined and readily available.
2.) It must have a simple spectrum.
3.) It must be chemically inert and not hygroscopic.
4.) It must have the ability to carry the sample into the arc over the entire arcing period.

In addition, a good buffer should enhance the lines of other elements and suppress the intensity of the Cyanogen band spectrum, which is always obtained

/ in the excitation of samples

in the excitation of samples contained in carbon or graphite electrodes. These bands emanate from their respective band heads at 3590, 3883 and 4216 A. They are very dense and their fine line structure extends throughout the 3500 to 4216 A region.

Harvey (65) listed the following elements in order of decreasing ability to enhance the lines of other elements÷ 1.) Lithium, 2.) Zinc, 3.) Bismuth, 4.) Barium, 5.) Tin, 6.) Cadmium, 7.) Lead, 8.) Silver, 9.) Copper and 10.) Platinum and recommended the use of Lithium carbonate as buffer for arc excitation of samples. As early as 1921 de Gramont (66) employed Lithium carbonate as a flux in the preparation of samples for spectrographic analysis. However, van Rooyen (67) claimed that Lithium carbonate is unsuitable for the determination of Cobalt, Molybdenum and Zinc because of its inability to enhance the lines of these elements.

Lithium carbonate, mixed in the ratio 1÷1 with SP 2 graphite powder, was chosen as the matrix in all the work described here. It is inadvisable to employ a matrix of the pure compound, since samples mixed with it were found to be rapidly ejected from the electrode during the first few seconds of the exposure. Lithium carbonate is available in "specpure" form and has a very simple spectrum. Because of its low ionisation potential (5.37 volt), Lithium provides an excellent buffer against temperature fluctuations in the arc. Like all alkali metal salts, Lithium carbonate causes a suppression of the Cyanogen band spectra by lowering the potential of the arc. This lowering is accompanied by a drop in the arc stream temperature which is then

/ insufficient to excite

insufficient to excite the Cyanogen radical to the level attained in a normal arc.

Heavy buffering with a 1÷1 mixture of Lithium carbonate and SP 2 graphite was found to produce a very steady burn of the electrodes in a 10 ampere d.c. arc during the first 60 to 90 seconds, while a certain amount of arc wander was usually experienced after this period. This was not the case when the trace elements were contained in a 1÷2 mixture of the alkali with graphite, as proposed by Strasheim, Eve and Fourie (49). During the first 20 to 30 seconds of the burn the arc was relatively steady; however, after about 60 seconds very erratic arc wandering persisted until the sample was consumed. This may be due to the weaker buffering capacity of such a mixture, most of the low boiling point alkali being volatilised during the earlier stages of the burn, leaving virtually only a graphite matrix. The effects of such erratic arc wander on the rate of emission of minor elements are demonstrated in the volatilisation curves, section 9.

7. MIXING OF THE ASHED EXTRACT WITH BUFFER.

In preliminary experiments the Chloroform extract was evaporated onto 40 mg. SP 2 graphite and ignited in a muffle furnace at 450°C. After cooling, 40 mg. "specpure" Lithium carbonate were added to the ignited sample contained in 3 inch length Pyrex test-tubes. Preliminary mixing was carried out by means of a Nickel spatula. This spatula was used for the transfer of the sample into a one inch perspex vial, containing a 3/8 inch nylon ball pestle. Final mixing was effected by vibrating the vial and contents for 20 seconds in a Wig-L-Bug amalgamator. The resultant mixture was sufficient to fill two electrodes.

The practice of evaporating the extract onto graphite powder was discontinued, however, because such samples caused serious spluttering out of the electrode during the first stages of the arcing period. The ejection of sample material occurred especially when standards containing higher concentrations of trace elements, e.g. Standards 1, la and 2 (see section 4) were arced. This problem will be more fully discussed in section 8.

In all subsequent experiments, the extract was ignited on its own and subsequently mixed with 80 mg. of a 1÷1 mixture of Lithium carbonate and SP 2 graphite as described above. The buffer mixture was weighed out on small sheets of glazed paper. In a few cases it was necessary to scrape the ignited oxides from the bottom of the Pyrex test-tube, in which the ignition was carried out. The nickel spatula used for this operation and also for the transfer of material was thoroughly cleaned by wiping several times with cottonwool swabs soaked in Ethyl alcohol and drying with cottonwool. Since the internal standards are incorporated in the ignited oxides of the extract, a complete recovery is not essential.

8. FILLING OF ELECTRODES AND ARCING.

The purified sample electrodes, described in Part I, section 3. were stored in a polythene container and transferred to an electrode stand made of bakelite for filling. This stand, shown in figure 17, had provision for storing 28 electrodes prior to arcing

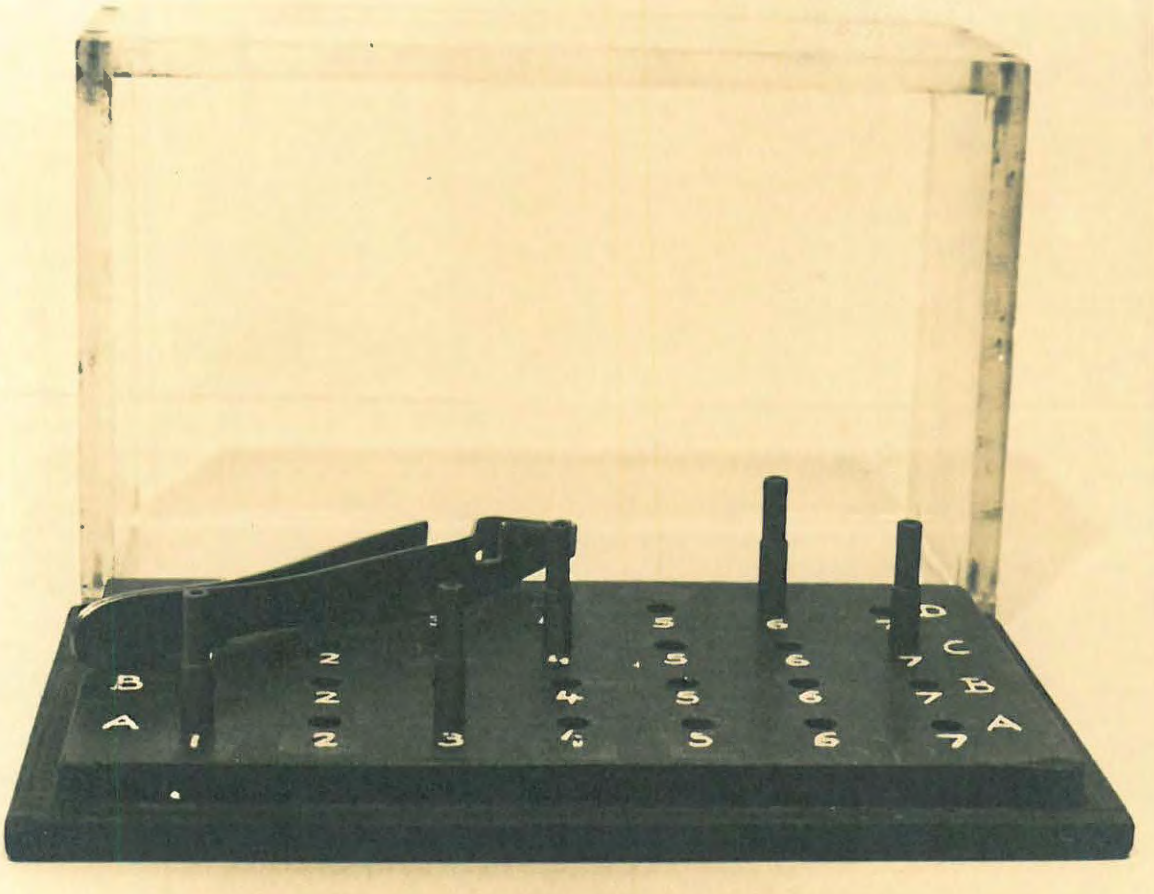


FIGURE 17.

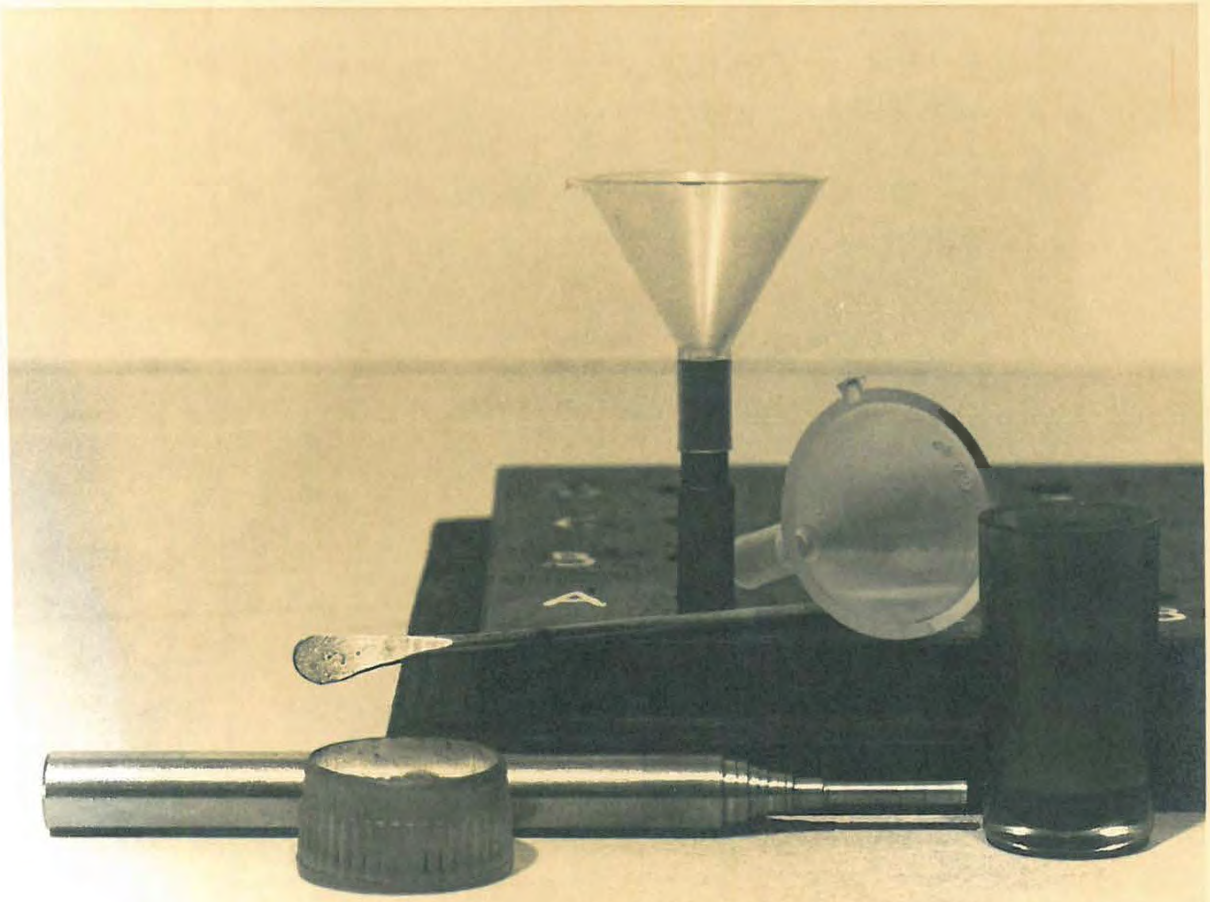


FIGURE 18.

and was protected from dust and other contamination by means of a perspex lid.

The packing of electrodes with the sample was accomplished as follows÷

A tight fitting polythene funnel, available commercially as an adjunct to the automatic "Elpac" electrode packing machine for the filling of electrodes of 4 mm outer diameter, was slipped over the part of the electrode containing the sample cavity. By means of a small platinum spatula, only used for this purpose, the sample material was transferred in small portions into the polythene funnel and tightly stamped into the electrode cavity with a stainless steel stamper. This stamper consisted of a rod of stainless steel, 5 cm long and 6 mm diameter, turned down to a diameter of just less than 2 mm over a length of approximately 12 mm. This part of the rod, employed in packing the sample material, snugly fitted the inner dimensions of the electrode. Figure 18 shows the equipment used for filling electrodes.

Mitchell (68) particularly stressed the importance of packing electrodes evenly and tightly for the attainment of reproducible results. From the experience gained during the work undertaken this point cannot be overemphasised. Initially the electrodes were filled by placing a slight excess of the sample into the funnel and stamping it down into the cavity by means of the stainless steel rod. Very soon it was found, however, that electrodes filled in this fashion sometimes caused ejection of the sample to take place during the arcing period. Subsequently the filling operation was carried out with great care, by transferring small amounts of sample into the funnel at a time and stamping it down tightly before the next portion was added. Although from 2 to 2½ minutes were spent in thus filling one electrode, the time expended was well worth while in view of the reproducibility of the burn and the final results obtained.

In order to check the reproducibility of the amount of material packed into the electrodes by the method described, 21 electrodes were accurately weighed on a semi-micro balance, filled with 1÷1 Lithium carbonate ÷ SP 2 buffer and weighed again. The weights, in mg., packed into the electrodes are tabulated below.

TABLE 11.

Weights of Buffer packed into Electrodes (in mg.)

Weight	Weight	Weight	Weight
31.74	29.14	30.96	30.92
29.71	31.32	31.43	30.83
31.40	31.56	31.74	31.59
32.25	31.94	31.04	31.59
32.15	30.93	31.86	31.09
			31.18

Mean÷ 31.26
Standard Deviation÷ 0.749
Coefficient of Variation÷ 2.4%

From the coefficient of variation obtained it may be seen that electrodes can be packed uniformly and evenly by hand if the necessary care is exercised.

After filling, the sample electrode was clamped in the lower position between the water-cooled jaws of the arc stand and a purified carbon counter-electrode was inserted in the top clamp. Anode excitation was employed in all cases. By means of the projection system described in Part I the position of the sample

/ electrode was adjusted

electrode was adjusted to 4 mm below the optical centre of the outer lens system of the spectrograph. The carbon counter-electrode was then brought into contact with the lower electrode and adjusted to touch only the electrode itself and not the sample. This alignment caused a slight lateral shift of one electrode with respect to the other, which did not affect the characteristics of the burn, such as arc wander.

Before making an exposure, the sample was heated for 30 seconds in the position described above, using a current of 20 amps. Sufficient resistance was then placed in series to reduce the current to approx. 6 amps. and the electrodes slowly separated to a total arc gap of 8 mm, while at the same time increasing the current slowly to its final value of 10 amps (shortcircuited). The arc gap was continually readjusted to 8 mm during the complete arcing period. Comparison of exposures of sample electrodes preheated in this manner and of electrodes arced without preliminary heating revealed that, with the limits of experimental error, no loss of volatile elements, such as Zinc, occurred during the initial heating period.

As mentioned in section 7, difficulties were experienced when extracts ignited with graphite powder and mixed with Lithium carbonate, were arced. Tight and uniform filling of the electrodes with the sample thus obtained did not produce any improvement, neither did a preliminary heating of the electrodes with the sample for approx. 1 hour at 110°C. In order to find the causes of the ejection of material in the arc the following three samples were prepared÷

1.) 7.2 mg. Ferric oxide (corresponding to approx. 5

/ mg. Iron)

mg. Iron) were mixed with 40 mg. SP 2 graphite in a 20 ml beaker and heated at 450°C in a muffle furnace overnight. When mixed with 40 mg. Lithium carbonate and filled into an electrode a perfect burn was obtained.

2.) 5 ml of 0.1% Iron solution were pipetted onto 40 mg. SP 2 graphite contained in a 20 ml beaker and evaporated to dryness in an air-oven at 110°C. To facilitate wetting of the graphite powder by the aqueous solution, two drops of Teepol were added. After drying, the mixture was similarly ignited at 450°C overnight, mixed with 40 mg. Lithium carbonate and arced. No spluttering of the sample took place. However, the same mixture, when allowed to stand for one day, was violently ejected from the sample electrode.

3.) Similar treatment to 2.) but the Iron solution was evaporated onto 20 mg. graphite and mixed with 60 mg. of 1÷1 Lithium carbonate÷SP 2 graphite buffer after ignition to 450°C. The sample burned smoothly, even after allowing to stand for one day.

When an actual ignited plant extract was found to splutter, 10 mg. SP 2 graphite powder were added to the remaining sample and, after mixing, a smooth burn resulted.

The behaviour of extracts, ignited with graphite powder, was so erratic, that no definite conclusions as to the reasons for spluttering could be drawn. It was, for instance, observed, that the ejection of sample in the arc was more pronounced on a humid day. Pre-heating of the electrodes in the manner described should have prevented spluttering if the presence of moisture in the sample material had been the cause.

/ 9. RATE OF EMISSION OF TRACE ELEMENTS,

9. RATE OF EMISSION OF TRACE ELEMENTS.

In spectrographic analysis it is important to know the rate at which elements are volatilised in an arc in order to determine the suitability of internal standards. Using a direct current arc for the excitation of a sample has the disadvantage, amongst others, that due to the intense heat produced in the arc, elements placed in the electrode are selectively volatilised. The rate at which an element emits radiation over a complete arcing period is proportional to the rate of volatilisation of this element into the arc stream, since mainly thermal excitation of the elements is produced with a direct current arc. The rate of volatilisation of an element is dependent on many factors, such as the chemical combination of the element, strength of the current, type of electrode and to a large extent on the nature and composition of the diluent. These considerations are less important when the so-called "Total Energy" method of analysis is used, the fundamental requirement of which is the arcing of a specimen to completion. Modern spectrographic analysis, however, usually employs cut-off procedures in conjunction with internal standards. In this procedure the integrated intensities of radiation emitted by the excited element and internal standard are recorded over a limited period of the arcing cycle, which does not take account of the rate at which both elements emit during this period. However, one of the basic requirements of a suitable internal standard for a particular element to be quantitatively determined is, that the rates at which both are volatilised out of the electrode (and hence, emit radiation) should be similar. To take an extreme example÷ It would be inadvisable to use the highly involatile element Zirconium as an internal standard for the determination of the very volatile Zinc. Ideally, therefore, emission of radiation from minor elements and internal standards in the matrix should take place uniformly during the total period for which the sample is excited. This ideal condition is never realised in practice, however, when working with the direct current arc; it may be closely approached by the correct choice of conditions of excitation, particularly so when employing an interrupted arc to volatilise the sample (63).

