A

CRITICAL STUDY

OF THE

DETERMINATION OF MOLYBDENUM

IN PLANT MATERIAL.

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

by

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SUMMARY.

1. Polarographic and spectrophotometric methods for the determination of molybdenum were reviewed.

2. The inclusion of salicylaldoxime in several supporting electrolytes was examined with a view to developing a polarographic procedure, suitable for routine analyses, which did not necessitate a preliminary time-consuming separation stage. A stable molybdenum wave was obtained from an electrolyte having a composition similar to the test solution obtained after the wet digestion of plant material. The height of the wave was found to be, over an optimum range, independent of the reagent concentrations. The wave was however distorted by a maximum, which rendered it unsuitable for quantitative analytical application.

3. The spectrophotometric procedure for molybdenum using thiocyanate is virtually specific and was examined critically. Modifications were found to improve the sensitivity of the procedure, and the stability of the chromatic compound. The accuracy and precision of the modified method was statistically determined on citrus leaf samples.

4. The modified spectrophotometric procedure is recommended for the routine determination of molybdenum in plant material.

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INTRODUCTION.

Certain field disorders in crop plants were known for nearly 40 years before the cause was traced to a deficiency of molybdenum in the nutrition of the plants. Within the last 20 years a great deal of research on molybdenum in the nutrition of plants has been carried out. Application of these findings to crop production has yielded such spectacular results that widespread interest has been aroused.

References to visual symptoms of molybdenum deficiency are numerous. Yellow spot in citrus, whiptail of cauliflower, leaf mottling, rolling and tip necrosis in tomatoes, withering or blackening of sugar beet, leaf scald in dwarf beans, cupping, chlorosis and marginal scorch in lettuce, stunted growth in clover and other affects are all attributed to molybdenum deficiency. Extensive work has revealed that the symptoms are remedied by the addition of molybdenised fertilisers. Excess molybdenum can also have a deleterious effect. Work in the U.S.A. has revealed that plants growing in soils which have a high molybdenum content are toxic to cattle.

The physiological role of molybdenum is complex and has as yet, not been completely established. Molybdenum deficiency is allied to the nitrogen supply. Characteristics of molybdenum deficiency are however unlike symptoms of nitrogen deficiency, and the addition of nitrogen or superphosphate alone creates only a temporary improvement. The first effect of the molybdenum is to increase symbiotic nitrogen fixation and growth.

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The nitrogen content is not a quantitative reflection of the molybdenum content. Though molybdenum deficiency decreases the percentage protein, non-protein and total nitrogen, it can also cause incomplete utilisation of nitrate nitrogen. In such cases the percentage protein nitrogen is decreased and the percentage non-protein nitrogen is increased, and the plants do not have a low percentage total nitrogen, but do exhibit symptoms associated with molybdenum deficiency. Responses to molybdenum are considerably affected by variation in the level of other elements, by differing absorptive capacity, seed reserves and nitrogen supply. The molybdenum requirement of each species is confined to a critical concentration range.

The aim of this investigation was to determine the most satisfactory procedure for the analysis of the molybdenum content of plant material. The first step of the investigation was to define the necessary requirements, for the ideal method:

1. The procedure must be both accurate and precise.
2. The procedure must be sensitive over the average molybdenum range in plants and soils. viz. 0.02 to 8 p.p.m.
3. The method must be specific and free from interference by the other constituents present in plant material.
4. The results must be independent of the concentration of the reagents and be stable for a practical period of time.
5. The procedure must satisfy the practical requirements of routine analysis of plant material. It must be inexpensive, rapid and simple.

The adoption of these criteria was found to eliminate certain possible techniques. For example the high sensitivity required rendered normal gravimetric and volumetric procedures unworthy of further study. Spectrographic procedures were not considered because of incomplete instrumental facilities. Polarographic and Spectrophotometric procedures were alone considered to be of value for further examination.

The investigation was divided into two parts: Part I, Polarographic Studies and Part II, Spectrophotometric Studies. Each part followed the same study programme. First a survey of the literature was undertaken to select that procedure which most closely fulfilled the criteria, or appeared most easily amenable to modification. The respective procedure was then examined experimentally in order to render it ideal. Finally the accuracy of the suitably modified procedure was established by statistical methods on actual leaf samples.

PART I/.....

PART I.

POLAROGRAPHIC STUDIES.

1. REVIEW OF THE PUBLICATIONS ON THE POLAROGRAPHIC DETERMINATION OF MOLYBDENUM.

The aim of this study was to determine a suitable polarographic procedure for the routine analysis of molybdenum in plant material.

The rapid increase in the number of quantitative polarographic procedures that have been described for the determination of molybdenum over the last ten years is an indication of the growing importance that is being placed in polarography as a method of analysis. In the majority of procedures mentioned, the polarogram was obtained by the reduction of Mo^{6+} ions at the dropping mercury cathode. A wide number of supporting electrolytes have been previously examined. These ranged from alkaline electrolytes to electrolytes containing weak organic acids and to strong acid electrolytes. As a basis of selection the following were considered to be desirable practical requirements of the procedure:

(i) The polarogram must be well defined and suitably formed for obtaining analytical measurements.

(ii) The polarogram must be reproducible with respect to time.

(iii) The polarogram must not be critically dependent upon the concentration of the supporting electrolyte.

(iv) The polarograms must yield a satisfactory and sensitive calibration curve for a plot of the height of the wave versus the concentration of molybdenum.

(v)/ .....

(v) The polarogram must be specific. It must be unaffected by the presence of the other substances occurring in the plant sample.

1.1 Critical examination of the existing procedures.

Uhl (1), Stackelburg et al (2), Kanevskii and Shvartsburd (3), Meites (4), Hokhstein (5), Johnson and Robinson (6), and Gokhstein (7) all reported that Mo^{6+} ions do not yield a reduction wave in alkaline or neutral supporting electrolytes. Recently Meites (4) reported a wave under these conditions from electrolytes containing citrate ions. In 0.1 M sodium citrate this author (4) obtained a single well-formed but irreversible wave for molybdenum between pH values ranging from 6.0 to 8.0. Though Carrit (8) established that Mo^{4+} ions were not normally produced by reduction of Mo^{6+} ions, Meites (4) found that this electrode reaction was the cause of the above wave. Meites (4) stated that the formation of a stable complex between citrate and Mo^{4+} ions enabled this reaction to occur and yield the wave.

The calibration curve obtained by Meites (4) was not linear. A major defect of these waves was the highly negative values of the half-wave potential. This made interference by several preceding waves possible. The half-wave potential became more positive as the sodium citrate concentration was increased from 0.1 M to 1 M. Unfortunately this increase in the sodium citrate concentration caused the wave to be distorted by a maximum. This maximum was not removed by the normal surface active agents (e.g. Triton X-100, gelatine etc.).

Several/ .....

Several authors examined electrolytes containing weak organic acids. Uhl (1) reported that the molybdenum waves were poorly developed from lactic acid electrolytes. Uhl (1), Milner (9) and Perry and Yakubik (10) reported that molybdenum waves in tartaric acid electrolytes were also poorly developed. The concentration range of tartaric acid examined varied from 0.5 M to 0.1 M. Two waves corresponding to the formation of Mo^{5+} and Mo^{3+} ions were obtained. The maximum on the second wave was caused by the desorption of the tartrate complex of the molybdenum poly-ion.

Milner (9) and Boltz et al (11) reported that the molybdenum waves were well developed in electrolytes containing citric acid. Milner (9) used 0.2 M citric acid, while Boltz et al (11) used an electrolyte containing 0.03 M disodium hydrogen phosphate, 0.09 M citric acid and 0.1 M potassium chloride. The reduction occurred in two stages in both these electrolytes.

Electrolytes containing acetic acid and acetate ions were examined by Uhl (1), Johnson and Robinson (6), Boltz, de Vries and Mellon (11), Nichols and Rogers (12), De Sesa et al (13) and Pribil and Blazek (14). Each of the above investigators reported that the waves for molybdenum were poor and malformed in acid-acetate buffers. Well-shaped waves were obtained by the reduction of complexes of molybdenum in this buffer. Pribil, Roubal and Zmotek (15) observed that the molybdate ions formed a stable complex with the disodium salt of 1,2, diaminocyclohexane N,N, N',N', tetra-acetic acid (complexone IV). This complex yielded a single wave in electrolytes containing an acetate buffer.

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The half-wave potential shifted to the negative direction as the pH was raised from 2.7 to 4.5. Well-developed waves for molybdenum were also obtained in acetic acid/acetate electrolytes containing E.D.T.A. Electrolytes containing the disodium salt of E.D.T.A. were examined by Pribil and Blazek (14) and Feltham and Martin (16). Pribil and Blazek (14) reported a single well-developed wave in 0.1 M E.D.T.A. (disodium salt) buffered at pH 4.8 with ammonium acetate. The number of waves obtained was dependent upon the pH. Feltham and Martin (16) reported a well-developed doublet wave when the pH was altered to 2.8. The shape of the waves obtained using E.D.T.A. was more satisfactory than those obtained using complexone IV. Neither of these electrolytes was suitable for simple analytical application because of interference by the preceding waves of Cu^{2+} and Fe^{3+} and other ions present in the plant sample.

In acid electrolytes in general the characteristics of the polarogram for molybdenum were markedly dependent on the nature and concentration of the acid. Hydrochloric acid was used as an electrolyte by Stackelburg et al (2). The affect of the concentration of this electrolyte was examined by Carritt (8) and Holje and Geyer (17). These investigators reported that the half-wave potential, the height and the number of waves obtained for molybdenum were each dependent on the concentration of hydrochloric acid. Holje and Geyer (17) stated that Mo^{6+} ions were reduced by the mercury of the anode if the acid concentration was greater than 2 N.

They/ .....

They recorded no waves for molybdenum in electrolytes with concentrations over 8 N hydrochloric acid. Holje and Geyer (17) recorded from one to three waves in the range 5 N to 0.1 N hydrochloric acid and stated that the different waves obtained corresponded to the following changes in oxidation number at the dropping mercury cathode:

$Mo^{6+} \rightarrow Mo^{5+}$ .....(i)

$Mo^{5+} \rightarrow Mo^{3+}$ (red) ....(ii)

$Mo^{5+} \rightarrow Mo^{3+}$ (green) ...(iii)

$Mo^{6+} \rightarrow Mo(OH)_3$ ....(iv)

According to Holje (17) the two forms of Mo^{3+} were not reversibly interconvertible and could coexist. The wave due to the change in oxidation number (iii) became predominant as the hydrochloric acid concentration was decreased. (Holje (17) also reported that the half-wave potential of the wave due to reaction (i) shifted in the negative direction as the acidity was decreased below 2 M. Carrit (8) examined the waves for molybdenum in the range 0.8 to 0.3 M hydrochloric acid and obtained three waves in 0.8 M hydrochloric acid, in contrast to the two reported by Holje (17). Carrit (8) employed controlled potential coulometry to show that the first two poorly-resolved waves were caused by the reduction to two states of Mo^{5+}, which co-exist in sluggish equilibrium. Gokhstein (7) proposed that the difference in the diffusion current values with the acid concentration was caused by the formation of polymolybdic acids.

Some/ .....

Some investigators coupled hydrochloric acid with salts. The waves reported by Boltz (11) in a potassium chloride/hydrochloric acid electrolyte at pH 1.1 and those reported by Nichols and Rogers (12) in an ammonium chloride/hydrochloric acid electrolyte at pH 1.1 were all poorly developed. Electrolytes containing hydrochloric acid were considered to be unsuitable for analytical application.

Phosphoric acid was examined as an electrolyte by Kanevskii and Shvartsburd (3), Boltz et al (11), Nichols and Rogers (12) and Holje and Geyer (17). Holje and Geyer (17) examined the waves for molybdenum in electrolytes for which the phosphoric acid concentration was increased from pH 2 to 20 N. They observed the number of waves to change from one to three, and that the half-wave potentials became more positive with this increase in acidity. The changes in oxidation number of the molybdenum at the cathode were the same as those reported by Holje and Geyer (17) for hydrochloric acid. Kanevskii and Shvartsburd (3) reported that waves due to both forms of Mo^{3+} were obtained in 10 N phosphoric acid, due to the weak nature of phosphoric acid. Nichols and Rogers (12) examined electrolytes containing 0.04 to 7 M phosphoric acid and reported that the waves were well-developed over the concentration range 2 to 3.5 M phosphoric acid, but did not recommend this electrolyte for the analysis of plant material.

Uhl (1), Hokhstein (5), Johnson and Robinson (6), Nichols and Rogers (12) and Cooke (18) examined nitric acid as a supporting electrolyte and reported that the waves for molybdenum were catalytic and poorly developed in this medium. The height of the wave was

reported/ ....

reported to be proportional to both the hydrogen and nitrate ion concentrations, and the diffusion current was governed principally by the rate of nitrate reduction rather than by the rate of diffusion. Uhl (1) observed that the addition of several reagents, like glycerol and mannitol, distorted the shape of the molybdenum wave and concluded that nitric acid by itself was unsatisfactory as a supporting electrolyte.

Uhl (1) and Saito (19) found that the height of the molybdenum waves obtained in electrolytes containing nitric and lactic acid and other reagents were less dependent on the concentration of nitric acid than waves obtained from nitric acid alone. The two molybdenum waves obtained were still poorly developed. Uhl (1) employed an electrolyte containing 0.04 N nitric acid, 0.2 N lactic acid, 0.15 M potassium oxalate, 0.1 M potassium chloride and 0.09 M ammonium nitrate. The second wave for molybdenum, used for quantitative measurements was distorted by a maximum. The addition of potassium oxalate eliminated interference by the iron wave. Saito (19) used a similar electrolyte but omitted chloride ions.

Nichols and Rogers (12) and Stern (20) examined electrolytes containing nitric acid and ammonium nitrate and observed that the wave for molybdenum was well developed if the electrolyte had a low acidity and a high ratio of nitrate to hydrogen ions. Both these factors had to be fulfilled for the wave to be satisfactory. In electrolytes containing greater than 3 M nitric acid, and in neutral electrolytes containing 2 N ammonium nitrate, Stern (20) observed that the waves for molybdenum were distorted by a maximum and were unsuitable for practical application. The half-wave potential was more

negative/ ....

negative in the neutral electrolyte than in the acid electrolyte. In both cases the maximum was found to be eliminated by 0.3% methyl cellulose. The electrolyte recommended by Stern (20) contained 0.25 M nitric acid and 3 M ammonium nitrate. Lactic acid was omitted from the electrolyte as it formed a maximum. Nichols and Rogers (12) determined molybdenum down to 1 p.p.m. in plant ash by employing an electrolyte containing 1 M ammonium nitrate at pH 1. Though the single wave obtained was well developed, the diffusion current increased with the nitric acid concentration over the pH range 0.5 to 4.7. Both iron and copper interfered with the method. The low sensitivity, the lengthy time required for the separation of the molybdenum and the critical reagent concentration were factors which made these authors (12) conclude that this polarographic method had no advantage over existing colorimetric procedures.

Sulphuric acid was used as an electrolyte by Stackelburg et al (2), Carrit (8), Nichols and Rogers (12), Holje and Geyer (17), Haight (21), Forss (22) and Vleck (23). According to Carrit (8), the waves in sulphuric acid were better shaped and less complex than those obtained in hydrochloric acid. Carrit (8), Nichols and Rogers (12), Holje and Geyer (17), Forss (22), Vlcek (23) and Stepien (24) all reported two well-formed waves in concentrations of sulphuric acid over 20 N, and stated that the anode mercury did not reduce Mo^{6+} at high sulphuric acid concentrations. These authors also stated that the diffusion current of the molybdenum waves was least dependent on the acidity at the higher sulphuric acid concentrations. In spite of the favourable linear calibration curve also reported, the author considered that such electrolytes were hazardous and therefore unsuitable for routine application. Stackelburg et al (2) recorded only one wave in 18 N sulphuric acid. These investigators employed a reference anode that had a more negative potential than that of the first wave and only recorded the second wave.

The waves for molybdenum in electrolytes containing 20 N to N/1000 sulphuric acid were examined by Holje and Geyer (17). These investigators found that the relative wave heights and the number of waves obtained for molybdenum varied with the sulphuric acid concentration in a similar manner to that observed for hydrochloric acid. The half-wave potentials of the waves due to changes in oxidation number (i) to (iv) each shifted in the positive direction as the concentration of acid was increased. Carrit (8) showed that the total diffusion current increased with decreasing sulphuric acid concentration according to the linear equation,

$$I = 4.24 - 0.25 M.$$

Where M = molarity of sulphuric acid over the range 0.2 to 14.4 M. and I = diffusion constant

$$= i_d/C m^{2/3} t^{1/6}$$

The latter relationship was obtained from the Ilkovic equation (Kolthoff and Lingane (25)). Although dilute sulphuric acid electrolytes were safer to manipulate, the molybdenum waves in this medium were not well formed and had a large space requirement.

Iron interfered by causing a preceeding wave. On taking these factors into account, together with the dependence of the total wave height on the acid concentration, the author concluded that the electrolytes containing less than 20 N sulphuric acid were also unsuitable for routine plant analysis.

Robinson and Johnson (6), Milner (9), Nichols and Rogers (12), Haight (21), Wolfson (26) and Pecsok and Parkhurst (27) reported more favourable waves for molybdenum in electrolytes prepared by coupling dilute sulphuric acid with salts and other reagents. Robinson and Johnson (6) and Nichols and Rogers (12) observed that the three waves obtained in an electrolyte containing 0.1 M sulphuric acid and 0.2 M sodium sulphate at pH 1.5 were irreversible and poorly developed. The height of the second wave was dependent on both the acid and the molybdenum concentration since the equilibrium between the two states of Mo^{3+} was sluggish. However the addition of 0.05 M sodium nitrate to the above electrolyte was observed by Robinson and Johnson (6) to decrease the total space requirement by causing the second and third waves to coalesce. According to these investigators a further improvement achieved by the addition of sodium nitrate was the greater sensitivity effected by the observed increase in the height of the second wave.

Haight (21) found that the enhancement was much larger in the presence of perchlorate ions and recommended an electrolyte containing 0.75 M sulphuric acid and 1 M perchloric acid. The height of the second wave for molybdenum in this electrolyte was increased tenfold as compared with that obtained in sulphuric acid alone, and Haight (21) used this electrolyte in the determination of 0.1 to 10 p.p.m. molybdenum in steels. Haight and Reynard (28) considered that Mo^{4+} ions were the intermediate catalyst which were immediately re-oxidised by the perchloric acid to the Mo^{5+} state. The total wave height was dependent on the reagent concentrations. As was expected by the catalytic nature of the electrode mechanism, Haight (21) observed that the height of the second wave was linearly proportional to the perchlorate ion concentration. Haight's observation that the half-wave potential shifted in the negative direction as the hydrogen ion concentration was decreased was in accordance with the effect described by Holje and Geyer (17) for perchloric and other acids.

Electrolytes containing a mixture of sulphuric and citric acids were examined by Milner (9), Wolfson (26) and Pecsok and Parkhurst (27). In all cases two well-formed waves were reported. Wolfson (26) used the second wave in an electrolyte containing 0.75 M sulphuric acid and 0.5 M citric acid for the determination of molybdenum in materials of the "Permalloy" type. The waves due to Fe^{3+} and Cu^{2+} ions interfered with the first wave for molybdenum. Pecsok and Parkhurst (27) recommended an electrolyte containing 0.1 M sulphuric acid and 0.5 M citric acid for the determination of molybdenum in plant materials.

Pecsok/ ....

Pecsok and Parkhurst (27) overcame the interference by the other elements present in plant material (Cr, V, Fe, Cu, Ni, W, Mn and Co) by the use of an ion exchange column (Dowex 50 - X12). The waves were well shaped at the recommended reagent concentrations. These authors (27) recommended analysis by the method of standard additions, because the reagent concentration was critical. The diffusion constant, I, increased with the acidity and with decreasing ionic strength. This relationship between wave height and acid concentration was contrary to that observed by Carrit (8) in electrolytes containing sulphuric acid alone. The concentration of citric acid was critical. Concentrations of citric acid less than 0.5 M caused distortion of the first wave and the formation of a large maximum. Pecsok (27) stated that the improvement in the form of the waves for molybdenum was probably due to the conversion of the polynuclear complexes in dilute sulphuric acid to simpler complexes in the presence of hydroxy acids. Tartaric, gluconic and other hydroxy acids were observed by Pecsok (27) to similarly improve the shape of the wave.

The essential properties of the wave for molybdenum in electrolytes containing perchlorate ions have already been discussed in the section dealing with sulphuric and perchloric acid mixed electrolytes. Milner (9) and Codell et al (29) examined perchloric acid as an electrolyte. Codell et al (29) investigated an electrolyte containing 2.1 N perchloric acid and 0.008% gelatine, while that investigated by Milner (9) contained 0.4 N perchloric acid. The half-wave potentials of the single

waves/ ....

waves obtained were -0.24 and -0.55 volt versus the s.c.e. respectively. Both these authors stated that the wave was catalytic and that the height of the wave was markedly dependent upon the concentration of perchlorate ion.

Holje and Geyer (17) examined the range of electrolytes containing 0.01 to 10 N perchloric acid. These authors (17) observed that the half-wave potential for a given wave became more positive with increasing acid strength. The values reported above concur with this directional trend. Holje and Geyer (17) also reported that the number of waves for molybdenum increased from one to three over this concentration range. Over the concentration range 0.1 to 5 N perchloric acid, two waves were reported. This finding did not concur with those of Milner (9) and Codell et al (29). The electrode mechanisms were similar to those reported by Holje and Geyer (17) for hydrochloric acid.

Jones (30) observed that sodium perchlorate produced the same catalytic effect on the molybdenum wave as did perchloric acid and recommended an electrolyte containing 0.75 M sulphuric acid and 1 M sodium perchlorate. This electrolyte was superior to those containing perchloric acid because careful control of the perchlorate ion was possible. The sodium perchlorate was added to the test solution containing molybdenum in sulphuric acid. The control of this variable made quantitative routine application of this electrolyte practical. Jones (30) reported a sensitivity of 0.02 μg. molybdenum per ml. and claimed that this sensitivity was several times that of the normal colorimetric procedures (refer Part II).

1.2/ ....

1.2 Discussion:

Several of the electrolytes described yielded waves which possess a satisfactory wave form. Many electrolytes were unworthy of further study because they were hazardous to handle (e.g. concentrated sulphuric acid) or else yielded waves that were critical on the reagent concentration (e.g. 0.1 M sulphuric acid, 0.2 M sodium sulphate and 0.05 M sodium nitrate, etc.). These have been fully discussed in the above review.

Three polarographic procedures have been published for the determination of molybdenum in plant material. These are (i) that of Nichols and Rogers (12) who employed an electrolyte containing nitric acid and ammonium nitrate, (ii) that of Pecsok and Parkhurst (27) who employed an electrolyte containing sulphuric and citric acid and (iii) that of Jones (30) who employed an electrolyte containing sulphuric acid and sodium perchlorate. The procedure of Jones (30) is superior to the other two in that the sensitivity is greater. The reagent concentration was critical for all three methods. The concentration of sodium perchlorate could however be readily controlled.

Several of the other substances occurring in plant material interfered with each of the above procedures. Normally such substances interfered by yielding a wave which preceeded that for molybdenum. When the concentration of these substances was in excess of that for molybdenum, which was usually the case, it was almost impossible to obtain quantitative measurements.

In each of the above procedures for the determination of molybdenum in plant material, the interferences were removed by a tedious preliminary separation stage. Nichols and Rogers (12) precipitated the molybdenum with α- benzoin-oxime. This procedure was inaccurate at low molybdenum concentrations. Peeck (27) separated the molybdate anions by the use of a cation exchange resin column. Jones (30) found that iron, copper and bismuth interfered with his procedure. α -Benzoinoxime was therefore added to the plant solution and the complex formed was extracted with chloroform. This extraction had a 100% efficiency when the test solution contained 1 to 2% sulphuric acid. The chloroform was subsequently removed by evaporation and the α-benzoinoxime was decomposed with nitric acid. The nitric acid was removed by heating to fumes with sulphuric acid. Neither iron, copper nor bismuth ions were extracted by the chloroform.

Each of the preliminary separation stages described was time consuming. This led to the statement by Nichols and Rogers (12) that the polarographic procedure possessed no advantage over the spectrophotometric procedure for the analysis of molybdenum in plant material. Since this statement was published, the sensitivity of the polarographic procedure has been increased (Jones (30)). The observation is nevertheless still valid, and the time requirement must necessarily be reduced.

Other separation techniques described include extraction, chromatography, ion exchange, reduction and complex forming procedures. Codell (29) extracted the molybdenum with 98% efficiency using ether. The test solution contained 4.7 N hydrochloric acid and 3.6 N sulphuric acid. The ether was removed by evaporation prior to the addition of the perchloric acid electrolyte. Codell (29) found the alternative ether extraction technique, from acidic solutions containing an alkali thiocyanate and stannous chloride, was more efficient. The co-extracted stannous ions were subsequently precipitated in the form of meta stannic acid on the addition of the perchloric acid electrolyte. Molybdenum was occluded in this precipitate. Codell (29) controlled possible anionic interference by evaporation with perchloric acid. Kolier (31) and Ghe and Fiorentini (32) separated the molybdenum by the use of paper chromatography. Cooke (18) and Alimarin (33) found the most rapid method for the elimination of interferences to be the use of ion exchange resins.

Procedures have been described in which the time consuming separation stage was entirely eliminated. Such procedures converted the interfering elements to polarographically inactive substances. Haight and Reynard (28) removed interference by reduction with zinc amalgam and stannous ions. Geyer (34) reduced the interfering ions with hydrazine sulphate. Uhl (1) used oxalic acid to complex the iron present. Parry and Yakubik (10) complexed chromium with sulphur dioxide. These complexes yielded a wave at a more negative potential than that for the molybdenum wave. Interference was thereby eliminated.

The simplest method of eliminating interference was the converse, i.e. to shift the half-wave potential of the molybdenum wave in the postive direction. Substances reduced at a significantly more negative potential do not interfere. Tucker (35) reported waves for molybdenum at zero volt versus the s.c.e. in an electrolyte containing salicylaldoxime and acetic acid. This procedure was therefore selected for further investigation. The possible elimination of the initial separation stage would reduce manipulative errors and significantly shorten the polarographic procedure.

1.3 The method of Tucker (35).

Tucker (35) developed a polarographic method for the determination of uranium and employed an electrolyte containing acetic acid and salicylaldoxime ($C_6H_4.OH.CHNOH$). The method was relatively free from interferences. Tucker (35) observed however that molybdenum produced a wave at zero volt versus the s.c.e. This wave was not sensitive to the salicylaldoxime concentration.

The practical details of the method are as follows:- Five ml. of the sample, containing 150 to 800 μg. uranium oxide (U_3O_8), were placed in a 10 ml. centrifuge tube and 1 ml. glacial acetic acid and 4 ml. salicylaldoxime were added. The tube was centrifuged at 1200 r.p.m. for two minutes to remove the copper precipitate. To a 5 ml. aliquot of the clear solution, 2 ml. of hydroxylamine hydrochloride were added. The solution was warmed gently on a water bath until the blue colour of the ferric complex had disappeared.

After removal of the oxygen by bubbling nitrogen through the solution, the polarogram was recorded.

Tucker found that 500 p.p.m. ferric iron, 400 p.p.m. ferrous iron, 100 p.p.m. manganous ions, 50 p.p.m. cupric ions, 10 p.p.m. nickelous ions, and 50 p.p.m. cobaltous, cobaltic, zinc, magnesium and plumbous ions, and anions such as nitrates, sulphates and chlorides did not interfere. This procedure therefore appeared well suited to determinations in a complex substance like plant material. Tucker reported a wave at -0.26 volt versus the s.c.e. for uranium. Antimony yielded a wave at -0.16 volt versus the s.c.e. Bismuth yielded a wave close to that obtained for molybdenum. The above three interferences may safely be disregarded since they normally occur only in minute traces in plant materials.

2. EXPERIMENTAL STUDIES ON THE POLAROGRAPHIC DETERMINATION OF MOLYBDENUM.

2.1 Description of the apparatus.

2.11 The polarograph.

The instrument used was a Leybold Nachfolger polarograph. The instrument was modified by the addition of a charging current compensator, since in most of this work it was necessary to operate at the maximum, or nearly the maximum sensitivity, (Eve (36)).

2.12 The thermostat.

All measurements were made at 25°C. The thermostat described by Eve (38) was modified slightly before use. A hollow copper spiral was incorporated inside the water bath. A flow of water ensured constant temperature even when the room temperature was above 25°C. A rack was constructed so that the reference anode as well as the series of five cells could be inserted simultaneously.

2.13 The electrolysis cells.

A new type of cell was designed. It was found to be robust, easily cleaned and excellently suited to the performance of a large series of determinations.

The reaction cell was a pyrex test tube, (figure 1). The tube was 10.0 cm. long and the diameter was 4.0 cm. It was stoppered by a rubber bung through which three holes had been drilled. The dropping electrode was inserted through one of these holes.

