

this solvent and replace it with one of the alcohols.

The apparatus is versatile in handling materials of varying volatilities. By raising or lowering the combustion tube in the furnace and adjusting the furnace temperature, materials ranging from vegetable oils to low-boiling alcohols or ethers may be burned. In the case of extremely volatile materials, the offset buret feed may be employed. Small samples may also be diluted with other solvents to obtain a mixture of medium volatility.

No explosions have occurred with the combustion apparatus, even when highly volatile materials such as ether-isopropyl alcohol mixtures were handled. The large air-to-sample ratio probably serves to prevent the formation of explosive mixtures.

Samples must be free from undissolved particles which impede sample flow. In the case of inorganic impurities, such as

boiling chips, a filtration is sufficient, but difficultly soluble organic materials may require heating or other solvents to bring about smooth sample flow.

Silicone stopcock lubricant was found to be of considerable value in sealing the spherical joints in the apparatus, particularly those affected by the heat of the furnace. The spherical joints should be cleaned occasionally to prevent a build-up of granular deposits which may allow outside air to bleed into the system.

A piece of Tygon tubing provides flexibility between the air purification train and combustion furnace assembly. However, this material should not be used for connections between absorbers or in connections which are subject to heat because of the danger of contamination.

The described apparatus has been in use for residue analyses in this laboratory for several years. It offers advantages over available equipment in regard to

simplicity and utility, especially where sample size is a limitation on sensitivity.

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ZINC DETERMINATION

Determination of Zinc in Plants and Soils

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A critical investigation of the reliability of the dithizone and polarographic methods, for the determination of zinc, is presented. A satisfactory modification of the Cowling and Miller dithizone photometric method was devised by the close study of the influence of pH on zinc extraction, transmittance curves, and accuracy of the technique. With this modified procedure, 24 zinc determinations per day could be carried out. A modified polarographic method, aimed especially at the elimination of a preliminary separation step was found to lend itself also to the simultaneous determination of copper, nickel, manganese, and possibly cobalt.

Study of Dithizone Photometric Method

ZINC IN BIOLOGICAL MATERIALS has been determined most commonly by either photometric procedures, based on the red complex which zinc forms with diphenylthiocarbazone, usually referred to as "dithizone," or polarographic methods. Both of these methods were selected for careful study.

The most popular dithizone method is that of Cowling and Miller (2), published in 1941. Since then many workers have presented modifications of this method (5-7, 14-17). The majority

of these were aimed at a reduction in the time of the lengthy extraction technique employed by Cowling and Miller. However, in many cases, a loss of accuracy was involved by the modification.

The essential feature of the Cowling and Miller method consisted in a three-stage extraction technique. First, the zinc and other metals forming dithizone complexes were separated from iron, aluminum, calcium, and other substances by extracting the sample solution, buffered at a pH of 8.5, with a dithizone-

carbon tetrachloride solution. The zinc was next separated from copper by extracting the organic phase with a dilute hydrochloric acid solution. Finally, the acid solution was neutralized, buffered at pH 8.5 with ammonium citrate-ammonia solution containing some sodium diethyl dithiocarbamate, and the zinc extracted with dithizone as before. Photometric measurements were carried out on a portion of the zinc dithizonate extract at a wave length of 530 to 540 $m\mu$ (17).

The critical points in this method which were selected for closer study were the effect of pH on the quantitative

extraction of zinc, the most suitable wave length at which the transmittance measurements should be made, and possible sources of error in the general technique. Several important modifications have been introduced in the original method and the modified method is described below.

Apparatus

All photometric measurements, unless otherwise stated, were carried out by means of a Beckman Model DU spectrophotometer, using 1-cm. Corex glass cells. A Beckman Model G pH meter was applied for the determination of hydrogen ion concentrations.