In order to study the rate of volatilisation of the trace elements and their respective internal standards, so-called volatilisation curves are plotted for each from intensity measurements during suitable fractions of the total arcing time. The method generally adopted is to initiate the arc, keeping the spectrograph shutter open, exposing a photographic plate for a certain interval, closing the shutter for a definite time while the plate is moved to a different position, exposing again for the same interval and repeating the procedure until the sample is consumed, this being signified by a change in the colour and sound of the arc. For this purpose it was found to be advisable to prepare a special mixture of the oxides of the trace elements and internal standards in the diluent under consideration, which, under the conditions of excitation and exposure chosen, would produce measurable spectral densities over the complete arcing period. The following concentrations were found to be suitable, when incorporated in a 1÷1 Lithium carbonate÷ SP 2 graphite base÷

1000 p.p.m. Cobalt and 5000 p.p.m. Cadmium
100 p.p.m. Copper 1000 p.p.m. Iron.
1000 p.p.m. Manganese
1000 p.p.m. Molybdenum
20000 p.p.m. Zinc

The following is a summary of the conditions used in recording volatilisation curves÷

Exposure Interval÷ 10 seconds
Shutter closed for÷ 5 seconds
Diaphragm opening÷ 2 mm
Arc gap÷ 8 mm
Fish-tail diaphragm (used in front of spectrograph slit)÷ 2 mm
Movement of photographic plate÷ 2 mm at a time.
Current strength÷ 10 amps (short-circuit value)
Photographic Plate÷ Kodak S.A. No. 1
Developer÷ Kodak D 153, diluted 1÷1
Development time and temperature÷ 4 min at 21.5°C, Brush development.

Since no automatic unit was available for the development of spectrographic plates, this operation was carried out in plastic trays as described in Part I, section 4. The plates were calibrated by recording four two-stepped Iron spectra of varying exposure on them and evaluating them by the preliminary curve method given in detail in Part I, section 7.

Three conditions of excitation and matrix were investigated÷

Condition 1÷

The oxides of the elements of interest were incorporated in a base of 1÷3 Lithium carbonate ÷ SP 2 graphite and arced at 10 amps, using a selenium rectifier. From the observations it was obvious that the buffering capacity of 25% Lithium carbonate was

/ insufficient to

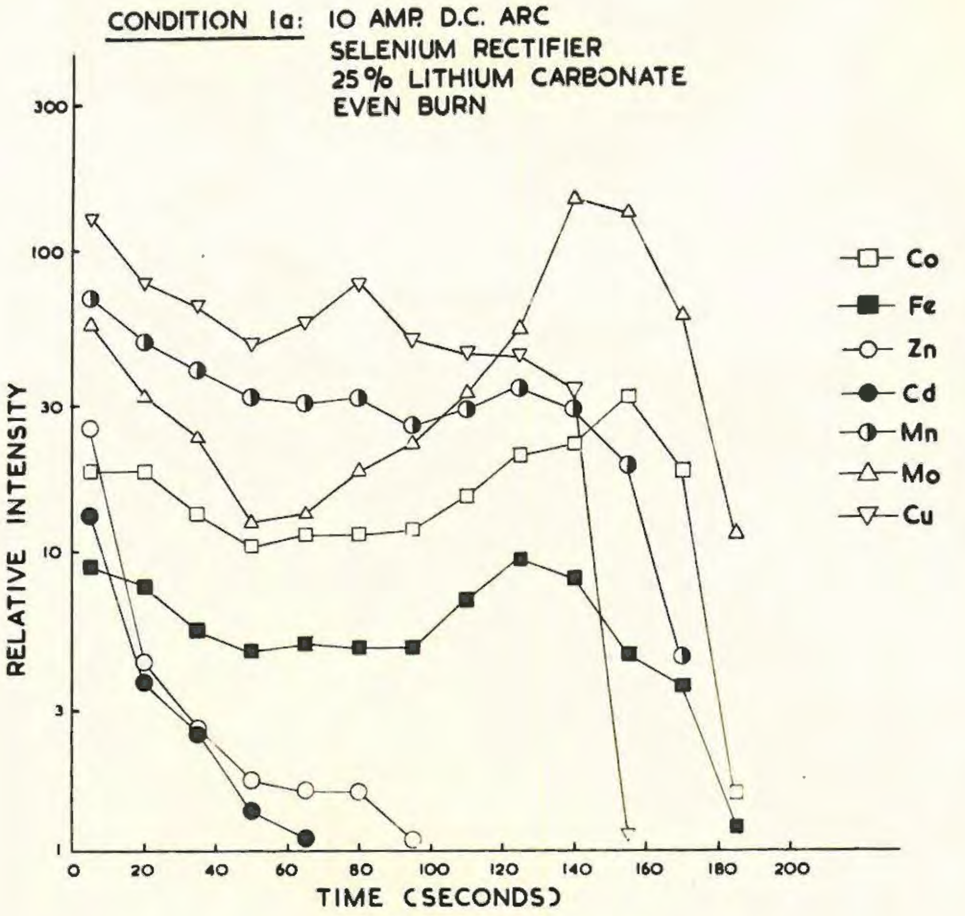


FIGURE 19.

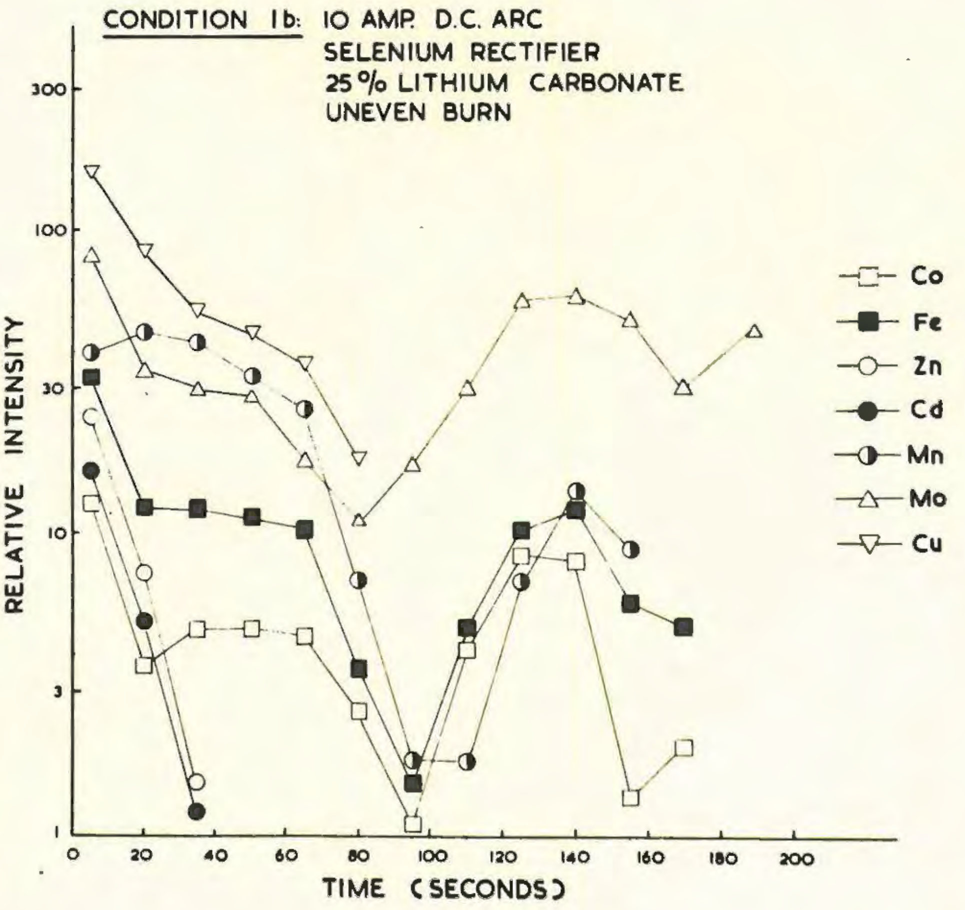


FIGURE 20.

insufficient to stabilise the arc and volatilise the elements evenly. Pronounced arc wandering occurred approx. 30 seconds after the electrodes were slowly separated and persisted until the sample was consumed. During this period the anode spot described erratic circular motions around the electrode, striking lower and lower while electrode walls and material were consumed at uneven rates. After approximately two minutes a pure carbon arc was obtained, which did, however, not signify the complete consumption of sample since at irregular intervals the characteristic red colour of Lithium reappeared for short periods of time. The uneven nature of the burn is illustrated by the erratic character of the volatilisation curves (fig. 19). Since it was thought, that the erratic behaviour of the arc was an exception and not a general phenomenon, several electrodes filled with the same sample were arced before the time-intensity curve was recorded. Similar irregular burning characteristics were, however, observed in each case.

A particularly serious example of an uneven burn was provided when some of the sample material was ejected during the initial 3 to 5 seconds (fig. 20) The rates at which elements were volatilised in this case hardly bear any resemblance to those of a relatively smoother burn in figure 19.

Condition 2÷

The oxides of the elements were mixed with 1÷1 Lithium carbonate ÷ SP 2 graphite buffer and arced at 10 amps, using a selenium rectifier. An even burning arc was obtained throughout the arcing period except for the last 15 to 20 seconds before the sample

/ was consumed,

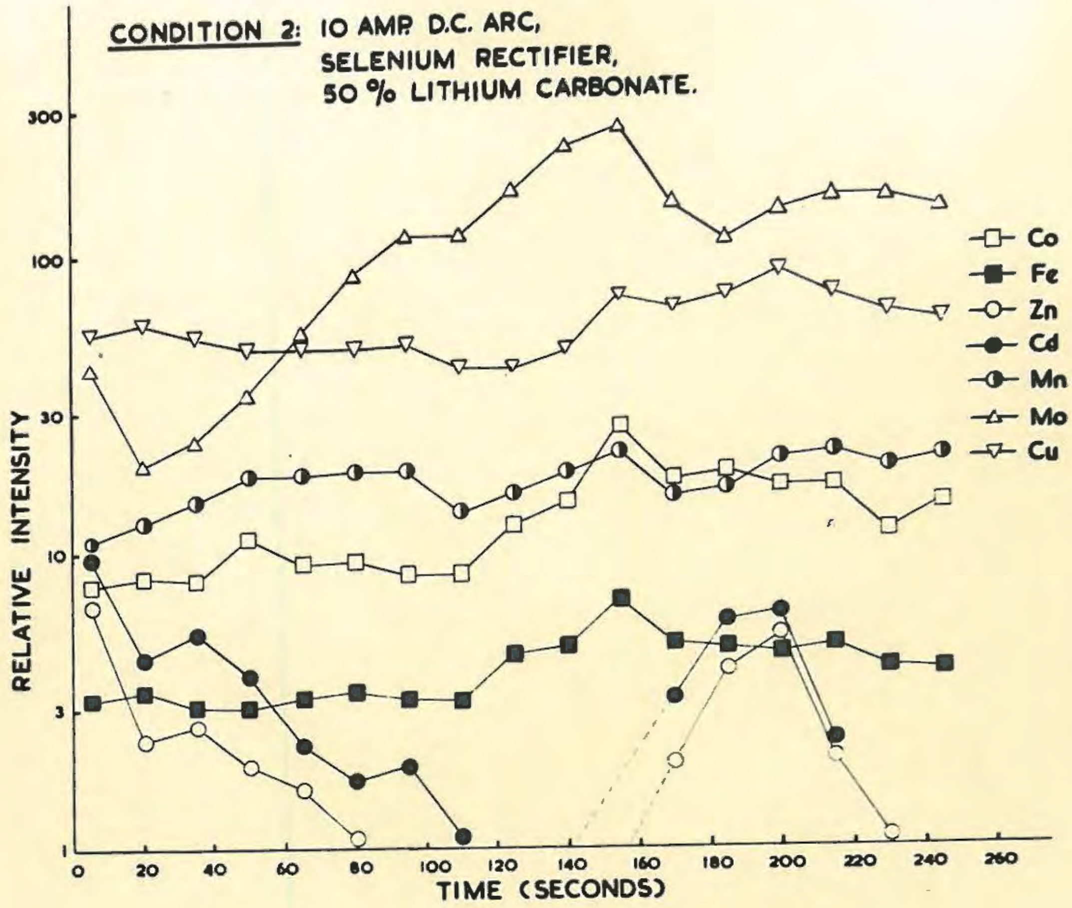


FIGURE 21.

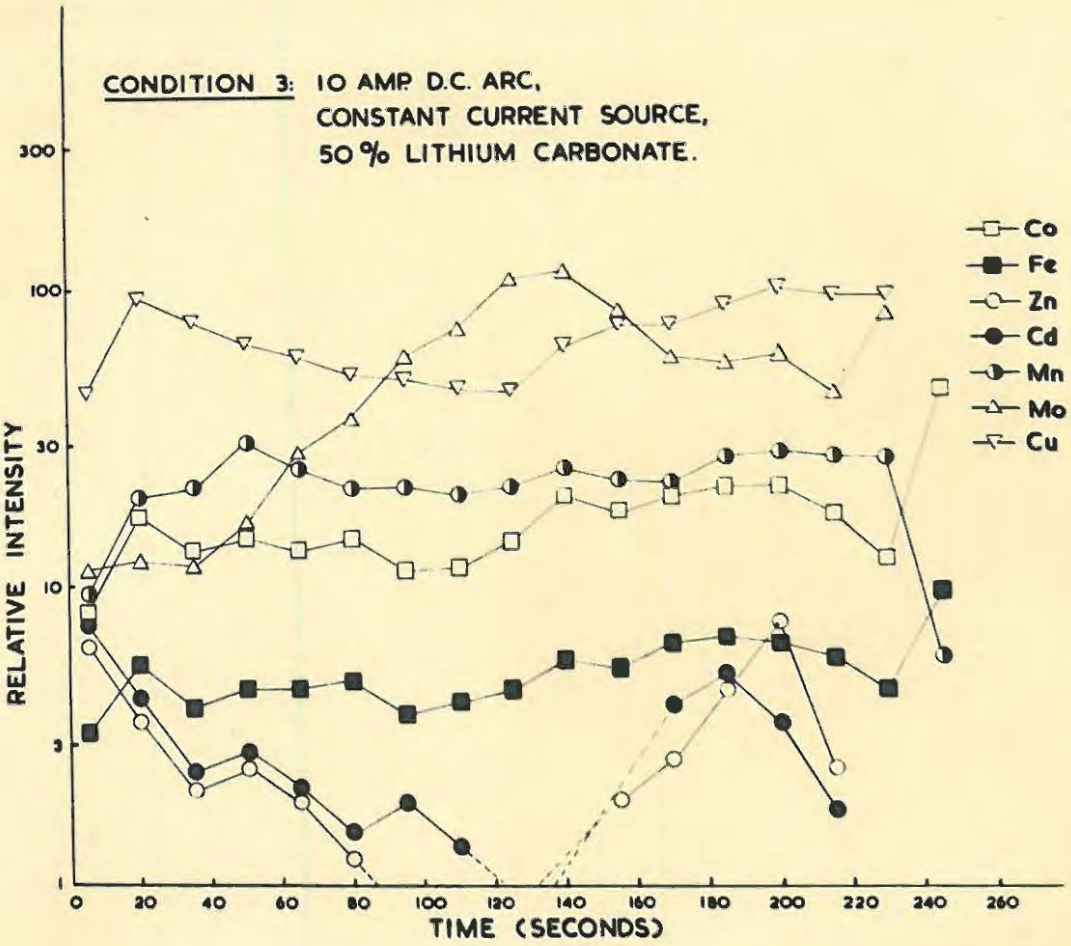


FIGURE 22.

was consumed, when erratic arc wandering occurred. Except for the very volatile elements Zinc and Cadmium and the highly involatile element Molybdenum the rates of emission of radiation of the elements Cobalt, Copper, Iron and Manganese were comparatively uniform. Most of the Zinc and Cadmium appeared to be volatilised during the first 60 seconds, but strong volatilisation of these elements again appeared to take place towards the end of the arcing period. An explanation for this phenomenon could not be found. The intensity of radiation emitted by Molybdenum was very small initially, but increased considerably during the later stages of the arcing period. Therefore a considerable increase in the sensitivity of the determination of Molybdenum may be obtained by recording only the later part of the arc burn. The volatilisation curves are shown in figure 21.

Condition 3÷

Similar to condition 2, however, a constant current rectifier, described in Part I, section 2, was employed for the excitation of the sample, which also produced a smoothly burning arc. The interesting result obtained in comparing the two source units was that an electronically controlled current strength produced little or no difference in the volatilisation of elements from the electrode. The volatilisation curves for this condition are shown in figure 22.

9.1. Choice of Internal Standards.

As mentioned earlier, modern spectrography employs internal standards for quantitative analyses. Instead of measuring intensities, intensity ratios are determined, that is, the ratio of the intensity of the analysis line to that of a line of an element present in constant amount. The use of the latter element, or internal standard, has the advantage that sources of error, such as incorrect development, poor control of arc wander across the spectrograph slit, variation of arc gap and failure to time the exposure exactly, are minimised to a large extent, while the necessity of arcing a weighed quantity of material in the electrode is eliminated. In addition to similar volatilisation rates of element and internal standard, several other factors govern the correct choice of a suitable internal standard. Of these, only similarity in the excitation potentials of the respective lines and their separation will be mentioned (69). Generally speaking, an arc or atom line of an element should not be compared with a spark or ion line of the internal standard and vice versa, so that variations in the temperature of the arc affect both lines to more or less the same extent. The second factor mentioned, namely a similarity in wavelength of the analysis pair, is important when measuring lines in regions of rapidly changing gamma.