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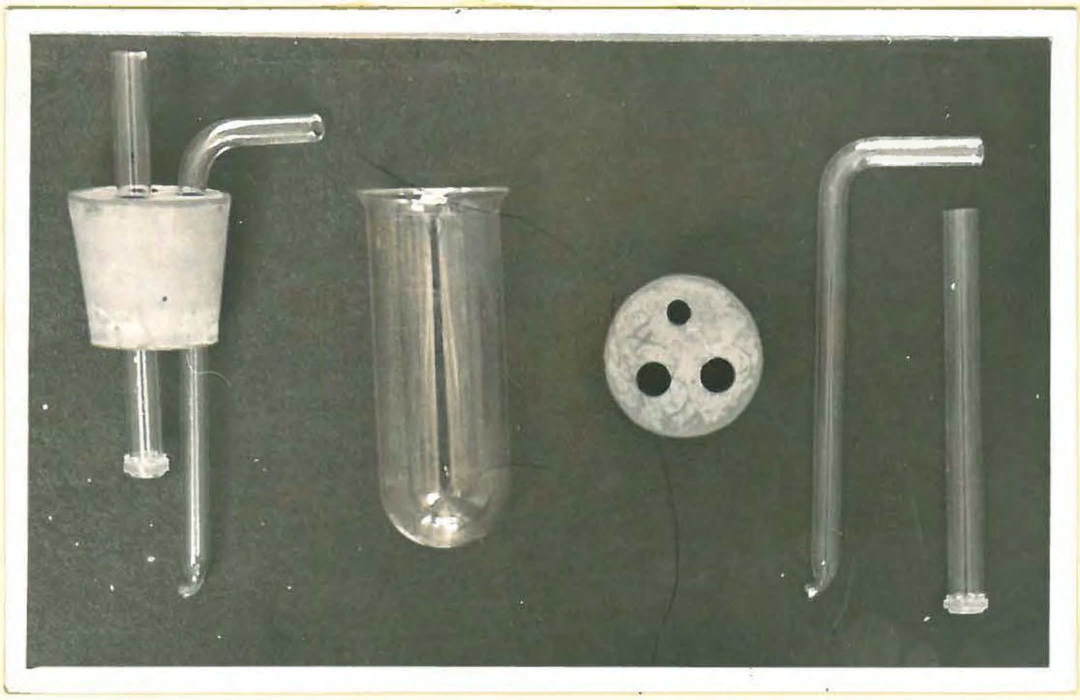

FIGURE I.

THE ELECTROLYSIS CELL

The same hole acted as an exit for the nitrogen. The nitrogen bubbler was fitted into the bung. A pyrex tube, 1 cm. in diameter, was inserted into the third hole. The ends of this tube had previously been ground smooth, and polished in a flame. This tube was sealed by cellophane, held in position by a ring cut from tygon tubing. The test solution was placed in this tube. The salt bridge from the anode was immersed into this tube. The anode compartment was thus separated from the main test solution, and diffusion by interfering ions was reduced to a minimum.

2.14 The reference electrode.

$Hg/Hg_2SO_4(s)$, 0.1 N H_2SO_4/Test solution/dropping electrode.

The non-polarisable merourous sulphate electrode was used as an external reference electrode.

The potential of this electrode is +0.2 volt versus the s.c.e. The mercury on the bottom of the cell was covered with a paste of pure Hg_2SO_4, and mercury. The solid phase was covered with 0.1N H_2SO_4, saturated with mercurous sulphate. The reference electrode was connected to the electrolysis cells by means of a salt bridge of inert tygon tubing. The tubing was connected to a pyrex tube which was sealed with agar and potassium nitrate (ref. Kolthoff (25)). The glass tube was bent so that it could easily fit into the electrolysis cell. The reference anode was kept in the water bath. By using this flexible salt bridge the reference electrode could be simply and rapidly connected to each of a series of electrolysis vessels in turn, under conditions of constant temperature.

2.15/ .....

2.15 Practical considerations.

(i) Normal dropping mercury electrodes were used. The drop time was two to three seconds per drop. The height of the mercury reservoir was maintained at a constant value. The reservoir was connected to the capillary tube with inert tygon tubing.

(ii) Oxygen was removed from the test solution by degassing with ordinary commercial nitrogen.

2.2 Preparation of reagent solutions.

All test solutions were prepared from deionised distilled water. All reagents used were A.R. quality. The salicylaldoxime used was Judex C.P. quality. The salicylaldoxime was not purified further before use. Scrupulous attention was paid to the cleanliness of all glassware used.

2.21 Standard molybdenum solution.

"Specpure" ammonium heptamolybdate (0.1822 g.) was dissolved in water and made to a litre. The solution was stored in a polythene container. 1 ml. of this solution contained 100 μg. molybdenum.

2.22 Salicylaldoxime solution.

Salicylaldoxime (1.00 g.) was dissolved in 5 ml. ethyl alcohol. This was added slowly to 95 ml. water.

2.3 A critical examination of several supporting electrolytes.

2.31 Reagent blanks.

It is necessary to distinguish between the polarographic characteristics caused by the presence of molybdenum in solution, and those caused by the supporting electrolyte itself. Current-voltage curves were therefore plotted for each of the supporting electrolytes prior to the addition of any molybdate ions.

The/ ....

The polarograms obtained have been reproduced in figure 2. In general two current variations were observed. The first occurred at zero applied potential, and the second occurred when a fairly large negative voltage was applied to the dropping mercury electrode.

Figure 2.1 is the polarogram obtained from an electrolyte containing 0.6 M sodium perchlorate and from 0.1 to 0.5 N sulphuric acid. The polarogram was recorded using a galvanometer shunt on 1/100. When the potential applied to the dropping electrode was more negative than -1.5 volts versus the sulphate anode, a current increase was observed. At such values, the hydrogen over-potential on mercury was exceeded and the current yielded was probably caused by the reduction of hydrogen ions at the cathode according to the reaction;

$$2\,H^{+} \;+\; 2\,e \longrightarrow H_2.$$

The polarogram obtained on the addition of 0.005% salicylaldoxime to the above electrolyte has been reproduced in figure 2.2. Comparison of the two polarograms revealed that the salicylaldoxime reagent yielded a wave with a half-wave potential of approximately -1.25 volts versus the sulphate anode, i.e. the wave occurred before the hydrogen wave.

A similar wave for the salicylaldoxime reagent was observed in supporting electrolytes containing acetic acid, sulphuric acid and also sodium sulphite.

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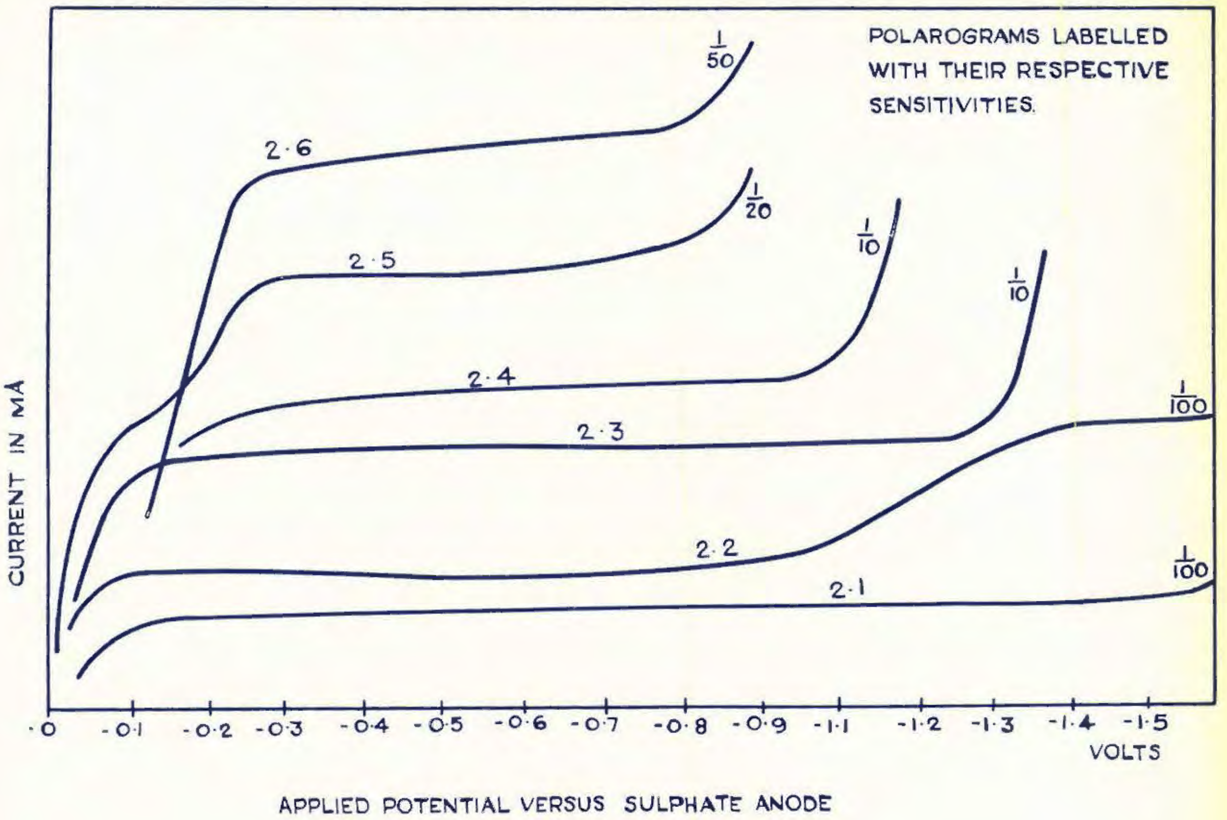


FIG. 2

POLAROGRAMS OBTAINED IN THE ABSENCE OF MOLYBDENUM.

This wave was not affected by the perchlorate ion concentration. Polarograms were recorded for test solutions containing 0.045% salicylaldoxime in 0.09 N hydrochloric acid and from 0.005 to 2.27 M sodium perchlorate. In each case the polarogram was identical to that reproduced in figure 2.4. The decomposition potential of this wave was however, dependent upon both the salicylaldoxime concentration and the acidity. Figure 2.3 was obtained for a test solution containing 0.05% salicylaldoxime in a sodium acetate, acetic acid buffer at pH 4.3. Figure 2.5 was obtained for a test solution containing 0.41% salicylaldoxime in 2.5 M perchloric acid. The decomposition potential of the wave due to the salicylaldoxime reagent occurred at a more positive value when the salicylaldoxime and acid concentration was increased.

The other current variation occurred at zero applied e.m.f. versus the sulphate anode. This phenomenon is clearly shown in figure 2.6. Figure 2.6 was obtained from an electrolyte containing 0.05% salicylaldoxime, 0.2 N perchloric acid, 0.15 N hydrochloric acid and 0.08 M sodium acetate. This current variation also occurred in electrolytes containing sulphuric acid, nitric acid, and in each of the electrolytes mentioned previously. The current was not influenced by the above ranges of salicylaldoxime, acetate ion or perchlorate ion concentration.

The magnitude of this initial current variation increased with the acidity. Electrolytes containing 5×10^{-5} to 2.5 M perchloric acid and 0.1 to 2 N sulphuric, nitric and hydrochloric acid each exhibited this tendency. According to Kolthoff and Lingane (25) this is an anodic current, caused by the anodic dissolution of the mercury from the dropping electrode.

In/

In subsequent investigations waves caused by the addition of molybdenum ions were examined. Such waves could only be examined between voltages governed by the above limits. For example, waves suitable for the analytical determination of molybdenum could not be employed if they occurred at potentials which were close to, or more negative than, the decomposition potential of the supporting electrolyte. For certain electrolytes, e.g. that used to obtain figure 2.6, the anodic current was large and full scale polarograms could not normally be obtained for the molybdenum wave. This was overcome by plotting the polarograms from a small negative initially applied potential and carefully aligning the photographic plate.

The above polarograms were also employed to determine the purity of the reagents used. A current-voltage curve was plotted each time a fresh electrolyte solution was prepared. No wave corresponding to molybdenum was detected, which indicated that the reagents were of satisfactory purity.

2.12 Salicylaldoxime - acetic acid electrolytes.

Aliquots of ammonium molybdate (Mo^{6+}) were added to the above supporting electrolyte, (Tucker (35)), and the resulting polarograms were examined. Possible interference by substances present in plant material was not examined at this stage. The polarograms, recorded against a mercury anode, are reproduced in figure 3.

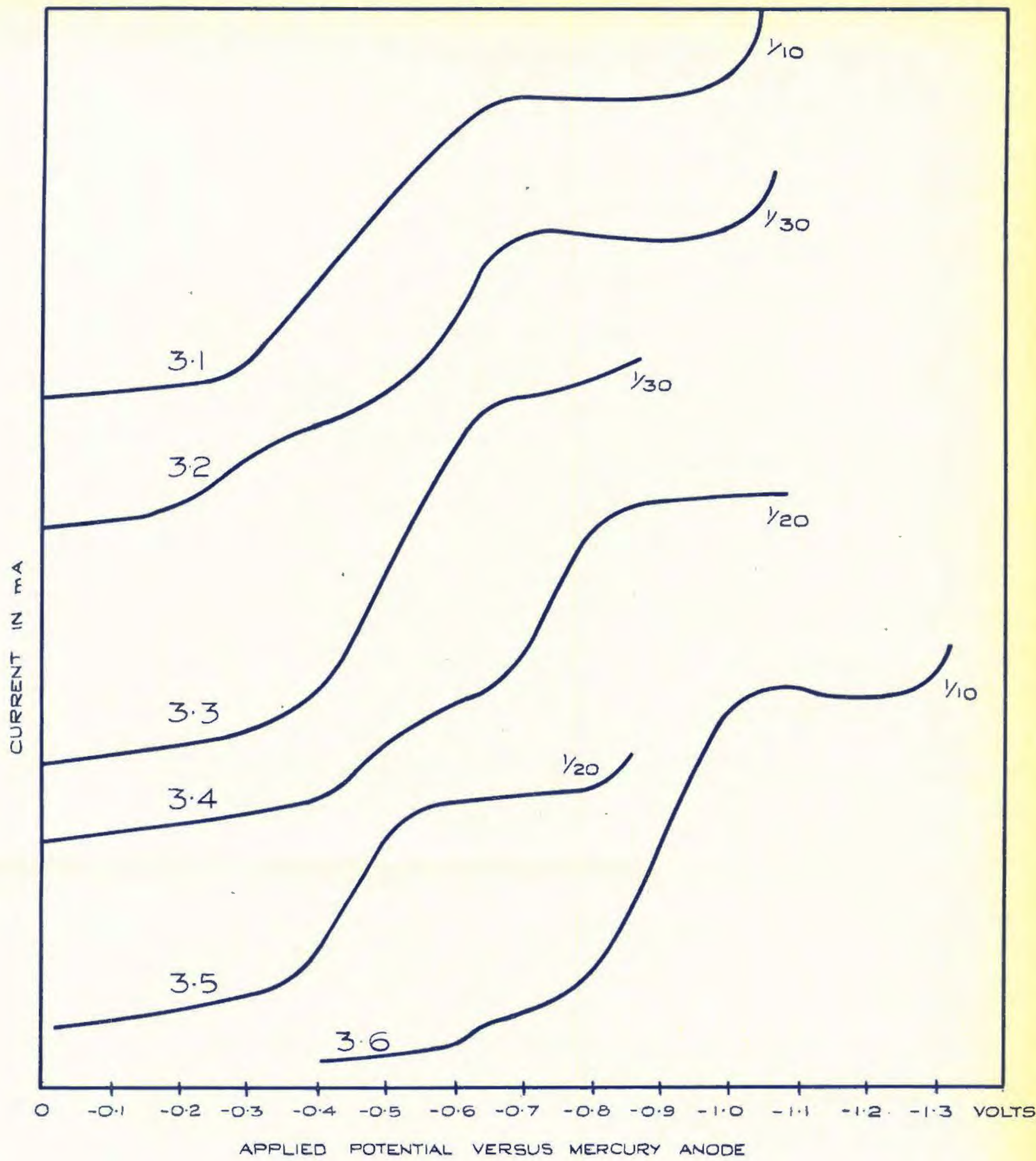
The first test solution had a bulk of 25 ml. and contained 2×10^{-4} M ammonium heptamolybdate in 0.75% salicylaldoxime and 0.7 M acetic acid. The molybdate ions were reduced to yield a double wave with a large space requirement. The half-wave potentials were approximately -0.3 and -0.6 volt versus the mercury pool.

The/ .....

The first wave was the smaller and was poorly defined. The second wave was distorted by a maximum. This maximum was diminished on the addition of gelatine (figure 3.2). The solution was stable and yielded identical polarograms for a minimum period of three days. The basic requirements of a polarogram suitable for quantitative measurements have been discussed previously in the literature survey. In view of the large space requirement and the negative half-wave potentials, this electrolyte was considered to be unsuitable for application to routine analysis.

On the addition of sufficient tartaric acid to the above test solution, a polarogram was obtained in which the molybdenum yielded a single wave. The polarogram obtained from a test solution to which 0.04% tartaric acid was added is reproduced in figure 3.3. No gelatin was present. This wave was more amenable to height measurements, but the half-wave potential was still fairly negative. The influence of the tartaric acid concentration on the polarogram was examined and was found to be critical. On increasing the tartaric acid concentration in the above electrolyte to 0.08%, the pre-wave was incompletely suppressed and polarograms similar to figure 3.2 were recorded. The height of the first wave was however considerably smaller.

The use of a supporting electrolyte containing 0.8% tartaric acid yielded two waves as is shown in figure 3.4. The half-wave potentials were respectively more negative than those observed from acetic acid - salicylaldoxime electrolytes. The waves did not possess a maximum. Similar waves have been reported by Milner (9) from less concentrated tartaric acid solution.



REAGENT CONCENTRATIONS.

THE TEST SOLUTION CONTAINS 2×10^{-4} g. Mo^{6+} ION PER LITRE PLUS THE FOLLOWING REAGENTS :-

TEST SOLUTION.	SALICYLALDOXIME %	ACETIC ACID NORMALITY.	TARTARIC ACID %	OTHER ACIDS.
3·1	0·68	—	0·07	0·005 $NHClO_4$
3·2	0·76	0·7	—	—
3·3	0·73	0·7	0·04	—
3·4	—	—	0·8	—
3·5	0·69	0·6	0·07	0·03 NHCl
3·6	0·72	—	0·07	0·03 NH_3PO_4

FIGURE 3.

POLAROGRAMS OF MOLYBDATE IONS IN SALICYLALDOXIME - ACETIC ACID ELECTROLYTES.

Upon the addition of 0.07% salicylaldoxime to the 0.8% tartaric acid electrolyte, a polarogram was obtained which resembled that reproduced in figure 3.2, rather than figure 3.4, i.e. the addition of salicylaldoxime rendered both half-wave potentials less negative. The reaction producing the above phenomenon is unknown, but may be connected with the formation of a molybdate-tartrate complex.

A stock solution (solution 1) containing 2×10^{-4} M ammonium molybdate in 0.7% salicylaldoxime and 0.07% tartaric acid and gelatin was prepared and examined polarographically. A single wave, similar to that reproduced in figure 3.3 was obtained. A comparison of the constituents of solution 1, with those used to obtain figure 3.3 indicated that the presence of acetic acid had no influence upon the half-wave potential of the molybdenum wave. The presence of acetic acid influenced the wave height. On the addition of 0.03 to 0.6 N acetic acid to solution 1, a progressive increase in the wave height was observed, though the half-wave potential remained constant.

The influence of the presence of several mineral acids upon the half-wave potential, the height and the form of the wave recorded may be observed from the polarograms reproduced in figure 3. The wave obtained subsequent to the addition of 0.005 N perchloric acid to solution 1 is reproduced in figure 3.1. That obtained subsequent to the addition of 0.03 N hydrochloric acid is reproduced in figure 3.5. In both cases the presence of the acid shifted the half-wave potential to a less negative value. A similar result was obtained when sulphuric acid was added to solution 1. The wave obtained subsequent to the addition of 0.03 *N* phosphoric acid has been reproduced in figure 3.6. Two waves were obtained. The pre-wave has approximately one tenth the height of the second wave.

Apart from these qualitative effects, the addition of these acids also increased the height of the molybdenum waves. This effect was examined for different concentration ranges of the above acids, viz., 0.005 to 0.42 *N* perchloric acid, 0.003 to 0.27 *N* hydrochloric acid, 0.005 to 0.03 *N* phosphoric acid and 0.005 to 0.29 *N* sulphuric acid. The height of the molybdenum wave obtained in the presence of 0.005 *N* perchloric acid (figure 3.1) was double that obtained from solution 1 alone (figure 3.3). The height increments obtained by the addition of the other acids to solution 1 were of a similar order of magnitude. Catalytic waves for molybdenum have been reported in perchloric acid electrolytes in the literature survey. Such waves were obviously not obtained in any of the above solutions. It appeared likely that a molybdenum-salicylaldoxime complex is formed which interferes with the catalytic mechanism at the electrode. This mechanism was not further investigated. Over each of the acid concentration ranges investigated the wave heights increased with the acidity. This increment was small in comparison with the height increment obtained by the addition of a mineral acid to solution 1. The addition of up to 0.6 *N* acetic acid to each of these solutions had no influence upon the half-wave potential.

As the phosphoric acid concentration was increased from 0.005 to 0.03 N, the pre-wave became better developed and increased slightly in height. The second wave developed a maximum. As the concentration of sulphuric acid and perchloric acid was increased there was an increasing tendency for a doublet wave to develop. A pre-wave occurred for sulphuric acid concentrations greater than 0.05 N and for perchloric acid concentrations greater than 0.09 N. As was the case for phosphoric acid, the pre-wave became better developed and the second wave tended to develop a maximum as the acidity was increased. The increase in the concentration of hydrochloric acid added distorted the wave.

None of the above electrolytes yielded a wave entirely suitable for the quantitative determination of molybdenum in plant materials. Since the plant solution usually contains inorganic acids, and electrolytes containing these acids have been shown to yield waves having greater sensitivity, electrolytes containing mineral acids and salicylaldoxime were examined further. Acetic acid was not added, since it's influence has been shown to be slight in the presence of mineral acids. Neither hydrochloric nor phosphoric acid electrolytes were examined further, since the above investigation revealed them to produce complex waves for molybdenum.

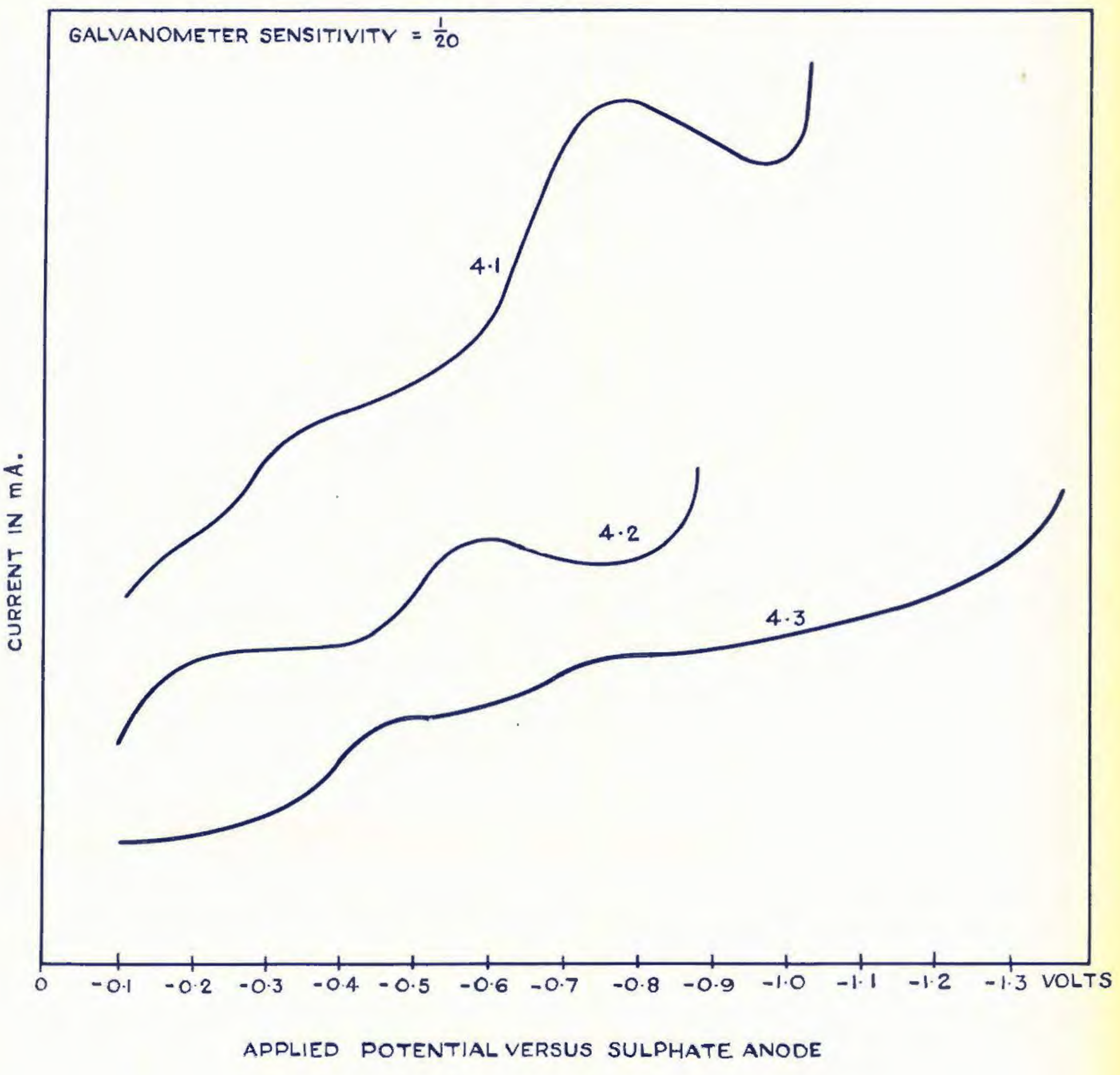
2.33 Salicylaldoxime - sulphuric acid electrolytes.

Supporting electrolytes containing salicylaldoxime and sulphuric acid were examined as follows: Polarograms were recorded for 6 p.p.m. molybdenum in 0.2% salicylaldoxime and concentrations of sulphuric acid covering the range 0.05 to 1.5 N. The polarograms for this, and for all subsequent electrolytes examined, were recorded versus the non-polarisable sulphate reference anode. In order to avoid recording the initial mercury current (figure 2), and to obtain full scale deflection, the polarogram was frequently recorded from an initial applied potential of -0.1 volt.

Two malformed waves occurred from electrolytes containing less than 0.1 N sulphuric acid (figure 4.1). The pre-wave was incompletely developed and the main wave had a maximum. This maximum was not removed by the addition of 0.012% gelatin. As the sulphuric acid concentration was increased, the potential of both waves became increasingly more positive. The polarogram recorded on the addition of 0.77 N sulphuric acid is reproduced in figure 4.2. For greater sulphuric acid concentrations than 1 N, one wave only was recorded. The maximum decreased with higher sulphuric acid concentration. The drift in the half-wave potential of the pre-wave was also encountered by Holje (17).

The molybdenum reduction waves in sulphuric acid electrolytes were also examined. Figure 4.3 was recorded from a test solution containing 4 p.p.m. molybdenum in 0.1 N sulphuric acid. Molybdenum was reduced in two stages in 0.1 N sulphuric acid. The waves were poorly developed. These findings concurred with the findings of Holje (17) and Haight (21) for sulphuric acid electrolytes. None of the waves reproduced in figure 4 were favourably shaped for quantitative measurements.

2.34/ ....



REAGENT CONCENTRATIONS

TEST SOLUTION	MOLYBDENUM (P.P.M)	SALICYLALDOXIME %	H_2SO_4 NORMALITY
4·1	5·7	0·19	0·08
4·2	3·9	0·19	0·77
4·3	4	—	0·1

FIG. 4

POLAROGRAMS OF Mo^{6+} ION IN SALICYLALDOXIME - SULPHURIC ACID.

2.34 Salicylaldoxime-nitric acid electrolytes.

The molybdenum reduction waves from this electrolyte were examined in a similar procedure to that described above. Test solutions containing 3.9 p.p.m. molybdenum in 0.2% salicylaldoxime and concentrations of nitric acid covering the range 0.08 to 1.5 N were polarographed. The waves obtained were similarly shaped to those previously obtained from salicylaldoxime-sulphuric acid. A prewave was recorded for nitric acid concentrations less than 0.4 N. The height of the pre-wave decreased with increasing acidity. Above 0.4 N, one wave only was obtained. The height of this wave was slightly higher than in a similar sulphuric acid electrolyte. The wave was distorted by a large maximum, which was not removed by gelatin. This made height measurement difficult.

Stern (20) discovered that an improvement in the wave form was obtained from solutions in which the ratio of nitrate to hydrogen ions in the electrolyte was increased. Test solutions containing 3.2 p.p.m. molybdenum in 0.35% salicylaldoxime and 0.53 M potassium nitrate over the range 0.033 to 1.0 N nitric acid were polarographed. Two waves were obtained as before. The addition of this concentration of nitrate ions did not improve the shape of the wave.