All glassware, particularly the separatory funnels, were scrupulously cleaned before use, first with chromic acid solution, thereafter with distilled water, and finally with zinc-free water. Stopcocks were reground so as to eliminate the use of grease. The use of rubber was strictly avoided, as rubber contains appreciable amounts of zinc. A special rack was constructed to hold two sets of six separatory funnels, one set above the other. Borosilicate glass was used throughout.

Reagents

All reagent solutions were stored in polyethylene bottles to avoid the possibility of ion exchange from glass vessels. This was particularly essential for storing the zinc standards.

All acids, alkalis, and water were redistilled from all-borosilicate glass stills. Only high-grade chemicals were used and these were further purified where necessary, as described below.

Standard Zinc Solution. Analytical reagent grade zinc oxide powder was heated to constant weight in a platinum crucible and 0.2488 gram of this was dissolved in 10 ml. of hot 1*N* sulfuric acid, neutralized with redistilled ammonia, and made up to 1000 ml. (200 p.p.m. of zinc). A 5-p.p.m. solution was made up from this for use in preparing calibration curves.

Carbon Tetrachloride. Technical grade carbon tetrachloride was dried with calcium chloride and redistilled from a borosilicate glass still. Used carbon tetrachloride was reclaimed by distillation with dilute sodium hydroxide-sodium thiosulfate solution, dried, and redistilled as before.

1*N* Ammonium Hydroxide. Prepared from redistilled ammonia.

1*N* Hydrochloric Acid. Prepared from redistilled hydrochloric acid.

Dithizone Reagent. Analytical reagent grade diphenylthiocarbazone, 0.1 gram, was dissolved in 500 ml. of carbon tetrachloride by shaking in a 2-liter separatory funnel. It was then shaken with 20 ml. of 1*N* ammonium

hydroxide in 1 liter of water to transfer the dithizone to the aqueous phase. The carbon tetrachloride was run off and the aqueous phase washed with several small portions of carbon tetrachloride. This was acidified with 50 ml. of 1*N* hydrochloric acid and shaken with 500 ml. of carbon tetrachloride to transfer the dithizone once more to the organic phase. The carbon tetrachloride phase was run off into an amber-glass bottle, diluted to 1 liter, and stored in a refrigerator.

Ammonium Citrate Buffer (0.5*M*). Dibasic ammonium citrate, 226 grams, was dissolved in 2 liters of water and the pH adjusted to 9 with redistilled ammonia. This solution was extracted with excess dithizone reagent and then washed with small portions of carbon tetrachloride until the washings were pure green.

Solution A. One liter of ammonium citrate buffer plus 150 ml. of 1*N* ammonium hydroxide were diluted to 4 liters.

Solution B. One liter of ammonium citrate buffer plus 320 ml. of 1*N* ammonium hydroxide were diluted to 4.5 liters.

Solution C. One volume of freshly prepared 0.2% sodium diethyl dithiocarbamate solution was mixed with 9 volumes of Solution B just before use.

0.02*N* Hydrochloric Acid. Prepared from 1*N* hydrochloric acid.

Procedure for Plant Material

Weigh out a portion of the powdered and dried plant material containing between 5 and 25 γ of zinc (usually 0.5 gram) into a wide-necked 150-ml. conical flask. Add a mixture containing 10 ml. of redistilled nitric acid plus 2.5 ml. of analytical reagent grade perchloric acid (60 to 70%) and cover with a reflux funnel. Digest on a hot plate at a very low heat until the vigorous reaction has subsided. Then boil gently until most of the material has dissolved. Remove the cover glass, rinse with water, and evaporate the contents of the flask to about 0.5 ml. At this stage, the contents should be colorless. If not, repeat the evaporation with a few drops of perchloric acid. Cool, dilute slightly, and filter through Whatman paper No. 40 which has been previously washed with hot, dilute perchloric acid. Wash with small portions of hot water and transfer to a 100-ml. separatory funnel. Add 1 ml. of 10% zinc-free ammonium citrate and 1 drop of 0.1% phenolphthalein, and titrate with redistilled ammonia until just pink. Then carry out the following extractions.