One way of assessing the quality of an analysis pair is to plot the intensity ratio vs. time curve from the volatilisation curves of both element and internal standard. In the ideal case, this plot would be a straight line parallel to the time axis, indicating that any fluctuations in the volatilisation affect the emission of radiation from both element and internal standard to the same extent. From an examination of figures 23 and 24, showing the intensity ratio vs. time relationship for the excitation

/ conditions 1, 2 and 3,

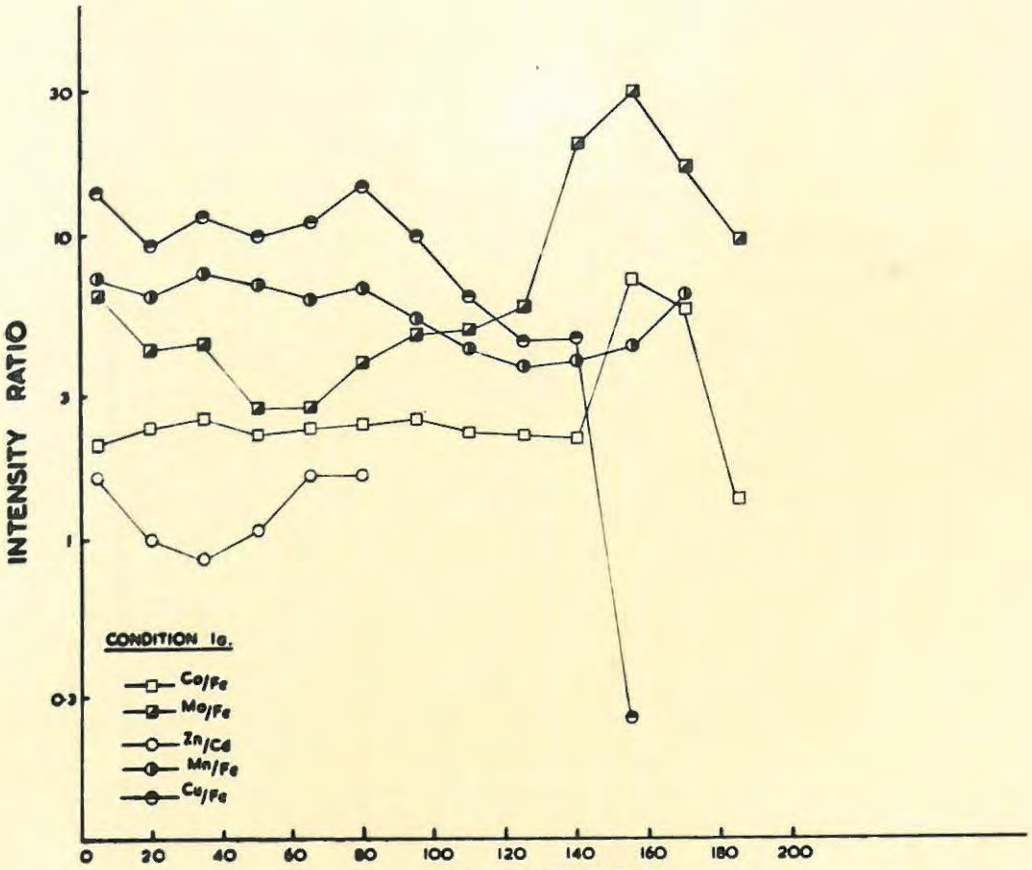


FIGURE 23.

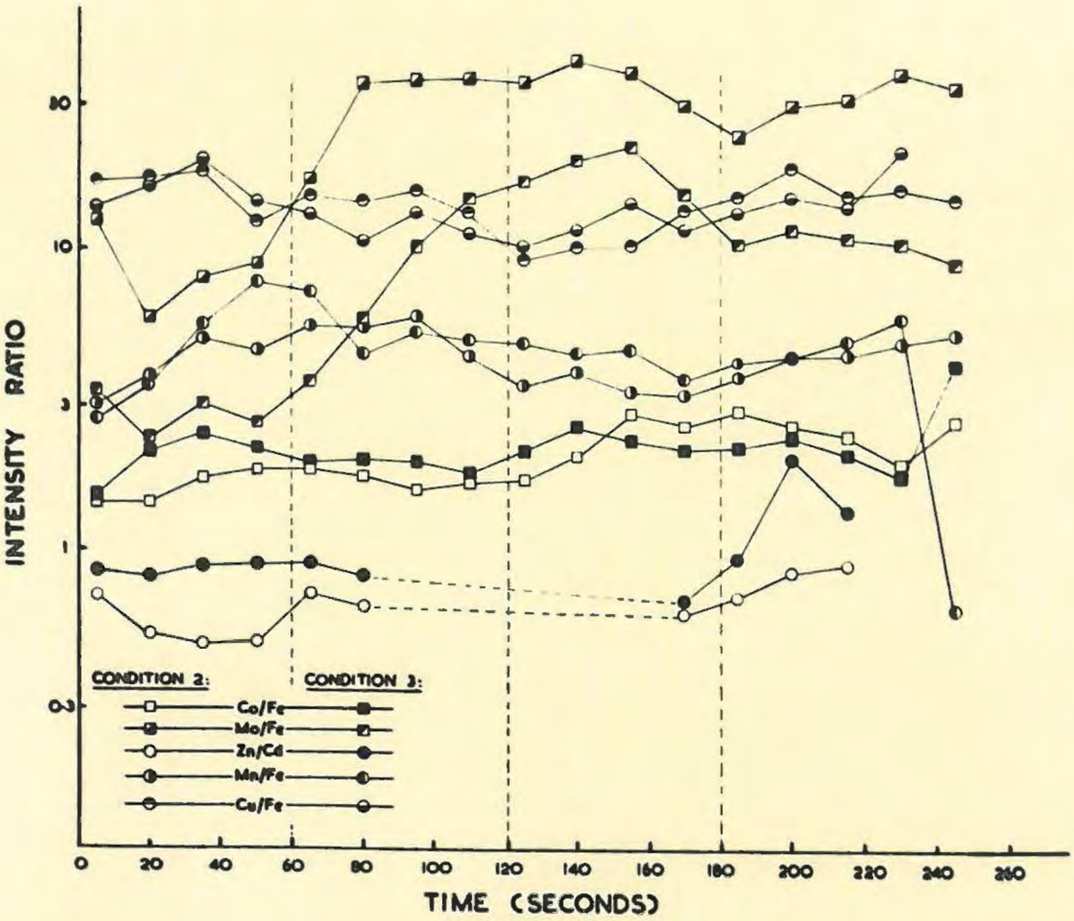


FIGURE 24.

conditions 1, 2 and 3, it may be seen that this ideal has been closely approached in the case of the Cobalt / Iron pair. This fact was confirmed by Mitchell (70), who reported close agreement in the relative intensities of the line pair Co 3453.51 A ÷ Fe 3451.92 A, although their absolute intensities varied appreciably. From a consideration of the difference in the ionisation potentials of these two elements (Co÷ 8.50 volts, Fe÷ 7.83 volts), this close agreement appears surprising, while the volatilisation curves show close similarity. This is an example of practical experience outweighing theoretical conditions when considering the suitability of an internal standard.

In the case of the Zinc / Cadmium pair, more uniform volatilisation occurred when a constant current rectifier was employed to excite the sample. Unfortunately this instrument was only acquired and put into operation towards the end of the work described here.

The same did not apply in the case of the intensity relationship of the Molybdenum / Iron analysis pair. The relative constancy of intensity ratio of this pair with time during the later stages of the arcing period, when compared with condition 3, is apparent from figure 24.

On examining volatilisation curves and intensity-ratio ÷ time plots the following excitation and recording conditions were chosen for the spectrographic analysis of trace elements÷

<u>Matrix÷</u> 1 ÷ 1 Lithium carbonate ÷ SP2 graphite.

<u>Excitation÷</u> 10 ampere (short circuit value) d.c. arc, obtained from a selenium rectifier, slow separation of electrodes.

Recording÷ 1.) Four-stepped spectrogram from 0 - 60 seconds and on the same film, racked down 6 mm,÷

2.) Four-stepped spectrogram from 120 - 180 seconds.

The intensities of the volatile elements Zinc and Cadmium were measured in the first spectrogram while the second was used for the determination of the remaining less volatile elements Cobalt, Copper, Manganese and Molybdenum, using Iron as internal standard. The increase in sensitivity obtainable, especially for Molybdenum, more than outweighed the disadvantage of having to record two exposures of the same electrode. The reduction in background of the second exposure as compared with the first was considerable, so that it was possible to measure the density of the Co 3453.51 A line at low concentrations of Cobalt without having to take recourse to background corrections.

The wavelengths of element and internal standard lines used in the quantitative evaluation of spectra are tabulated below÷

TABLE 12.

Analysis Pairs used.

Element	Internal Standard	Approx. Range, p.p.m.*
CoI 3453.51 A	FeI 3450.53 A	0.03 - 3
CuI 3273.96 A	FeI 3286.76 A	0.05 - 10
(CuI 2824.37 A	FeI 2835.46 A	10 - 100)
(Mn 2798.27 A	Fe 2808.32 A	0.5 - 5)
(Mn 3044.57 A	Fe 3045.08 A	1 - 100)
Mn 2933.06 A	Fe 2918.03 A	5 - 300
(Mn 2914.60 A	Fe 2918.03 A	30 - 1000)
Mo 3170.35 A	Fe 3166.44 A	0.03 - 3
ZnI 3345.02 A	CdI 3261.06 A	5 - 300

* Referred to 10 g. of plant material.

/ The spectrum lines

The spectrum lines given in brackets are alternatives, which may be used for higher or lower concentrations, respectively. The concentration range over which a particular analysis pair may be used, is only approximate.

Additional information on recording conditions adhered to in all subsequent work, is summarised in table 13.

TABLE 13.

Recording Conditions.

Diaphragm Opening÷ 1 mm
Spectrograph Slit Width÷ 15μ calibrated, 9μ on scale.
Arc Gap÷ 8 mm, kept constant during arcing period.
Emulsion÷ Kodak S.A. No. 1, 35 mm film.
Development÷ Kodak D 153, diluted 1÷1, automatic,
4 min at 21.5°C.
Fixing÷ "Amfix", 90 seconds.
Washing÷ 30 min, running water.
Densitometer Slit Width÷ 0.375 mm.

10. THE ANALYTICAL WORKING CURVES.

In section 4 the preparation of plant standards was described in detail. These standards were used in the construction of working curves for the trace elements Cobalt, Copper, Manganese, Molybdenum and Zinc.

For this purpose, 10 ml of each standard were extracted in duplicate as described in section 5.3. and four series of spectra were recorded. The deflection of each analysis pair was measured on the densitometer, using as many steps of the four-stepped spectra as possible and tabulating the readings on specially printed working sheets, an example of which is shown in table 14.

/ TABLE 14.

TABLE 14.

Example of Working Sheet.

(Exposures C3I and C3II).

Step	Element	Int. Stand.	Element		Int. Stand.		Intensity Ratio	Mean Intensity Ratio	conc. p.p.m.
			θ_1	I_1	θ_2	I_2			
1	Co 3453	Fe 3450	16.5	5.10	10.9	7.00	.728		
2	"	"	35.5	2.55	26.8	3.47	.735		
1	"	"	11.3	6.62	7.7	9.20	.720	.727	0.316
2	"	"	27.7	3.36	18.8	4.64	.725		
1	Zn 3345	Cd 3261	16.8	5.35	7.9	10.90	.492		
2	"	"	33.3	2.68	17.0	5.40	.495		
3	"	"	46.4	1.32	33.5	2.66	.495		
1	"	"	15.5	5.65	7.7	11.25	.502	.497	31.6
2	"	"	32.6	2.73	16.6	5.50	.496		
3	"	"	45.3	1.46	31.3	2.90	.504		
3	Cu 3274	Fe 3287	2.05	46.5	8.5	10.30	4.52		
4	"	"	3.70	23.5	18.5	4.97	4.74		
3	"	"	1.90	50.5	6.5	11.60	4.35	4.48	10.0
4	"	"	3.40	26.0	14.7	6.15	4.24		
1	Mo 3170	Fe 3166	11.5	9.50	26.5	3.85	2.47		
2	"	"	22.3	4.65	42.0	1.96	2.38		
1	"	"	8.7	12.75	21.3	4.86	2.62	2.51	0.316
2	"	"	17.2	6.15	37.6	2.38	2.58		
1	Mn 2933	Fe 2918	11.9	9.35	17.2	6.20	1.51		
2	"	"	22.7	4.52	31.6	3.02	1.50		
3	"	"	37.0	2.36	44.3	1.56	1.51		
1	"	"	9.2	12.6	14.3	7.60	1.66	1.58	31.6
2	"	"	17.0	6.30	26.2	3.80	1.66		
3	"	"	31.4	3.06	41.5	1.86	1.65		
1	Cu 2824	Fe 2835	48.2	1.05	11.0	10.25	.103		
2	"	"						.105	10.0
1	"	"	47.1	1.22	10.0	11.40	.107		
2	"	"							

The spectrogram of an actual plant sample extract, showing the position of analysis and internal standard lines, is reproduced in figure 25.

/ the relative

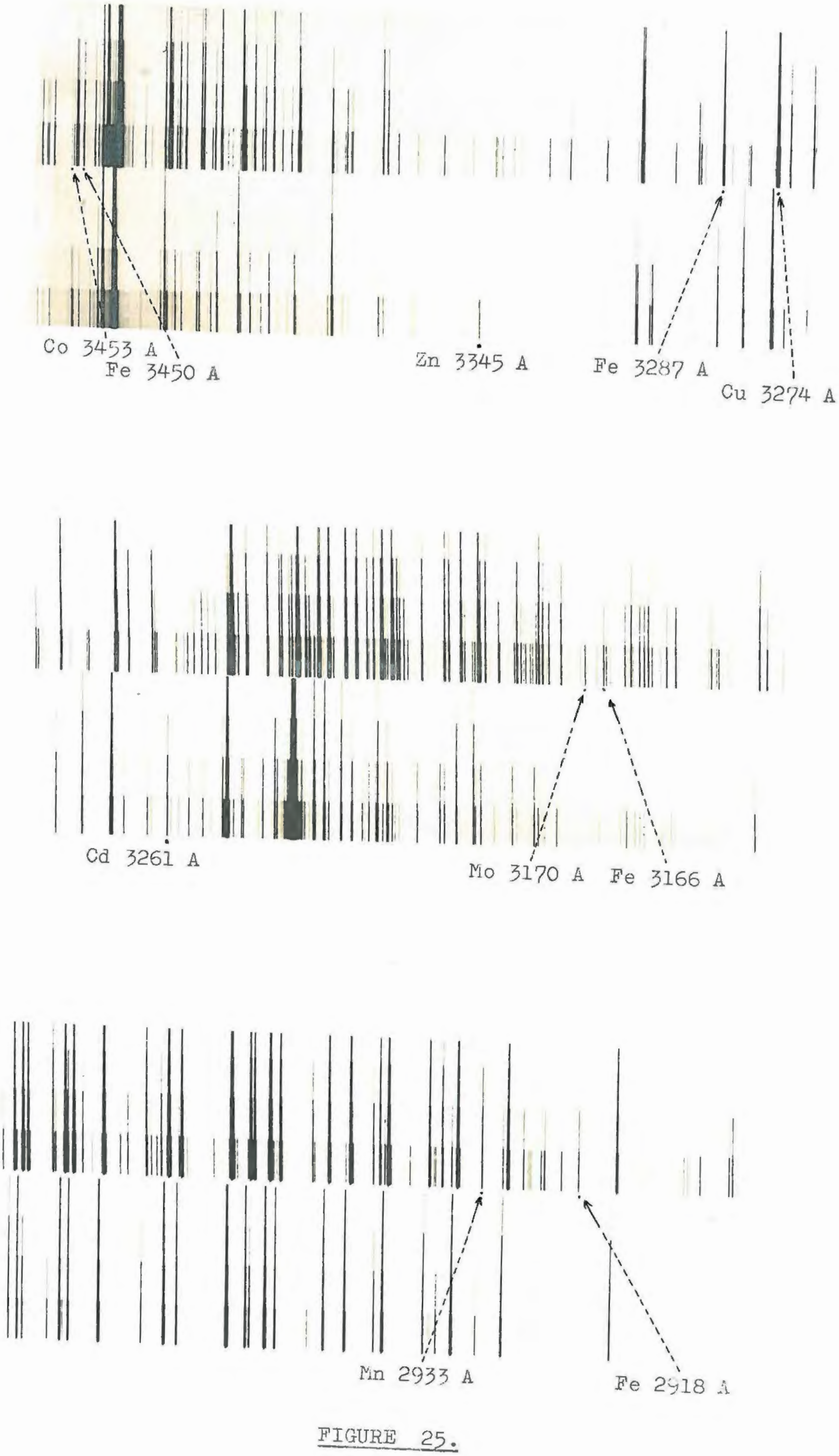

Co 3453 A
Fe 3450 A
Zn 3345 A
Fe 3287 A
Cu 3274 A
Cd 3261 A
Mo 3170 A
Fe 3166 A
Mn 2933 A
Fe 2918 A

FIGURE 25.

The relative intensities of each element and internal standard line were read off from the calibration curve and inserted into the appropriate column of the working sheet. Intensity ratios of each measured step of every analysis pair were obtained with the aid of a slide rule. The mean intensity ratio from a number of steps was noted down under the appropriate heading. A general average of the mean intensity ratios of the four spectra were taken for each plant standard and plotted on 2 cycle vs. 3 cycle logarithmic graph paper against the concentration of the particular element. One working sheet was sufficient to contain all the density measurements and calculated intensity ratios for a duplicate determination.

From the intensity ratios obtained the use of the analysis pair Mn 3044.57 A / Fe 3045.08 A and of Mn 2914.60 A / Fe 2918.03 A resp. was immediately eliminated. The gamma value for these two Manganese lines and hence the calibration curves, differed considerably from the gamma of this region of the spectrum. An explanation for this difference was found in Harrison's Table of Wavelengths (71) in which both lines are classified as "wide and hazy". The deviation of intensity ratios for these two Manganese / Iron analysis pairs from the mean was considerable.

The working curves for all elements were straight lines with more or less unit slope, sometimes exhibiting a slight toe, except in the case of Copper. The working curves for the elements are reproduced in figures 26 and 27.

When using the CuI 3273.96 A / FeI 3286.76 A analysis pair to plot the analytical curve, the slope of this plot was found to be considerably smaller than unity. The working curve is linear up to a value of approx. 10 p.p.m., then it deviates sharply to become nearly parallel to the log. concentration axis. Two important factors may contribute to working curves having slopes differing from unity÷ Firstly, the incomplete extraction of this element, mentioned in section 5.4. and secondly, the reversal of the Cu 3273.96 A line at higher concentrations of Copper. Both factors, however, have opposite effects on the value of the slope, so that it may be concluded that the large deviation from unit slope was due to self-reversal of the analysis line.