2.35 Perchloric acid electrolytes.

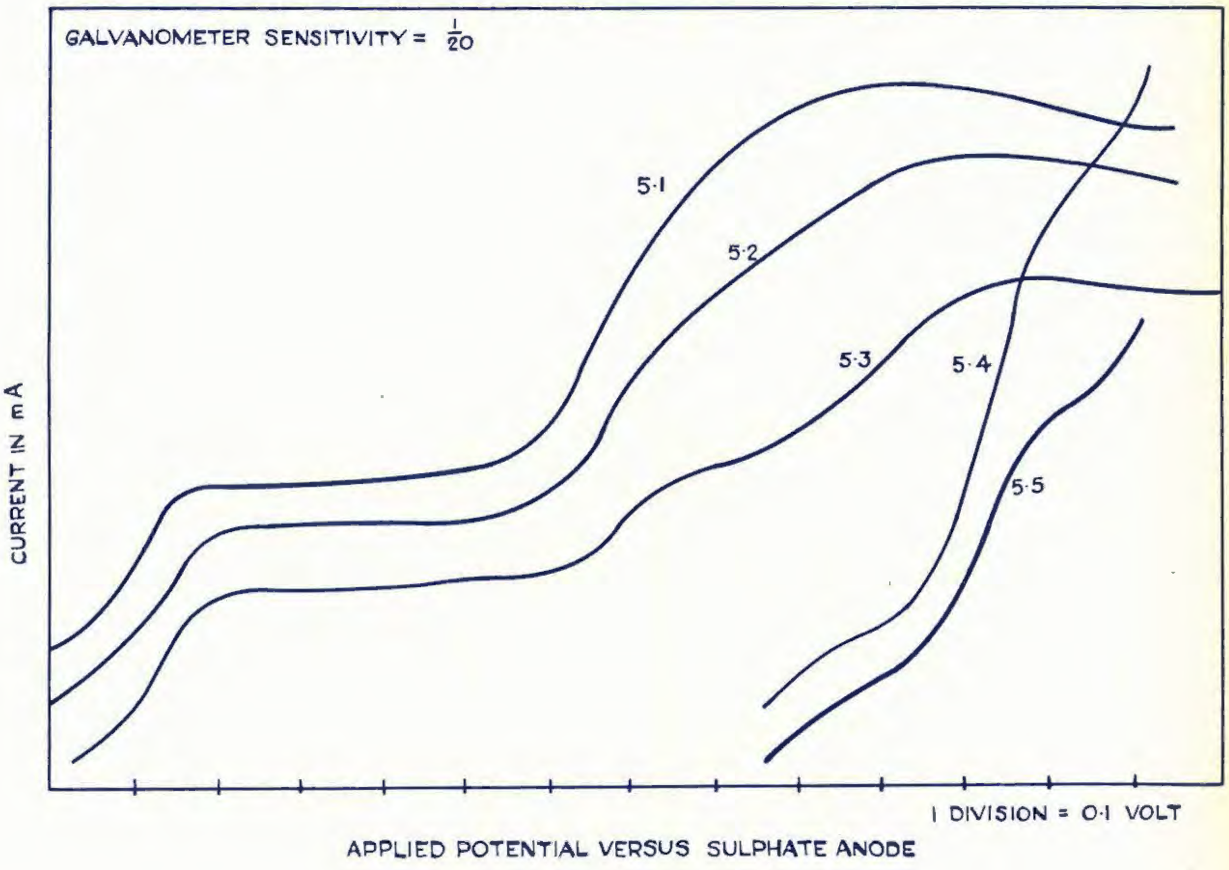
Perchloric acid was used in the wet oxidation of the plant material (Steyn (37)). The test solution obtained contained molybdenum, plus the other substances present in plant material, in perchloric acid. Nearly all the other anions were removed during the digestion stage. The use of electrolytes containing perchloric acid was examined. It was found that the height and the number of molybdenum waves obtained varied with the acidity.

The polarograms obtained for molybdenum in electrolytes containing 0.0008 to 0.8 *N* perchloric acid were examined. In 0.4 *N* perchloric acid, the molybdenum yielded a single wave, similar to that reproduced in figure 5.1. This result concurred with the findings of Milner (9). In 0.8 *N* perchloric acid the reduction occurred in two stages and the waves were poorly developed. The total wave heights for 8 p.p.m. molybdenum were observed to increase by 50% as the perchloric acid concentration was increased from 0.4 to 0.8 *N*. The catalytic nature of the molybdenum wave in this electrolyte was shown by the fact that the wave height was several times that observed in sulphuric acid electrolytes. In electrolytes where the reduction occurred in two stages, the height of the second wave increased with the acidity, while that of the first wave was constant. This is in accordance with the findings of Haight (21) who stated that Mo^{4+} ions acted as an intermediate catalyst.

The polarograms obtained over the range 0.0008 to 0.08 *N* perchloric acid have been reproduced in figures 6.1 to 6.3. The reduction occurred in two stages. The first wave was small. The second wave was poorly developed. Kolje (17) recorded only one wave in 0.01 *N* perchloric acid.

The molybdenum content was increased from 5 p.p.m. to 30 p.p.m. in 0.5 *N* perchloric acid. The height of the single wave obtained increased with the molybdenum concentration. Accurate height measurements were however impossible because the wave was poorly developed.

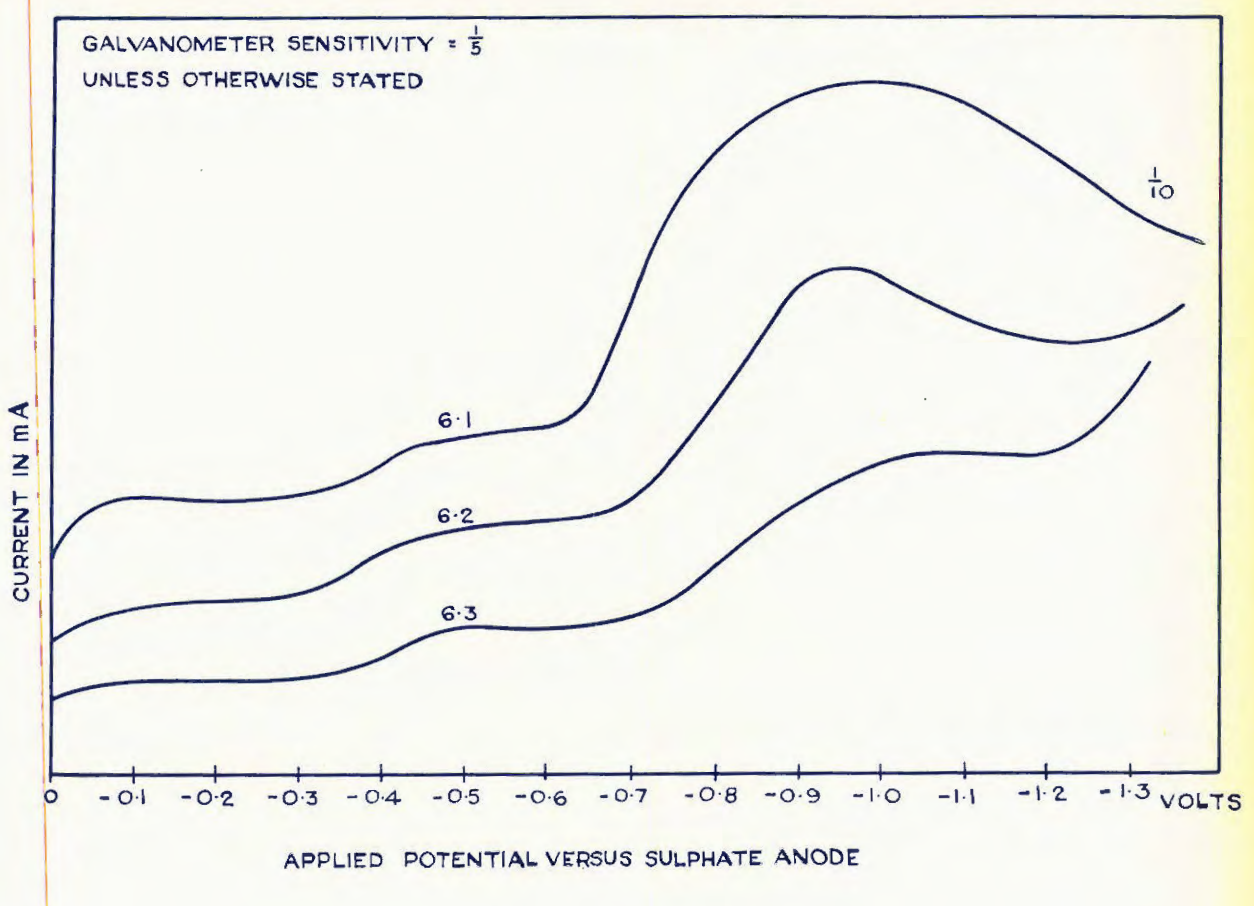
Electrolytes/ ..



THE TEST SOLUTIONS CONTAIN 5 p.p.m. MOLYBDENUM IN 0·1 M $HClO_4$ AND 0·5 M $NaClO_4$

FIG. 5.

POLAROGRAMS FOR Mo^{6+} ION IN PERCHLORIC ACID.



THE TEST SOLUTIONS CONTAIN 8 p.p.m MOLYBDENUM IN THE FOLLOWING $HClO_4$ CONCENTRATIONS :-

TEST SOLUTION	6·1	6·2	6·3
$HClO_4$ CONCENTRATION	0·08N	0·008N	0·0008N

FIG. 6.

EFFECT OF PERCHLORIC ACID CONCENTRATION ON POLAROGRAMS FOR Mo^{6+} ION.

Electrolytes were also examined in which the ratio of perchlorate ion concentration to hydrogen ion concentration was increased. Stern (20) had improved the poorly-shaped waves obtained by Uhl (1) from nitric acid solutions by increasing the ratio of nitrate to hydrogen ions. Polarograms were recorded from solutions containing 5 p.p.m. molybdenum in 0.1 N perchloric acid and 0.5 N sodium perchlorate. The polarogram obtained from a freshly prepared solution has been reproduced in figure 5.1. The current variation at zero volt was caused by the anodic dissolution of mercury. The molybdenum was reduced in one stage. The maximum was removed with gelatin. The addition of this concentration of perchlorate ions did not improve the shape of the wave.

If the test solution was left in contact with mercury the form of the polarogram obtained was modified. Polarogram 5.1 was recorded two hours after mixing the reagents. The polarogram was reproducible for several days if a fresh aliquot of the test solution was added to the electrolysis cell. Figures 5.2 and 5.3 were recorded from the identical solution used to obtain figure 5.1. Mercury was present from the dropping electrode. Figure 5.2 was recorded 4 hours after recording figure 5.1 and figure 5.3 was recorded 28 hours later. The single wave initially obtained from a test solution in contact with mercury gradually dissociated into two waves with time. Figures 5.4 and 5.5 were recorded after 24 hours and 48 hours respectively. The first wave became better developed with time. It appeared possible that this phenomenon would explain the difference between the number of waves recorded by Milner (9) and by Holje (17) in 0.4 N perchloric acid.

The/ .....

The above electrolytes were unsuitable for quantitative purposes. Few of the requirements listed in the literature survey are fulfilled.

3. A critical examination of salicylaldoxime - perchloric acid electrolytes.

Initial experiments on the above electrolyte indicated that the shape of the polarogram recorded for molybdate ions was markedly dependent upon the concentration of both reagents. The effect of the concentraion of each of the reagents on the height, the shape and the stability of the wave was examined in turn, with a view to determining the optimum concentration ranges.

A test solution containing 8 p.p.m. molybdenum in 0.41 M perchloric acid and 0.004% salicylaldoxime was freshly prepared and the polarogram recorded. This has been reproduced in figure 7.5. A double wave was obtained. Neither wave was well developed. The addition of the salicylaldoxime modified the single reduction stage previously obtained for an electrolyte containing 0.4 N perchloric acid, (figure 5.1). On comparison of polarogram 7.5 with polarogram 2.2, it was observed that the potential of the second wave was close to that observed for salicylaldoxime. The height of the second wave (figure 7.5) increased linearly with the salicylaldoxime concentration. The height of both waves (figure 7.5) was observed to increase with the molybdenum concentration. The height of the second wave increased when the perchloric acid concentration was increased to 0.73 N.

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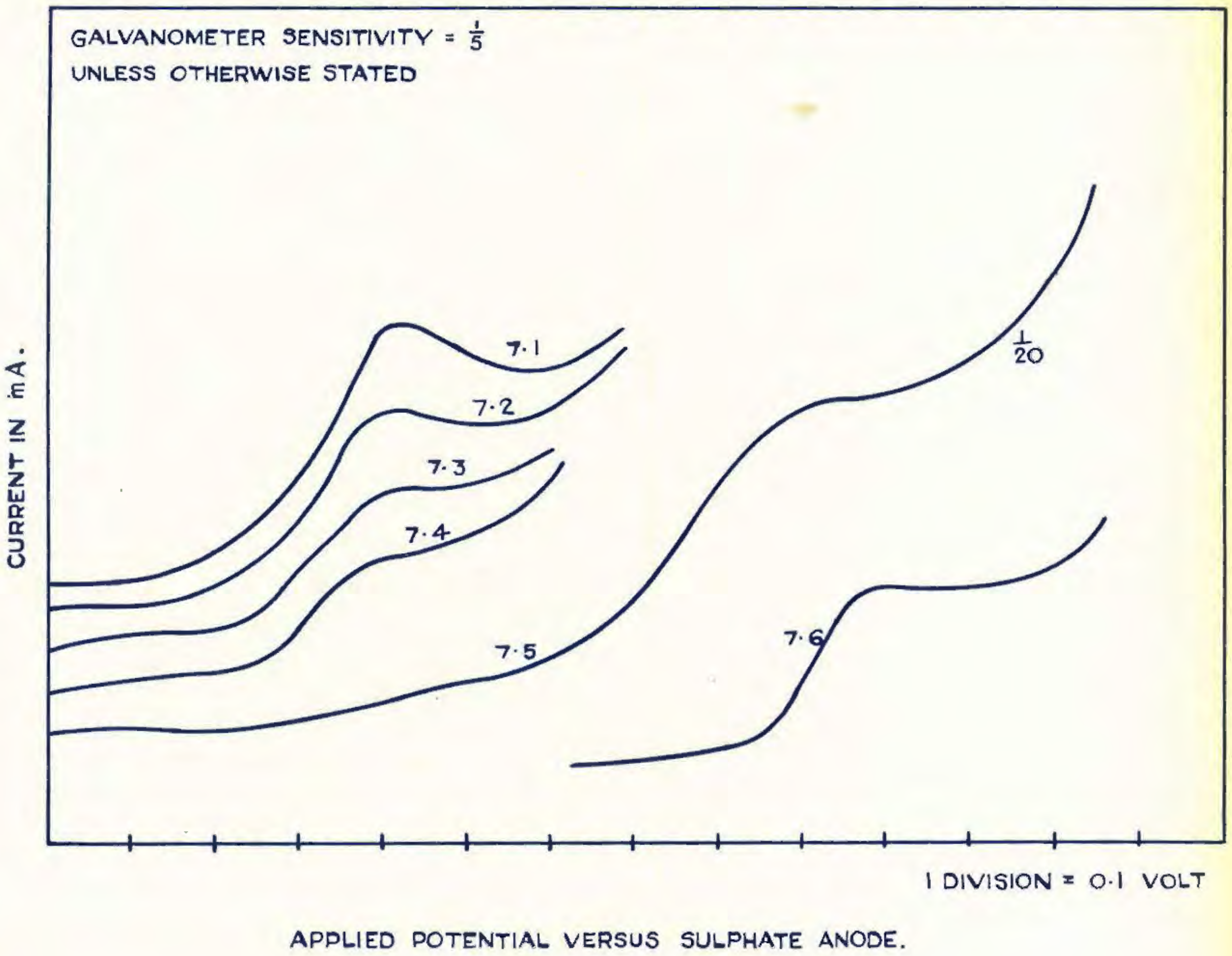


THE TEST SOLUTIONS CONTAIN 8 p.p.m. MOLYBDENUM IN 0·41 N $HClO_4$ AND THE FOLLOWING SALICYLALDOXIME CONCENTRATIONS:

TEST SOLUTIONS	7·1	7·2	7·3	7·4	7·5	7·6
% SALICYLALDOXIME	0·180	0·042	0·020	0·012	0·004	0·064

EACH POLAROGRAM WAS RECORDED FROM AN INITIAL, APPLIED POTENTIAL OF − 0·3 VOLT VERSUS THE SULPHATE ANODE.

FIG. 7.

EFFECT OF SALICYLALDOXIME CONCENTRATION ON THE POLAROGRAMS FOR Mo^{6+} ION.

In view of the above observed properties, the second wave obtained in figure 7.5 was considered to be two superimposed waves. The one wave was caused by reduction of the molybdenum ions and the other by the salicylaldoxime. The dependence on the salicylaldoxime concentration rendered this second wave unsuitable for the quantitative determination of molybdenum.

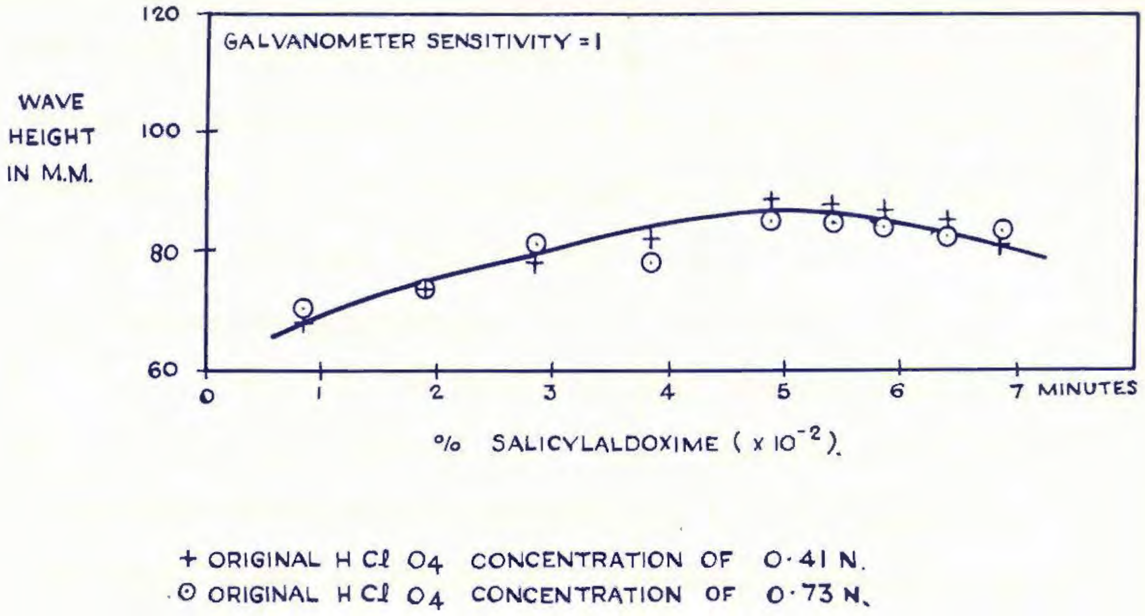
3.1 Salicylaldoxime concentration.

The influence of the concentration of this reagent on the shape of the first wave was initially examined qualitatively. Aliquots from 0.004 to 0.45% salicylaldoxime were added to a freshly prepared solution containing 8 p.p.m. molybdenum in 0.41 M perchloric acid. Polarograms were recorded within an hour of the addition of the salicylaldoxime reagent. These polarograms have been reproduced in figures 7.1 to 7.5. As the salicylaldoxime concentration was increased from the lower value (figure 7.5) the wave became better developed (figure 7.4). Addition of further salicylaldoxime produced a maximum. The height of this maximum increased progressively as the salicylaldoxime concentration was increased above 0.02%. The maximum reached a limiting height on the addition of 0.2% salicylaldoxime. The addition of further salicylaldoxime had no influence on the maximum.

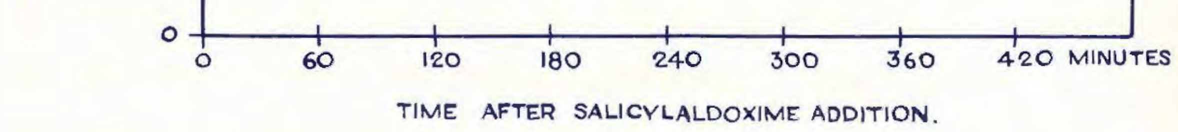
The concentration of salicylaldoxime optimum for a suitably-shaped wave was found to be dependent upon the concentration of the perchloric acid. A similar series of polarograms were recorded for a test solution containing different concentrations of salicylaldoxime in a test solution containing 8 p.p.m. molybdenum and 0.5 N perchloric acid. At this acid concentration the wave was distorted by a maximum when the salicylaldoxime concentration exceeded 0.032%. This value is greater than the concentration value of 0.02% salicylaldoxime observed for 0.41 N perchloric acid. This inter-relation was used to eliminate the maximum. The maximum obtained by the addition of 0.042% salicylaldoxime (figure 7.2) was eliminated by increasing the perchloric acid concentration from 0.41 N to 0.87 N. A well-shaped wave was recorded.

The quantitative influence of the salicylaldoxime concentration on the wave was determined by examining the height of the wave at -0.6 volt. In addition to the previous recordings for 0.41 N perchloric acid, polarograms were also recorded for test solutions containing 8 p.p.m. Mo in 0.73 N perchloric acid and from 0.004 to 0.45% salicylaldoxime. The heights of the waves obtained were plotted as a function of the salicylaldoxime concentration (figure 8.1). The curve increased to a maximum value and then decreased with increasing salicylaldoxime concentration. The above determinations were performed by the addition of subsequent aliquots of 1% salicylaldoxime to 40 ml. of test solution originally containing 8 p.p.m. molybdenum. Graph 8.1 would therefore be expected to have a small negative gradient caused by the dilution effect, if the salicylaldoxime reagent did not influence the molybdenum wave.

Figure 7.6 was recorded from a test solution containing 8 p.p.m. molybdenum in 0.73 N perchloric acid and 0.64% salicylaldoxime. This wave is ideal for height measurements. Several other such waves were obtained.



8·1 EFFECT OF SALICYLALDOXIME CONCENTRATION ON THE HEIGHT OF THE WAVE.



8·2 EFFECT OF THE AGE OF THE TEST SOLUTION ON THE HEIGHT OF THE WAVE.

FIG. 8.

FACTORS INFLUENCING THE WAVE HEIGHT.

This investigation indicated that the optimum salicylaldoxime concentration could not be selected without an examination of the influence of the perchloric acid concentration upon this factor.

3.2 Reduction mechanism.

The wave at -0.6 volt was smaller than the wave at -1.07 volts (figure 7.5). This indicated that the electrode reaction was non-catalytic. The electrode reactions given by Kolje (17) for this concentration of perchloric acid indicated that the wave was caused by the reaction:

$$Mo^{(6+)} + e \longrightarrow Mo^{(5+)}$$

The dependence of the shape of this wave on both the salicylaldoxime and perchloric acid concentrations, indicated that the reduction mechanism was probably more complex than the simple equation would indicate.

3.3 Stability.

The reproducibility of the shape and height of the wave at -0.6 volt was examined with respect to time subsequent to the addition of the different concentrations of perchloric acid and salicylaldoxime. Polarograms recorded at suitable time periods from a solution containing 7.4 p.p.m. molybdenum in 0.40 N perchloric acid and 0.08% salicylaldoxime have been reproduced in figures 9.1 to 9.7. The polarograms recorded from a test solution containing 8.9 p.p.m. molybdenum in 0.47 N perchloric acid and 0.32% salicylaldoxime have been reproduced in figures 9.8 to 9.12. The initial potential of the polarograms was -0.3 volt versus the sulphate anode. The time of recording in minutes, subsequent to the addition of salicylaldoxime is printed on each polarogram. The time of salicylaldoxime addition was taken as zero time.

Figures/ ...

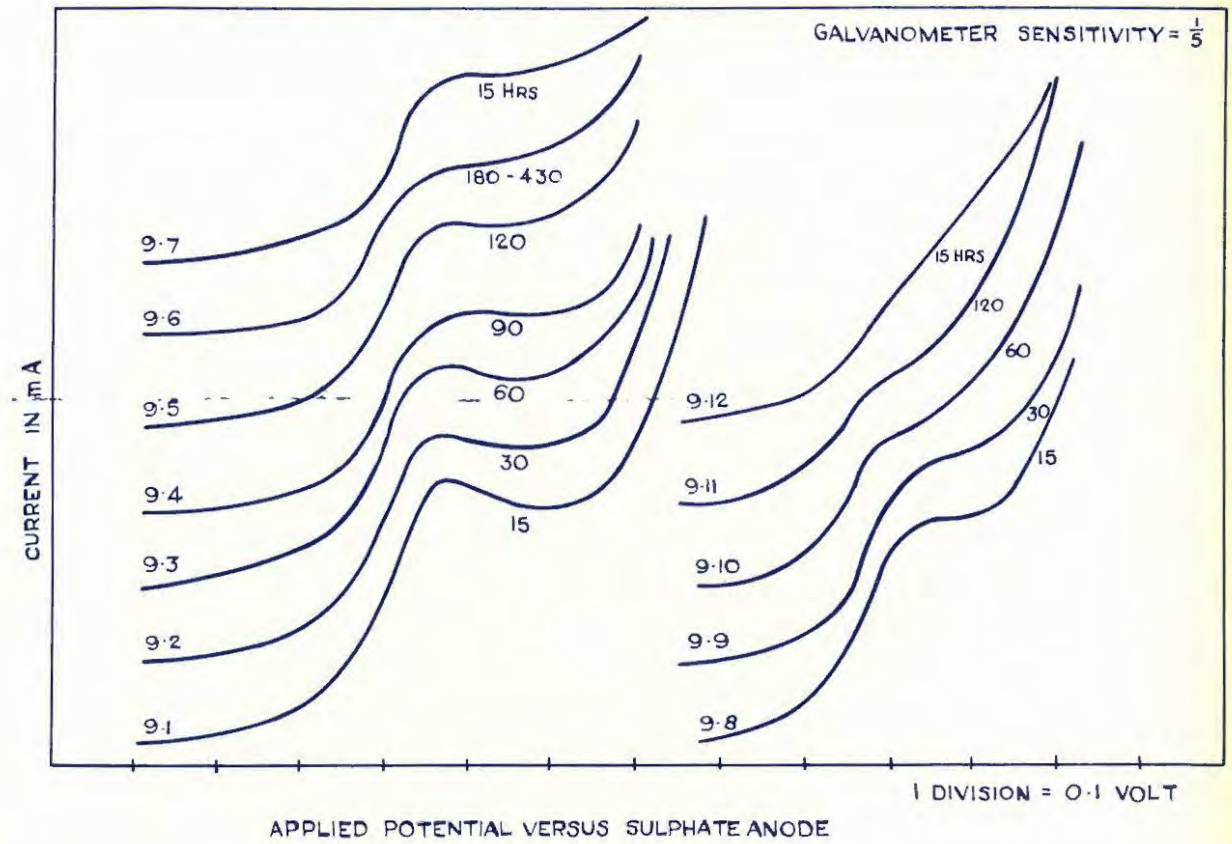


FIG. 9

EFFECT OF AGE OF TEST SOLUTION ON THE POLAROGRAMS FOR Mo^{6+} ION.

figures 9.7 and 9.12 were each recorded 15 hours subsequently to the addition of the salicylaldoxime reagent.

The waves in these concentrations of perchloric acid and salicylaldoxime were unstable with respect to time. The maximum tended to disappear (figures 9.1 to 9.7) and the wave became malformed on standing (figures 9.8 to 9.12). Similar results were obtained whether the salicylaldoxime reagent was dissolved in methanol or in ethanol. The height of the reduction wave from 8.1 p.p.m. Mo in 0.44 N perchloric acid and 0.058% salicylaldoxime was plotted against the recording time (figure 8.2). The height of the wave was found to decrease with time at first, and then to attain a constant value. This height instability occurred during the recording of the values reproduced in figure 8.1. Consideration of figures 8.1 and 8.2 together, indicated that the wave height increased with the salicylaldoxime concentration up to an optimum value, and that thereafter the wave height was probably independent of the salicylaldoxime concentration. The similar values obtained for 0.41 N and 0.73 N perchloric acid indicated that the wave height was also independent of this change in the perchloric acid concentration (figure 8.1).

Polarograms were recorded at suitable time intervals for 8 p.p.m. molybdenum in:-

(1) 0.47 N perchloric acid and 0.032% salicylaldoxime,

(2) 0.44 N perchloric acid and 0.058% salicylaldoxime and

(3) 0.40 N perchloric acid and 0.078% salicylaldoxime.

Waves which could be used for height measurements were obtained over the following time intervals subsequent to salicylaldoxime addition:

(1)/ .....

(1) 0 to 20 minutes,

(2) 1 to 18 hours, and

(3) 2 to 16 hours.

These results indicated that the wave instability was dependent upon the concentration of both reagents. It is necessary that the polarogram recorded must be reproducible for a period of time sufficient to obtain the wave height. This criterion also had to be considered in the determination of the optimum reagent concentrations.