First Extraction (separation of zinc and other dithizone complex-forming metals from iron, aluminum, calcium, and other substance). Pipet 40 ml. of

solution A and 10 ml. of dithizone reagent into the separatory funnel and shake vigorously for 2 minutes. The aqueous layer should now be yellow-orange, indicating excess of dithizone. Allow the carbon tetrachloride layer to separate and run this phase into a second separatory funnel, being careful not to let the aqueous layer enter the bore of the stopcock. Wash down with three 2-ml. portions of carbon tetrachloride, withdrawing each washing into the second funnel. Then add 5 ml. of carbon tetrachloride and shake for 0.5 minute. The carbon tetrachloride layer should now be pure green. Run this layer into the second funnel and flush with a small volume of carbon tetrachloride as before. Discard the contents of the first funnel.

Second Extraction (separation of zinc from other dithizone complex-forming metals). Pipet 40 ml. of 0.02*N* hydrochloric acid into the second funnel, shake vigorously for 2 minutes, allow layers to separate, and run off the carbon tetrachloride layer (keep for reclaiming). The drops on the surface and the remaining colored carbon tetrachloride are flushed out as before. Add 5 ml. of carbon tetrachloride, shake for about 15 seconds, and run out. This removes any impure carbon tetrachloride which may stick to the sides of the funnel.

Final Extraction (to recomplex the zinc for photometric measurement). Pipet 40 ml. of Solution C and 10 ml. of dithizone reagent into the separatory funnel. Shake vigorously for 2 minutes, allow to separate, and run the organic phase into a 50-ml. volumetric flask. Flush out with three 2-ml. portions of carbon tetrachloride to remove all of the colored phase. Make up to the mark with carbon tetrachloride and measure the transmittance of the solution at 520 $m\mu$.

Calibration Curve. Treat standard solutions containing 0 to 25 γ of zinc exactly as above. Plot a calibration curve of micrograms of zinc *vs.* per cent transmittance.

Procedure for Soils

Soil solutions for total zinc were prepared from 100-mesh samples according to the hydrofluoric-perchloric evaporation technique of Sherman and McHargue (15). Soil extracts for acid-soluble zinc were prepared from 1-mm. samples according to the 0.1*N* hydrochloric acid-extraction procedure of Wear and Sommer (23).

An aliquot of the soil solution containing 5 to 25 γ of zinc is treated as above.

Experimental Results

Influence of pH on Extraction of Zinc. Cowling and Miller (2) reported that zinc could be quantitatively extracted as dithizonate from aqueous solutions at a pH of between 8 and 9,