The difficulty of determining trace and minor constituents of plants simultaneously when chemical concentration methods are employed, has already been mentioned. This problem is less serious in the case of Manganese, whose line-rich spectrum allows the choice of several spectral lines for analysis. However, Copper has a relatively simpler spectrum, of which only two self-reversed lines, namely Cu 3273.96 A (3000 R) and Cu 3247.54 A (5000 R) may be used at low concentrations of Copper in the concentrate. This was found to be possible by measuring the density of the Cu 3273.96 A line only in the 4th step of the four-stepped spectrogram for amounts above 50 μg. and below 100 μg. Copper in the electrode, corresponding to concentrations of 5 and 10 p.p.m. in 10 g. of plant sample, since the intensity of the Copper line is considerable in this concentration region. However, in practice, the 3rd step of this line was also measured and used in calculating the average intensity, if the density was smaller than approx. 1.5 (corresponding to deflections larger than 1.5). At concentrations lower than 5 p.p.m. more steps can, of course, be measured.

Unfortunately the problem of determining Copper simultaneously with the other trace elements in the concentration range above 10 p.p.m. may only be partially solved by measuring the intensity of the very much weaker, but non-reversed Cu 2824.37 A (1000) line. This line becomes measureable, although only just, at approx. 100 µg. Copper in the electrode, corresponding to 10 p.p.m. Copper in 10 g. of dried plant leaves.

A second solution to the problem would be, for instance, to make the Chloroform extract obtained in the chemical concentration step, up to a certain volume, say 20 ml, and to determine Copper on a small aliquot of 2 ml. However, this would involve separate ignition to the oxides and the recording of a separate spectrum for Copper alone. The alternative mentioned above was not investigated. Relatively larger errors may therefore be encountered in determining concentrations of approx. 10 p.p.m. Copper when duplicate arcings of the complete extract are recorded.

The initial working curve for Molybdenum was found to exhibit a marked toe at low concentrations of Mo. Plant standards 1, 1a, 2, 2a, etc. were used for its construction. The appearance of a toe in a working curve at low concentrations is often due to either or both of two factors÷ The presence of a constant amount of this element in all standards, i.e. contamination of the plant blank, or of one or more of the reagents used in the chemical enrichment procedure. The second factor is the neglect of a correction for background,

/ when measuring the

when measuring the line density of this element. The second set of working curves drawn up, eliminating the plant blank and using only aqueous solutions 1b, 2b etc., revealed that the plant blank was contaminated to the extent of approx. 0.03 p.p.m. Mo. Re-examination of a spectrum obtained from the plant blank led to the discovery of a very low-density Mo 3170.35 A line, which had previously been overlooked.

Although a slight difference was also obtained in the two Zinc working curves,for all the other elements close agreement existed between the two sets of analytical curves.

These observations would suggest that synthesis of plant solution with respect to the major elements is not necessary when preparing plant standards for extraction; aqueous solutions of the trace elements in graded concentrations being sufficient for plotting working curves. In fact, it is preferable to employ aqueous solutions for this purpose, since the danger of contamination with one or more of the elements to be determined is minimised.

The old and the new analytical working curves of the elements, pertaining to fully synthesised plant standards and aqueous solutions of the elements, resp., are compared in figures 26 and 27. All analytical results for the elements were at first obtained from the first set of working curves. Those for Zinc and Molybdenum were subsequently re-calculated using the second set of analytical curves.

The average values of intensity ratios for each element at different concentrations, are listed in table 14. These averages were obtained from four

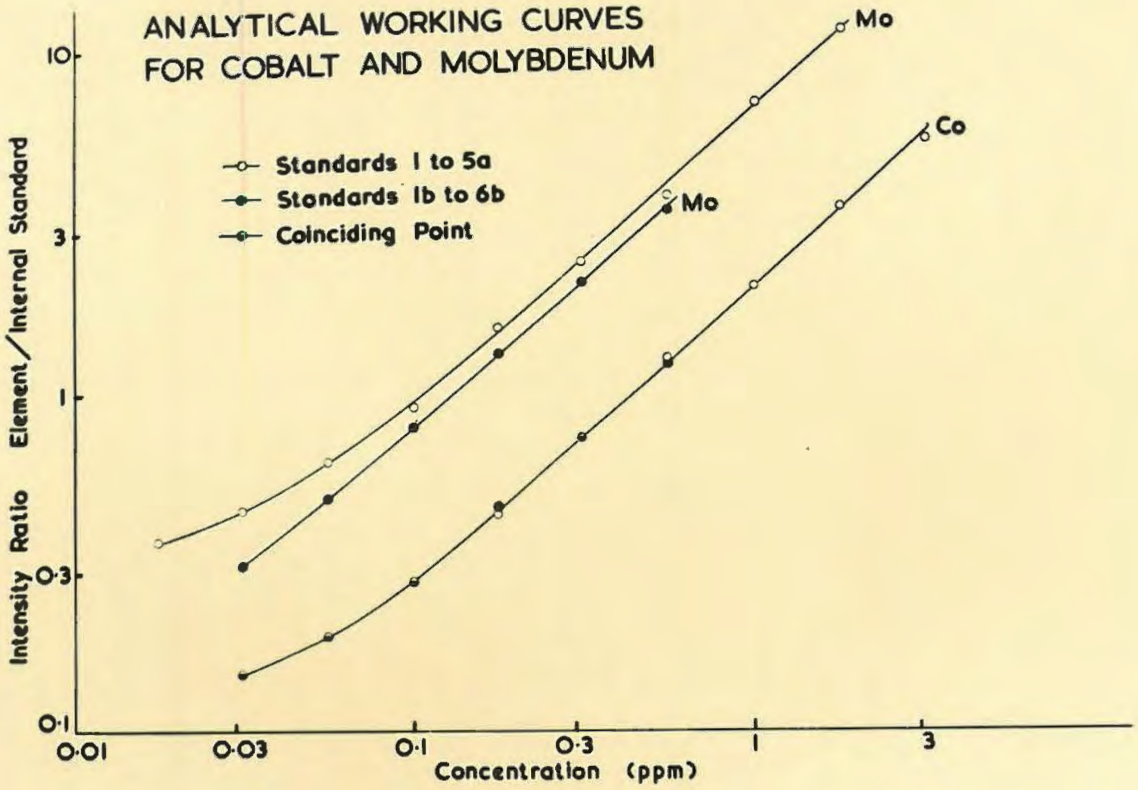


FIGURE 26.

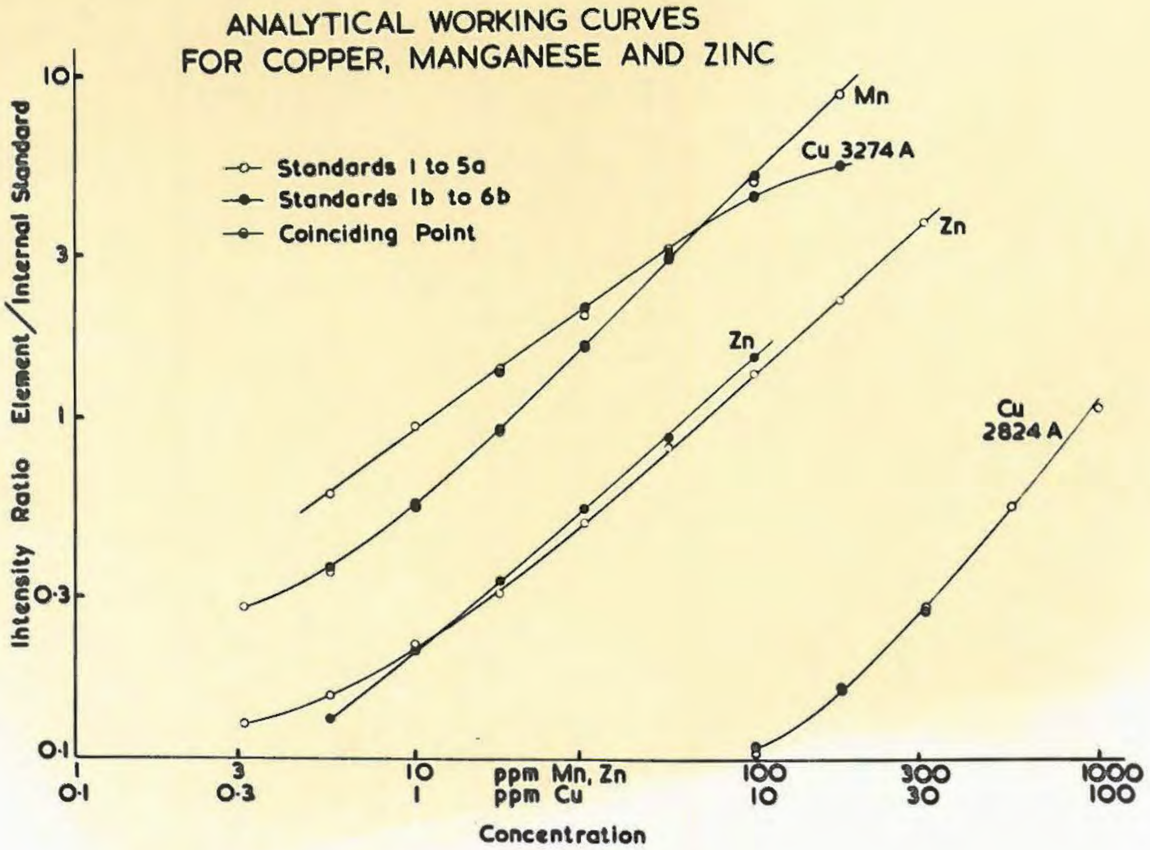


FIGURE 27.

spectra for each concentration level, corresponding to duplicate extractions. Each extract was arced in duplicate. In cases where spectral lines could be measured in all four steps of a spectrogram, the mean of 16 density measurements for each element and internal standard line yielded the average intensity ratio.

TABLE 14.

Average Intensity Ratios used for plotting Working Curves.

Conc. p.p.m.	Cobalt		Molybdenum	
	Old Standards	New Standards	Old Standards	New Standards
3.16	5.40	-	16.43	-
1.77	3.43	-	11.58	-
1.00	2.02	-	6.92	-
0.56	1.25	1.20	3.75	3.40
0.316	0.730	0.735	2.43	2.10
0.177	0.441	0.463	1.55	1.30
0.100	0.283	0.285	0.912	0.795
0.056	0.195	0.195	0.630	0.490
0.0316	0.150	0.150	0.456	0.315
0.0177	-	-	0.369	-
	Zinc		Manganese	
316	3.65	-	16.15	-
177	2.12	-	8.63	-
100	1.32	1.45	4.77	5.00
56	0.841	0.864	2.89	2.85
31.6	0.495	0.540	1.58	1.61
17.7	0.301	0.335	0.895	0.920
10.0	0.220	0.210	0.545	0.559
5.6	0.154	0.132	0.368	0.352
3.16	0.127	-	0.278	-
	Cu 3274 A		Cu 2824 A	
100	-	-	1.06	-
56	-	-	0.547	-
31.6	-	-	0.277	0.268
17.7	5.40	5.45	0.165	0.160
10.0	4.47	4.52	0.105	0.110
5.6	3.02	3.05	-	-
3.16	1.85	2.08	-	-
1.77	1.38	1.35	-	-
1.00	0.928	-	-	-
0.56	0.610	-	-	-

/ 11. SUMMARY OF THE ANALYTICAL PROCEDURE.

11. SUMMARY OF THE ANALYTICAL PROCEDURE.

11.1. Sample Preparation.

Weigh out exactly 10.000 g. (± 0.001 g.) of washed, oven-dried and crushed plant material into a 100 ml silica basin. Ignite the sample overnight at 450°C in a muffle furnace. (Section 1.1.). After cooling, transfer the resultant ash to a 100 ml Pyrex beaker, moisten with a few drops of water and carefully add 20 ml constant boiling point (6.5N) HCl (section 1.2.). Evaporate to dryness on a hotplate (avoid boiling). Take up residue in 10 ml 6.5N HCl and 10 ml water, warm and filter through No. 41 Whatman filter paper into a 100 ml volumetric flask; wash well. Transfer filter paper and contents into a platinum crucible and ignite. Take up ignited residue in approx. 5 ml A.R. HF and 3 to 4 drops conc. A.R. H_2SO_4. Evaporate to SO_3 fumes on a hotplate. Add 2 ml 6.5N HCl and 2 ml water and filter into the same 100 ml volumetric flask mentioned above. Make flask up to volume.

11.2. Iron Determination.

Pipette 1 ml of the plant solution into a 50 ml volumetric flask, add 1 ml 10% Hydroxylamine hydrochloride and 5 ml 1 M Sodium acetate. Pipette 5 ml 0.1% α-α'-Bipyridyl in 0.2N HCl into the flask, make up to volume and allow to stand for 1 hour. Measure the absorbance of the solution on a spectrophotometer at 522 mμ in 4 cm absorption cells. Interpolate the Iron concentration from a calibration curve (Section 3.)

11.3. The Extraction.

From the colorimetric Iron determination

/ calculate the volume

calculate the volume of standard 0.1% Iron solution necessary to produce a final concentration of exactly 5 mg. Iron in the plant solution. Pipette this volume into the remainder of the plant solution (99 ml), now contained in a 250 ml beaker. Add the following reagents (purified as described in the appropriate sections)÷

2 ml 0.01% Cadmium solution
20 ml 12.5% Sulphosalicylic acid (Section 2.4.3.)
2 ml Sodium acetate buffer (Section 2.4.5.)

Using a pH meter, adjust the pH of the solution to 4.8 by adding conc. and dilute NH_4OH drop by drop (Section 2.4.2.). Transfer the solution to a 250 ml separating funnel, heat over an open flame to between 50 and 60°C (the temperature is estimated without a thermometer) and add 10 ml of freshly prepared 2.5% Sodium pyrrolidine dithiocarbamate solution, shaking the funnel vigorously. Cool the separating funnel under running water and dry the outside with a clean towel. Wash the inside of the stem with "pure" water and dry with a rolled-up piece of filter paper (Section 5.3.). Extract the precipitated carbamates once with 10 ml redistilled Chloroform, running the extracts into a 3" long, 1" diameter Pyrex boiling tube and placing the tube into the waterbath (figure 15) between extractions. Add 2 ml 1÷10 (approx. 0.7N) HCl to the solution in the separating funnel and carry out a final extraction with 5 ml of Chloroform. Carefully evaporate the combined extracts on the waterbath (Section 5.5.). Remove the rubber band, place the boiling tube into a labelled 100 ml beaker (figure 16). Dry the extract for 30 to 60 minutes in an oven at 110°C and ash the dried

/ residue overnight

residue overnight in a muffle furnace at 450°C. (Section 5.5.)

11.4. The Spectrographic Analysis.

Weigh out 80 mg. of a 1÷1 mixture of Lithium carbonate and SP 2 graphite on a small piece of glazed paper and transfer to the ashed extract in the boiling tube. Carry out preliminary mixing with a Nickel spatula, used only for this purpose, if necessary scraping the bottom of the tube to loosen as much of the ignited oxides as possible. Transfer as much of the material as possible into a 1" Perspex vial containing a 3/8" Nylon ball pestle and vibrate for 20 seconds in a Wig-L-Bug amalgamator. Fit a plastic funnel over a purified electrode and stamp the sample tightly into the cavity using a small platinum spatula for the transfer of the material and a stainless steel stamper for the filling operation (Section 8.). The equipment used in this procedure is shown in figure 18. Fill two electrodes with the sample. Clamp a purified carbon counter-electrode and the sample electrode in the arc stand, align their position on the scale and move the counter electrode down to touch the rim of the electrode containing the sample. Do not allow a direct contact with the sample itself. Chose a diaphragm opening of 1 mm, select the opening of the Hartmann Diaphragm allowing four steps to be recorded, switch on the step sector and open the spectrograph shutter and the dark slide of the film attachment. Check the plate holder rack reading (42). Heat the electrodes to a red glow with a current of 20 amps for 30 to 40 seconds, decrease the current to 6 amps. Slowly

/ separate the electrodes

separate the electrodes at 6 amps, while at the same time increasing the current gradually to a final value of 10 amps (short-circuited). Expose for 60 seconds from the time of separation of the electrodes, close the spectrograph shutter and rack the film attachment to position 48. After 120 seconds (timed from the separation of the electrodes) open the shutter and expose the second half of the film for 60 seconds. Switch off the current, close the spectrograph shutter and the dark slide of the film attachment. Unclamp the film holder and wind the exposed strip of film into the cassette, clamp it in the slotted tube of the cassette and turn the sprocket wheel 2½ times with the left hand while at the same time turning the base of the cassette in an anticlockwise direction. Again clamp the film into the focal plane and carry out a second set of similar exposures with the duplicate sample electrode, unclamp and turn the sprocket wheel 3½ turns, clamp and cut off the strip of film

Measure out 50 ml each of solutions A and B of the developer Kodak D 153 into the developing tray and add 100 ml of water, check the temperature of the developing solution and the levels of both stop-bath and fixer in their respective trays. Set the automatic timer to 4 minutes. In absolute darkness stretch the exposed length of film across the film holder, immerse it in the developer and start the timer. When the alarm rings, transfer the film into the stop-bath tray for 10 to 15 seconds and then immerse it in Amfix fixing solution for 2 minutes. Place the film, still on its holder, into the automatic washing unit and wash for 30 minutes. After a final rinse with distilled water, remove the film from its holder and stretch it out across the glass surface of the infrared drier. Dry the film for 2 to 3 minutes.

When dry, cut the two exposed lengths of film and insert one strip into the film adaptor of the densitometer. Focus the spectrum and measure the galvo deflections for both analysis and internal standard lines (Part I, section 5). Measure as many steps as possible of the following line pairs in the second spectrogram (i.e. recorded from 120 to 180 seconds.)÷

Co 3453.51 A / Fe 3450.53 A
Cu 3273.96 A / Fe 3286.76 A
Mo 3170.35 A / Fe 3166.44 A
Mn 2933.06 A / Fe 2918.03 A
Cu 2824.37 A / Fe 2835.46 A

and the following line pair in the first spectrogram (recorded from 0 - 60 seconds)÷

Zn 3345.02 A / Cd 3261.06 A.