3.4 The acid concentration.

The influence of the perchloric acid concentration on the characteristics of the molybdenum wave was examined at constant salicylaldoxime concentration. The stability was not examined at this stage.

A test solution was prepared containing a final concentration of 5 p.p.m. molybdenum in 0.05% salicylaldoxime and from 5×10^{-8} to 2.5 M perchloric acid. A buffer was added to each test solution in order to minimise possible variations in the curves caused by fluctuations in the hydrogen ion concentration during lengthy examinations at the lower acidities. A sodium acetate-acetic acid buffer containing 0.08 g. acetate ions per litre was employed at the lower perchloric acid concentrations (figure 11). A 0.08 M sodium acetate-hydrochloric acid buffer was used for higher acidities (figure 10). Sodium chloride (0.06 M) was added to the test solution containing 2.5 M perchloric acid.

The/ ....

The efficiency of the buffers was determined by frequent use of a pH meter and was found to be satisfactory. The presence of the buffers did not influence the molybdenum wave. Identical waves were recorded when the buffer was absent.

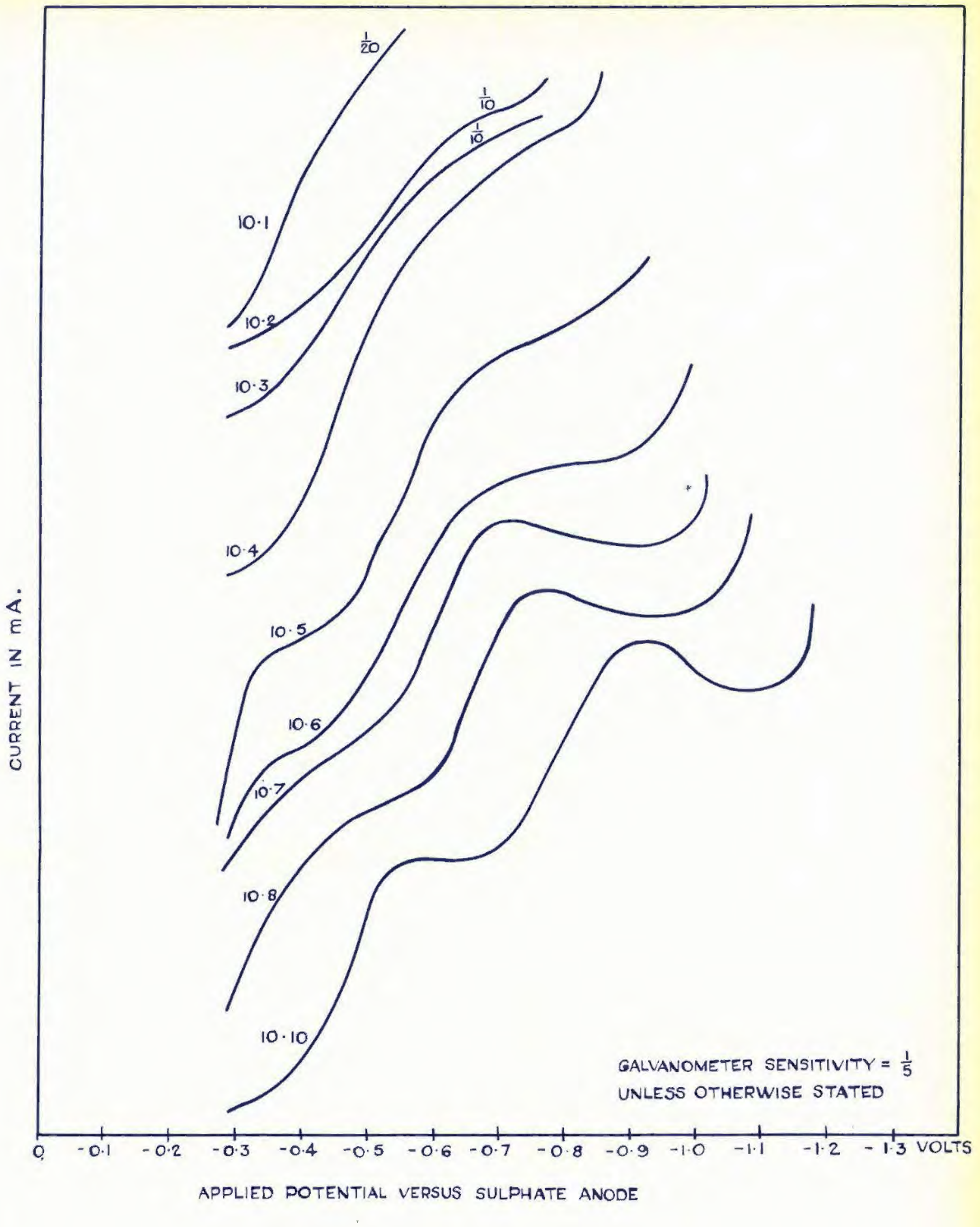
The characteristics of the polarograms recorded for this range of acidities have been summarised in Table I. The wave heights (in m.m.) were recorded prior to the addition of gelatin. The galvanometer sensitivity employed was 1/10.

TABLE I.

VARIATION OF THE WAVE HEIGHT AND HALF-WAVE POTENTIAL WITH THE ACIDITY.

Test Solution.	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	11.1	10.10	11.2	11.3	11.4
p^H	—	--	--	--	1.2	1.3	1.8	2.5	3.4	4.1	4.4	5.4	5.8
Half-Wave Potential	0.6	0.6	0.6	0.6	0.6	0.6	0.64	0.68 --	0.7 0.35	0.7 0.4	0.8 0.42	0.8 0.44	0.83 0.43
Wave Height	—	32.5	33.0	33.5	31	24	14	15.5	11 22.8	-- --	7.5 20	3.5 9.5	4.2 8
Total Height.	--	32.5	33.0	33.5	31	24	14	15.5	33.8	—	27.5	13	12.2

Note:- The half-wave potentials are all negative values.



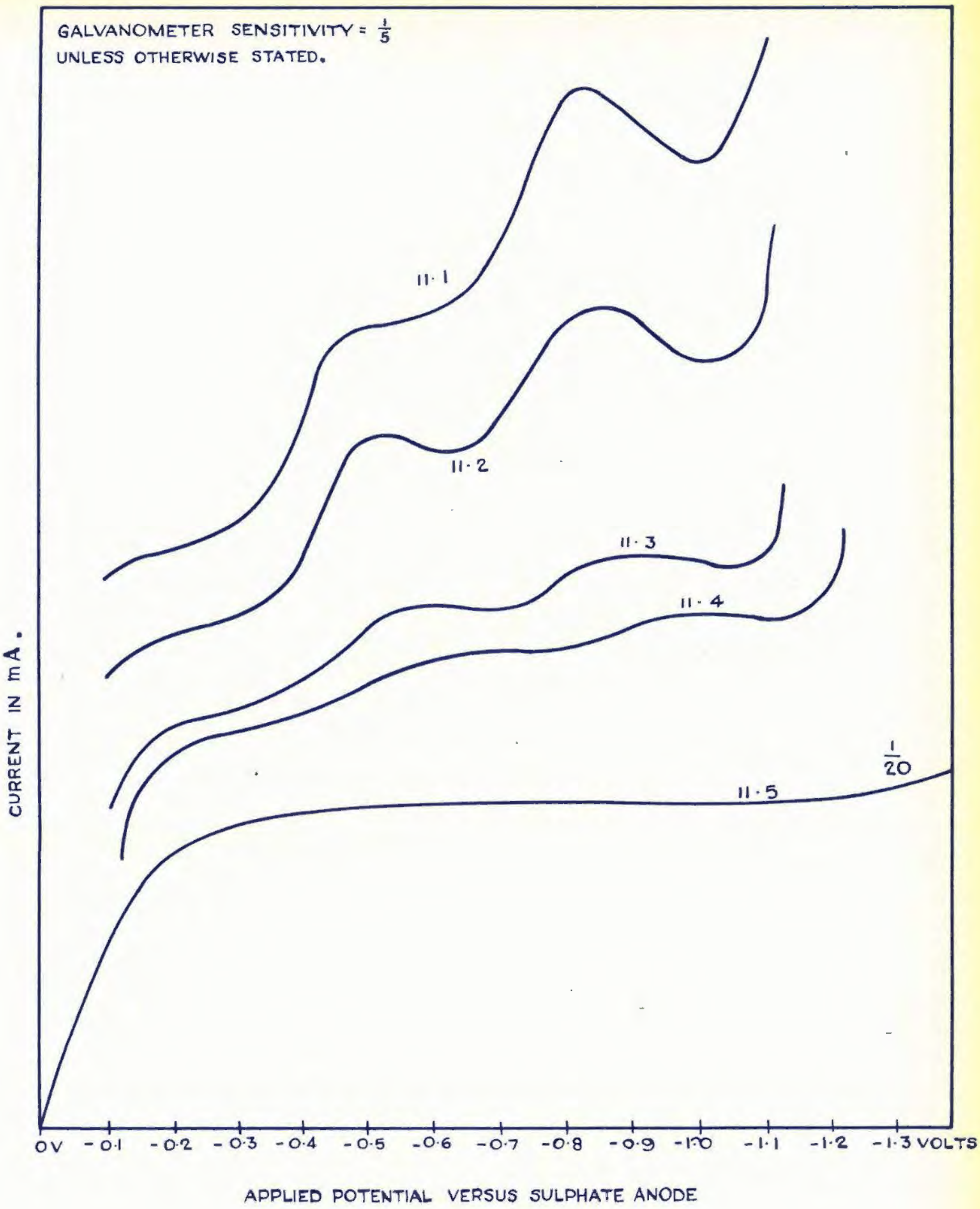
GALVANOMETER SENSITIVITY = $\frac{1}{5}$
UNLESS OTHERWISE STATED

THE TEST SOLUTIONS CONTAIN 5 p.p.m. MOLYBDENUM, 0·05 % SALICYLALDOXIME, 0·08 M. SODIUM ACETATE, 0·015 % GELATINE AND THE FOLLOWING ACID CONCENTRATIONS:

TEST SOLUTION	10·1	10·2	10·3	10·4	10·5	10·6	10·7	10·8	10·10
$HClO_4$ NORMALITY	2·5	0·25	0·2	0·15	0·1	0·05	5×10^{-3}	5×10^{-4}	$5 \times 10^{-}$
HCl NORMALITY	-	0·15	0·14	0·14	0·10	0·09	0·08	0·08	0·05
pH	-	-	1·0	0·9	1·2	1·3	1·8	2·5	4·1

FIG. 10

EFFECT OF THE ACID CONCENTRATION ON POLAROGRAMS FOR Mo^{6+} ION.



THE TEST SOLUTIONS CONTAIN 5 p.p.m. MOLYBDENUM, 0·05 %. SALICYLALDOXIME, 0·08 g. ACETATE ION PER LITRE, 0·006 % GELATINE AND THE FOLLOWING ACID CONCENTRATIONS:

TEST SOLUTION	11·1	11·2	11·3	11·4	11·5
$HClO_4$ NORMALITY	5×10^{-4}	5×10^{-5}	5×10^{-6}	5×10^{-7}	5×10^{-8}
ACETIC ACID NORMALITY	0·08	0·06	0·02	0·004	—
pH	3·4	4·4	5·4	5·8	7·6

FIG. 11.

EFFECT OF THE ACID CONCENTRATION ON POLAROGRAMS

FOR Mo^{6+} ION.

Figures 10 and 11 are the reduction waves obtained subsequent to the addition of 0.01% gelatin to the above solutions. The gelatin was found to decrease the height of the maxima slightly. From these figures it was observed that waves for molybdenum occurred in acid, but not in alkaline electrolytes. The variation in the current observed in figure 11.5 was due to anodic mercury and to salicylaldoxime. The waves obtained from acid electrolytes varied with respect to:-

1) The half-wave potential and the number of waves recorded,

2) the wave height and

3) the gradient of the diffusion current.

Examination of Table I and figures 10 and 11 revealed a relationship between these properties and the acidity of the test solution.

(1) At pH values from 5.8 to 3.4 a doublet wave was obtained. The potential of both waves became more positive with the increase in acidity. One complete wave only, was recorded from electrolytes with pH values less than 2.5. No pre-wave was recorded for perchloric acid concentrations greater than 0.1 M. The number of reduction stages obtained corresponded to the number reported by Holje (17) for perchloric acid electrolytes. However, though Holje (17) reported only one wave from 0.01 N perchloric acid, the reduction was observed to occur in two stages at this acidity in the above electrolyte, and at this acidity of an electrolyte containing perchloric acid alone (figure 5.2.).

For .....

For perchloric acid concentrations greater than 0.05 M the half-wave potential of the polarogram was constant at -0.6 volt versus the sulphate anode, (Table I). Haight (21) investigated the influence of the perchloric acid concentration in sulphuric acid electrolytes and found that the potential became more positive as the hydrogen ion concentration was increased from 0.05 to 1.0 M.

(2) The height of both the pre-wave and the main wave increased with the perchloric acid concentration (Table I). The main wave height increased to a maximum and almost constant value for acidities greater than 0.1 N. The total wave height at pH 3.4 was close to this value.

A minimum concentration of 0.1 N perchloric acid was selected as the optimum concentration value. At such values a single wave was recorded, and the half-wave potential and wave height were only to a small extent dependent upon the acidity.

(3) The shape of the wave obtained for electrolytes containing perchloric acid concentrations in excess of 0.1 N were however unsatisfactory for height measurements (figures 10.1 to 10.5). The section of the wave that is controlled by the diffusion current altered it's shape regularly from a maximum (figure 10.10) to a value with a positive gradient (figure 10.1) as the perchloric acid concentration was increased. The trend occurred for both the pre-wave and the main wave (figure 11).

The previous examination indicated that the gradient of this section of the curve was controlled by the salicylaldoxime to perchloric acid concentration ratio (figure 7). A perfectly-formed wave, well suited for height measurements, was obtained from 2.5 N perchloric acid when the salicylaldoxime concentration was increased to 0.41%. These results indicated that a satisfactorily shaped wave could be obtained for electrolytes containing 0.1 N perchloric acid or more, by increasing the salicylaldoxime concentration above 0.05%.

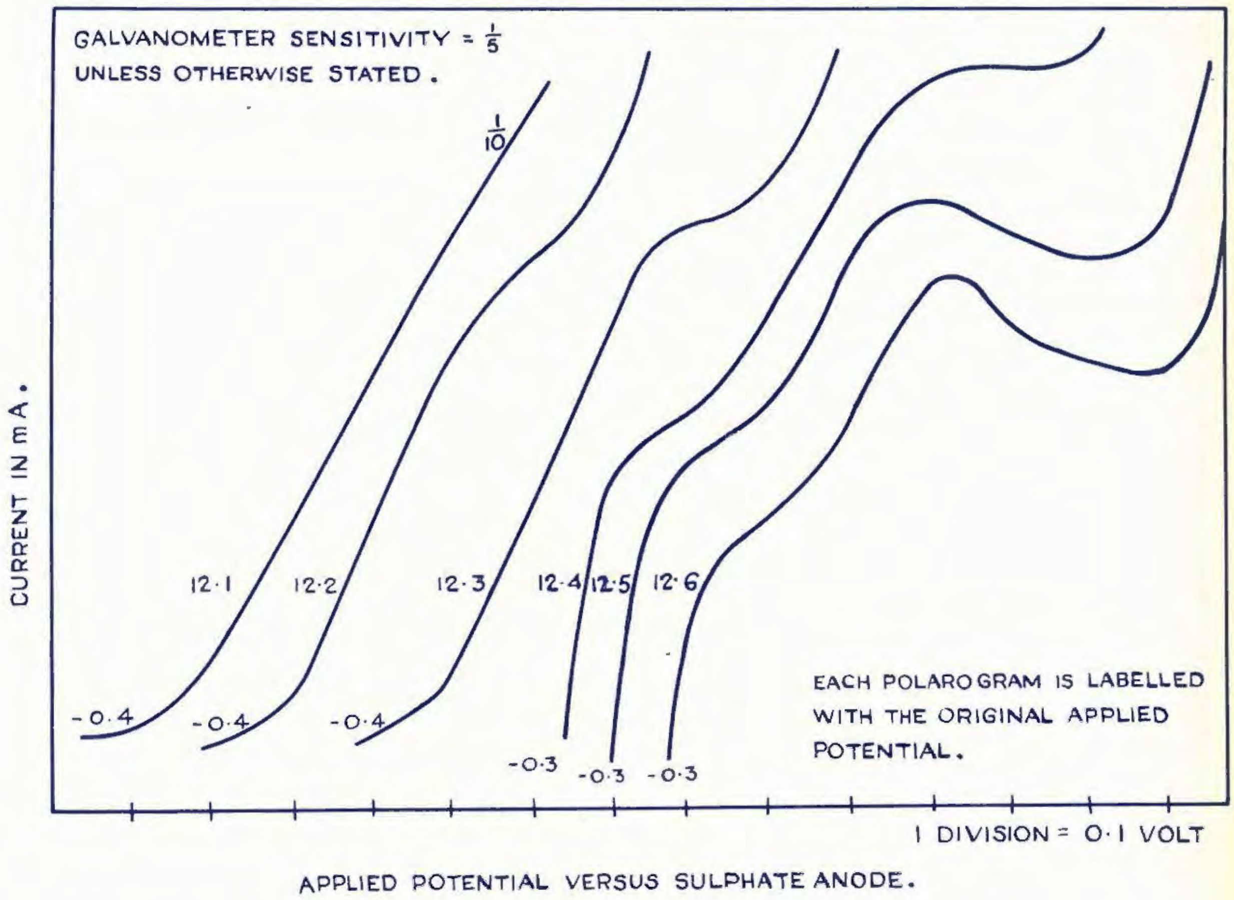
3.5 The concentration of perchlorate ions.

Haight (21) reported that the hydrogen ion concentration had no effect upon the wave height and stated that the height was markedly dependent upon the perchlorate ion concentration over the range 0 to 0.1 g. perchlorate ion per litre. The increase in the wave height noted above was likely to be caused by the increase in the perchlorate ion concentration. The influence of the concentration of perchlorate ions and salicylaldoxime was therefore examined in 0.1 N acid electrolytes. It is necessary that the extent of the dependence upon the perchlorate ion concentration be negligible, since the concentration of perchlorate ions present in plant solutions after wet digestion cannot be accurately controlled. The following procedure was used to determine the optimum perchlorate concentration range over which the wave for molybdenum was unaffected.

Aliquots of sodium perchlorate, to cover the range 4.5 x 10^{-6} to 2.27 g. perchlorate ion per litre, were added to a test solution containing 4.5 p.p.m. molybdenum in 0.045% salicylaldoxime and 0.094 N hydrochloric acid (vide section 3.4). The polarograms recorded have been reproduced in figure 12. The wave heights were observed to increase with an increase in the perchlorate ion concentration. This is an agreement with the findings of Jones (30) who stated that sodium perchlorate produced a similar catalytic effect upon the molybdenum wave to that produced by perchloric acid.

The section of the wave that is governed by the normally constant diffusion current was found to be influenced progressively by the perchlorate ion concentration. At negligible perchlorate ion concentrations the wave exhibited a maximum (figure 12.6). As the concentration of perchlorate ions was increased, the maximum became less pronounced and the wave became better developed. The addition of excess perchlorate ions caused the wave to be incompletely formed. In the presence of 0.23 M sodium perchlorate the wave obtained (figure 12.3) was suitable for height measurements. The optimum concentration of perchlorate was critical.

The reason for the dependence of the shape of the curve upon the ratio of perchlorate ions to salicylaldoxime concentration was now apparent. An increase in the perchlorate concentration modified the wave in a manner which is opposite in action to the modification produced by an increase in the salicylaldoxime concentration. Suitable diffusion current gradients could be



THE TEST SOLUTIONS CONTAIN THE FOLLOWING CONCENTRATIONS OF ClO_4^- ION:

TEST SOLUTIONS	12·1	12·2	12·3	12·4	12·5	12·6
g. ClO_4^- ION PER LITRE	2·27	0·45	0·23	0·045	0·005	$4 \cdot 5 \times 10^{-4}$ $4 \cdot 5 \times 10^{-5}$ $4 \cdot 5 \times 10^{-6}$

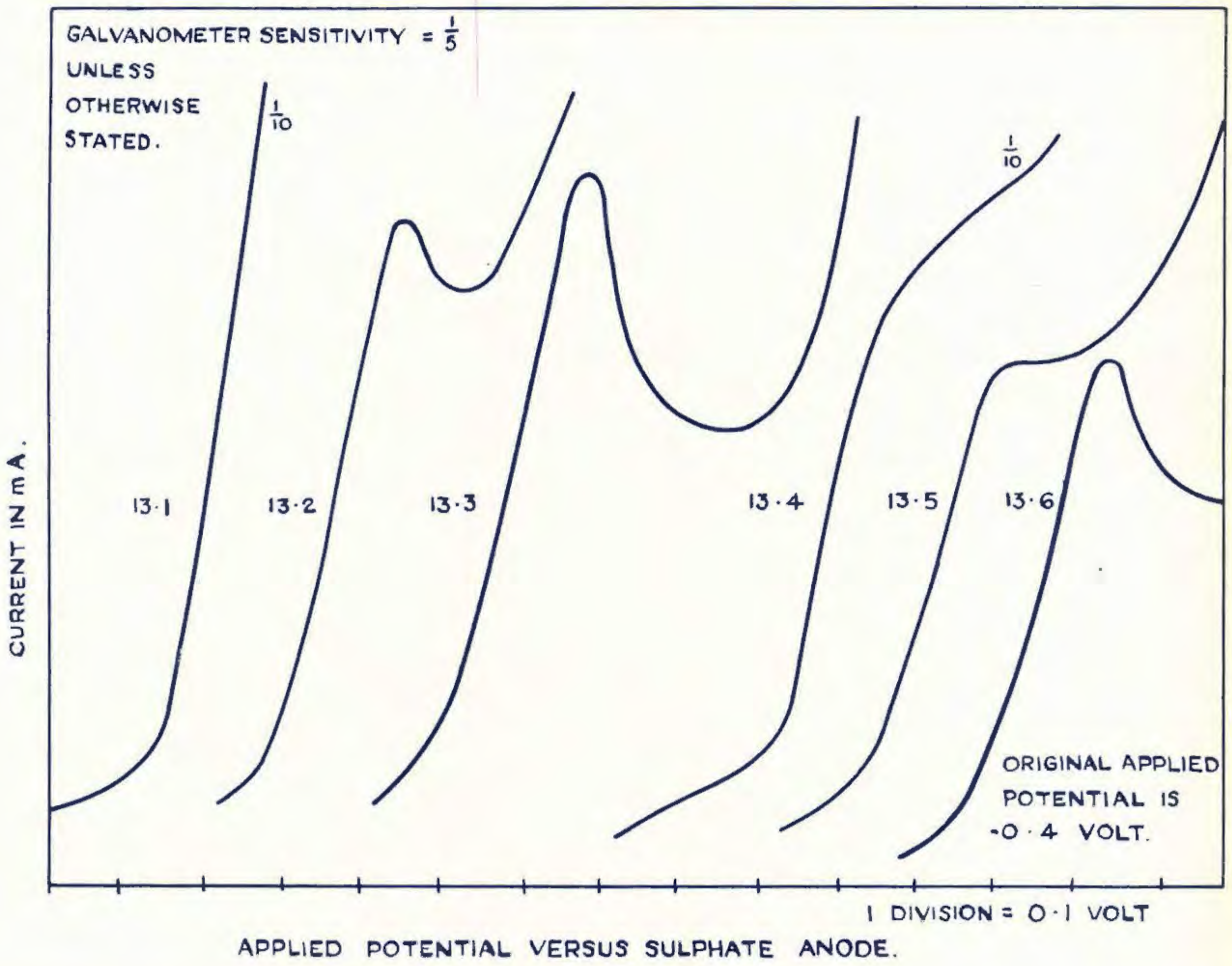
FIG. 12.

EFFECT OF PERCHLORATE ION CONCENTRATION ON POLAROGRAMS FOR Mo^{6+} ION.

obtained by balancing the two influences. The shape of the waves obtained at increasing salicylaldoxime concentrations was therefore examined. A test solution was prepared which contained a final concentration of 4.5 p.p.m. molybdenum in 0.09 N acid and 2.27 g. perchlorate ions per litre. The salicylaldoxime concentration was increased from zero to 0.1 M. Similar test solutions were also examined in which the perchlorate ion concentration was 0.45 g. ions per litre. The polarograms obtained have been reproduced in figure 13. In each series, the diffusion current gradient became less positive and a maximum developed with increasing salicylaldoxime concentration, in agreement with the previous findings on the influence of this reagent. The diffusion current gradient was greater in figure 13.1 than in figure 13.4, which was expected, since the perchlorate ion concentration was larger in the former case. Comparison of figures 13.3 and 13.6 and figures 13.2 and 13.5 revealed the anomaly that the maximum was bigger for the test solution having the higher perchlorate ion concentration. Polarograms 12.1 and 13.2 and polarograms 12.2 and 13.5 were recorded from solutions which had identical reagent concentrations. Comparison of these graphs revealed that the polarograms obtained were not identical, and that the maximum was apparently not reproducible.

The inconsistent results were found to be caused by the variation of the shape of the maximum with time. The instability was revealed by the following experiment. Polarograms were recorded at suitable time intervals from test solutions containing 4.8 p.p.m. molybdenum in 0.1 N perchloric acid, 0.46 M sodium

perchlorate, ...



THE TEST SOLUTIONS CONTAIN THE FOLLOWING SALICYLALDOXIME AND PERCHLORATE ION CONCENTRATIONS :

TEST SOLUTIONS	13·1	13·2	13·3	13·4	13·5	13·6
g. ClO_4 ION PER LITRE	2·27	2·27	2·27	0·45	0·45	0·45
% SALICYLALDOXIME	-	0·045	0·12	-	0·045	0·12

FIG. 13 .

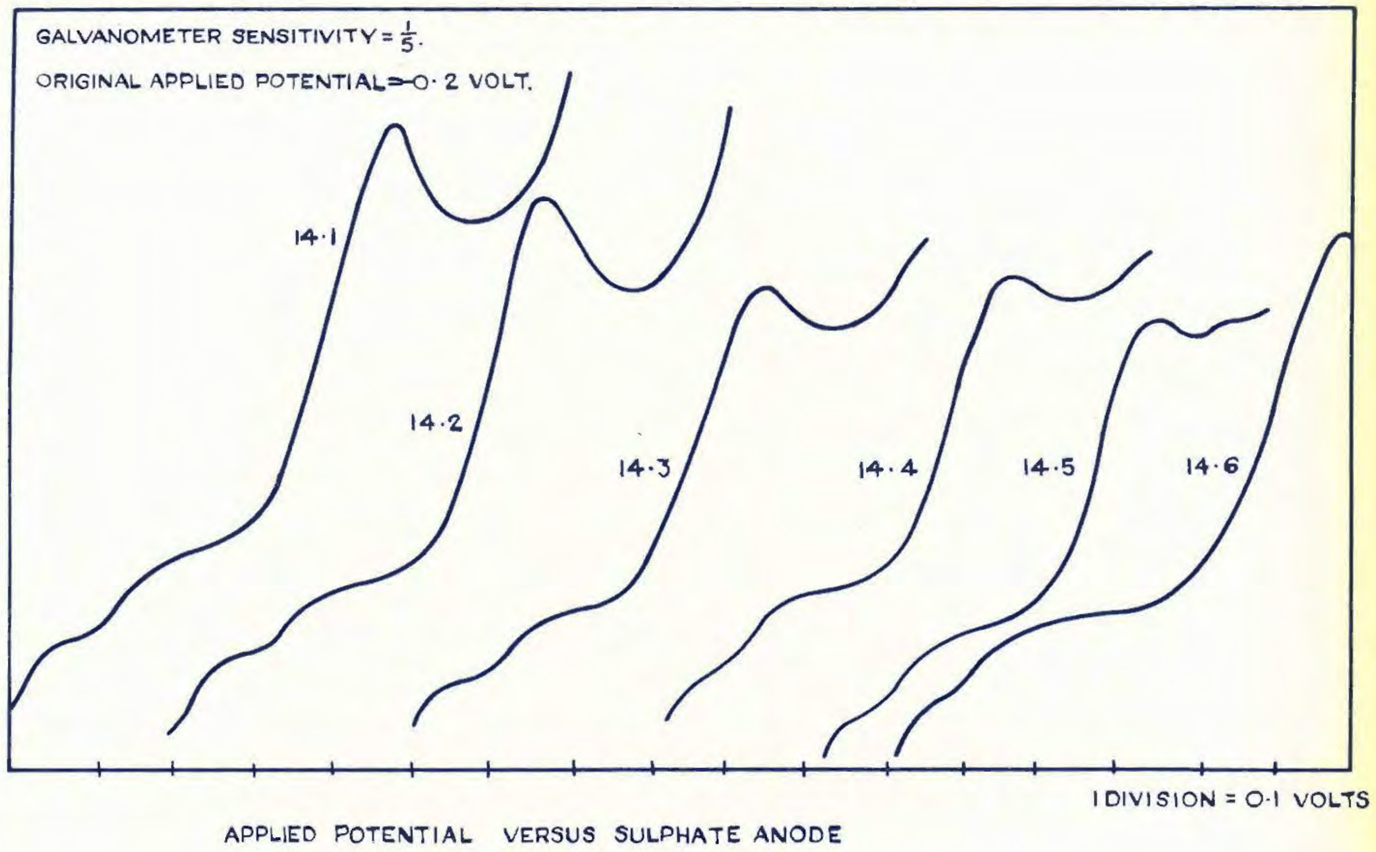
EFFECT OF PERCHLORATE ION AND SALICYLALDOXIME CONCENTRATIONS ON POLAROGRAMS FOR Mo^{6+} ION.

perchlorate and 0.042% salicylaldoxime. The reagents were added in the above sequence. The polarograms were recorded from an initial potential of -0.2 volt and are reproduced in figure 14. The graphs had an initial current increase caused by anodic mercury, and the molybdenum was reduced in two stages. The second and larger wave was distorted by a large maximum when the polarogram was recorded from a freshly prepared solution, (figure 14.1). This maximum decreased on standing over 30 minutes. The maximum on this wave was thereafter virtually constant for a minimum 24 hour period, (figure 14.4). For the concentration of salicylaldoxime shown the maximum did not disappear entirely. The height of both the pre-wave at -0.34 volt and main wave at-0.57 volt decreased slightly over the period 0 to 40 minutes subsequent to salicylaldoxime addition. Both wave heights were however constant for the subsequent 2 day period.