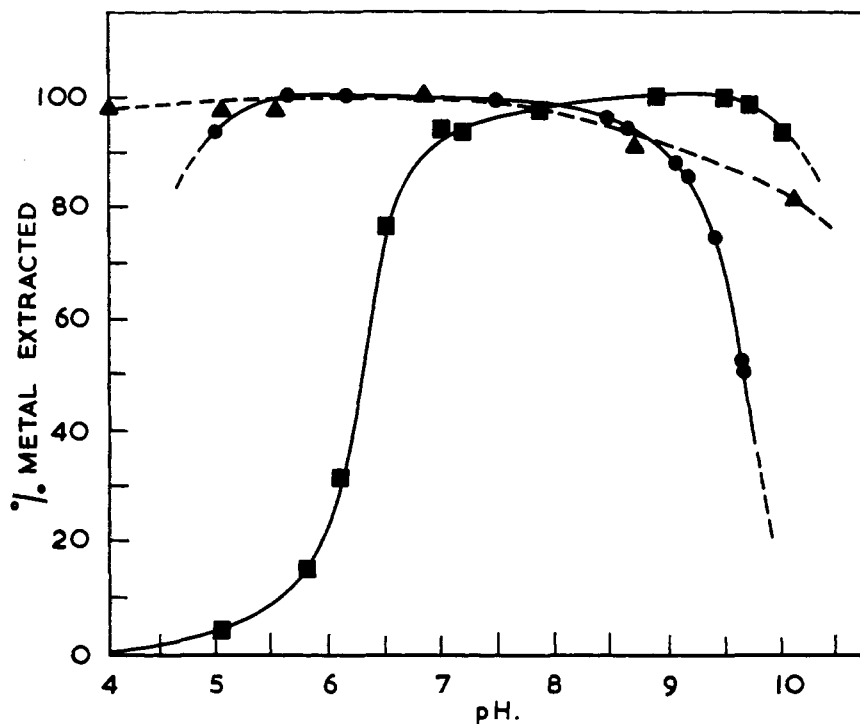


Figure 1. pH-Extraction relationships

- Extraction of Zn with dithizone in CCl₄
- Extraction of Zn with carbamate and CCl₄
- ▲ Extraction of Cu with dithizone in CCl₄

and, also, that in the presence of carbamate, unless extreme care was taken to keep the conditions absolutely standard throughout, variable proportions of the zinc were extracted. Cholak, Hubbard, and Burkey (7) reported 100% extraction of zinc dithizonate from ammonium citrate solutions at pH 8.3, with a rapid decrease in extraction efficiency above this pH. On the other hand, Walkley (22) and Hibbard (6)

reported complete extraction of zinc dithizonate from similar solutions between pH 7 to 10.

Because of these anomalies, a full investigation was carried out of the pH-extraction relationships of zinc-dithizone-carbon tetrachloride, of copper-dithizone-carbon tetrachloride, and of zinc carbamate. The results are shown in Figure 1.

These curves showed that the extrac-

tion of zinc dithizonate from ammonium citrate solution approached a maximum between pH 9 and 9.5, the amount of copper dithizonate extracted decreased appreciably above pH 9, and the extraction of zinc by carbamate fell off rapidly above pH 9.

On the basis of these results, all zinc dithizone-carbon tetrachloride extractions were carried out at a pH of between 9 to 9.5 rather than 8.5 to 9.0 as recommended by other workers. This modification ensured complete recovery of the zinc and minimized the danger of possible interference from copper. Moreover, Walkley (22) reported that the adsorption of zinc by suspended silica was appreciable at pH 8 but negligible at pH 9.5.

Transmittance Curves. Shirley and coworkers (17) published transmittance curves for dithizone and zinc dithizonate in carbon tetrachloride and, as a result of this work, recommended that photometric measurements of zinc dithizonate in carbon tetrachloride should be carried out at a wave length of between 530 and 540 m μ .

The results of a similar study carried out by the present authors are shown in Figure 2.

The curve for zinc dithizonate shows that maximum adsorption took place at about 535 m μ . For mixed-color methods, it is usually recommended that measurements be made at the wave length of maximum separation between the transmittance curves of pure reagent in solvent and metal complex in solvent. However, in the extraction procedure as described above, not all the excess of dithizone remains in the carbon tetrachloride phase—a proportion of it entering the ammoniacal aqueous phase. Thus this may not be regarded as a true mixed-color method in which the concentration of excess reagent is inversely proportional to the metal being determined. It is, therefore, more important to select a wave length at which absorption due to unchanged reagent is negligibly small.

In order to assist in the selection of the most suitable wave length at which measurements should be made, Table I was drawn up.

A wave length of either 515 or 520 m μ would be the most suitable (Table I). Although the separation between the curves was not as great at 520 as at 515 m μ , 520 m μ was finally selected because the difference is only slight while the sensitivity towards zinc dithizonate was greater.

All measurements were made at a wave length of 520 rather than at 535 m μ , as recommended by previous workers. An examination of the statistics in Table III showed that the authors were justified in making this modification.