Record the deflection readings in a working sheet (table 13) and read off the corresponding relative intensities from the appropriate calibration curve. Find the intensity ratio of element to internal standard by means of a slide rule, for all steps of the spectra. Obtain the mean intensity ratio for each analysis pair and interpolate the concentration of the particular element from the appropriate analytical working curve. Take the mean of a duplicate determination.

/ 12. ACCURACY AND REPRODUCIBILITY OF ANALYSIS METHOD

12. ACCURACY AND REPRODUCIBILITY OF ANALYSIS METHOD.

12.1. Reproducibility of the Analysis Method.

In order to examine the errors involved in each step of the spectrographic analysis for trace elements such as sampling, extraction and spectrographic errors, a bulk sample of citrus leaves was obtained from Richardson Citrus Ltd., Kirkwood, and used in all subsequent experiments. This sample of approximately 10 lbs was washed and dried in an air oven and crushed by hand. Dry ashing of the dried plant leaves was effected in stages in large silica basins, taking all the precautions enumerated in section 1.1. For this purpose the muffle furnace had to be placed into a fume cupboard since copious fumes were produced in the early stages of the ashing process, i.e. when the temperature was slowly raised.

From 2072.1 g. of dried plant leaves, 301.3 g. of ash were finally obtained, when most of the organic constituents had been volatilised by completing the ashing for 48 hours at a controlled temperature of 450°C.

The ash content of the sample of citrus leaves corresponded to 14.541%, so that for each solvent extraction 1.4541 g. (± 0.0005 g.) were weighed out and treated as described under the appropriate heading.

12.1.1. The Spectrographic Error÷ Elimination of Sampling and Extraction Errors.

This series of experiments (Series I), was designed to investigate purely spectrographic errors, i.e. the reproducibility of the final, instrumental analysis step, which included the following sources of errors:

1.) Mixing of the ignited extract with 80 mg. buffer,
2.) Filling of electrodes,
3.) Excitation of the sample electrodes in a d.c. arc, probably the most serious source of error,
4.) Recording and Development of spectra,
5.) Densitometric evaluation of spectral lines, and
6.) Interpolation errors involved in converting densitometer deflection readings into relative intensities and intensity ratios, and reading off concentrations from the working curves.

Some of these errors have already been investigated separately and reported on, such as the reproducibility of intensity ratios with time of mixing (Part II, section 2), when the sample was mixed in an agate mortar. The effectiveness of mechanical mixing using plastic vials and ball pestles and its dependence on time of vibration in a Wig-L-Bug amalgamator was not examined. It is claimed that the use of plastic materials, such as Perspex vials and Nylon ball pestles leads to the generation of electrostatic charges which prevent efficient homogenisation of some samples.

The reproducibility of filling electrodes, as indicated by the standard deviation of the amount packed into the electrode, was investigated in Part III, section 8, while the reproducibility of development, measured by the standard deviation of the contrast factor gamma, was examined in Part II, section 1.

For this series of experiments, fifteen times the weight of plant ash corresponding to 10 g. of dried citrus leaves, i.e. 15 × 1.4541 g. = 21.8115 g. were weighed out on glazed paper and dissolved

/ according to Piper's

according to Piper's method, described in section 1.2. The filtrate was diluted to 750 ml, using 500 and 250 ml volumetric flasks. 50 ml of filtrate therefore corresponded to 10 g. of dried plant material. After mixing the contents of the flasks in a 1 litre beaker, exactly 625 ml were measured out, using volumetric flasks and pipettes and transferred to a second one litre beaker. The Iron concentration in the plant solution thus obtained was determined colorimetrically (see section 3) on three 1 ml aliquots of the remaining 125 ml. The determinations yielded a mean of 195 p.p.m. Iron, calculated on 10 g. of dried plant leaves. This corresponded to 1.95 mg. Iron in 1.4541 g. of plant ash. Therefore, for each aliquot corresponding to the above amount of plant ash, 3.05 mg. Iron had to be added. In other words, 12½ × 3.05 ml of 0.1% Iron solution (= 38.1 ml) were buretted into 625 ml of plant solution. Twelve and one half times the following amounts were added÷

2 ml 0.01% Cadmium solution,
2 ml Sodium acetate buffer, and
20 ml 12½% Sulphosalicylic acid,

and the resultant solution was diluted to approximately 1500 ml. The pH of this solution was adjusted to 4.8 with concentrated (8N) re-distilled Ammonium hydroxide. The dilution was carried out to avoid any possible formation of an emulsion in the subsequent solvent extraction, because of the relatively high concentrations of macro-elements (see section 5.3.). The enrichment of trace elements was effected in two 1 litre separating funnels, adding 70 ml of freshly prepared 2.5% Sodium pyrrolidine dithiocarbamate solution to each funnel to precipitate the trace metals. The organic complexes were extracted with 20 ml portions of Chloroform, the extracts combined in a 250 ml volumetric flask and diluted to the mark with redistilled Chloroform.

Twelve aliquots of the Chloroform extract of 20 ml each were pipetted into Pyrex boiling tubes (section 5.5.), the Chloroform evaporated off and the remaining organic metal complexes ashed overnight at 450°C.

To each ignited extract 80 mg. of a 1÷1 Lithium carbonate ÷ SP 2 graphite buffer were added, superficially mixed in the test tube with a nickel spatula and transferred to 1 inch plastic vials containing one 3/8 inch Nylon ball pestle. Final mixing was effected by shaking each vial for 20 seconds in a Wig-L-Bug amalgamator.

Two electrodes could be filled comfortably with the contents of one vial, with some material to spare.

Twenty-four sets of spectra were recorded, using the excitation conditions given in section 9.1. and then evaluated. The results obtained, in p.p.m. of the respective element, are tabulated in table 16. The mean of a duplicate analysis was used in calculating the standard deviation from the overall mean of all 12 results. The results of a duplicate analysis were recorded on one working sheet, similar to that described in section 10. An example of a typical working sheet used in the calculations is reproduced in table 15. A typical spectrogram obtained in this series of experiments (Series I), and showing the analysis and internal standard lines used, is reproduced in figure 25.

/ Table 15.

TABLE 15.

Example of a Working Sheet.
(Series III, Exposures 33 a and 33 b)

Step	Element	Int. Stand.	Element		Int. Stand.		Intensity Ratio	Mean Intensity Ratio	conc. p.p.m.
			θ_1	I_1	θ_2	I_2			
1	Co 3453	Fe 3450	36·1	2·46	8·2	8·75	·278		
2	"	"	47·4	1·225	21·3	4·20	·282	·280	·095
1	"	"	35·8	2·50	7·8	9·10	·275		
2	"	"	47·1	1·280	13·2	4·74	·270	·272	·090
1	Zn 3345	Cd 3261	24·7	3·77	9·5	9·15	·413		
2	"	"	42·3	1·775	21·2	4·35	·408	·410	22·7
3	"	"							
1	"	"	24·7	3·77	9·9	8·80	·428		
2	"	"	42·7	1·73	22·2	4·17	·415	·421	23·3
3	"	"							
3	Cu 3274	Fe 3287	2·25	42·0	7·2	11·95	3·51		
4	"	"	4·60	18·5	16·6	5·48	3·38	3·44	6·3
3	"	"	2·10	45·5	6·2	13·80	3·30		
4	"	"	4·30	19·9	15·3	5·90	3·37	3·33	6·1
1	Mo 3170	Fe 3166	21·5	4·82	22·6	4·57	1·05		
2	"	"	38·4	2·28	38·9	2·22	1·03	1·04	·133
1	"	"	20·6	5·05	20·9	4·98	1·01		
2	"	"	38·2	2·31	38·7	2·25	1·03	1·02	·132
1	Mn 2933	Fe 2918	8·3	14·3	12·1	9·20	1·55		
2	"	"	16·8	6·40	24·6	4·10	1·56	1·55	30·5
3	"	"	31·5	3·06	40·6	1·97	1·55		
1	"	"	8·0	14·9	12·1	9·20	1·62		
2	"	"	16·7	6·43	25·1	4·02	1·60	1·61	32·0
3	"	"	31·7	3·01	41·5	1·37	1·61		
1	Cu 2824	Fe 2835	/	/	/	/			
2	"	"	/	/	/	/			
1	"	"	/	/	/	/			
2	"	"	/	/	/	/			

/ TABLE 16.

TABLE 16.

Results of Series I - Concentration of Trace Elements.

COBALT		ZINC		COPPER	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.088 0.088	0.0880	19.2 19.3	19.25	6.9 6.1	6.50
0.086 0.089	0.0875	18.1 17.2	17.95	6.0 6.5	6.25
0.086 0.089	0.0875	17.0 19.0	18.00	6.4 6.1	6.25
0.085 0.086	0.0855	18.6 18.5	18.55	6.1 6.5	6.30
0.085 0.080	0.0825	19.9 17.8	18.35	5.7 6.3	6.00
0.077 0.080	0.0795	19.3 15.7	17.50	5.5 6.3	5.90
0.080 0.084	0.0820	17.7 17.3	17.50	7.4 6.4	6.90
0.085 0.086	0.0855	14.4 19.8	17.10	5.8 5.7	5.75
0.090 0.086	0.0880	14.7 19.3	17.00	6.3 6.9	6.60
0.087 0.086	0.0865	17.4 17.1	17.25	6.8 6.0	6.40
0.086 0.088	0.0870	18.7 19.2	18.95	(10.0) 6.8	6.80
0.087 0.088	0.0875	15.9 17.9	16.90	5.5 6.1	5.80
Mean÷	0.0855		17.86		6.29
Standard Deviation÷	0.00275		0.771		0.376
Coefficient of variation÷	3.21%		4.31%		6.00%

/ TABLE 16. (contd.)

TABLE 16.

Results of Series I - Concentration of Trace Elements.

COBALT		ZINC		COPPER	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.088 0.088	0.0880	19.2 19.3	19.25	6.9 6.1	6.50
0.086 0.089	0.0875	18.1 17.2	17.95	6.0 6.5	6.25
0.086 0.089	0.0875	17.0 19.0	18.00	6.4 6.1	6.25
0.085 0.086	0.0855	18.6 18.5	18.55	6.1 6.5	6.30
0.085 0.080	0.0825	19.9 17.8	18.35	5.7 6.3	6.00
0.077 0.080	0.0795	19.3 15.7	17.50	5.5 6.3	5.90
0.080 0.084	0.0820	17.7 17.3	17.50	7.4 6.4	6.90
0.085 0.086	0.0855	14.4 19.8	17.10	5.8 5.7	5.75
0.090 0.086	0.0880	14.7 19.3	17.00	6.3 6.9	6.60
0.087 0.086	0.0865	17.4 17.1	17.25	6.8 6.0	6.40
0.086 0.088	0.0870	18.7 19.2	18.95	(10.0) 6.8	6.80
0.087 0.088	0.0875	15.9 17.9	16.90	5.5 6.1	5.80
Mean÷	0.0855		17.86		6.29
Standard Deviation÷	0.00275		0.771		0.376
Coefficient of variation÷	3.21%		4.31%		6.00%

/ TABLE 16. (contd.)

TABLE 16. (contd.)

Results of Series I - Concentration of Trace Elements.

MOLYBDENUM		MANGANESE	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.113 0.130	0.1215	30.0 28.5	29.25
0.140 0.127	0.1335	29.0 31.0	30.00
0.140 0.123	0.1315	30.5 32.0	31.25
0.126 0.136	0.1310	30.8 29.0	29.90
0.120 0.150	0.1350	34.3 31.7	33.00
0.146 0.150	0.1480	32.0 29.3	30.65
0.127 0.142	0.1355	37.2 31.0	34.10
0.150 0.150	0.1500	29.2 29.8	29.50
0.118 0.140	0.1290	31.5 31.5	31.50
0.133 0.127	0.1300	33.0 31.3	32.15
(0.073) 0.129	0.1290	(55.0) 29.7	29.70
0.132 0.143	0.1395	33.2 31.5	32.35
Mean÷	0.1345		31.18
Standard Deviation÷	0.00809		1.546
Coefficient of Variation÷	6.02%		4.96%

The standard deviations from the mean concentration, calculated on 11 degrees of freedom, for each element, are shown in table 16. The coefficient of variation, which is a measure of the reproducibility of the analytical results, is also listed in the same table. The reproducibility of the instrument-

/ -al analysis step

al analysis step therefore lies between 3.2% (for Cobalt) and 6.0% (for Molybdenum and Copper)

During the arcing of one of the electrodes of this series considerable spluttering occurred, the results obtained from the spectrum are given in brackets. They were rejected when taking the mean of a duplicate analysis. It was noted in cases where the sample was ejected during the later stages of the burn, that the results for Manganese and Copper were always considerably higher and those for Molybdenum appreciably lower than those obtainable from a smoothly burning electrode, while those for Cobalt and Zinc usually remained unaffected, within the limits of experimental error.

12.1.2. The Spectrographic and Extraction Errors÷ Elimination of the Sampling Error.

The following series of experiments (Series II) was designed in order to determine the variabilities inherent in the combined extraction and spectrographic steps. The results of this series will therefore give a measure of the reproducibility of the complete analytical method, including the chemical concentration step.

For this purpose aliquots were taken of a standard plant solution and separate extractions carried out. The details of the procedure will be described briefly.

Since a certain quantity of the plant solution was subsequently required for tests of the accuracy of the analytical method (section 12.2.), 25 times 1.4541 g. of plant ash were dissolved in two lots of 21.8115 g. each as described in section 12.1.1. The filtrates were diluted to 1250 ml in 500 and 250 ml volumetric flasks, so that again 50 ml of the plant solution corresponded to 10 g. of dried plant leaves. After mixing the solutions in a 2 litre beaker, the Iron concentration was again determined colorimetrically on three 1 ml aliquots, yielding a mean of 193 p.p.m. Iron.

Twelve 50 ml aliquots of the plant solution were pipetted into 250 ml beakers and the following reagents were added to each beaker÷

3.07 ml 0.1% Iron solution,
2.0 ml 0.01% Cadmium solution,
2.0 ml Sodium acetate buffer and
20 ml 12.5% Sulphosalicylic acid.

After dilution to approximately 150 ml, each aliquot was extracted separately in 250 ml separating funnels and the extracts were dried, ashed and arced in duplicate as described in section 12.1.1.

The results of the evaluation of spectrograms of series II are listed in table 17.

When comparing the results of the standard deviations from the mean and especially the coefficients of variation of series II with the corresponding values of series I, it will be apparent that a decrease in the reproducibility was obtained. This decrease was in all cases very small and insignificant and it may therefore be concluded that the chemical enrichment procedure was accurate and reproducible, if all samples are treated in exactly the same way.

Scharrer and Judel (24), using a similar enrichment procedure, but different excitation conditions, reported the following coefficients of variation, obtained in duplicate determinations on 140 plant samples, The values of series II are shown in brackets.

Co÷ 3.2% (3.5%)
Zn÷ 4.6% (4.5%)
Cu÷ 4.9% (6.5%)
Mo÷ 2.5% (6.7%)
Mn÷ 2.8% (5.7%)

TABLE 17.

Results of Series II.
Concentration of Trace Elements.

COBALT		ZINC		COPPER	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.097 0.082	0.0895	18.5 17.8	18.15	6.1 5.3	5.70
0.085 0.087	0.0860	19.0 17.7	18.35	5.8 6.4	6.10
0.084 0.082	0.0830	16.8 18.0	17.40	6.2 7.0	6.60
0.085 0.083	0.0840	15.3 21.5	18.40	8.5 4.9	6.70
0.081 0.084	0.0825	17.2 19.8	18.50	5.5 5.9	5.70
0.078 0.084	0.0810	17.8 20.0	18.90	7.1 5.8	6.45
0.088 0.088	0.0880	19.5 17.3	18.40	5.6 6.4	6.00
0.087 0.083	0.0850	18.7 16.7	17.70	5.8 5.9	5.85
0.078 0.080	0.0790	19.0 17.8	18.40	6.2 5.6	5.90
0.082 0.079	0.0805	17.3 17.3	17.30	6.5 6.0	6.25
0.090 0.078	0.0840	19.8 19.8	19.80	6.5 6.6	6.55
0.087 0.079	0.0830	19.0 21.0	20.00	7.7 6.0	6.85
Mean÷	0.0846		18.44		6.22
Standard Deviation÷	0.00296		0.828		0.406
Coefficient of Variation÷	3.50%		4.49%		6.53%

/ TABLE 17. (contd.)

TABLE 16. (contd.)

Results of Series II.
Concentration of Trace Elements.

MOLYBDENUM		MANGANESE	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.136 0.138	0.1370	32.5 26.8	29.65
0.145 0.137	0.1410	31.5 29.0	30.25
0.130 0.150	0.1400	32.5 35.5	34.00
0.142 0.137	0.1395	29.5 30.0	29.75
0.143 0.152	0.1485	32.5 28.5	30.50
0.126 0.142	0.1340	31.5 28.0	29.75
0.118 0.142	0.1300	33.0 30.0	31.50
0.093 0.157	0.1250	38.0 31.0	34.50
0.123 0.145	0.1340	33.0 26.5	31.50
0.143 0.142	0.1425	33.0 32.5	32.75
0.123 0.107	0.1150	34.0 32.5	33.25
0.136 0.118	0.1270	26.8 32.0	29.40
Mean÷	0.1345		31.40
Standard Deviation÷	0.00901		1.801
Coefficient of Variation÷	6.70%		5.74%

In an earlier publication (51) the same authors examined the reproducibility of their method by carrying out 33 duplicate analyses on a soil sample. The following coefficients of variation were obtained÷

Co÷ 1.8% (3.5%)
Zn÷ 5.0% (4.5%)
Cu÷ 3.2% (6.5%)
Mo÷ 5.4% (6.7%)
Mn÷ 7.1% (5.7%)

/ Scharrer and Judel

Scharrer and Judel did not, however, state what concentrations these standard deviations referred to. Considering the very low concentrations of some of the trace elements determined, e.g. Cobalt and Molybdenum, the reproducibility of the analytical method as judged from the results of series II, was good.

Examination of several sets of duplicate determinations of some of the elements, particularly Zinc, reveals that, although a single exposure may vary considerably from its duplicate, the mean of the two usually falls within the standard deviation of the overall average for this particular element. This is exemplified by the coefficient of variation obtained on considering each single determination, which is usually appreciably higher than the one calculated on duplicate exposures. In mixing just sufficient buffer with the ashed extracts to fill two electrodes, with a small amount to spare, inefficiencies in mixing, such as possible separation of some trace metal oxides due to electrostatic charges, were virtually eliminated.