The age of the salicylaldoxime reagent did not influence the shape of the polarograms obtained. Identical polarograms were recorded from freshly prepared and two week old salicylaldoxime reagent, provided the graphs were recorded immediately subsequent to the addition of the salicylaldoxime reagent.

The polarogram was observed to alter significantly when the test solution was two days old. The larger wave was observed to divide into two reduction stages (figure 14.5). The new wave formed was small and had a half-wave potential of -0.7 volt. The total height of figure 14.5 was identical to that obtained for figures 14.3 and 14.4 and indicated that the molybdenum reduction had divided into three stages.

3.6/ .....



THE POLAROGRAMS WERE RECORDED AT THE FOLLOWING TIMES SUBSEQUENT TO THE ADDITION OF SALICYCALDOXIME:

TEST SOLUTION	14·1	14·2	14·3	14·4	14·5
TIME	15 MINS	36 MINS	80 MINS	27 HRS.	2 DAYS

FIG. 14

STABILITY OF THE POLAROGRAMS FOR Mo $^{6+}$ ION.

1.6 Stability reaction mechanism.

A study of the variation of the characteristics of the wave with time revealed that on standing the maximum exhibited a progressive trend which was similar to that obtained by a decrease in the salicylaldoxime concentration. The assumption was made that the decrease in the height of the maximum was caused by a decrease in the salicylaldoxime concentration with time. This decrease was possibly caused by a slow reaction with the perchloric acid.

The assumption was tested by the following examination: Stability tests were performed upon test solutions containing 8 p.p.m. molybdenum, 0.036% salicylaldoxime and concentration of perchloric acid more dilute than 0.1 N. The polarograms obtained have been reproduced in figure 15. Each polarogram is labelled with the time, in minutes, at which it was recorded subsequent to the addition of salicylaldoxime. In the presence of 0.085 N perchloric acid (pH 1.3) the alteration in the curve was slight over a 340 minute period (figures 15.1 and 15.2). The polarograms obtained from electrolytes having perchloric acid concentrations corresponding to the pH range 2.3 to 4.3 (figures 15.3 to 15.5), were stable for a minimum period of 320 minutes subsequent to the salicylaldoxime addition. The fact that the waves were stable, for a practical period of time, at these lower acidities indicated that the above assumption probably has valid grounds.

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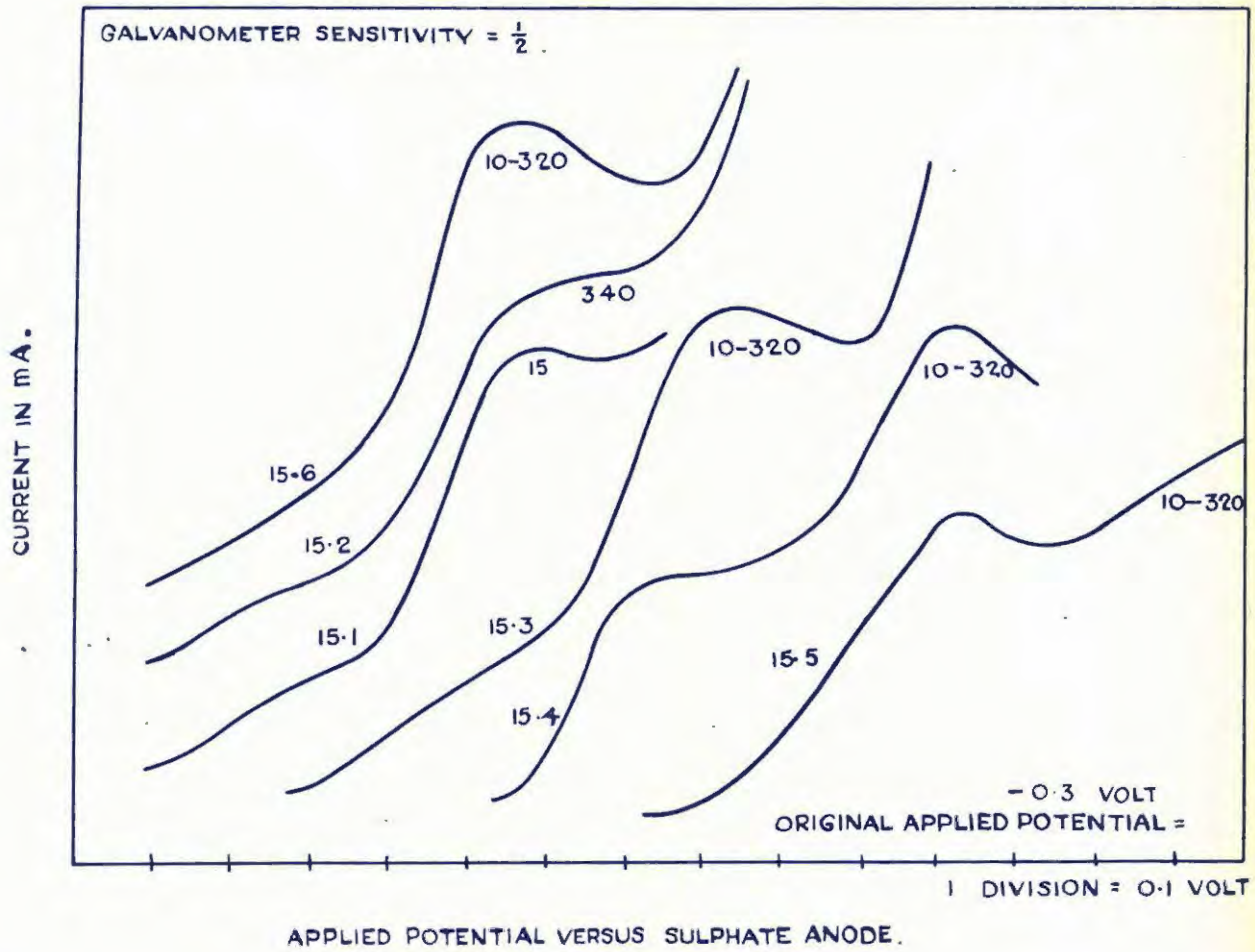


THE TEST SOLUTIONS WERE AT THE FOLLOWING pH VALUES:

TEST SOLUTION	15·1	15·2	15·3	15·4	15·5	15·6
pH	1·3	1·3	2·3	3·3	4·3	1·3

FIG. 15.

EFFECT OF THE pH ON THE STABILITY OF POLAROGRAMS FOR Mo^{6+} ION.

The inconsistency of the results reproduced in figures 12 and 13 was explained if this assumption was taken as being correct. The polarograms reproduced in figure 12 were recorded from test solutions in which the order of the addition of the reagents was: Acid, perchlorate solution, salicylaldoxime and finally the molybdate ions. The graphs in figure 13 were obtained from test solutions in which the order of reagent addition was: Molybdate, perchloric acid, sodium perchlorate and finally, the salicylaldoxime. In the latter sequence of reagent addition, the salicylaldoxime and the acid had less time for possible interaction prior to the recording of the polarogram. The polarograms obtained by the latter addition sequence would therefore be expected to exhibit the greater tendency towards the formation of a maximum. Comparison of the polarograms obtained from test solutions with equivalent concentrations in figures 12 and 13 revealed this to be the case.

The latter sequence of reagent addition is the natural sequence that would occur in plant analysis. All subsequent polarograms were recorded using the sequence that most closely approximated routine plant analyses.

Polarograms obtained from electrolytes having acidities corresponding to the pH range 1.3 to 4.3 were reproducible for a practical period of time. Several test solutions were examined in this range. At these acidities the shape of the wave was unsuitable for accurate height measurements.

It appeared likely that satisfactory reproducibility would be obtained by increasing the salicylaldoxime concentration. If this assumption is correct, the decrease in the salicylaldoxime concentration caused by interaction with the acid was, under such conditions, negligible in comparison with the total salicylaldoxime concentration.

The stability of a test solution containing 8 p.p.m. molybdenum in 0.085 N perchloric acid and 0.072% salicylaldoxime was examined. Except for the higher salicylaldoxime concentration, the reagent concentrations were identical to test solution 15.1. The polarogram obtained (figure 15.6) had a larger maximum than figure 15.1, due to the excess salicylaldoxime, but the graph was reproducible for a minimal 320 minute period.

This method of achieving reproducible results was examined further.

3.7 The influence of the salicylaldoxime concentration on the stability.

The optimum concentration range of salicylaldoxime necessary for the recording of waves reproducible with respect to time was determined as follows: An acid molybdate test solution (solution II) was prepared containing 4.8 p.p.m. molybdenum in 0.1 N perchloric acid and 0.47 M sodium perchlorate. Aliquots of salicylaldoxime covering the range 0.01 to 0.09% were added to the freshly prepared test solution. The polarograms of these solutions were recorded at suitable time intervals. Examples of the polarograms obtained have been reproduced in figure 16.

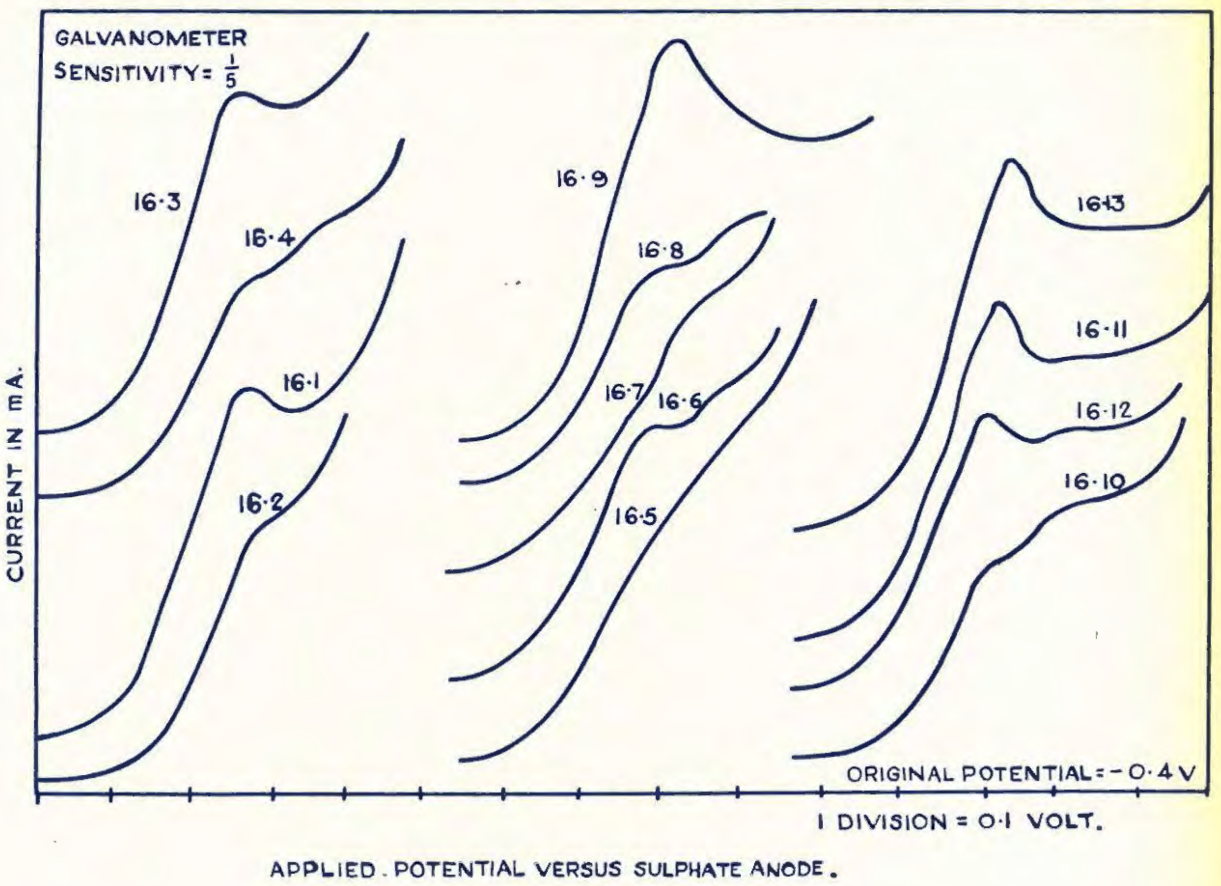
The/ .....

The wave obtained upon the addition of 0.015% salicylaldoxime to the freshly prepared acid molybdate solution has been reproduced in figure 16.1. This wave, recorded immediately after the addition of the salicylaldoxime, had a maximum. This maximum decomposed completely when the same solution was examined twelve minutes later (figure 16.2). On increasing the concentration of salicylaldoxime in this solution to 0.022%, the maximum reappeared (figure 16.3). The maximum from this concentration of salicylaldoxime (0.022%), decreased only slightly over a further 30 minute period. The wave was reproducible for several hours when the salicylaldoxime concentration was increased to 0.04%. Both the wave height and shape were found to be independent of the salicylaldoxime concentration over the range 0.04 to 0.09%.

3.8 <u>The influence of the age of the test solution</u>.

The test solution containing 0.022% salicylaldoxime (figure 16.3) was also recorded 22 hours after the addition of the salicylaldoxime. The polarogram obtained (figure 16.4) revealed that the graph had divided into a doublet wave. The total wave height of figure 16.4 was the same as the height of figure 16.3. The small wave at -0.7 volt had been observed previously (figure 14.5). A similar wave had appeared from perchloric acid solutions on standing (figure 5.3). These previous results indicated that the wave with the more negative half-wave potential was independent of the age or the presence or absence of the salicylaldoxime reagent. The following experiment revealed that this second wave (i) was governed solely by the age of solution of molybdate in perchloric acid (ii) was stable for a practical period of time and (iii) was independent of the salicylaldoxime concentration.

Aliquots/ ....



THESE POLAROGRAMS WERE RECORDED FROM TEST SOLUTIONS, CONTAING Mo^{6+} IN $HClO_4$, WHICH HAD STOOD FOR THE FOLLOWING PERIODS:

TEST SOLUTIONS	16·1 – 16·4	16·5 – 16·9	16·10 – 16·13
AGE OF ACID MOLYBDATE SOLUTION	FRESHLY PREPARED	ONE DAY	TWO DAYS

FIG. 16

EFFECT OF SALICYLALDOXIME CONCENTRATION ON THE STABILITY OF POLAROGRAMS FOR Mo^{6+} ION.

Aliquots containing from 0.002 to 0.09% of salicylaldoxime were added to the acid molybdate solution (solution II) prepared on the previous day. Examples of the polarograms obtained have been reproduced in figures 16.5 to 16.9. The wave was incompletely developed when the polarogram was recorded immediately subsequent to the addition of 0.002% salicylaldoxime, (figure 16.5). Two reduction waves were obtained when the polarogram was recorded immediately after the addition of 0.01% salicylaldoxime (figure 16.6). The half-wave potentials of the two waves were identical with the values obtained in figure 16.4, verifying statement (i). When the test solution containing 0.01% salicylaldoxime was recorded 75 minutes after the salicylaldoxime addition (figure 16.7), the maximum of the first wave disappeared and the shape of the second wave remained unchanged over this period. On increasing the salicylaldoxime concentration to 0.016%, and recording the polarogram (figure 16.8), it was observed that the increase in the salicylaldoxime concentration influenced the first wave, but did not alter the shape of the second wave. These latter findings verified statements (ii) and (iii). On increasing the salicylaldoxime concentration to 0.087% the maximum on the first wave (figure 16.9) was so large as to mask the second wave completely.

This second wave became better developed as the age of solution II was increased. Aliquots of salicylaldoxime from 0.004 to 0.09% were added to solution II prepared two days previously. Examples of the polarograms obtained are reproduced in figures 16.10 to 16.13.

Figures/ ...

Figures 16.10 and 16.11 were recorded immediately subsequent to the addition of 0.004% and 0.025% salicylaldoxime respectively. Polarogram 16.12 was recorded from the same solution as was used to obtain figure 16.11, but after a time interval of 26 minutes. Figure 16.13 was the polarogram recorded from test solutions containing from 0.038 to 0.065% salicylaldoxime. The first wave was influenced by, but the second wave was independent of, the salicylaldoxime concentration as before. The diffusion current plateau of the second wave was better developed in the acid molybdate solution that had stood for the longest period. Because of this improved development, accurate height measurements could be made on figures 16.10 to 16.13 even though the maximum was present. The total wave height was found to be independent of the salicylaldoxime concentration in excess of a minimum 0.02%. The maximum was reproducible for several hours over the concentration range 0.04 to 0.09% salicylaldoxime (figure 16.13). Figures 16.9 and 16.13 were reproducible for a minimum period of 24 hours. This salicylaldoxime concentration range was selected as being the optimum value. In this range the wave was stable and the wave height independent of the salicylaldoxime concentration. On performing the analysis upon a two day old test solution, a polarogram was obtained which was well suited to height measurements even though a maximum was present.

3.9 Elimination of the maximum.

Although the test solution obtained by the wet digestion of the plant material may well stand for lengthy periods prior to polarographic analysis, the incorporation of this stage as an integral part of the analysis procedure was considered too lengthy. A method which produced

a/ .....

a polarogram independent of the age of the plant solution was required. For such a procedure, the large salicylaldoxime concentration necessary for the production of a stable wave produced a maximum which made height measurements difficult. The elimination of this maximum was therefore investigated.

In spite of extensive experiments in this field, it was found that the maximum could not be eliminated by any of the reagents normally found effective in the literature. The addition of capillary-active electrolytes and non-electrolytes (Kolthoff (25))and non-capillary active ions (Heyrovsky (39)) did not remove the maximum obtained from a test solution containing 5 p.p.m. molybdenum in 0.1 *N* perchloric acid, 0.5 *M* sodium perchlorate and 0.042% salicylaldoxime. Amongst the reagents examined were agar agar, tartaric acid methyl red, methyl green, potassium thiocyanate, succinic acid, potassium ferricyanide, tannic acid, citric acid, oxalic acid and gum arabic. Most of the reagents were without effect. Gelatin in excess of 0.019% distorted the wave. Tartaric acid, in excess of 0.026% only partially decreased the height of the maximum.

4. Conclusions.

The above study revealed that the wave for molybdenum in an electrolyte containing 0.1 *N* perchloric acid, 0.5 *M* sodium perchlorate and 0.04% salicylaldoxime was reproducible with respect to time. The wave height was not critically dependent upon the reagent concentrations in this range. The wave was distorted by a maximum which made accurate height measurements difficult.

The reduction wave has previously been observed to possess a maximum. Both Meites (4) and Peocok (27) observed a maximum in electrolytes containing citrate ions. Meites (4) observed that the maximum, obtained from neutral electrolytes containing concentrations greater than 0.1 M sodium citrate, was not removed by either Triton X-100 or gelatin. Meites (4) evolved an analysis procedure in which analytical measurements were made upon the waves distorted by a maximum. In the case of the above electrolyte, the form of the wave was such that the presence of the maximum could not be ignored.

It was concluded that the use of an electrolyte containing perchloric acid and salicylaldoxime was unsatisfactory for the routine determination of molybdenum in plant material. None of the polarographic procedures available in the literature was examined, for the reason stated before. The lengthy preliminary separation required rendered polarographic analysis procedures too time-consuming to be considered where a shorter colorimetric procedure was available. Spectrophotometric methods for the determination of molybdenum in plant material were studied (Part II).

PART II.

SPECTROPHOTOMETRIC STUDIES.

1. REVIEW OF THE PUBLICATIONS ON THE COLORIMETRIC DETERMINATION OF MOLYBDENUM.

1.1. Existing procedures.

An examination of the literature revealed that several methods for the determination of molybdenum by colorimetric reactions were available. One of the oldest and most commonly used colorimetric procedures for molybdenum depends upon the use of the thiocyanate ion as the colour developing agent. This procedure has been used subsequent to the original published work of Braun (41) by such workers as Hurd and Allen (42), Sandell (43), Evans, Purvis and Bear (44) and several others. Dick and Bingley (45) (46), Purvis and Peterson (47), Chapman (48) and other workers, used this procedure for the determination of molybdenum in plant material. The thiocyanate procedure has the advantage of being virtually specific for molybdenum. The method is recommended by the Association of Official Agricultural Chemists. The procedure involves the addition of a reducing agent to an acid solution of molybdenum containing an alkali thiocyanate. The Mo^{5+} ions produced, react with the thiocyanate to form an orange coloured complex. The sensitivity of the procedure can be improved by extracting the complex into an organic solvent.

A recent method uses dithiol (4 methyl-1:2-dimercaptobenzene) as a colour developing reagent (references (49) to (60)). The dithiol procedure is 60% more sensitive than the thiocyanate procedure.

The procedure involves the formation of an insoluble green molybdenum mercaptide in dilute acid solution. The precipitate is then dissolved. Piper and Beckworth (50) recommended the use of iso-amyl acetate as an extractant. Bickford et al (52) extracted the precipitate with butyl acetate, while Miller (55) recommended butyl acetate and carbon tetrachloride. Wells and Pemberton (56) and Bagshawe and Truman (57) extracted the precipitate with iso-amyl alcohol. Hamence (58) dissolved the complex in a 1:1 mixture of amyl alcohol and ethyl ether or in excess ammonia. The extracted molybdenum complex had a maximum absorption at 670 to 690 mμ. A mild reducing agent must be present for complete colour development, the most common in use being thioglycollic acid. Perchloric acid interfered with the procedure.

The dithiol procedure is not specific. Copper, iron and tin interfere. Lüders and Huser (49) and Piper and Beckworth (50) used dithiol for the determination of molybdenum and described methods for the elimination of interference by the other substances occurring in plant material. Copper was first removed by extraction with dithizone and carbon tetrachloride. The molybdenum could then be selectively extracted with cupferron and chloroform. The chloroform was evaporated and the cupferron was destroyed by acid digestion prior to the formation of the insoluble mercaptide. Williams (51) separated the molybdenum as the thiocyanate complex. This complex also had to be destroyed prior to the addition of the dithiol. Jeffery (53) separated the molybdenum from the interfering substances by the addition of ethanolic α-benzoinoxime to the test solution, and repeated extraction with chloroform.

Scharrer and Eberhardt (54) separated the molybdenum as the sulphide in the presence of excess bismuth. Clark and Axley (59) eliminated interference by iron with potassium iodide and sodium thiosulphate. Each of these preliminary stages was time consuming.

Another colorimetric procedure uses phenylhydrazine hydrochloride. Ayres and Tuffly (61) used this reagent to determine molybdenum in rocks and soils. The reagent forms a stable red coloured complex with Mo^{6+} ions which is soluble in 50% acetic acid. Mineral acids interfere with the procedure. The maximum absorption occurs at 505 mμ. Goldstein (62) recommended the use of citric acid instead of acetic acid, especially when tungsten is present in the sample. The sensitivity of the method is low, approximately 2 to 10 μg. molybdenum per ml. Sandell (43) stated that this procedure was unsuitable for the determination of molybdenum in plant material because the large sample weight of 150 to 270 g. was impractical.

Waterbury and Bricker (63) described a method using chloranilic acid. The procedure using chloranilic acid was more sensitive than the phenylhydrazine method, but not specific for molybdenum. Mo^{6+} ions react directly with chloranilic acid to form a stable coloured complex with maximum absorption at 350 mμ. Waterbury and Bricker (63) separated the molybdenum by a lengthy series of extractions, in order to overcome interference by iron and copper. The molybdenum was extracted into 4 methyl-2-pentanone, and then back extracted into water.

Iron/ .....

Iron was removed by precipitation as the hydroxide. MacKaveney and Freiser (64) selectively extracted the molybdenum from ferrous materials with a 1:1 mixture of carbon tetrachloride and acetyl acetone. Chloranilic acid was added after the extractant was destroyed.

Petrovsky (65) described a method using catechol. This reagent forms an orange coloured complex with molybdenum which shows maximum absorption at 430 mμ. Ferric and many other ions cause interference.

Goldstein et al (66) recently used quercetin for the colorimetric determination of molybdenum. This reagent produces a yellow complex with molybdenum which has a maximum absorption at 420 mμ. The reagent is not specific and the procedure requires that the molybdenum be extracted prior to developing the complex. Goldstein (66) achieved this by precipitation of the molybdenum from strong acid solutions with α-benzoinoxime and extraction into chloroform. The coloured complex is formed in the extract by the addition of an ethanolic solution of quercetin to the chloroform. Unfortunately the concentration of α-benzoinoxime is critical and interferes markedly with the colour intensity.

1.2. <u>The thiocyanate method</u>.

The thiocyanate procedure was selected for further study for a number of reasons:

i) <u>The sensitivity</u>. A study of the literature revealed that the two most sensitive procedures were those involving the use of dithiol and thiocyanate. According to Williams (51) the former was the more sensitive.

Procedures/ ...

Procedures other than the above two were eliminated by this factor alone.

ii) Rapidity and simplicity.

The dithiol procedure was tedious. Piper and Beckworth (50) stated that complete precipitation of the mercaptide required 30 minutes and that the complex took 4 to 6 hours to dissolve completely in the organic extractant. In comparison the thiocyanate procedure was far more suitable for routine plant analysis.

iii) Freedom from interferences.

The major advantage of the thiocyanate procedure over the other existing methods lay in it's simplicity. Williams (51) showed that the thiocyanate procedure was almost specific. Substances normally occurring in plant materials interfered with all the other procedures examined. Such interferences could be eliminated by an additional stage as discussed above. Normally such a stage required the destruction of some extractant, which was time consuming. Purvis and Peterson (47) "found the dithiol procedure to be too tedious and time consuming for routine work. The possibility of error introduced by the increased number of operations more than offset the greater sensitivity of the dithiol procedure above the thiocyanate method". The author preferred the thiocyanate procedure as it did not require a preliminary extraction stage.

iv) Stability.

Piper and Beckworth (50) stated that the dithiol reagent was unstable and subject to aerial oxidation. Crouthamel (67) stated that the thiocyanate reagent was stable for several weeks. The stability of the molybdenum thiocyanate complex was however not as satisfactory as the molybdenum dithiol complex.

In/ .....

In order to obtain optical density values that were reproducible, many workers who adopted the thiocyanate procedure recommended a strict adherance to a definite technique and a rigid time schedule. This is not always practical in routine analysis. The experimental work was therefore aimed at improving the stability of the thiocyanate complex of molybdenum.

For simplicity, the literature on the thiocyanate procedure will be discussed under different sub-headings. Each sub-heading corresponds to a separate stage in the procedure.

1.21. Preparation of standard molybdenum solutions.

From the literature it appeared that ammonium molybdate was the most convenient standard to use. This reagent was used by Sandell (43), Dick and Bingley (45), Stanfield (68), Marmoy (69), Cox and Pollit (70) and by Ellis and Olson (71). Though the salt may be obtained in a pure state, it is not a good primary standard because of the variable water of crystallisation. The solutions were therefore standardised gravimetrically as lead molybdate before use (Vogel (91)).

1.22. The alkali thiocyanate reagent.

The most commonly used alkali thiocyanate is, according to the literature, the potassium salt. King (72) and Maag and McCollum (73) used this reagent for the analysis of molybdenum in steels, while Sandell (43) and Robinson (74) used it to determine molybdenum in rocks. The potassium salt was also used by Dick and Bingley (45), Stanfield (68), Marmoy (69), Parks et al

(75)/ .....

(75), Nichols and Rogers (76), Barshad (77), Grigg (78) and Johnson and Arkley (79) for plant analysis.

Evans, Purvis and Bear(44) and Hope (80), recommended the use of the ammonium salt for test solutions containing perchloric acid. This avoided possible loss of the molybdenum from solution by co-precipitation or adsorption upon the potassium precipitate. Johnson and Arkley (79) and Crouthamel and Johnson (67) described purification procedures for this salt by recrystallisation from methanol.

The literature was rather inconsistent in reference to the effect of the thiocyanate concentration upon the optical density. Marmoy (69) and Hurd and Allen (42) for example, showed that the colour stability and intensity did not vary for concentration above 0.6%, but that the solutions became unstable at lower thiocyanate concentrations. On the other hand Dick and Bingley (46) and Johnson and Arkley (79) showed the optimum concentration range to lie between 0.2 and 10%. This range must be fully examined prior to adaptation of the method to routine analysis.

1.23. The acid and acid concentration.

It was apparent from the work of Hope (80) that molybdenum is lost from alkaline solutions. Previous workers therefore studied the effect of different acids and different acid concentrations upon the stability and the intensity of the molybdenum thiocyanate in solution. There was general agreement that hydrochloric acid is the most suitable acid medium and it was used for the analysis of a wide variety of samples. Sandell (43), and Robinson (74) used hydrochloric acid for the analysis of molybdenum in rocks. Stanfield (68), Marmoy (69), Nichols and Rogers (76) and Johnson and Arkley (79) used hydrochloric acid for the analysis of plant material. This acid was unlikely to interfere with the oxidation reduction equilibrium. However, King (72), Stanfield (68) and Maag and McCollum (73) and all the above workers showed that fading occurred if the incorrect hydrochloric acid concentration was used. There was a lack of consistency for the optimum acid concentration range. Hurd and Allen (42) stated that the optimum hydrochloric acid concentration of 5% yielded a colour stable for 40 minutes. The test solutions contained 1% thiocyanate and 0.8% stannous chloride. On the other hand Dick and Bingley (45) and Johnson and Arkley (79) determined the optimum hydrochloric acid concentration to be 3%.

Mineral acids other than hydrochloric acid were normally removed. Hurd and Allen (42), Stanfield (68), Maag and McCollum (73), James (81) and Codell, Mikula and Norwitz (82) concurred that nitrate ions must be driven off for reproducible results. They achieved this by heating to fumes of sulphuric acid, or, in the case of the latter two references, to fumes of perchloric acid. Hurd and Allen (42) showed, that provided the sulphuric acid concentration was less than 0.5%, it did not interfere. Dick and Bingley (46), Parks et al (75), Grigg (78) and Ward and Johnson (83) argued that perchloric acid might interfere as well as the nitric acid. They removed the last traces of both these acids by heating to dryness, and took up the residue in hydrochloric acid. Evans, Purvis and Bear (44) eliminated the oxy-acids by repeated evaporation with hydrogen peroxide. None of these workers gave any data describing perchloric acid interference.

Barshad/ ...

Barshad (77) showed conclusively that the elimination of nitrate ions was unnecessary. He found that the addition of sodium nitrate to the aqueous test solution stabilised the complex for 30 minutes and doubled the optical density. The mechanism of this phenomenon was given by Evans et al (44), who indicated that nitrate ions prevented reduction of molybdenum below the 5+ state. Further, the nitrate ions oxidised any such molybdenum back to the 5+ state, thereby intensifying the colour. The fading noticed by the previous workers only occurred when the nitrate concentration exceeded the stannous chloride concentration.

Other workers studied the influence of other acids. Burd and Allen (42) showed that the addition of greater than 0.5% sulphuric acid to the optimum 5% hydrochloric acid caused rapid initial fading. In the absence of chloride, maximum stability was obtained in 5 to 10% sulphuric acid. Cox and Pollit (70) on the other hand found the complex to reach maximum development rapidly in 2% perchloric acid and 10 to 15% sulphuric acid, but reported that the time requirement was inconveniently long, and that fading occurred outside these limits.

The evaporation stages employed to eliminate ions other than chloride were time consuming and, possibly, unnecessary. It has been shown that nitrate ions need not interfere. Further, up to 0.5% sulphate may be tolerated. The method used by Steyn (37) at Rhodes University for the dissolution of plant material involved wet digestion with nitric and perchloric acids.

It/ .....

It seemed probable that the lengthy evaporation stage in the analysis procedure could be eliminated. The sole remaining reason for the inclusion of the evaporation step was the possible interference by perchlorate ions.

Few facts were available on the possible interference by perchloric acid. Cox and Pollit (70) mentioned that a stable colour was developed within a practical period of time if the perchloric acid concentration was 17% with no other acid present. These authors (70) also stated that up to 2% perchloric acid did not interfere with the colour developed in sulphuric acid medium.

There appeared to be reasonable grounds for examining whether perchlorate ions did in fact cause instability of the molybdenum thiocyanate complex in hydrochloric acid medium. If this was so, the lengthy evaporation stage could be eliminated.

It was also considered necessary to establish the optimum hydrochloric acid concentration range required to record transmittancy values constant for a practical period of time.

These two aspects were investigated experimentally as described under "Experimental Studies".

1.24. The reducing agent.

The coloured complex is formed by reaction between thiocyanate ions and molybdenum ions possessing an oxidation number of five. Molybdenum at other oxidation states does not form a coloured complex. The prime requirement of the reducing agent was quantitative conversion of the molybdenum to an oxidation state of five.

The reducing agent must be efficient. Incomplete reduction caused interference by the formation of molybdenum blue. This reagent must in addition eliminate interference by the coloured ferric thiocyanate complex. Iron must be present for maximum chromatogenic development, but ferric interference is eliminated by reduction to the ferrous state. The reduction must be rapid, and the resultant solutions must remain stable.

Crouthamel and Johnson (67) studied the reduction mechanism of several reducing agents in different acid solutions and showed that the reaction with stannous chloride in hydrochloric acid was quantitative in the presence of iron and nitrate ions. This reductant was widely used. King (72), Maag and McCollum (73), James (81) and Grimaldi and Wells (84) used stannous chloride for the determination of molybdenum in steel, while Sandell (43) and Robinson (74) used the reagent for silicate rock analysis. Evans, Purvis and Bear (44), Dick and Bingley (45), Stanfield (68), Marmoy (69), Parks et al (75), Nichols and Rogers (76), Barshad (77) and Johnson and Arkley (79) used stannous chloride for the analysis of plant material. The speed of colour development and the resultant intensity and stability depended on the stannous chloride concentration. Johnson and Arkley (79) gave the optimum concentration range to be 0.02 to 0.2 M, while Hurd and Allen (42) stated the range to be 0.04 to 0.6% stannous chloride in 5% hydrochloric acid. Dick and Bingley (45) found that in the presence of iron and 3% hydrochloric acid, the optimum conditions for colour development lay between 0.6 and 2% stannous chloride.

Ellis/ ...

Ellis and Olson (71) reduced Mo^{6+} ions to Mo^{5+} ions by heating with hydrochloric acid and acetone. This produced a stable complex, but the reaction time of 20 minutes was unfavourable. The precipitation of salts and the interference by iron, which occurs in plant material, rendered this reagent unsuitable. Crouthamel and Johnson (67) recommended the use of a single electron reductant, as the reduction occurred directly to the Mo^{5+} state. Iron interfered with the cuprous reagent recommended. This reagent, as prepared, contained stannic ions which, according to Hurd and Allen (42) caused instability. Ginsberg and Luré (85), Hope (80) and Chernikov and Dobuna (86) used solid potassium iodide and removed the iodine produced with freshly prepared sodium sulphite. The colour stability and speed of reaction were dependent upon the acid concentration and the temperature.

Stannous chloride was therefore selected as the simplest and most suitable reagent to use. The presence of ferric, and nitrate ions ensured that the reaction was quantitative and that the molybdenum was not reduced below the 5+ state. The previous advantage of single electron reductants was no longer valid. The experimental studies were aimed at determining the optimum stannous concentration range with nitrate ions present. The previous data were given for nitrate free solutions.

1.25. The extractant.

In view of the low concentration of molybdenum in plant material, a necessary step in the analysis procedure was the extraction of the molybdenum thiocyanate complex. Greater sensitivity was achieved by the concentration of the molybdenum traces. Crouthamel

and/ .....

and Johnson (67) stated further that the method was more sensitive in organic than in aqueous medium. For example Ellis and Olson (71) obtained greater sensitivity over the procedure of Grimaldi and Wells (84) by the use of a greater concentration of acetone. The author considers that these phenomena may have been caused by the change in dielectric constant. It was also highly desirable that the selected extractant should stabilise the complex for a suitable period of time, that it be convenient to handle and that it be non-volatile.

Ether was used as an extractant by Brown (41), Sandell (43), Evans et al (44), Marmoy (69) and King (72). Nichols & Rogers (76), and Barshad (77) recommended ether for the determination of molybdenum in plant material. The complex has a maximum absorption at 475 mμ in ether. Sandell (43), Parks et al (75) and Nichols and Rogers (76) and others stated that before use the ether must be freshly shaken with the stannous chloride and thiocyanate reagents. The two phases are then separated. Ether not so treated had a tendency to impart a reddish hue to the normal yellow-brown of the complex. A similar saturation procedure was recommended for the other extractants. A disadvantage of using ether was the inflammability and high vapour pressure which, according to Sandell (43) and Maag and McCollum (73) lead to back pressure in the separating funnel. Marmoy (69) and King (72) designed special cells to minimise the error due to evaporation.

Stannic/ ....

Stannic ions render the separation of the two phases difficult. Ether being less dense than the aqueous phase was another drawback. Hurd and Allen (42), Sandell (43) and Marmoy (69) showed that the complex was not perfectly stable in ether.

James (81), Hurd and Reynolds (87) and Grimaldi and Wells (84) replaced ether with butyl acetate because of the latter's lower volatility. Because of the greater solubility of the complex, only one extraction was necessary. Dick and Bingley (45) and Stanfield (68) however, recommended several extractions when this reagent was used for plant analysis. Stanfield (68) recommended that any turbidity of the extract be removed by a few drops of ethyl alcohol. As in the case of ether, the butyl acetate must be saturated with stannous chloride and thiocyanate before use, according to Stanfield (68), Barshad (77), Codell et al (82) and Wrangell (88). The complex gives maximum absorption at 465 mμ . Unfortunately, as discovered by Hurd and Reynolds (87) and Hurd and Allen (42), the hydrolysis products of butyl acetate promoted colour fading and the complex was even less stable in this medium than in ether. Other extractants that have been used include isopropyl ether by Evans, Purvis and Bear (44) and Robinson (74) and a 1:1 mixture of iso-amyl alcohol and amyl acetate by Ward and Johnson (63).

Hurd and Reynolds (87) obtained excellent stability by the use of cyclohexanol. Hurd and Allen (42) showed that the reproduction of the optical density with time was superior to either ether or butyl acetate.

In addition the optical density of the complex was more intense in this reagent than in ether. The increased solubility of the complex enabled smaller volumes to be used which increased the sensitivity further. The complex exhibited maximum absorption at 465 mμ. Unfortunately the physical properties of cyclohexanol were unfavourable. Hurd and Allen (42), Dick and Bingley (45) and Grimaldi and Wells (84) found that the reagent sometimes solidified in the laboratory and that the viscous liquid was difficult to handle. On the other hand this factor made the volatility negligible. Cyclohexanol separated slowly from the aqueous phase and turbidity sometimes developed.

Stability was achieved by the use of iso-amyl alcohol as an extractant, but Johnson and Arkley (79) encountered errors due to the high solubility of this reagent. Dick and Bingley (46) and Grigg (78) reduced the solubility and expansion effects by equilibriating the solutions to a constant temperature before use. These latter workers and Gotô and Ikeda (89) found the complex to have maximum absorption at 335 and 468 mμ in this reagent when stannous chloride was used as the reductant.

Johnson and Arkley (79) developed an extractant mixture of iso-amyl alcohol and carbon tetrachloride. The use of this mixture improved the sensitivity and accuracy above that of the alcohol alone. The favourable density ratio improved the phase separation and simplified the ease of manipulation, with a resultant saving in time. The complex was stable in this reagent for several hours. Maximum absorption was at 470 mμ.

Johnson/ ...

Johnson and Arkley (79) overcame the variable solvent solubility by saturating the test solution with the mixed extractant prior to thiocyanate and stannous chloride addition. Unfortunately this reagent was very volatile and the results given for plant samples with low molybdenum content indicated incomplete recovery. Johnson gave the optimum reagent concentration ranges for complex development in the aqueous phase to be 0.72 to 1.8 N hydrochloric acid, 0.05 - 1.8 M potassium thiocyanate and 0.02 - 0.08 M stannous chloride.

The simplification of the procedure by the use of an extractant denser than water had obvious advantages. In spite of the stability obtained, the use of the iso-amyl alcohol, carbon tetrachloride mixture was not entirely satisfactory, because of the solubility, the volatility and doubtful extraction efficiency. The author considered that the thiocyanate procedure could be improved by the use of an extractant with more suitable physical properties. The selection of an improved extractant was examined in the experimental studies.

1.26. Interfering elements.

Warmoy (69) stated that none of the elements normally present in plant material, interfere with the thiocyanate procedure.

Iron is necessary for complete colour development. Warmoy (69), Robinson (74), Nichols and Rogers (76), Barshad (77) and Johnson and Arkley (79) found that the presence of iron doubled the colour intensity and increased the accuracy and reproducibility of the method. Maximum enhancement occurred when molybdenum and iron were present in equi-molar quantities. Addition of further iron had no affect upon the intensity. For this reason, Nichols and Rogers (76) recommended the addition of 10 mg. ferric iron to both the blank and the standard solutions. Dick and Bingley (45) explained this phenomenon. They noted that the absorption spectra of the molybdenum thiocyanate complex was the same whether iron was present or not. On this and other grounds they postulated that the molybdenum occurred in both the chromatogenic and in the non-chromatogenic parts of the molecule. With iron available the molybdenum in the non-chromatogenic section of the molecule was replaced and was then available for colour intensification. The generally accepted structures are $Mo_2\ (Mo\ O(CNS)_5)_3$ and $Fe\ (Mo\ O(CNS)_5)$. Copper had a similar affect to iron. It was however less marked.

According to Barshad (77) the intensity is independent of the ferric concentration when the ratio of the number of equivalents ferric iron to molybdenum exceeds unity. The presence of a large excess of iron, however, causes interference. Ferrous thiocyanate is co-extracted together with the molybdenum complex and aerial oxidation produces an interfering colour. Dick and Bingley (46) and Grigg (78) overcame this by washing the extract with stannous chloride. Hurd and Allen (42) found that stannic tin caused the intensity of aqueous solutions to fade with time. For this reason, the stannous chloride solutions are prepared fresh each day. Evans, Purvis and Bear (44) stated that it was necessary to remove all traces of organic material.

Destruction of the organic material was achieved by prolonged acid digestion, by several evaporations to dryness, and by evaporation with hydrogen peroxide.

1.3. The method of Purvis and Peterson.

In 1956 Purvis and Peterson (47) published a spectrophotometric procedure for the analysis of trace amounts of molybdenum in plant material. A description of the method follows:

Digestion of the plant sample.

The authors stated that one of the analyst's major obstacles lay in the digestion of the initial samples. In view of the large amounts of reagents required during this stage, they recommended purification of the water and the acids by distillation before use, and stressed the importance of reagent blanks.

Digestion procedure.

A 2 g. sample of the oven-dried, finely ground plant material was weighed into a tall 200 ml. beaker. Concentrated nitric acid (15 ml.) was added and the beaker was covered with a watch glass before being heated on a hot plate at low heat. When the solid matter disappeared, 2 ml. of 70% perchloric acid were added and the solution was digested slowly to dryness. This stage took a minimum of three hours. The watch glass was then removed, and 5 ml. concentrated nitric acid and 1 ml. of 70% perchloric acid were added. The solution was evaporated carefully to dryness and spattering was avoided, by heat control. Two ml. of 30% hydrogen peroxide were added and the solution was again brought to dryness. After cooling, approximately 70 ml. of distilled water were added and this mixture was heated to boiling for 1 minute. Ten ml. of concentrated hydrochloric acid were added, the solution was filtered and was made up to 100 ml.

Determination of the molybdenum.

Reagents.

All reagents should be of satisfactory low molybdenum content.

Stannous chloride.

20 g. $SnCl_2$ were dissolved in 20 ml. of concentrated hydrochloric acid by heating below the boiling point. The solution was made to 200 ml. with distilled water and was prepared fresh daily.

Ammonium thiocyanate.

A 10% solution was prepared.

Ferric chloride.

49 g. of $FeCl_3.6H_2O$ were dissolved in distilled water and made up to 1 litre.

Sodium nitrate.

42.5 g. of $NaNO_3$ were dissolved in distilled water and made up to 100 ml.

Isopropyl ether.

Reagent grade isoproyl ether was placed in a glass stoppered separatory funnel. One tenth it's volume of a mixture containing one third each of stannous chloride and ammonium thiocyanate solutions and one third water was then added. After shaking, the phases were separated. This was prepared fresh daily.

Standard molybdenum solution.

0.150 g. of C.P. MoO_3 was dissolved in 10 ml. of 0.1 N sodium hydroxide. The solution was made slightly acid with hydrochloric acid, and diluted with distilled water up to 1 litre. Twenty ml. of this solution was made to 1 litre to produce a solution containing 2 p.p.m. molybdenum.

Procedure/ ...

Procedure.

One hundred ml. of a test solution (prepared as described under the digestion procedure) were transferred to a 150 ml. glass stoppered separatory funnel. One ml. of ferric chloride solution, 1 ml. of sodium nitrate, 5 ml. of ammonium thiocyanate and 5 ml. of stannous chloride solution were added in turn with thorough shaking between each addition. Ten ml. of isopropyl ether were added and the funnel was shaken 100 times. The liquids were allowed to separate and the aqueous phase was drawn off. The ether was delivered into a glass stoppered container. The transmittance was determined at 475 mμ. After subtraction of the reading for the reagent blank, the molybdenum content of the test solution was determined by reference to a standard curve.

A standard curve was prepared by following the above procedure with six or more aliquots of standard molybdenum solution containing 0 to 20 μg. of the element.

Purvis and Peterson (47) obtained good recovery with this procedure. No attempt was made to examine the method statistically. The procedure had to be rigidly adhered to, and no data on the optimum concentration ranges or the stability were given.

2. EXPERIMENTAL STUDIES ON THE THIOCYANATE METHOD.

2.1. Description of the apparatus and reagents.

2.11. Instruments.

All readings were recorded using 1 cm. cells on a Cenco Sheard Spectrophotelometer. (Cat. No.12315). The stable light source developed has been described by Davidson (90). Two large capacity 6 volt batteries in parallel with a battery charger ensured a constant voltage supply to the light source.

2.12. Reagents.

All reagents used were of A.R. quality. The nitric acid used for plant digestion, and the carbon tetrachloride were redistilled before use. The cyclohexanol was of B.D.H. quality, but purification by vacuum distillation was found to be unnecessary. Reagent solutions were prepared using water which had been purified by passing freshly distilled water through an ion exchange column containing Amberlite MB 3 (mixed bed) resin.

The molybdenum solution was prepared from 'specpure' ammonium molybdate, which had been standardised by the gravimetric procedure, described by Vogel (91). The molybdenum content was determined to be 54.37%; the theoretical content was 54.34% molybdenum.

From the literature survey it was obvious that ammonium thiocyanate was the best salt to use, since perchloric acid is used for the digestion of the plant material. The C.P. reagent available was unsatisfactory and the crystals were moist in appearance. Similar crystals had previously been reported by Crouthamel and Johnson (67) to yield poor colour stability. The following modified technique of recrystallation from methanol was found to be shorter and more satisfactory than the existing method.

The methanol was warmed to 55°C in a water bath, and was saturated with the ammonium salt. While hot, the solution was filtered through a warm coarse porosity sintered glass buchner funnel. Cooling produced an even crop of crystals. The cold mix of crystals and supernatent liquor were separated by filtration through the cold buchner funnel. The crystals were washed with sufficient cold re-distilled methanol to remove the last iron thiocyanate traces. Thirty minutes suction dried the crystals sufficiently for them to be transferred to a vacuum desiccator where they were stored until required. A 10% solution in de-ionised distilled water was found to be stable for a minimal period of three months.

2.13. Glassware.

The standard molybdenum solution was stored in a polythene container. Pyrex glassware, standard to all laboratories, was used for the investigation. All the glassware was meticulously cleaned first with acid, and then with teepol. The apparatus was rinsed with tap and de-ionised distilled water before use. The use of automatic pipettes, as described by Eve (36) is recommended, especially for extractant addition. No graduated separating funnels were available. The normal funnel was therefore etched with hydrofluoric acid at the 100 ml. mark, and was found to be completely satisfactory. Photographs of the glassware used are reproduced in figures 17 and 18. Figure 17 shows details of the micro burette and automatic pipettes. Figure 18 shows details of the etched separating funnels and kjeldahl flasks. Where available, the latter should be of silica.

2.?/ .....

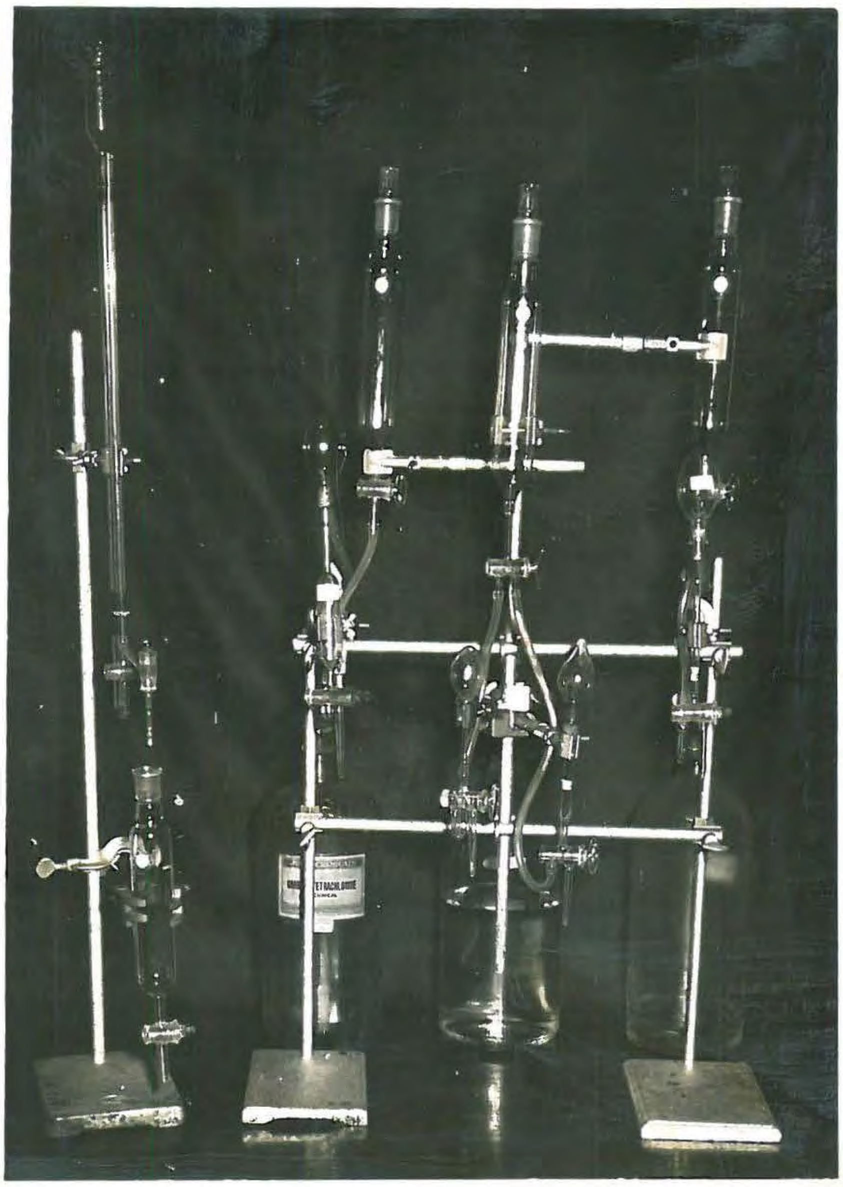


FIGURE 17.

GLASSWARE.

FIGURE 18.

GLASSWARE.

2.2. A critical examination of suitable extractants.

Examination of the literature revealed that the ideal extractant must possess the following properties:

(a) The extraction must be quantitative.

(b) The extractant should preferably be denser than water.

(c) The two phases must be separated by a sharp interface.

(d) The extractant must contain no trace of turbidity.

(e) The complex must be stable in the extractant.

(f) The extractant must be non-volatile.

Various extractants were tested employing the standard conditions described below. The complex was developed in the aqueous phase exactly as recommended by Purvis and Peterson (47).

Aliquots containing 0 to 100 µg. molybdenum were taken. Sufficient concentrated hydrochloric acid was added to ensure a final hydrochloric acid concentration of 5%. The individual test solutions were standardised against sodium carbonate, to ensure that possible transmission variation was not caused by variable acid content. The solution was diluted to 100 ml., and transferred to a 150 ml. separating funnel. To this was added 10 mg. iron (as $FeCl_3$), 1 ml. 42.5% sodium nitrate, 5 ml. 10% ammonium thiocyanate and 5 ml. freshly prepared 10% stannous chloride in dilute hydrochloric acid. The solution was shaken between each addition.

Subsequent/ ...

Subsequent work revealed that the use of this volume of thiocyanate reagent caused the complex to undergo slight fading in the aqueous phase. This did not interfere with the initial examination of the different extractants as the extraction was immediately performed.

The first extractant examined was isopropyl ether.

2.21. Isopropyl ether.

In the initial experiments 10 ml. of equilibriated isopropyl ether were used as the extractant. The transmittance was determined at 475 $m\mu$. These experiments revealed that this reagent was not entirely suitable. The intensity of the coloured complex in ether began to fade after 28 minutes. The time of addition of the stannous chloride to the aqueous phase was taken as zero time.

The extract was frequently turbid. This turbidity did not disappear upon standing. A clear extract was obtained when the concentration of hydrochloric acid in the aqueous phase was reduced to 3.3%, and also when the extract was washed with 1.8% hydrochloric acid. The former procedure caused instability in the aqueous phase, and the latter produced a marked instability of the complex in the organic phase. The addition of either carbon tetrachloride or acetone to the extracted organic phase clarified the ether immediately. Because of the more favourable density ratio attention was directed upon:

2.22/ .....

2.22. Isopropyl ether and carbon tetrachloride.

This reagent was prepared by mixing isopropyl ether and carbon tetrachloride in the ratio 2:3 giving a solution with a specific gravity of 1.25 at 20°C. Prior to use 100 volumes of the reagent were shaken with a mixture of 3 volumes 10% ammonium thiocyanate, 3 volumes stannous chloride and 4 volumes water. The phases were then separated. This procedure was necessary in order to saturate the extractant with the reagents used. Aliquots containing 50 µg. molybdenum were taken. The complex was developed in the aqueous phase exactly as described previously, but this reagent was used as the extractant instead of the ether. The use of the heavier-than-water extractant gave sharper separation and simplified the extraction manipulation. The extractant was inefficient. Two 10 ml. extractions were found to be insufficient to quantitatively remove all the molybdenum from the aqueous phase. The extract was turbid unless the acid concentration was controlled, and the colour intensity tended to fade.

In order to achieve stabler transmittancy readings, 20 ml. of acetone were added to the aqueous phase prior to the dilution to 100 ml. The resultant extracted colour was stable for forty minutes, and the extract did not have any trace of turbidity. The presence of acetone in the aqueous phase was found to increase the solubility of the extractant. The aqueous phase was therefore saturated with the extractant prior to thiocyanate addition. The volume of extractant subsequently recovered was constant, but unfortunately molybdenum traces were detected in the initial washings.

2.23/ ....

2.23. Cyclohexanol and carbon tetrachloride.

From the literature survey it was clear that cyclohexanol would extract the molybdenum complex, and that the colour was phenomenally stable in this medium. The previous workers discarded this reagent because of the unsatisfactory physical properties. The author overcame this latter difficulty by the addition of carbon tetrachloride to the cyclohexanol.

The extractant was prepared by mixing equal volumes of B.D.H. cyclohexanol and re-distilled carbon tetrachloride, giving a clear solution with a specific gravity of 1.28 at 20°C. Before use, the extractant was saturated with the reagents: 100 volumes of the mixed extractant were shaken with 3 volumes of ammonium thiocyanate and 7 volumes of stannous chloride reagent. During the initial tests on the mixed extractant, the complex was developed in the aqueous phase exactly as recommended by Purvis and Peterson (47). Ten ml. of the above extractant were however used instead of isopropyl ether.

Separation of the phases was sharp and was quickly established. The first few tests concurred with the literature on cyclohexanol. The mixed reagent recovered after extraction was also turbid. This turbidity was not removed by washing with concentrated hydrochloric acid. The extract clarified immediately and completely on the addition of either acetone or ethanol. The latter reagent was therefore added to all subsequent extracts.

Preliminary readings on the stability of the molybdenum complex in the optically clear extractant were unsatisfactory. The transmittancy readings recorded in Table II, were obtained from different aliquots of molybdenum. The complex was extracted twice with 10 ml. of the extractant. The extracts were combined, and the turbidity was removed with 5 ml. of ethyl alcohol. The solutions were allowed to stand for varying periods of time and the percentage transmission was then determined at 475 mμ. versus a water blank. The extracts from both the test solution and the blank exhibited a colour which faded rapidly with time.

The addition of 1 ml. of 10% stannous chloride to the extract reduced the colour observed in the blank and also stabilised the colour.