12.1.3. Reproducibility of the Analytical Method÷ Inclusion of the Sampling Error.

In order to examine the magnitude of the error involved in sampling and dissolving portions of plant ash, series III of 24 exposures was evaluated. A different sample of plant leaves was ashed and used in this series of experiments. Twelve portions of the plant ash, corresponding to 10 g. of plant leaves, were separately dissolved according to Piper's method and were extracted and arced in duplicate. The results of this series are shown in table 18.

/ TABLE 18.

TABLE 18.

Results of Series III. Concentration of Trace Elements.

COBALT		ZINC		COPPER	
cond. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.082 0.089	0.0855	24.5 22.2	23.35	6.4 5.9	6.15
0.083 0.092	0.0875	22.5 22.2	22.35	6.2 6.5	6.35
0.088 -	0.0880	21.3 -	21.30	6.7 -	6.70
0.086 0.090	0.0880	25.7 21.5	23.60	5.8 6.2	6.00
0.083 0.084	0.0820	21.0 25.0	23.00	6.9 6.7	6.80
0.098 0.096	0.0970	21.5 21.3	21.40	7.3 7.2	7.25
0.093 0.090	0.0915	18.3 24.5	21.40	7.2 6.6	6.90
0.090 0.092	0.0910	20.0 22.3	21.15	7.8 6.2	7.00
0.095 0.090	0.0925	22.7 23.3	23.00	6.3 6.1	6.20
0.088 0.089	0.0885	20.2 26.5	23.50	6.4 7.2	6.80
0.091 0.086	0.0885	21.0 26.0	23.50	6.1 7.0	6.55
0.095 0.083	0.0890	21.2 18.7	19.95	7.7 7.5	7.60
Mean÷	0.0891		22.28		6.69
Standard Deviation÷	0.00372		1.193		0.472
Coefficient of Variation÷	4.18%		5.35%		7.05%

/ TABLE 18. (contd.)

TABLE 18. (contd.)

Results of Series III. Concentration of Trace Elements.

MOLYBDENUM		MANGANESE	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.125 0.140	0.1325	35.5 29.5	32.50
0.118 0.134	0.1260	39.0 28.5	33.75
0.115 -	0.1150	30.8 -	30.80
0.118 0.137	0.1275	34.0 33.5	33.75
0.128 0.145	0.1365	33.5 29.0	31.25
0.104 0.117	0.1105	32.5 30.5	31.50
0.130 0.148	0.1390	32.7 29.8	31.25
0.146 0.130	0.1380	30.5 28.5	29.50
0.133 0.132	0.1325	30.5 32.0	31.25
0.127 0.145	0.1360	35.0 28.5	31.75
0.123 0.115	0.1190	31.5 30.0	30.75
0.110 0.122	0.1160	41.0 32.5	36.75

	Molybdenum	Manganese
Mean÷	0.1274	32.07
Standard Deviation÷	0.00999	1.993
Coefficient of Variation÷	7.83%	6.21%

The coefficients of variation for all elements in series III are higher than the corresponding values for series II, and in some cases the difference is considerable. Therefore, appreciable errors may be incurred in sampling and dissolving plant ash. The

/ ashed plant material

ashed plant material did not appear to be homogeneous, probably due to the method employed in the destruction of the organic matter. Some of the centre ribs of the leaves, which had not been cut away before ashing, were only charred and appeared as a black residue on the filter paper.

12.2. The Accuracy of the Analytical Method.

The accuracy of the spectrographic analysis method was tested firstly by standard additions of the respective elements and secondly by independent analyses of some of the trace elements.

A test of the accuracy of a particular analysis method is only significant if it is performed at concentrations of the elements equal to or approximating those of the actual plant sample. This is particularly true in spectrographic trace analysis, when in spite of chemical enrichment procedures the densities of spectral lines of some elements may be very low and an increase in the concentration of these elements would lead to corresponding increases in the density of these analysis lines. Generally speaking it is a fact, that stronger signals can be measured more accurately than weaker ones. In spectrography this is equally true, the signal being the density of a line. Recovery tests performed on a plant sample to which known amounts of trace metals have been added are not as significant as those carried out on fractions of the sample which received appropriate amounts of the trace metals to approximate the original concentration in the complete sample. This was borne in mind in the following two series of experiments, series IVa and IVb.

The plant solution, prepared and analysed to determine the reproducibility of the analytical method (section 12.1.2.) was employed for the recovery tests. Appropriate volumes of standard solutions of the trace metals were added to aliquots of the plant solution, which contained one half and one third of the original trace element concentrations, respectively.

Approximately the same quantities of trace metals contained in 250 ml of the plant solution were added to this volume and diluted to 500 ml in a volumetric flask. Six 50 ml aliquots were extracted and arced in duplicate (Series IVa). The trace element solutions added were the same as those employed in making up the standard solutions for the second set of working curves (Section 4.). A brief summary of the calculation is shown in table 19.

TABLE 19.

Quantities of Trace Elements in Plant Solution IVa.

	Original conc. (p.p.m.)	In 250 ml, before Addition (μg.)	Quantity added (μg.)	In 500 ml, after Addition (μg.)	New conc. (p.p.m.)
Co	0.0846	4.230	5.0	9.230	0.0923
Mo	0.1345	6.725	5.0	11.725	0.1173
Cu	6.22	311.0	300.0	611.0	6.11
Zn	18.44	922.0	1000.0	1922.0	19.22
Mn	31.40	1570.0	1500.0	3070.0	30.70
Fe	193	9.65 mg.	40.35mg.	50.0 mg.	500

A similar procedure was adopted for series IVb. 167 ml of plant solution were buretted into a 500 ml flask and diluted to the mark after measured volumes of the respective trace element solutions had been added. The calculations are shown in table 20.

/ TABLE 20.

TABLE 20.

Quantities of Trace Elements in Plant Solution IVb.

	Original conc. (p.p.m.)	In 167 ml, before Addition (μg.)	Quantity added (μg.)	In 500 ml, before Addition (μg.)	New conc. (p.p.m.)
Co	0.0846	2.82	7.5	10.32	0.1032
Mo	0.1345	4.48	7.5	11.98	0.1198
Cu	6.22	207.0	400.0	607.0	6.07
Zn	18.44	615.0	1500.0	2115.0	21.15
Mn	31.40	1047.0	2000.0	3047.0	30.47
Fe	193	6.43 mg.	43.57mg.	50.0 mg.	500

The results of the spectrographic analyses of the two standard addition series IVa and IVb are summarised in tables 21 and 22. The mean concentrations obtained from six duplicate arcings were compared with the predicted values of each series. The averages usually agreed well with the calculated concentrations; in most cases they were within the standard deviation, except for Zinc in the second and Molybdenum in the first series. This might have been due to inaccuracies in measuring the volumes of standard solutions of these elements. The percentage agreement is shown for each element.

/ TABLE 21.

TABLE 21.

Results of Series IVa.

Concentration of Trace Elements.

COBALT		ZINC		COPPER	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.091 0.088	0.0895	17.3 19.7	18.50	6.5 5.8	6.15
0.092 0.096	0.0940	21.0 21.2	21.10	6.0 6.4	6.20
0.102 0.092	0.0970	19.7 18.2	18.95	7.3 6.3	7.05
0.095 0.088	0.0915	18.6 20.8	19.70	5.6 6.1	5.85
0.093 0.100	0.0965	19.2 19.3	19.25	5.2 6.9	6.05
0.102 0.102	0.1020	21.2 20.3	20.75	6.5 6.3	6.40

Mean÷	0.0951 ± 0.044	19.71 ± 1.03	6.28 ± 0.42
Predicted÷	0.0923	19.22	6.11
Agreement÷	3.0%	2.5%	2.7%

MOLYBDENUM		MANGANESE	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.112 0.127	0.1195	34.0 29.2	31.60
0.142 0.135	0.1385	32.0 28.5	30.25
0.133 0.112	0.1225	33.0 33.0	33.00
0.120 0.118	0.1190	29.5 29.5	29.50
0.130 0.125	0.1275	32.0 32.5	32.25
0.118 0.133	0.1255	38.0 27.5	32.75

Mean÷	0.1254 ± 0.072	31.56 ± 1.48
Predicted÷	0.1173	30.70
Agreement÷	6.7%	2.7%

/ TABLE 22.

TABLE 22.

Results of Series IVb.
Concentration of Trace Elements.

COBALT		ZINC		COPPER	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.096 0.100	0.0980	21.5 18.2	19.85	7.9 5.7	6.80
0.103 0.093	0.0980	19.5 18.7	19.10	6.2 5.9	6.05
0.114 0.102	0.1080	17.7 19.8	18.75	6.7 5.6	6.15
0.098 0.099	0.0985	19.8 18.5	19.15	6.0 5.7	5.85
0.100 0.102	0.1010	22.3 19.9	22.10	7.0 6.4	6.70
0.100 0.090	0.0950	19.5 21.5	20.50	5.8 5.7	5.75

Mean÷	0.0998 ± 0.044	19.83 ± 1.07	6.25 ± 0.45
Predicted÷	0.1032	21.15	6.07
Agreement÷	3.3%	6.4%	2.9%

MOLYBDENUM		MANGANESE	
conc. p.p.m.	Mean conc. p.p.m.	conc. p.p.m.	Mean conc. p.p.m.
0.102 0.113	0.1075	29.5 29.0	29.25
0.118 0.127	0.1225	29.0 34.0	31.50
0.128 0.116	0.1230	25.0 31.6	28.30
0.126 0.118	0.1230	38.2 24.2	31.20
0.109 0.102	0.1055	30.0 32.0	31.00
0.124 0.117	0.1205	28.0 29.7	28.85

Mean÷	0.1170 ± 0.082	30.02 ± 1.38
Predicted÷	0.1198	30.61
Agreement÷	2.4%	2.0%

/ The two solutions

The two solutions from series IVa and IVb were analysed independently for Copper, Manganese and Zinc in the Soil Analysis Laboratory of the Chemistry Department, Rhodes University. Copper was determined colorimetrically, using Diethyldithiocarbamate while atomic absorption methods were employed for the estimation of Manganese and Zinc, using a Hilger Uvispec spectrophotometer with atomic absorption attachment. The results are compared with the spectrographic data in table 23.

TABLE 23.

Comparison of Analytical Results (in p.p.m.)

	Spectrographic	Atomic Absorption or Colorimetric
Cu	6.28	5.8
	6.25	6.0
Mn	31.56	29.0
	30.02	28.0
Zn	19.71	19.0
	19.83	21.6

The agreement of the analyses, performed by independent methods and operators was in the region of 4 - 10%.

/ PART IV.

PART IV.

1. EXCITATION IN A CONTROLLED ATMOSPHERE.

In an attempt to increase the sensitivity and accuracy of spectrographic d.c. arc analysis various methods have been developed and perfected in the past. Increased sensitivity is particularly important in trace element determinations. It implies, among other factors, the raising of the ratio of the intensity of an analytical spectral line to that of the background associated with it. The reduction of background by incorporating the sample in an alkali matrix, thus lowering the temperature of the arc with an associated lowering of the density of Cyanogen band spectra, has already been discussed. This matrix is also effective in producing absolute increases in the emission of some elements, particularly spectral lines of low excitation potential being enhanced. The two factors which are mainly responsible for the relatively low precision of direct current arc analysis, as compared to e.g. the high voltage spark excitation, are the irreproducibility of the arc, which includes arc wandering, and the selective volatilisation of elements from the electrode. Several suggestions have been put forward, and successfully applied, to improve the reproducibility of arc excitation, such as slowly rotating the sample electrode, confining the arc in a rotating magnetic field, or using double arcs. One of the most successful developments has proved to be stabilisation of the arc discharge by blowing an annular curtain of gas upwards around the arc column to restrain arc wander.

This was first suggested by Stallwood (72) and the device, known as the Stallwood jet, is now used in many laboratories. Hawley and co-workers (73), using a Stallwood air jet, claimed that sharper lines and reduced Cyanogen band spectra were obtained, selective volatilisation was eliminated and the linearity of working curves was improved. Another advantage of surrounding the sample electrode with a stream of gas is a reduction or even elimination of self-absorption of the so-called self-reversed lines of elements. Self-reversal occurs mainly at higher concentrations of the elements in the electrode, when atoms in low energy states, present in the cooler outer fringe of the arc column, absorb some of the radiation given off by excited atoms in the hot central part of the column. It has been shown earlier that this phenomenon was responsible for the limitation in the use of the Cu 3274 A line for the analysis of this element when amounts exceeding approximately 100 μg. Copper were present in the electrode. An annular jet of gas reduces the concentration of these low energy state atoms in the outer layers of the arc discharge; thereby minimising the degree of self-reversal of a line, which may then be used to determine higher concentrations of the particular element. An improved volatilisation of the elements is attributed to the stream of gas around the sample electrode that continually cools the lower portion of the electrode. This temperature gradient permits volatilisation of only the uppermost portion of the sample. A similar solution was suggested by Hoens and Smit (74) who placed a small collar with cooling water, closely fitting around the sample electrode at a constant distance below the burning spot.

An important reason for employing an annular stream of gas surrounding the arc column is the reduction of background associated with burning graphite or carbon electrodes in air, which is achieved by excluding atmospheric Nitrogen from the discharge. Cyanogen band spectra are thereby reduced considerably in intensity. Several gases have been used in conjunction with a Stallwood jet, e.g. Oxygen (75), Carbon dioxide (76), pure Argon (77) and mixtures of Argon and Oxygen (78). The last two cases will be discussed more fully later.

In order to provide more constant surroundings and to exclude atmospheric Nitrogen more effectively from the arc discharge, the Stallwood jet is often enclosed in a special arcing chamber.

The effect of noble gas atmospheres, e.g. Argon and Helium on d.c. arc discharges has been the subject of many investigations. Vallee and co-workers and Thiers (79, 80) reported that the background of spectra was substantially lowered, that the rates of volatilisation of the elements were altered considerably (81, 82) and that the absolute intensity was appreciably increased for some elements (83).

In order to investigate the increase in sensitivity and accuracy provided by using different atmospheres, a Stallwood type jet in conjunction with an arc chamber was fitted to the arc stand described in Part I. Both jet and chamber, shown in figures 28 and 29, were designed in the National Physical Laboratories of the South African Council for

/ Scientific and

ARC CHAMBER

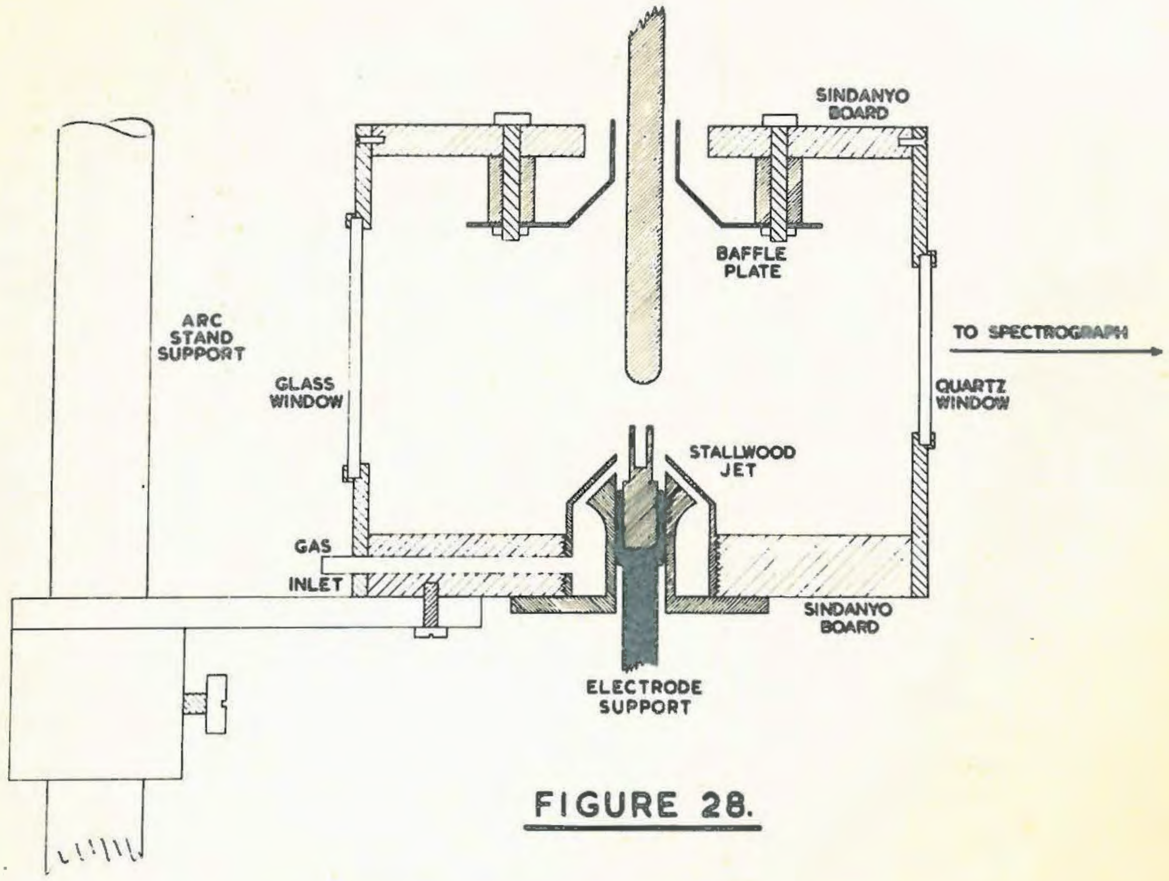


FIGURE 28.