TABLE II.

EFFECT OF STANNOUS CHLORIDE ADDITION ON THE STABILITY.

Percentage transmission on standing for varying periods.

μg. Mo Added.	Time of Standing in minutes.							
	Without $SnCl_2$				With 1 ml. 10% $SnCl_2$			
	20	25	30	40	20	30	40	90
0.0	88.6	89.9	90.9	92.1	--	99.3	99.3	99.2
5.0	--	74.1	78.8	87.1	92.9	93.0	92.8	92.8
12.5	--	--	--	--	87.3	87.2	87.0	87.0
25.0	--	--	--	--	76.2	76.1	76.1	76.0

The interfering colour obtained in the absence of stannous chloride was apparently caused by ferric thiocyanate. Though ferric ions were not present in the aqueous phase just prior to the extraction, the ferrous ions extracted were soon oxidised. The coloured ferric complex increased the reagent blanks. The interference was increased by the absence of stannous chloride, or by bubbling oxygen through the extract. Dick and Bingley (46) removed ferric ions formed by aerial oxidation, by successive washing with dilute stannous chloride. The above method of adding the stannous chloride to form one homogeneous phase is more simple and far shorter. The addition of stannous chloride also eliminated possible interference by traces of iron present in the extractant itself.

2.24. Stability of the molybdenum thiocyanate complex in cyclohexanol/carbon tetrachloride.

The above data indicated that this mixed extractant could be adapted into an analysis procedure; the turbidity being removed by the addition of ethyl alcohol, and interference by aerial oxidation of the iron eliminated by the addition of stannous chloride. The author therefore studied the stability of the molybdenum thiocyanate complex in this extractant. Aliquots of molybdenum to cover the range 0 to 25 µg. were taken, and the complex was developed in the aqueous phase exactly as described by Purvis and Peterson (47). The solution was extracted twice with 10 ml. of the reagent, and the two extracts were combined. The addition of

10 ml./ ....

10 ml. ethanol clarified the solution and interference was eliminated by the addition of 1.6 ml. of stannous reagent. The transmittance was determined at 475 mμ at five minute intervals for two hours. Some of the results obtained are recorded in Table III. These results showed that the stability is excellent.

TABLE III.

THE STABILITY OF THE MOLYBDENUM THIOCYANATE COMPLEX IN CYCLOHEXANOL/CARBON TETRACHLORIDE EXTRACT.

Time of Standing (hours).	Percentage Transmission.					
	0 μg.Mo	5 μg.Mo	10 μg.Mo	15 μg.Mo	20 μg.Mo	25 μg.Mo
⅓	99.7	93.5	88.1	83.1	77.7	73.2
½	99.9	93.0	87.9	81.1	77.2	70.9
2¼	100.0	93.9	87.9	81.1	77.1	73.2
26	99.8	93.5	86.8	83.2	--	73.2
⅓ (x)	99.4	--	88.8	--	79.6	--
26 (x)	99.2	--	88.8	--	79.4	--

(x) Results obtained with the solubility of the extractant in the aqueous phase reduced to a minimum.

Subsequent work showed that if kept in stoppered flasks, the molybdenum complex was stable in this medium for a minimum of five days. Constant transmission readings were obtained over this period, (Table IV).

The/ ....

The possible influence of laboratory temperature variation upon the reproducibility of the results was also examined at this stage. Cyclohexanol is soluble to the extent of 3.6 parts in 100 parts of water at 20°C. No data were available for the solubility in 5% hydrochloric acid, but it was considered to be a similar value. This solubility may vary with the temperature, and cause the method to be non-reproducible. The total effect of the solubility of the extractant was determined by the procedure below:

Aliquots of up to 20 μg. of molybdenum were taken, and the procedure of Purvis and Peterson (47) was followed as before. Prior to the addition of the thiocyanate and stannous reagents, the aqueous phase was saturated with cyclohexanol by shaking with 10 ml. of an unequilibriated mixture of cyclohexanol and carbon tetrachloride. No molybdenum could be detected in the washings under these conditions. After separating the washings, the complex was developed by the addition of thiocyanate and stannous chloride as before. The complex was extracted twice with 10 ml. extractant, and the extracts were combined. The transmission was determined at 475 mμ. after the addition of 10 ml. ethyl alcohol and 1.6 ml. stannous chloride. The results are recorded in Table III, and are marked with a (x). Comparison of these results with transmission values which were obtained without this presaturation, revealed that the total solubility effect was small. The transmission values were slightly higher for those determinations in which the solubility of the extractant was reduced.

This/ ....

This increase was caused by the greater volume of extractant recovered. Since the total solubility effect was itself small, the variation in solubility caused by temperature could be considered as insignificant.

Temperature expansion effects were not considered.

2.25. Extraction efficiency.

No molybdenum could be detected in the aqueous phase subsequent to two 10 ml. extractions with cyclohexanol/carbon tetrachloride. Tests were carried out in order to decrease the volume of extractant used, and thereby increase the sensitivity, without changing the quantitative recovery. The complex was formed in the aqueous phase by following the procedure described in section 4. The complex was extracted with different volumes of extractant. Addition of stannous chloride and ethyl alcohol and further cyclohexanol/carbon tetrachloride to the extract ensured that the transmittance was determined from a constant final volume. The results are given in Table IV.

TABLE IV.

EFFICIENCY OF THE EXTRACTION.

Time of Standing.	Percent Transmission.		
	Two 10 ml. Extractions.	One 10 ml. Extraction.	One 5 ml. Extraction.
20 mins.	92.1, 92.4	92.0, 92.2	92.1, 92.2, 92.7 (x).
5 days.	92.2, 92.4	92.1, 92.2	91.5, 92.2, 92.7 (x).

(x) perchlorate ions present.

The/ ....

The results clearly showed that a single 5 ml. extraction was as efficient as two 10 ml. extractions. The complex was therefore extracted once with 5.0 ml. extractant. The volume of alcohol added was reduced to 5.0 ml. and aerial oxidation was stopped by the addition of 1 ml. 20% stannous chloride.

2.26. The shaking time.

The minimum period of shaking that would efficiently extract the complex from the aqueous phase was found to be at least one minute. A 10 µg. aliquot of molybdenum was buretted into the separating funnel. The colour was developed in the aqueous phase following the procedure described in section 4. The complex was first extracted into 4 ml. and then into 1 ml. of extractant, by shaking for different period of time. The results are given in Table V.

TABLE V.

COMPARISON OF SHAKING TIMES.

Test Number	µg. Mo Added.	Shaking Time in minutes.	µg. Mo Found.
1	10.0	1	10.1
2	10.0	2	9.4
3	10.0	3	9.5
4	10.0	4	10.1

The recovery after a shaking time of one minute was therefore satisfactory. It is adviseable to leave the solutions at least three minutes in order to allow the two liquid layers to separate.

2.3/ .....

2.3. The optimum concentrations of the reagents for colour development.

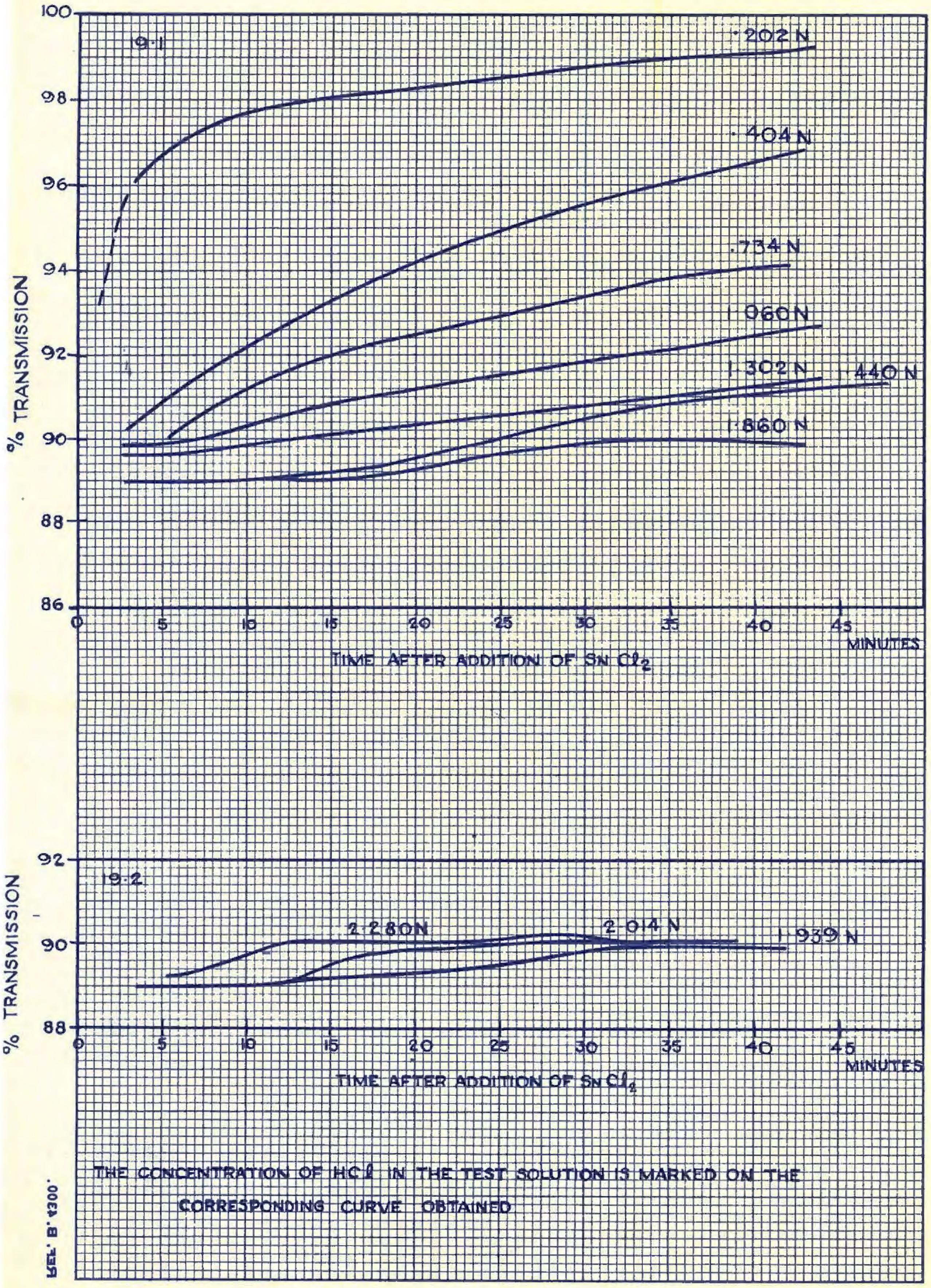
The previous tests established the efficiency of the extraction stage. Attention was now directed to the formation of the complex in the aqueous phase. The reagent concentration ranges necessary for maximum colour development and stability were determined in turn.

2.31. The hydrochloric acid concentration.

From the literature survey it was observed that the colour stability of the complex was markedly dependent upon the acid concentration, but there was disagreement as to the optimum concentration to be used. The author therefore determined transmission-time curves for a series of hydrochloric acid concentrations. The concentration of molybdenum was kept constant.

Aliquots containing 110 µg. molybdenum were placed in 100 ml. volumetric flasks and 0 to 18 ml. of concentrated hydrochloric acid were added. Then a few ml. of purified water, 1 mg. ferric iron, 1 ml. of 42.5% sodium nitrate and 5 ml. of 10% ammonium thiocyanate were added to each flask. The flasks were shaken after each addition. The solution was diluted to 100 ml. and the colour was formed by the addition of 5 ml. 10% stannous chloride in 5% hydrochloric acid. Transmittancy readings were taken on the aqueous phase at 475 mµ. versus the water blank, using 1 cm. cells. The acid concentration was determined by titration against sodium carbonate. The results are recorded in figure 19.

Rapid/ ....



THE CONCENTRATION OF HCl IN THE TEST SOLUTION IS MARKED ON THE CORRESPONDING CURVE OBTAINED

FIG. 19.

EFFECT OF THE HYDROCHLORIC ACID CONCENTRATION ON THE COLOUR INTENSITY AND STABILITY.

Rapid fading occurred when the only acid present was that added with the reducing agent. (final concentration of 0.202 N hydrochloric acid). The solution became more stable as the acid concentration was increased. Over the optimum range 1.44 to 2.01 N hydrochloric acid, the complex was stable for 15 minutes. This range corresponded to the addition of 11 to 16 ml. concentrated hydrochloric acid. At yet higher acid concentrations, both fading and reduction in intensity increased.

The concentration of 1.7 N hydrochloric acid was selected as the value which was optimum for constant transmission.

The period, for which constant transmission values were recorded, was not completely satisfactory. In routine analysis it was possible that the period of standing prior to the extraction of the molybdenum complex might exceed 15 minutes. Further improvements were studied to increase the period of reproducible readings.

2.32. The sodium nitrate concentration.

The optimum concentration of this reagent was determined in the same way as described. To each test solution, 13.5 ml. of concentrated hydrochloric acid were added. The volumes of sodium nitrate used were 0 and 2 ml. The transmission was determined at different times as above.

In the absence of sodium nitrate, fading was marked and the transmission-time graph obtained resembled that reproduced for 1.06 N hydrochloric acid in figure 19.1. The addition of 2 ml. of sodium nitrate yielded stability curves identical with those reproduced in

figure/ ....

figure 19. The optimum reagent concentration was concluded to be reached by the addition of between 1 and 2 ml. of 42.5% sodium nitrate. This value agreed with Purvis and Peterson (47). The use of sodium nitrate reagent which was four months old yielded results identical with the freshly prepared reagent.

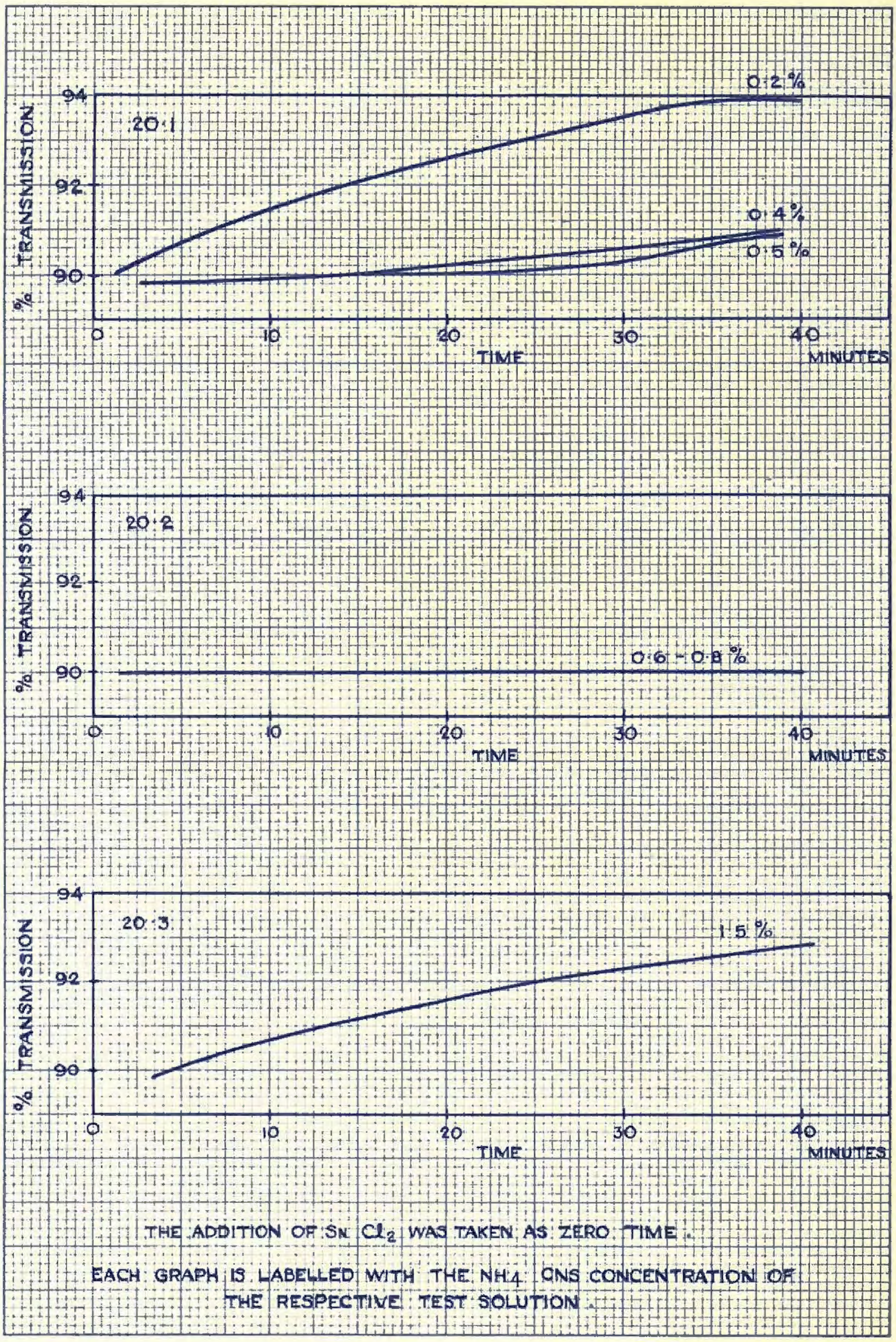
2.33. The ammonium thiocyanate concentration.

A marked increase in the stability of the coloured complex in the aqueous phase was obtained following the determination of the optimum concentration range of the above reagent.

Transmission-time curves were determined at 475 mμ. for concentrations of thiocyanate covering the range 0.2 to 1.5%. The same procedure as described above was followed. The optimum concentrations of hydrochloric acid and sodium nitrate were used for each test, i.e. 13.5 ml. concentrated hydrochloric acid and 1 ml. 42.5% sodium nitrate for a final volume of 110 ml. The results obtained are recorded in figure 20, and revealed that over the concentration range of 0.6 to 0.8% thiocyanate, the complex was stable for 40 minutes. It was subsequently discovered that in this range of 0.6 to 0.8% thiocyanate the complex was stable for a minimum of 12 hours.

The thiocyanate concentration recommended by Purvis and Peterson (47) did not lie in this optimum concentration range. For this reason the strength of the thiocyanate reagent in the final recommended procedure (section 4) was increased to 14%.

Similar results to those recorded above were obtained with ammonium thiocyanate solution that was two months old. This illustrated that the reagent



THE ADDITION OF $SnCl_2$ WAS TAKEN AS ZERO TIME.

EACH GRAPH IS LABELLED WITH THE NH_4 CNS CONCENTRATION OF THE RESPECTIVE TEST SOLUTION.

FIG. 20

EFFECT OF THE THIOCYANATE CONCENTRATION ON THE STABILITY.

itself was stable, (ref. Table VI).

2.34. The stannous chloride concentration.

The influence of the stannous chloride concentration was determined by constructing transmission-time curves for various concentrations of stannous chloride. The other reagents were added in the optimum concentrations as found above, and the final solution contained from 0 to 10 ml. of 10% stannous chloride in 5% hydrochloric acid in a total volume of 110 ml. The total hydrochloric acid concentration was maintained at 1.7 N by the addition of further hydrochloric acid. The transmission was found to be largely independent of the stannous chloride concentration. The use of less than 4 ml. did not remove ferric interference, and the optimum concentration was chosen to lie between 4 and 10 ml.

The possible influence of the age of the stannous chloride reagent itself was also examined. The results obtained by the use of 5 ml. stannous chloride of different ages are recorded in Table VI . The results indicated that this reagent could safely be used 48 hours after preparation, if it was kept in a well-stoppered container. Longer periods of storage are not recommended.

TABLE VI.

THE INFLUENCE OF THE AGE OF THE REAGENTS USED.

Age of Ammonium Thiocyanate Reagent.	Age of Stannous Chloride Reagent.	µg. Mo Added.	µg. Mo Found.
Fresh	2 Days	10.0	9.9, 9.8
Fresh	Fresh	15.0	15.0, 14.8
Fresh	1 Day	15.0	14.7
Fresh	2 Days	15.0	14.8
2 Months	Fresh	15.0	15.0, 14.4

2.4. General Observations.

2.41. Interference by foreign ions.

In the analysis of plant material there was the possibility of interference by other substances. In addition to the major and minor elements normally found in plant material, perchloric acid will also be present in the final plant solution. Possible interferences were tested for as follows: A 'synthetic' plant solution was prepared containing the following elements in the proportions given:

700 parts N; 30 parts P; 100 parts Na; 20 parts Al; 100 parts K
500 parts Ca; 250 parts Mg; 50 parts B; 100 parts Fe
5 parts Mn; 2 parts Zn; and 2 parts Cu.

To each of 6 replicate solutions 0.15 parts of molybdenum (equivalent to 15 µg.Mo) and 5 ml. 70% perchloric acid were added. The acidity was controlled at 1.7 N with hydrochloric acid. The molybdenum content was determined. Reference tests, on solutions free from perchloric acid, and other possible interferences, were run simultaneously

on/ .....

on 15 μg. molybdenum. The percentage recoveries are recorded in Table VII.

TABLE VII

PERCENTAGE RECOVERIES.

Synthetic plant solution.			Control test.		
μg. Mo Added.	μg. Mo Recovered.	% Recovery.	μg. Mo Added.	μg. Mo Recovered.	% Recovery
15.0	15.3	102.0	15.0	15.2	101
15.0	15.0	100	15.0	13.4	89.3
15.0	13.9	92.7	15.0	15.0	100
15.0	16.0	106.8	15.0	14.4	96
15.0	17.4	116	15.0	15.9	106
15.0	15.0	100	--	--	--

These results revealed that none of the elements normally occurring in plant material interfered with the procedure, in the proportions usually present. The above quantity of perchloric acid did not interfere either, provided the total acid concentration was controlled. The maximum absorption was found to be unchanged by the presence or absence of these ions.

2.42. Maximum absorption of the complex.

The transmission versus the wavelength curve, shown in figure 21, was obtained for the molybdenum thiocyanate complex in cyclohexanol/carbon tetrachloride. This revealed the molybdenum complex to have maximum absorption at 470 mμ. This value was therefore used for subsequent determinations.

Hurd/ .....

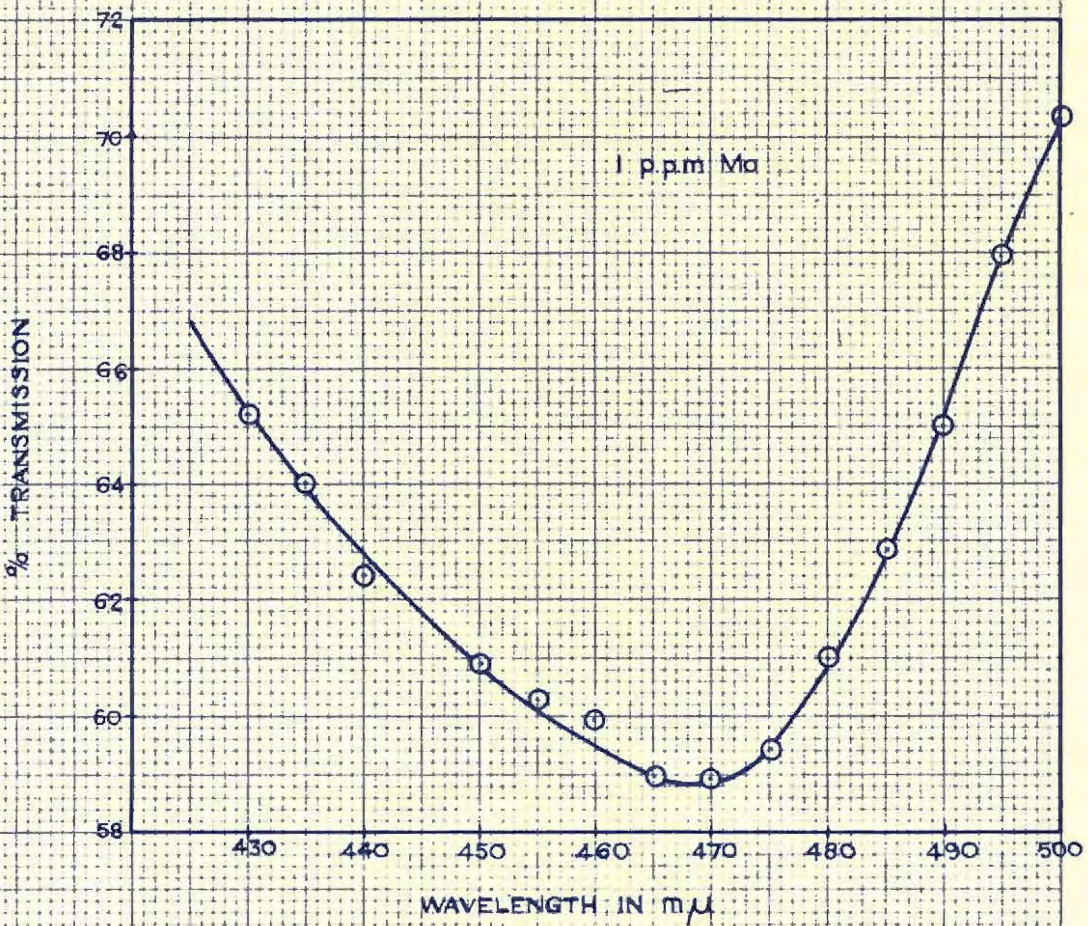


FIG.21

TRANSMISSION WAVELENGTH CURVE FOR MOLYBDENUM THIOCYANATE IN CYCLOHEXANOL - CARBON TETRACHLORIDE.

Hurd and Reynolds (87) found the complex to have maximum absorption at 465 mμ. in cyclohexanol. The 5 mμ. increase in wavelength as found by the author appeared to be in accord with the work reported by Johnson and Arkley (79), who found that the maximum absorption in iso-amyl alcohol/carbon tetrachloride was also at 470 mμ. while the absorption in isoamyl alcohol alone was at 465 mμ. (Dick and Bingley (46)).

2.43. Adsorption of the molybdenum by the glassware.

The wet ashing stage in the analysis of plant material is fairly prolonged and not always reproducible with respect to time. The author therefore investigated possible loss of molybdenum during this stage by way of adsorption on the glass vessels used. The test was carried out by placing 10.00 μg. molybdenum in a 100 ml. pyrex volumetric flask and adding 3 ml. of 70% perchloric acid. The vessels were stoppered and kept for two weeks. Ten ml. of hydrochloric acid were then added, and the complex was developed by the procedure detailed in section 4. The results are reproduced in Table VIII.

TABLE VIII.

PERCENTAGE RECOVERIES.

μg. Mo Added.	μg. Mo Found.	% Recovery.
10.00	10.02	100.2
10.00	10.08	100.8
10.00	9.80	98.0

In/ .....

In view of these results it was concluded that possible adsorption of the molybdenum by the glassware could be safely ignored if perchloric acid was present, even though the test solution had stood for a period of 14 days.

In the recommended procedure for the wet digestion of plant material, a final volume of between 2 and 5 ml. was obtained in the kjeldahl flask. This medium consists mainly of perchloric acid. The addition of 10 ml. of concentrated hydrochloric acid to either of these volumes yielded a final total acid concentration, on diluting to 100 ml, that lay between 1.4 and 2.0 N. This acid concentration range has already been shown (using hydrochloric acid) to stabilise the molybdenum complex in the presence of sodium nitrate. The possibility of using a mixture of perchloric and hydrochloric acids was examined.

2.44. The use of perchloric acid as a stabilising agent.

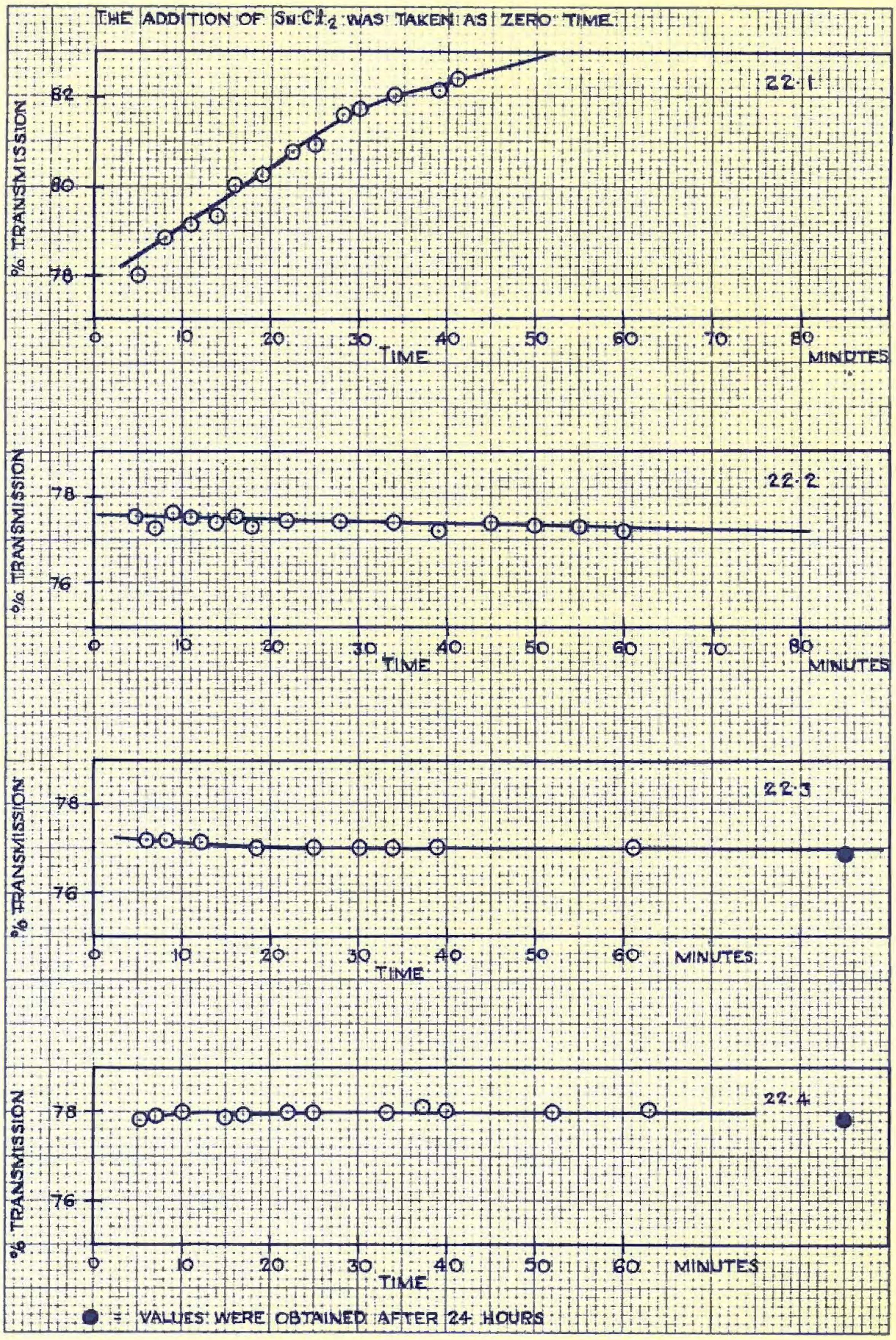
As reported in the literature survey several workers removed perchloric acid by repeated evaporation to fumes. The above results (Table VIII) indicated that in fact perchloric acid did not cause interference in hydrochloric acid medium. It therefore appeared to be unnecessary to include this lengthy evaporation stage in the analysis procedure, merely to remove perchloric acid. The author realised further, that perchlorate ions might behave similarly to nitrate ions and prevent reduction of the molybdenum below the 5+ oxidation state. If the perchlorate ions re-oxidised the molybdenum back up to the 5+ st , these ions could be used as the stabilising agent and thus avoid the use of sodium nitrate in the analysis procedure. The following tests were performed in order to examine this possibility:

Aliquots/ ...

Aliquots of molybdenum were taken to give a final concentration of 1 p.p.m. Concentrated hydrochloric acid was added so that the total acidity lay between the optimum concentration of 1.4 to 2.0 N. The previously determined optimum concentrations of ferric iron, sodium nitrate and ammonium thiocyanate were added with shaking after each addition. The volume was made to 100 ml. and the colour developed by the addition of 5 ml. of stannous chloride reagent. The transmission of the aqueous phase was determined. The point at which the stannous chloride reagent was added was taken as zero time, and a transmission-time curve was drawn. The results, for the different conditions, are recorded in figure 22.

Figure 22.1 revealed that the complex is unstable in the absence of both nitrate and perchlorate ions. Figure 22.2 revealed that stability was obtained in the presence of both perchloric acid and sodium nitrate. The acidity was kept at the optimum value by hydrochloric acid addition. Figures 22.3 and 22.4 revealed that in the presence of 2 to 5 ml. of 70% perchloric acid the complex was stabilised for a minimum of 24 hours, even though no sodium nitrate was present.

As a result of these findings, the author considered it unnecessary to use the sodium nitrate reagent if perchloric acid was already present. This acid effectively stabilised the complex, and the time of extraction was no longer so critical. The complex should however not be extracted until five minutes after the addition of the stannous reagent, to ensure complete colour development.



THE CURVES WERE OBTAINED FROM THE FOLLOWING TEST SOLUTIONS

TEST SOLUTIONS	TOTAL ACIDITY	Ml 7% $HClO_4$	Ml 5M $NaNO_3$
22·1	1·6N	NIL	NIL
22·2	1·8N	5·0	1·0
22·3	1·8N	5·0	NIL
22·4	1·7N	2·0	NIL

FIG. 22

STABILITY GRAPHS FOR MOLYBDENUM THIOCYANATE IN AQUEOUS SOLUTION.

3. THE DETERMINATION OF MOLYBDENUM IN CITRUS LEAF MATERIAL.

3.1. Preparation of the sample.

Approximately 800 leaves were picked from twelve citrus trees growing in the grounds of the Rhodes University Botany Department. The samples were taken from different heights of each tree and from different positions on the branch. Spring cycle leaves were not specifically selected. The sampling was performed at sunset. Apart from being slightly withered by the summer heat, no marked peculiarity was noticed about the leaves. The leaves were then prepared for analysis as described by Steyn (37):-

Immediately after picking, the leaves were transferred to a polythene bag and were stored overnight in a refrigerator. Each leaf was then washed individually in order to remove any surface dirt or adhering soil.

Five polythene dishes were taken. About 750 ml. 0.1% teepol were placed in the first and the same volume of distilled water was placed in the second the third dishes. 750 ml. deionised water were placed in the fourth dish, and the final dish was used to collect the leaves. Each leaf was sponged with cotton wool in the teepol solution and then rinsed consecutively in the three sets of water. The main ribs of the leaves were then removed with a pair of stainless steel scissors. The leaf-halves were then placed in suitably sized muslin bags. The bags were closed with a rubber band and were placed inside a forced draught oven set at 65°C. The sample was dried in this manner for 48 hours, and then finely ground in an agate ball mill. The sample was then mixed intimately and stored in a screw-capped polythene bottle. Before analysis the sample was dried for a further period of 24 hours at 65°C.

3.2. Preparation of leaf solutions.

An aliquot of the powdered sample was dried for 24 hours at 65°C. Approximately 3 g. were weighed into a 200 ml. Kjeldahl flask. Then 25 ml. of acid digestion mixture were added, (20 ml. concentrated redistilled nitric acid and 5 ml. 70% perchloric acid). After the initial reaction had subsided, the material was rendered to clear solution by gentle boiling on a sand bath. Evaporation to fumes eliminated the nitric acid, and left a clear colourless solution with a final volume between 2 and 5 ml.

3.3. The addition of sodium nitrate to plant solutions.

The previous results obtained for 'synthetic' plant solutions revealed that perchloric acid stabilised the molybdenum-thiocyanate complex in aqueous medium and that the addition of sodium nitrate was unnecessary if this acid was present. However, the investigations upon plant solutions revealed that the use of sodium nitrate was advisable if incompletely decomposed organic material was still present.

The leaf samples were treated as described in 'Preparation of sample' (section 3.1). 3 g. of plant material were brought into solution as described in section 3.2. The molybdenum thiocyanate was developed and extracted into cyclohexanol-carbon tetrachloride as described in 'Recommended Procedure' (section 4). One ml. of ferric reagent was added, but the addition of sodium nitrate was omitted. The transmittance measurements were not reproducible. Aliquots of the standard molybdenum solution were therefore added to the plant

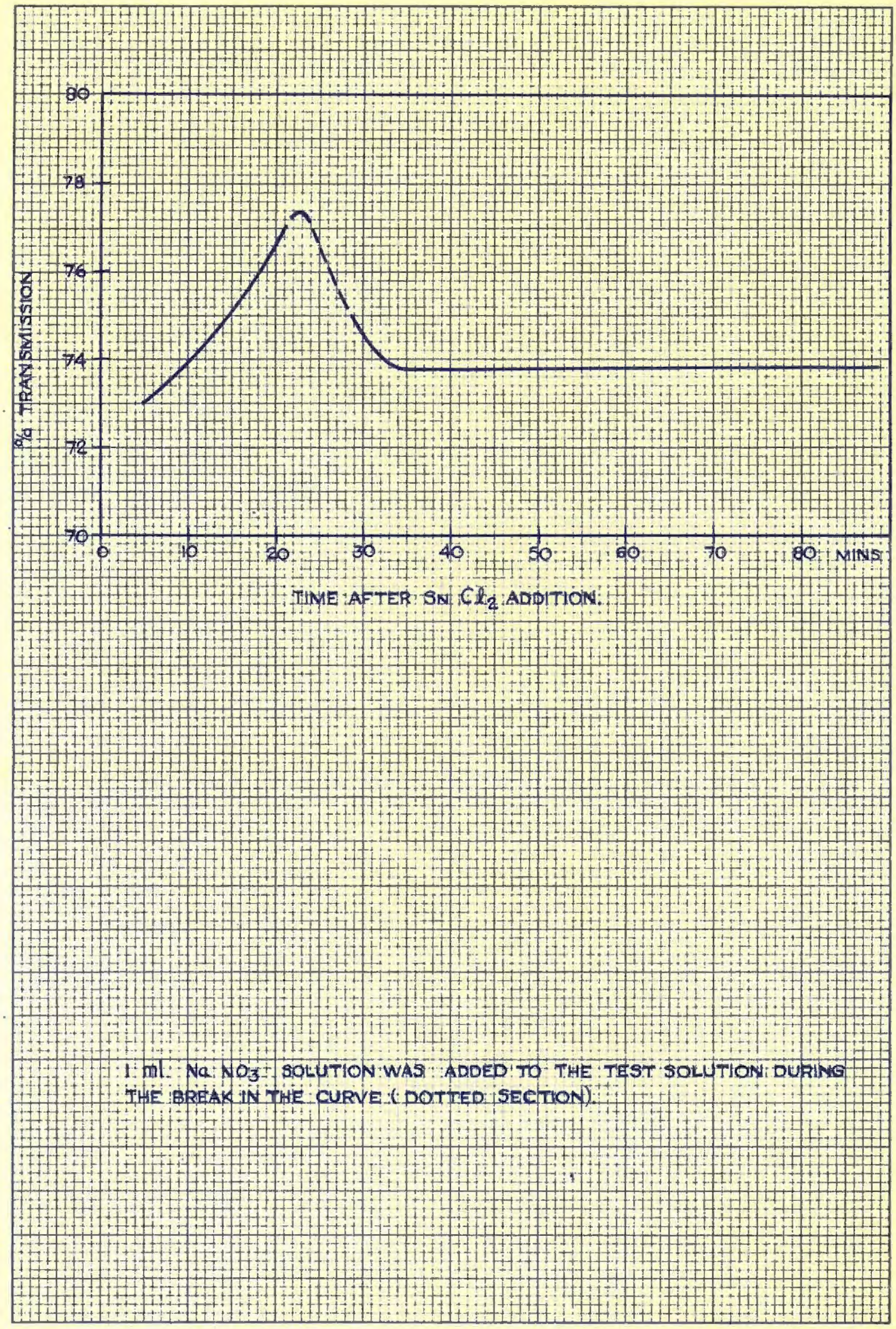
solution/ ..

solution to obtain suitable transmission values in the aqueous phase. Fading was found to occur in this medium, whether iron was present or not.

In order to overcome this instability, 3 g. plant material were brought into solution as described in section 3.2., and the resultant solution was treated in a similar manner to that recommended by Chapman (48). The solution was heated to dryness. Hydrogen peroxide was added and the solution was again taken to dryness. After being taken up in water, 3 ml. 70% perchloric acid were added followed by aliquots of the standard molybdenum solution,to obtain significant readings. The complex was developed by the procedure recommended in section 4. No sodium nitrate was added. The transmission values were stable for a minimal period of one hour. In a similar series of experiments, no molybdenum was added to the plant solution and the complex was extracted as described. These results were reproducible. The author therefore concluded that incompletely oxidised organic material upset the oxidation-reduction equilibrium and caused instability.

The addition of sodium nitrate was found to increase the stability in the aqueous phase. Three g. of plant material were brought into solution as described in section 3.2. The final solution had a bulk of 3 ml. and was tinted yellow by the trace of organic material present. One hundred μg. molybdenum were added to give suitable transmittance readings. The complex was developed in the aqueous phase by the procedure described in section 4. Neither sodium nitrate nor ferric ions were added. The transmittance readings were recorded at 470 mμ. and plotted as a function of time. The time

when/ .....



1 ml. $NaNO_3$ SOLUTION WAS ADDED TO THE TEST SOLUTION DURING THE BREAK IN THE CURVE (DOTTED SECTION).

FIG. 23.

TRANSMISSION VALUES FOR MOLYBDENUM THIOCYANATE IN AQUEOUS SOLUTION.

when stannous chloride was added was taken as zero time. The results have been reproduced in figure 23. Section A of the graph revealed that the volume of perchloric acid used (3 ml.) was insufficient, and that the complex was unstable. Section B of the graph was obtained for the same solution after the addition of 1 ml. 42.5% sodium nitrate. The transmittance was stabilised and the complex was regenerated. The author therefore included this reagent in the final procedure.

The stability of transmission measurements in cyclohexanol/carbon tetrachloride medium for plant material was also examined. Three g. of plant material were dissolved as described in section 3.2. After the nitric acid had been driven off, the solution was boiled vigorously for an hour. The complex was developed and extracted by the procedure recommended in section 4. The transmission readings obtained are reproduced in Table IX.

TABLE IX.

STABILITY OF THE COMPLEX IN CYCLOHEXANOL/CARBON TETRACHLORIDE.

Time after first extraction (minutes).	20	25	30	40	50	60	70	80
Percentage Transmission.	93.8	93.8	93.8	93.9	93.9	94.0	94.1	94.0

These values revealed that the complex developed from plant solutions, was stable for a suitable period of time. It is recommended that the digestion solution be boiled for at least an hour after the nitric acid has been driven off. Multiple evaporations to dryness were obviously unnecessary and laborious.

4./ .....

4. RECOMMENDED PROCEDURE FOR THE DETERMINATION OF MOLYBDENUM IN PLANT MATERIAL.

Reagents.

Use deionised distilled water for the preparation of all solutions.

1. Nitric Acid. Purify C.P. HNO_3 by distillation from an all glass still.
2. Perchloric Acid. 70% A. R. $HClO_4$. No purification required.
3. Dissolving Acid Mixture. Prepare by mixing acids 1 and 2 in the proportions 20 volumes to 5 volumes.
4. Hydrochloric Acid. A. R. concentrated HCl.
5. Ferric Chloride. Dissolve 49 g. $FeCl_3.6H_2O$ in water and make to 1 litre. This solution has a concentration of 10 mg. Fe/ml.
6. Sodium nitrate. Dissolve 42.5 g. A. R. $NaNO_3$ in water and make to 100 ml.
7. Ammonium thiocyanate. Dissolve 14 g. of dry recrystallised ammonium thiocyanate in water and make to 100 ml.
8. Stannous chloride. Achieve solution with the minimum amount of heating. Dissolve 20.0 g. $SnCl_2$ in 13.5 ml. concentrated hydrochloric acid by stirring. Heat gently to remove turbidity. Do not boil. Cool and dilute to 100 ml. Prepare fresh daily.
9. Carbon Tetrachloride. Purify by distillation.
10. Cyclohexanol. B.D.H. reagent.
11. Extractant. Prepare an equi-mixture containing 20 ml. carbon tetrachloride and 20 ml. cyclohexanol. The use of automatic pipettes avoids noxious carbon tetrachloride vapours. Equilibriate with 3 ml. stannous chloride reagent and 2 ml. ammonium thiocyanate.

Collect/ ....

Collect the organic phase in a reservoir to which is connected a 4 ml. and a 1 ml. automatic pipette. Prepare fresh daily.

12. Ethyl Alcohol. 95%.

13. Standard molybdenum solution. Solution A. Dissolve 0.1822 g. of 'specpure' ammonium molybdate ($(NH_4)_6 Mo_7O_{24}.4H_2O$) in water and make to a litre. Store in a polythene container. The solution contains 100 p.p.m. Mo. Solution B. contains 1 p.p.m. Mo. Prepare by dilution of solution A.
    The molybdenum content of fresh batches of ammonium molybdate should be checked gravimetrically as lead molybdate.

PROCEDURE.

Prepare the leaf material as described under 'Preparation of the sample'. Weigh an aliquot of the sample into a 200 ml. kjeldahl flask and proceed as described under 'Preparation of leaf solutions'. Ensure that the final volume lies between 2 and 5 ml. by the addition of perchloric acid if necessary. Add 10 ml. of water and mix by swirling. Smear a thin film of pure grease on the outside lip of the flask. Filter the solution through a 9 cm. Whatman No.40 paper, which has been previously washed with a hot solution containing 10% hydrochloric acid, into a graduated 100 ml. separating funnel containing 10 ml. concentrated hydrochloric acid. Transfer the contents of the flask quantitatively, and wash the silica residue on the filter with hot water until the volume in the funnel is about 80 ml. Shake the funnel to mix. Cool to room temperature.

Add/ .....

Add 1 ml. sodium nitrate and 5 ml. ammonium thiocyanate. Shake after each addition. Dilute to 100 ml. Add 3 ml. stannous chloride and shake. After 5 minutes extract the molybdenum thiocyanate with 4 ml. extractant from an automatic pipette. Shake for 1 minute. Collect the residue in a 50 ml. conical flask. Repeat the extraction using 1 ml. extractant. Combine the extracts. Add 4 ml. ethyl alcohol and 1 ml. stannous chloride, again using automatic pipettes. Determine the transmission at 470 mμ.

A reagent blank must be performed. Filter 3 ml. perchloric acid into the stated separating funnel containing 10 ml. hydrochloric acid and proceed as above.

Prepare a calibration graph by taking aliquots of solution B to cover the range 0 to 10 μg. molybdenum. Add 3 ml. perchloric acid and proceed as above. Before adding the sodium nitrate, add 1 ml. ferric solution. The plant solution contains sufficient iron for this addition to be unnecessary.

5. THE STATISTICAL ACCURACY AND PRECISION OF THE MODIFIED PROCEDURE.

The modified colorimetric method was subjected to a rigorous statistical test in order to evaluate the accuracy and precision under routine laboratory conditions.

5.1. Calibration curve.

A calibration curve was drawn following the experimental conditions defined above. The results obtained are recorded in Table X.

Table X/.....

TABLE X.

CALIBRATION CURVE.

Molybdenum μg.	Percent Transmission at 470 mμ.	Molybdenum μg.	Percent Transmission at 470 mμ.
0.0	99.1	4.0	81.1
0.3	98.8	6.0	73.0
0.6	97.7	8.0	65.5
1.0	95.9	10.0	58.9
1.2	95.5	10.2	58.8
1.4	94.9	10.4	57.9
1.6	93.8	10.6	57.1
2.1	91.8	11.0	56.3

These results have been reproduced in figure 24.

The calibration curve obtained is a straight line.

5.2. Accuracy of the method.

The absolute accuracy of the method was examined by the addition of molybdenum to citrus leaf samples, as follows: Twenty three 3 g. aliquots of the homogeneous plant sample, prepared as detailed in section 3.1, were weighed separately. Ten μg. molybdenum were added to fifteen of the samples. Each of the samples was brought into solution, following the procedure detailed in section 3.2. The molybdenum content of each sample was determined as described in section 4. The results obtained are reproduced in Table XI.

Table XI/ .....

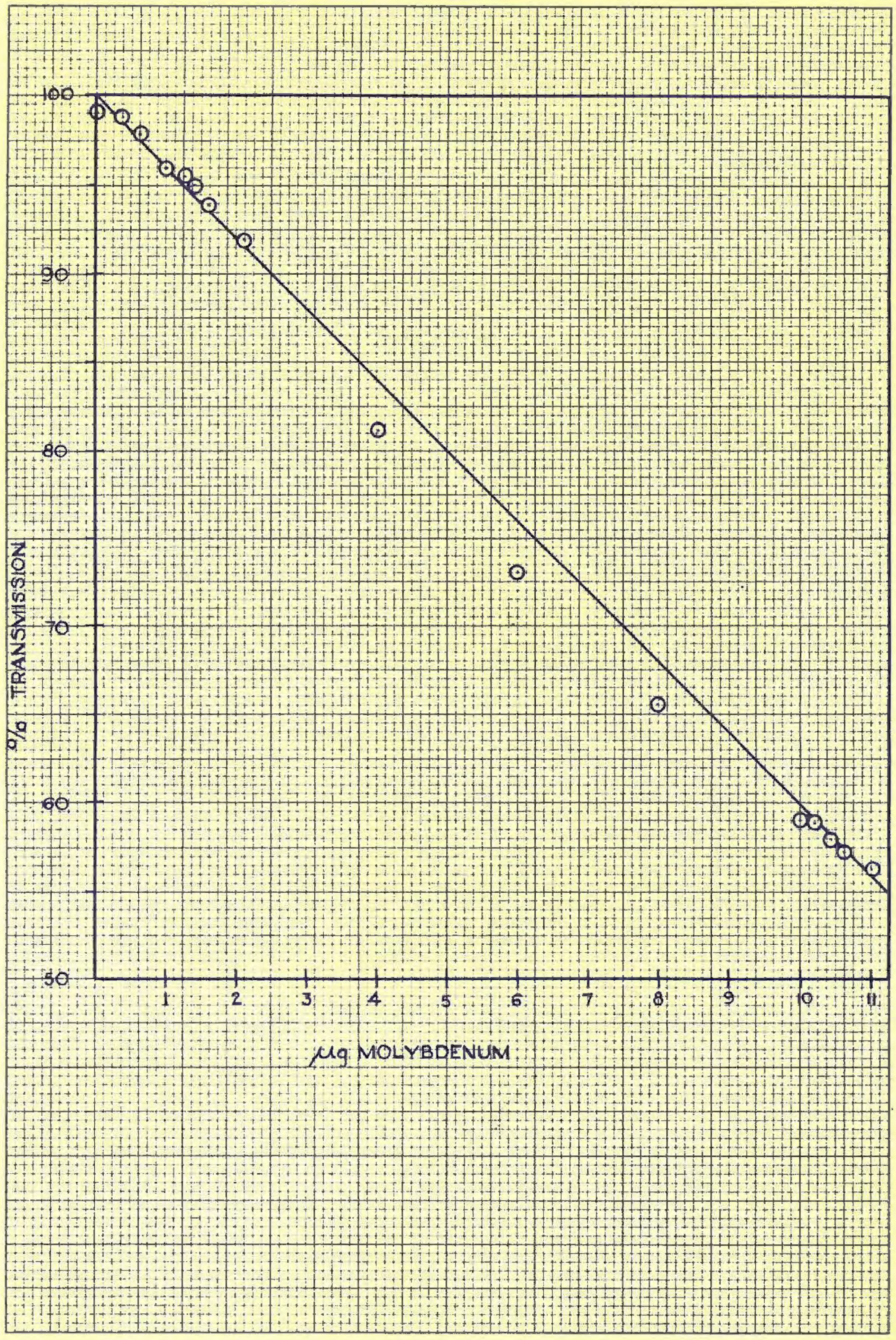


FIG.24

CALIBRATION GRAPH

TABLE XI.

THE MOLYBDENUM CONTENT OF CITRUS LEAF MATERIAL.

Determination Number.	Weight plant material g.	Molybdenum Added μg.	Molybdenum found μg.	p.p.m. Molybdenum.	% Recovery.
1'	3.05	--	1.69	0.55	189
2'	2.99	--	0.74	0.25	86
3'	3.06	--	0.73	0.24	83
4'	3.05	--	0.77	0.25	86
5'	3.05	--	0.74	0.24	83
6'	3.00	--	0.74	0.25	86
7	3.03	--	0.73	0.24	83
8	3.06	--	0.93	0.30	103
9	2.99	10.0	10.74	0.25	86
10	3.06	10.0	11.04	0.34	117
11	2.98	10.0	11.04	0.35	120
12	3.06	10.0	11.02	0.33	114
13	3.04	10.0	10.77	0.25	86
14	3.04	10.0	11.00	0.33	114
15	3.01	10.0	11.24	0.41	141
16	3.05	10.0	11.02	0.33	114
17	3.03	10.0	11.00	0.33	114
18	3.04	10.0	10.90	0.30	103
19	3.01	10.0	11.50	0.50	172
20	3.05	10.0	10.95	0.31	107
21	3.03	10.0	10.95	0.31	107
22	3.06	10.0	11.05	0.34	117
23	3.04	10.0	10.85	0.28	97
			Average	0.29	114%

The / .....

The molybdenum content of the plant material in p.p.m. was calculated from the μg. molybdenum determined and the sample weight. The 10 μg. molybdenum aliquot added was deducted where this applied. The results are recorded in column five. The average of the twenty three determinations was found to be 0.29 p.p.m. molybdenum. The ratio of each individual determination to this average was calculated as a percentage. This value is given in column six. From these data it was concluded that this procedure gave a true reflection of the molybdenum content of plant material.

5.3. The precision of the method.

The precision of the method was evaluated as follows: A homogeneous citrus plant sample was prepared. This was dried in an oven at 65° and twenty three 3 g. samples were weighed out. Each sample was individually dissolved in the acid mixture and the molybdenum content of the resulting solution was determined by the recommended procedure.

This series of tests was performed under conditions closely approximating those in a routine laboratory. The tests were performed over a period of several days. The room temperature varied. Fresh reagents were prepared where recommended. The results are recorded in Table XII. The sample weights marked with an asterisk (') contained incompletely oxidised organic material. This was evidenced by the decided yellow solution obtained in the kjeldahl flask.

Table XII/ ....

TABLE XII.

THE PRECISION OF THE MODIFIED PROCEDURE.

No. (n)	Sample Weight g.	p.p.m. Mo	M - Y	$(M - Y)^2$
1'	3.021'	0.12	0.05	0.0025
2'	3.032'	0.17	0.00	0.0000
3'	2.995'	0.17	0.00	0.0000
4'	3.044'	0.18	0.01	0.0001
5'	3.063'	0.17	0.00	0.0000
6'	2.997'	0.11	0.06	0.0036
7	2.996	0.18	0.01	0.0001
8	3.064	0.17	0.00	0.0000
9	3.054	0.16	0.01	0.0001
10	2.987	0.13	0.04	0.0016
11	2.965	0.17	0.00	0.0000
12	2.976	0.18	0.01	0.0001
13	3.000	0.16	0.01	0.0001
14	2.988	0.17	0.00	0.0000
15	3.022	0.26	0.10	0.0100
16	3.017	0.18	0.01	0.0001
17	3.017	0.26	0.09	0.0081
18	3.062	0.15	0.02	0.0004
19	3.046	0.18	0.01	0.0001
20	3.039	0.18	0.01	0.0001
21	3.030	0.18	0.01	0.0001
22	3.028	0.18	0.01	0.0001
23	2.961	0.17	0.00	0.0000
$n - 1$ $= 22$		0.17 (Mean).		0.0272 $\Sigma (M - Y)^2$

Standard deviation $(\sigma) = \sqrt{\frac{\Sigma(M - Y)^2}{n - 1}} = 0.03(5)$ p.p.m.

Coefficient of Variation (Percent Standard Deviation)

$= \frac{0.035}{0.17} \times 100$

$= 20\%$.

These results revealed that the procedure could determine down to 0.17 p.p.m. molybdenum in plant material with a coefficient of variation of 20%. The presence of incompletely oxidised organic material did not influence the results.

CONCLUSIONS.

A method for the routine determination of molybdenum in plant materials has been developed which is considered to be superior to the existing procedures.

i) Speed. Not the least of the advantages of the thiocyanate procedure lay in it being virtually specific for molybdenum. In consequence the procedure is simpler, presents few difficulties with regard to technique and the results are obtained more rapidly than by other procedures.

ii) Reagent concentration. The procedure is insensitive to a variation in the reagent concentrations over a wide range. The experimental study established that in the optimum concentration ranges of 1.4 to 2.0 N hydrochloric acid, 0.42 to 0.85% sodium nitrate, 0.4 to 1.0% stannous chloride and 0.6 to 0.8% ammonium thiocyanate, the optical density values were reproducible for several days. The study also revealed that far from interfering, the presence of up to 3.5% perchloric acid actually stabilised the coloured complex in aqueous solution. This observation shortened the procedure further by eliminating the necessity of several evaporations.

iii) Stability of the reagents. With the exception of the stannous chloride reagent, which is freshly prepared, the reagents could be stored for several months before use.

iv) Improved extractant. The extractant, developed in this study, is considered to be superior to those developed previously. The excellent stability of the complex in this medium (5 days) rendered it unnecessary to follow a rigid time schedule for each analysis. Because the density

of/ .....

of the reagent is greater than unity, it could be handled with ease and the phase separation was sharp and rapidly obtained. The extractant may be recovered by distillation. This reduces the cost.

(v) Accuracy and precision. The statistical examination revealed that there is no fundamental error in the procedure. The standard deviation of 0.03 p.p.m. molybdenum was considered to be satisfactory for the levels of molybdenum occurring in plant materials. The sensitivity may be further improved either by taking a larger sample weight or by using a 4 cm. light path. Such cells were not available for the above study.

In view of the greater stability and other improvements, it is recommended that the above modified procedure be adapted to the routine determination of molybdenum in plant materials.

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