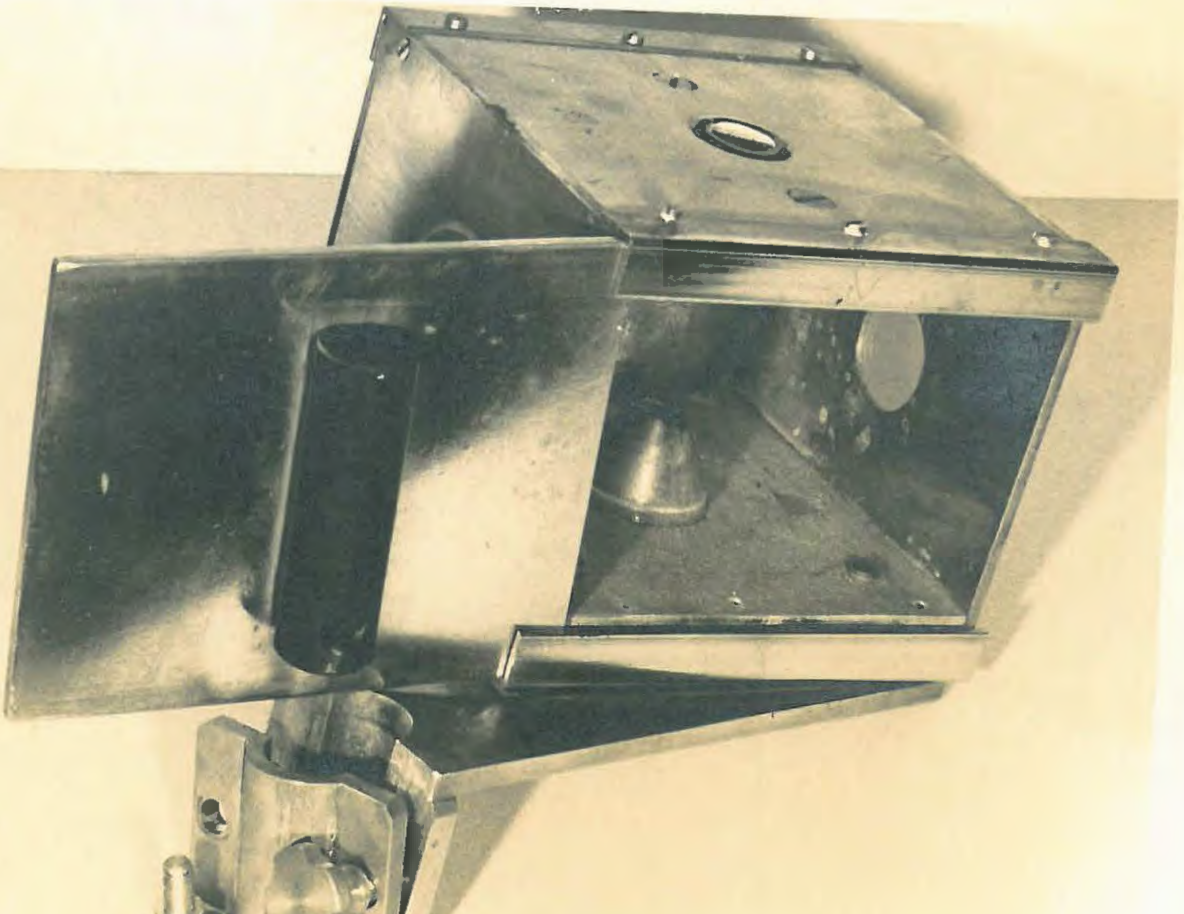


FIGURE 29.

Scientific and Industrial Research and constructed in the workshop of the Chemistry Department, Rhodes University. Argon and Oxygen, and mixtures of the two gases, were investigated.

The experiments performed in this section were only designed to assess the possibilities of using rare gas environments for trace element analysis. Since the necessary equipment was only available during the later stages of the project, no actual analysis on a plant sample was carried out.

A large quantity of a synthetic plant extract, containing approximately the same concentrations of trace elements as the actual plant sample extract, was incorporated in a 1÷1 Lithium carbonate ÷ SP 2 graphite matrix and used in all subsequent experiments. For this purpose a solution containing÷

0.06 mg. Cobalt,
0.08 mg. Molybdenum,
0.25 mg. Copper,
15.0 mg. Zinc,
15.0 mg. Manganese
and 10.0 mg. Cadmium

was extracted with 0.5 g. Sodium pyrrolidine dithiocarbamate. It was then ashed and mixed with 178.7 mg. Ferric oxide (= 125 mg. Iron) and 4.000 g. of buffer. The concentrations of trace elements in this synthetic plant extract, calculated on 10 g. of dried plant material, were as follows÷

Cobalt÷ 0.12 p.p.m.
Molybdenum÷ 0.16 p.p.m.
Copper÷ 0.50 p.p.m.
Zinc÷ 30.0 p.p.m.
Manganese÷ 30.0 p.p.m.
Cadmium÷ 20.0 p.p.m.
and Iron÷ 500.0 p.p.m.

/ A low concentration

A low concentration of Copper (0.5 p.p.m.) was chosen in order to measure the densities of the Cu 3274 A line more accurately without the use of a step sector to reduce its intensity.

A simple U-tube flowmeter was constructed and calibrated to measure the volume of gas flowing through the Stallwood jet per minute.

Preliminary exposures, using a flowrate of 2 litres per minute of pure Argon gas, revealed that the Cyanogen band spectra were still very much in evidence, although precautions were taken to exclude air from the arc chamber by allowing Argon to flow strongly for about 15 seconds before the exposure. Thiers (80) suggested that these band spectra might be due to Nitrogen impurities in the graphite electrodes. This might well have been the case, considering the method used for purifying all sample electrodes by extraction with hot concentrated Nitric acid, as described in Part II.

The time necessary to consume most of the sample and electrode in a 10 ampere d.c. arc was increased from approx. 4 minutes in air to 17 minutes in Argon gas with an accompanying considerable increase in background density over the whole region of the spectrum. Increasing the current to 18 amperes did not result in an expected large reduction of arcing time and background, 12 minutes elapsing before the electrode was consumed.

Following a practice adopted at the National Physical Laboratories of the C.S.I.R., graphite counter electrodes, sharpened to a fine point in a pencil sharpener, were employed in all experiments.

/ They were purified

They were purified by burning in pure Oxygen in a 10 amp. arc for 15 seconds. Their use proved to be very disappointing, since considerable wandering of the cathode spot was observed, in spite of the stabilisation provided by the Stallwood jet. Because of lack of suitable lengths of carbon electrodes, the use of graphite cathodes had to be continued. An explanation for the pronounced arc wander, which did not occur in experiments carried out in the National Physical Laboratories, could not be found. Increasing the flow-rate of Argon to 5 litres / minute did not reduce the effect in any way.

The synthetic plant extract was arced in air, using the standard exposure conditions (recording the first and third minute of the burn, Exposure 1) and subsequently in pure Argon (2 litres / minute) for 5 minutes (Exposure 2). The results obtained for each element are discussed below.

Cobalt÷ A significant decrease of approximately 50% in the intensity of the Co 3453 A line resulted when compared with exposure 1. This confirmed the findings of Thiers (80) who compared the relative intensities of the Co 3261 A line, using combinations of different gases and matrices, but identical exposure conditions. With a Lithium carbonate matrix in Argon the sensitivity was decreased to 1/8th of that in air. In spite of the presence of Cyanogen band spectra, the background density in the neighbourhood of the Co 3453 A line was more or less the same as in exposure 1. The use of a Lithium carbonate matrix in Argon gas is therefore not recommended for the determination of traces of Cobalt.

Molybdenum÷ Similar considerations apply to the determination of this element. For Molybdenum the decrease in the sensitivity of the Mo 3170 A line was even more pronounced, being only about 1/5th of that in air. It appears that Argon does not enhance the spectrum of less volatile and involatile elements such as Cobalt, Iron and Molybdenum in an alkali matrix.

Iron÷ The reduction in sensitivity was similar to Cobalt, so that the intensity ratio Co 3453 / Fe 3450 A was hardly affected, but not so in the case of Molybdenum. The ratio Mo 3170 / Fe 3166 A was reduced from 1.3 in air to 0.3 in Argon.

Copper÷ A significant increase of approx. 100% in the intensity of the Cu 3274 A line was obtained in Argon. The enhancement of the Spectrum of a more volatile element, such as Copper was confirmed by Vallee and co-workers (83, 84).

Manganese÷ The sensitivity increase was even more marked for this element. The intensity of the Mn 2933 A line was approximately four to five times that in air. Since Manganese is not a very volatile element, an explanation for the pronounced enhancement of the Mn 2933 A line has to be found elsewhere. Majkowski and Schreiber (85) give a value of 12.85 eV for the excitation potential of the Mn 2933 A line, which corresponds approximately to the lowest excited state of Argon (11.6 eV). Baker, Adelstein and Vallee (86) stated that maximal enhancement of ion lines in Argon is caused by resonance excitation if the excitation potential of the particular line is similar to that of Argon in its lowest state of excitation. This factor was probably mainly responsible for the

/ high intensity

high intensity of the Mn 2933 A line.

Zinc and Cadmium÷ Both the Zn 3345 A and Cd 3261 A lines were considerably enhanced in an Argon atmosphere, the former line to a higher degree than the latter. The increase in intensity amounted to 200 - 300% causing an increase in the intensity ratio from 0.33 to 0.74.

Since an arcing time of 5 minutes is excessive for spectrographic analysis, exposures of 2 minutes in a stream of Argon (2 litres / minute) were carried out and the resultant sensitivities of the elements measured. The result was discouraging, since neither Cobalt nor Molybdenum lines were discernible in the spectrum.

An increase in exposure to 3 minutes and current to 18 amps resulted in the production of a strong background of density 0.23 in the vicinity of the Co 3453 A line. The intensities of the Zinc, Copper and Manganese lines were enhanced to such an extent, that their measurement became impossible in the full exposure step of the 4-stepped spectrogram. The possibility of determining low concentrations, particularly of Zinc, directly on the plant ash by using the above excitation conditions, was not examined. It did, however, appear feasible. Unfortunately the increase in current strength did not lead to increased sensitivity as far as Cobalt and Molybdenum were concerned.

In order to increase the sensitivity of these two elements, a three minute exposure at 10 amps in a mixture of 80% Argon and 20% Oxygen (Total flow rate÷ 2.5 litres / minute) was recorded. The resulting background was higher than that obtained under identical conditions in an environment of pure Argon. The addition of Oxygen to the gas did not lead to the expected sensitivity increase in the case of Iron, Cobalt and Molybdenum. The intensities of their spectra were still considerably lower than those in air, while the analytical lines of Copper and Manganese were slightly enhanced. The intensities of the Zn 3345 A and Cd 3261 A lines were depressed, relative to air. The ratio of intensities of spectral lines in an 80% Argon + 20% Oxygen atmosphere to those obtainable in air, were÷ Cobalt÷ 0.67, Iron÷ 0.67, Molybdenum÷ 0.25, Zinc÷ 0.63, Cadmium÷ 0.34, Copper÷ 1.5, Manganese÷ 1.3.

When the proportion of Oxygen was increased to 50% (Total flow rate÷ 2 litres / minute), the time in which both sample and electrode were consumed was reduced to 1½ minutes. This exposure resulted in depression of all intensities.

Arcing a sample to completion in an atmosphere of 100% Oxygen (Total flow rate÷ 2 litres / minute) at 10 amp produced very weak spectra for all elements. Exposure time÷ 45 seconds.

The ratios of intensities of all elements, obtainable in the different atmospheres, to those in air, are summarised in tables 24 and 25.

TABLE 24.

Conditions of Excitation.

Condition No.	Current Strength	Atmosphere	Flowrate litres/ min.	Exposure
1	10 amps	100% A	2	5 min.
2	10 amps	100% A	2	2 min.
3	18 amps	100% A	2	3 min.
4	10 amps	80% A + 20% O_2	2.5	3 min.
5	10 amps	50% A + 50% O_2	2	1½ min.
6	10 amps	100% O_2	2	45 sec.

/ TABLE 25.

TABLE 25.

Ratios of Intensity obtainable in constant Atmosphere to those obtainable in Air.

Condition No.	Co	Mo	Fe	Zn	Cd	Cu	Mn
1	0.68	0.15	0.63	2.0	1.2	2.1	4.6
2	-	-	0.45	2.1	0.8	1.2	1.3
3	0.43	0.21	0.45	3.0	0.42	1.6	8.7
4	0.67	0.25	0.67	0.63	0.34	1.5	1.3
5	0.51	0.18	0.49	0.51	0.20	0.85	0.30
6	0.38	±0.05	0.27	0.33	0.13	0.48	0.22

From tables 24 and 25 it can be seen that except for Zinc, Copper and Manganese, sufficient increase in sensitivity was not obtained to warrant the excitation of the sample in an Argon atmosphere, or in mixtures of Argon and Oxygen. However, since only an alkali matrix was investigated, definite conclusions as to the suitability of rare gas atmospheres for trace element determinations could not be drawn.

Volatilisation curves of the elements, obtained from moving plate studies as described in Part III, section 9, in atmospheres of 100% Argon and 80% Argon + 20% Oxygen, respectively, were plotted and are reproduced in figures 30 and 31. These two atmospheres were at one stage thought to be most suitable for the determination of trace elements. For this purpose the concentration of Molybdenum in the arcing mixture (see Part III, section 9) had to be increased to 10000 p.p.m. and exposure intervals prolonged to 20 and 15 seconds, resp., in order to obtain measurable densities of the Mo 3170 A line. The sample was arced at 10 amps, using the constant current rectifier

/ described in Part I,

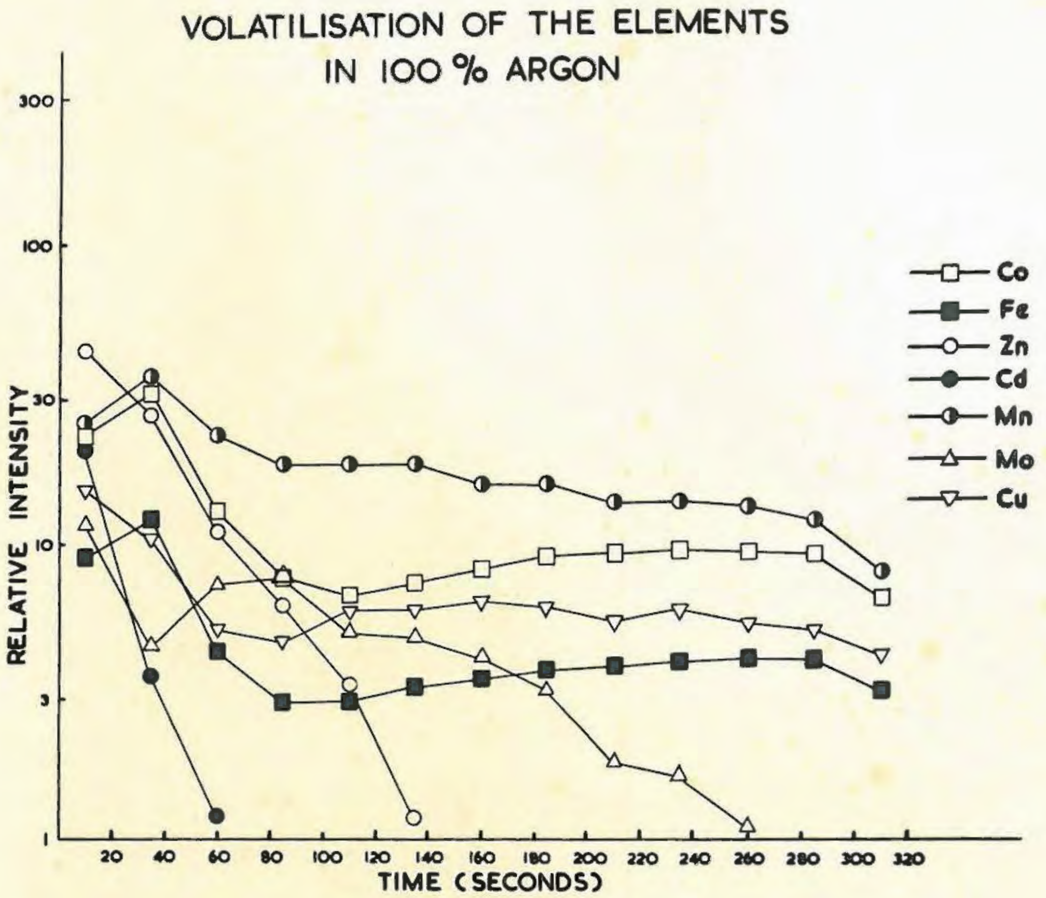


FIGURE 30.

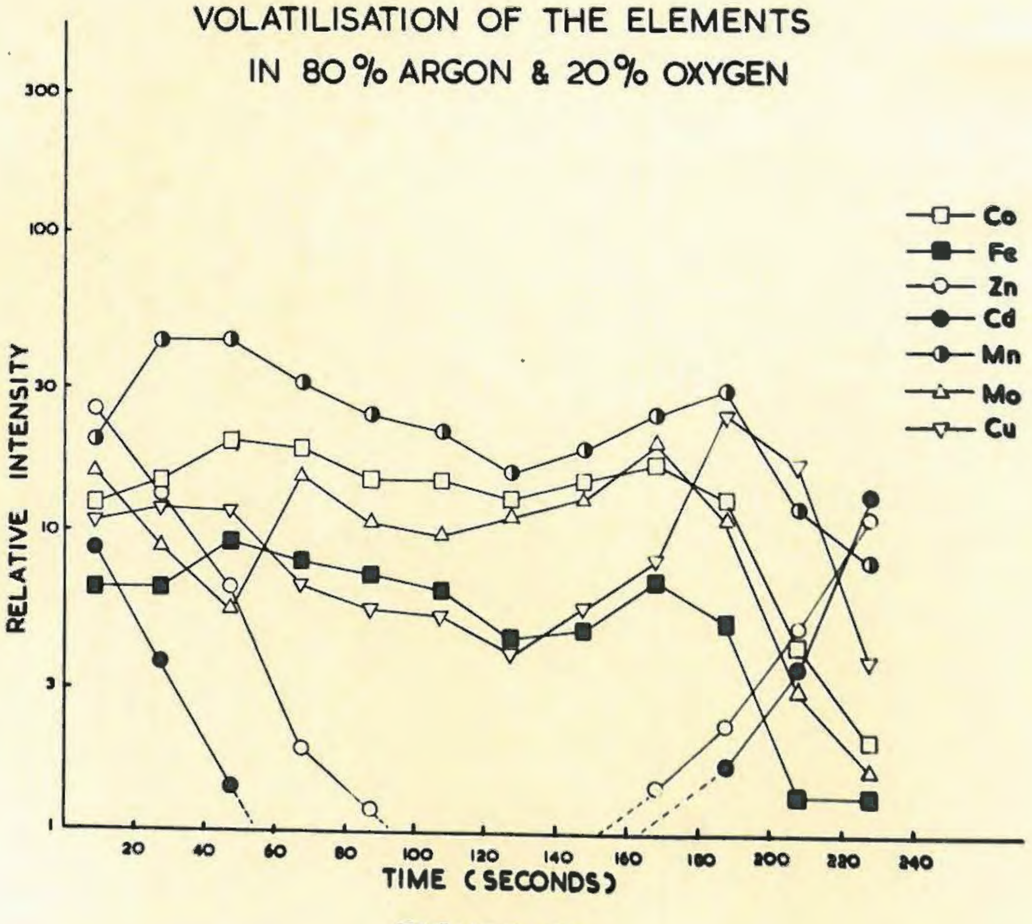


FIGURE 31.

described in Part I, section 2. A short discussion of the rates of volatilisation recorded in the two different atmospheres is given below.

<u>100% Argon÷</u> Successive exposures of 20 seconds each were made for a total of 7 minutes, with the spectrograph shutter closed for intervals of 5 seconds while the plate was racked to the next position. After approx. 5 minutes the colour of the arc changed from the characteristic red of Lithium, to an intense carbon arc. Three millimeter of the electrode walls were observed to have burnt down very evenly after seven min., the crater was still filled to the top with material. However, upon examining the series of exposures, it was noticed that emission of all elements stopped after 5 minutes. No spluttering occurred during the arcing, however, the material in the electrode could be compressed to a small volume. A possible explanation for this phenomenon is the lowering of the arc temperature from 5000°K in air to 2600°K in Argon, which is not high enough to volatilise carbon or graphite very readily. The trace elements, however, distill off together with the Lithium carbonate buffer, leaving behind the other constituents of the matrix, namely graphite powder. On repeating a moving plate study under identical conditions, similar results were obtained.

From an examination of the intensity-time curves in figure 30 it was found that most elements volatilised more evenly from the electrode, except for Zinc and Cadmium, which distilled off during the early stages of the arc burn. Selective volatilisation was therefore only decreased to a small extent, which might have been due to the small cooling effect provided by the low rate of gas flow. It was surprising to note that the volatilisation pattern of Molybdenum resembled that of a more volatile element in air.

80% Argon + 20% Oxygen÷ In contrast to an environment of 100% Argon, both sample and electrode were consumed evenly and uniformly during the whole arcing period. Arc wander, particularly climbing of the cathode spot on the graphite counter electrode, was very pronounced, resulting in rather erratic behaviour of most elements. Figure 31 shows that the volatilisation pattern in a mixture of 80% Argon + 20% Oxygen was similar to that obtained in air, particularly as far as the strong emission of Zinc and Cadmium towards the end of the burn was concerned. It was also observed that the emission of Molybdenum was more uniform than in air.

2. COMPARISON OF REPRODUCIBILITY OF INTENSITY RATIOS.

In order to assess the merits of using an electronically stabilised current strength for the excitation of elements, the reproducibility of the intensity ratios of the various analysis pairs employed in the spectrographic analysis of trace elements, **was** determined. The result was compared with the reproducibility of the same intensity ratios obtainable under identical conditions, except that an ordinary selenium rectifier was used to excite the sample. Furthermore, a series of exposures in a 100% Argon atmosphere (Flowrate÷ 2 litres / minute) was recorded, using the stabilised source unit, and the reproducibility of the three analysis pairs÷ Cu 3274 A / Fe 3286 A, Mn 2933 A / Fe 2914 A, Zn 3345 A/Cd 3261 A

/ were compared

were compared with that obtainable in air.

2.1. Reproducibility of Intensity Ratios - Excitation with Selenium Rectifier.

Twenty-four electrodes, filled with the synthetic plant extract, described in Part IV, section 1, were arced using the standard exposure conditions of recording the first and third minute of the burn. These spectra were not stepped since the concentrations of the elements were chosen to yield easily measurable line densities at full exposure. In this way three electrodes, i.e. 6 spectra, could be recorded on one strip of film. The excitation was provided by a selenium rectifier, run at 10 amps.

The density of all relevant analysis pairs, namely÷

Co 3453.51 A / Fe 3450.53 A,
Zn 3345.~~57~~02 A / Cd 3261.06 A,
Cu 3273.96 A / Fe 3286.76 A,
Mo 3170.35 A / Fe 3166.44 A and
Mn 2933.06 A / Fe 2918.03 A

were measured with the microphotometer and converted to intensity ratios. The mean of each set of 24 ratios and the standard deviation from the mean, were calculated on 23 degrees of freedom. The individual intensity ratios are reproduced in table 26.

Unfortunately it was only noticed when the films were developed that mixing of such a large sample of approx. 4 g. could not be effected very efficiently in three inch vials when the Wig-L-Bug amalgamator was employed. The experiments were, however, not repeated since only a relative comparison of the reproducibility of intensity ratios obtainable on the same sample under different conditions of excitation, was

/ required.

required. The results did, however, show that it is very important to employ thoroughly homogeneous samples when carrying out spectrographic analysis. The amount of sample filled into one electrode is so small (between 30 and 35 mg.) compared with the bulk of 4 g. employed in this instance, that any inefficiency in mixing immediately becomes apparent in the poor reproducibility of intensity ratios. In Part III it was shown that errors of sampling were virtually eliminated in spectrographic analysis by mixing the ashed plant extract with just sufficient diluent to fill two electrodes.

TABLE 26.

Intensity Ratios of Analysis Pairs.
Selenium Rectifier Excitation.

	Co/Fe	Zn/Cd	Cu/Fe	Mo/Fe	Mn/Fe
	0.297	0.400	0.703	1.00	1.53
	0.329	0.376	0.783	1.45	1.46
	0.299	0.382	1.050	1.32	1.22
	0.297	0.370	0.880	1.31	1.29
	0.304	0.378	0.955	1.30	1.31
	0.296	0.340	0.708	1.42	1.58
	0.305	0.374	1.000	1.38	1.62
	0.289	0.352	0.928	1.77	1.60
	0.289	0.344	0.695	1.12	1.13
	0.254	0.350	0.925	1.47	1.15
	0.255	0.309	0.900	1.34	1.48
	0.285	0.327	1.070	1.42	1.44
	0.304	0.357	0.800	1.45	1.87
	0.302	0.380	0.873	1.35	1.18
	0.314	0.383	0.935	1.18	1.35
	0.298	0.394	0.860	1.41	1.76
	0.289	0.364	0.810	1.38	1.63
	0.308	0.348	0.880	1.56	1.84
	0.313	0.314	0.820	1.16	1.38
	0.337	0.339	0.726	1.83	1.97
	0.290	0.375	0.860	1.13	1.43
	0.276	0.410	0.702	1.17	1.29
	0.290	0.368	0.890	1.36	1.59
	0.304	0.376	0.973	1.29	1.59
Mean÷	0.297	0.363	0.864	1.36	1.48
Standard Deviation÷	0.0186	0.0256	0.1174	0.190	0.229
Coefficient of Variation÷	6.27%	7.07%	12.54%	13.96%	15.44%

/ 2.2. Reproducibility

2.2. Reproducibility of Intensity Ratios - Excitation with Constant Current Rectifier.

The effect on the reproducibility of intensity ratios of the analysis pairs when an electronically stabilised current of 10 amps was used for the excitation of the identical sample was investigated. The exposure conditions were identical to those described in section 2.1., that is, the first and third minute of the burn of each electrode were recorded. An additional stabilisation of the burning arc was provided by the higher voltage of the constant current source unit, namely 300 volts as compared to 220 volts of the selenium rectifier. This also resulted in higher intensities of emission for most elements and in corresponding changes in the mean values of intensity ratios. The rates of volatilisation of the elements from the electrode appeared to be effected by the excitation energy provided by the stabilised source unit (3.0 Kilowatt) as compared to the selenium rectifier (2.2 Kilowatt), proving that the same analytical working curves could not be used for both instruments.

The influence of current control on the accuracy of d.c. arc analysis was investigated by Mackenzie(87), who reported that significant reduction of the analytical error could be obtained for involatile elements, while it had little or no effect on elements of low or medium volatility. Mackenzie arced sulphated plant ash samples to completion at 15 amps, the current was maintained at this value for the duration of the arcing period by means of a sliding resistance in series with the arc gap.

The results of 24 exposures with controlled current excitation are reproduced in table 27. The standard deviations from the mean intensity ratios and the coefficients of variation were calculated on 23 degrees of freedom.

TABLE 27.

Intensity Ratios of Analysis Pairs.
Constant Current Excitation.

	Co/Fe	Zn/Cd	Cu/Fe	Mo/Fe	Mn/Fe
	0.320	0.383	0.746	1.05	1.78
	0.311	0.334	0.880	1.23	1.89
	0.302	0.374	0.835	1.05	1.84
	0.290	0.367	0.597	1.06	1.89
	0.304	0.365	0.668	1.03	1.98
	0.306	0.375	0.695	1.16	1.54
	0.267	0.320	0.955	1.12	2.04
	0.286	0.347	0.853	1.20	1.92
	0.286	0.399	0.953	1.26	2.03
	0.272	0.337	0.910	1.31	2.19
	0.278	0.358	0.770	1.36	1.55
	0.280	0.338	0.820	1.33	1.74
	0.297	0.349	0.832	1.18	1.70
	0.271	0.367	0.820	1.33	1.74
	0.294	0.374	0.878	1.17	1.93
	0.264	0.324	0.875	1.26	1.95
	0.276	0.388	0.833	1.16	1.91
	0.307	0.378	0.805	1.32	1.72
	0.270	0.351	0.910	1.06	2.16
	0.272	0.384	0.910	1.03	2.38
	0.314	0.404	0.865	1.03	2.38
	0.296	0.355	0.910	1.31	2.31
	0.260	0.386	0.853	1.27	1.90
	0.280	0.377	0.953	1.30	2.37
Mean÷	0.287	0.363	0.857	1.21	1.94
Standard Deviation÷	0.0179	0.0229	0.0919	0.126	0.246
Coefficient of Variation÷	6.22%	6.30%	10.72%	10.38%	12.65%

It was significant that the control of current strength during the arcing period had relatively little effect on the reproducibility of the analysis pairs÷ Co 3453 A / Fe 3450 A and Zn 3345 A / Cd 3261 A, the former being recorded in the third

/ minute and

minute and the latter in the first minute of the arc burn, indicating the favourable choice of Iron and Cadmium as internal standards for Cobalt and Zinc, resp. A considerable advantage was, however, gained for the determination of the elements Copper, Manganese and Molybdenum, by controlling the current. Of these three elements, only Molybdenum is involatile. The relative constancy of intensity ratios was shown by their lower coefficient of variation when compared with the previous series of experiments. The controlled current appeared to create more reproducible volatilisation conditions during the later stages (i.e. in the third minute) of the arcing period.

2.3. Reproducibility of Intensity Ratios - Excitation in an Argon Atmosphere.

The time-intensity curves for the elements in an Argon atmosphere (figure 30) indicated that volatilisation of most elements occurred more uniformly than in air. It would seem that the choice of an internal standard is therefore not as critical, and that intensity ratios may be reproduced more accurately. In order to test this assumption 24 electrodes were filled with the same sample used in the two previous series of experiments and arced in a slow stream of Argon (Flowrate÷ 2 litres / minute). The special arc chamber with built-in Stallwood jet was employed for this purpose and the constant current rectifier used to excite the sample at 10 amps. The first two minutes of each arc burn were recorded and the intensity ratio of the analysis pairs÷

/ Zn 3345 A / Cd 3261 A,

Zn 3345 A / Cd 3261 A,
Cu 3274 A / Fe 3286 A, and
Mn 2933 A / Fe 2918 A

were calculated as described earlier. Unfortunately the densities of the Cobalt and Molybdenum analysis lines were either too low or zero and could therefore not be measured. Since in this series of experiments graphite counter electrodes were employed the arc behaved very erratically as mentioned in section 1. The cathode spot had a tendency to climb up the counter electrode at irregular intervals which led to the poor reproducibility of intensity ratios obtained. The intensity ratios obtained are shown in table 28.

TABLE 28.

Intensity Ratios of Analysis Pairs.
Argon Atmosphere Excitation.

	Zn/Cd	Cu/Fe	Mn/Fe
	0.848	1.07	10.30
	0.836	1.39	11.45
	0.723	1.43	10.30
	0.807	1.16	10.80
	0.717	1.24	11.34
	0.795	1.05	11.70
	0.888	1.10	9.12
	0.835	1.12	11.40
	0.743	1.08	11.38
	0.838	1.34	11.80
	0.837	1.41	12.35
	0.743	1.40	10.00
	0.835	1.35	12.58
	0.810	1.52	14.85
	0.952	1.24	10.90
	1.031	1.09	11.37
	0.908	1.49	11.28
	0.908	1.24	10.60
	0.916	1.43	10.20
	0.882	1.44	9.03
	0.812	1.55	12.45
	0.774	1.43	14.38
	0.973	1.25	15.13
	1.000	1.40	12.75
Mean÷	0.846	1.30	11.55
Standard Deviation÷	0.0861	0.158	1.582
Coefficient of Variation÷	10.2%	12.2%	13.7%

/ From the

From the time-intensity curves for Zinc and Cadmium it was not immediately apparent that the rates of volatilisation of these elements in Argon had changed to such an extent that the latter element could no longer be regarded as a very suitable internal standard for Zinc. By changing the arcing conditions, e.g. using carbon counter electrodes and high tension spark initiation of the d.c. arc, and possibly by redesigning the Stallwood jet, better precision may be obtained.

In table 29 the coefficients of variation of intensity ratios obtainable under the various excitation conditions are compared.

Condition 1÷ Recording of first and third minute of the arc burn, excitation at 10 amps, using a selenium rectifier.

Condition 2÷ Recording of first and third minute of the arc burn, excitation at 10 amps, using a constant current rectifier.

Condition 3÷ Recording of the first 2 minutes of the arc burn in Argon (Flowrate÷ 2 litres / minute), using a constant current rectifier at 10 amps.

TABLE 29.

Comparison of Reproducibilities of Intensity Ratios.

Condition	Co/Fe	Zn/Cd	Cu/Fe	Mo/Fe	Mn/Fe
1	6.27	7.07	12.54	13.96	15.44
2	6.22	6.30	10.72	10.38	12.65
3	-	10.2	12.2	-	13.69

/ 3. CONCLUSION.

3. CONCLUSION.

Although this brief survey on the use of a rare gas atmosphere for the excitation of plant extracts had not been thorough enough to permit any definite conclusions to be drawn as regards its suitability for trace element analysis, some results may be useful for further investigations. In this connection the large increase in sensitivity obtained for some elements, such as Zinc, may be mentioned, which may allow this element to be determined directly on plant ash. The decrease in sensitivity of the less volatile elements in an Argon atmosphere was very disappointing; the choice of more suitable matrices may, however, change the picture considerably.

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SUMMARY.

For this thesis a study of a spectrographic method for the simultaneous analysis of certain trace and minor elements in plants has been undertaken. Some of the errors inherent in this type of analysis were investigated and attempts were made to improve sensitivity and accuracy of the established method by exciting the sample in a noble gas atmosphere.

In Part I a detailed description of the apparatus employed in spectrographic excitation, recording and measurement is given. The advantages and disadvantages of using 35 mm film instead of plates are discussed. The methods used to standardise and calibrate the equipment are described and because of the importance of accurate conversion of spectral line densities to relative intensities a chapter is devoted to the techniques employed in the calibration of emulsions.

Part II deals with some general sources of error in spectrographic analysis and methods to reduce these errors. An attempt was made to gain a measure of the purity of graphite electrodes obtainable by extraction with different acids and mixtures of acids. The optimum time necessary to obtain a homogeneous sample by mixing in an agate mortar was investigated. Experiments were undertaken to determine and improve the reproducibility of development condition and contrast factor gamma.

Part III deals with the development of the spectrographic analytical method. The preparation of the plant sample by dry ashing and different methods of dissolving the ashed sample are discussed. A critical review is given of procedures used to isolate trace

/ constituents

constituents from the macro-elements in plant material. These procedures comprise electrolysis, ion exchange chromatography, carrier precipitation and solvent extraction. Particular emphasis is placed on a discussion of the latter. The advantages and disadvantages of either removing Iron from the plant solution or using this element as an internal standard at a predetermined concentration, are discussed. The solvent extraction method originally described by Stetter and Exler, who used Sodium pyrrolidine dithiocarbamate to precipitate the trace metals, and the application and modification of this method by Scharrer and Judel to the spectrographic analysis of trace elements in biological specimens, are given in detail. This method was adopted and refined to concentrate the following elements in citrus leaves÷ Cobalt, Copper, Manganese, Molybdenum and Zinc. Stress was laid on the necessity for using very pure reagents in the chemical operations and methods of purification of these reagents. The spectrophotometric determination of Iron is dealt with briefly, and the preparation and composition of synthetic plant standards is fully described. The volatilisation rates of the trace elements in different matrices, using different source units for excitation, were determined from moving plate studies. The methods of evaporating and ashing Chloroform extracts of precipitated metal carbamates and the design and construction of a special waterbath to handle small volumes and prevent "creeping" of the extract, are described in detail. The reasons for incorporating the ashed plant extract in an alkali matrix and the wavelengths of analysis and internal standard lines are given. The reproducibility of the analytical method was tested step by step by eliminating sampling and extraction variations and thus determining the purely spectrographic error. A second series of exposures revealed that the extraction error was negligible while a third series showed that sample variations of trace element concentrations in a sample of plant ash could be quite considerable. The accuracy of the combined concentration and spectrographic analysis method was tested in two ways. Firstly, by determining the percentage recovery of two series of standard additions of solutions of the trace elements and secondly by independent spectrophotometric and atomic absorption analyses for Copper, Manganese and Zinc. These experiments showed that the spectrographic analysis method was reproducible as well as accurate to within between 4 and 10%.

In Part IV a brief survey of the possibility of exciting plant extracts in an atmosphere other than air was carried out. It was hoped that a combination of more uniform volatilisation of the elements from the electrode and enhancement of line intensities would lead to greater accuracy and sensitivity in the spectrographic determination of trace elements. A partial success was achieved in increasing the sensitivity for the relatively volatile elements Copper, Manganese and Zinc.

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