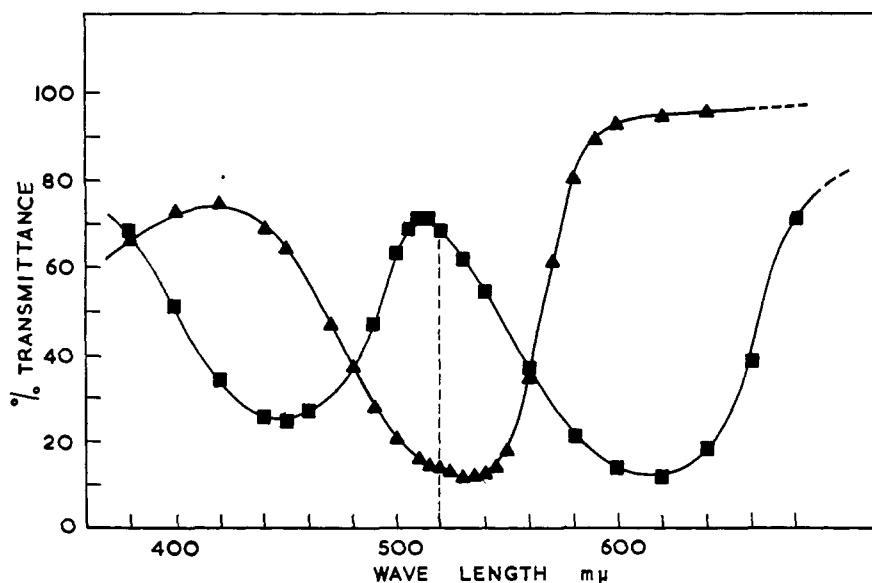


Figure 2. Transmittance curves

- ▲ Transmittance curve for zinc dithizonate in CCl₄
- Transmittance curve for dithizone in CCl₄

Table I. Quantitative Examination of Transmittance Curves

Wave Length, $m\mu$	Transmittance of Dithizone (T), %	Transmittance of Zn Complex (T), %	Separation ($T' - T$), %	Loss in Sensitivity ($T - T \text{ Min.}^a$), %
505	68.5	17.5	51.0	5.7
510	71.0	15.9	55.1	4.1
515	70.8	14.2	56.6	2.4
520	68.2	13.3	54.9	1.5
525	65.0	12.6	52.4	0.8
530	61.9	12.0	49.9	0.2
535	57.5	11.8	45.7	0.0

^a T min. equals transmittance of Zn complex at 535 $m\mu$.

Technique. In the Cowling and Miller method, zinc was first extracted from the sample solution by shaking for 0.5 minute with 10 ml. of dithizone reagent, followed by repeated 0.5-minute extractions with 5-ml. portions of carbon tetrachloride until the organic phase had a pure green color. The present authors found, however, that by shaking for 2 minutes after addition of the dithizone, complete extraction of up to 25 γ of zinc could be obtained, and that the first extraction thereafter with carbon tetrachloride gave a pure green solution. Equilibrium had not been reached after shaking for only 0.5 minute, several additional extractions being necessary. For the sake of uniformity and to ensure complete equilibrium, the shaking time for all three extraction stages was increased to 2 minutes. By increasing the shaking time in this way, the total time for the analysis, as well as the total volume of carbon tetrachloride consumed, was actually reduced.

Instead of making a 5-ml. aliquot of the final extract to 25 ml. before photometric measurement, as described by Cowling and Miller, the authors decided that it would be safer and less time-consuming to transfer the complete carbon tetrachloride phase at the end of the final extraction to a 50-ml. volumetric flask. By the washing procedure as described, the complete colored phase, including the drop on the surface, could readily be transferred. This procedure was found to be more rapid and precise.

Previous workers (17) recommended the use of amber-glass separatory funnels and volumetric flasks in order that the colored extract should not deteriorate during extraction and before measurements can be carried out. The present authors carried out tests on the stability of the zinc dithizonate-carbon tetrachloride zinc. No deterioration could be detected in solutions which were left standing for 48 hours in the diffused light normally present in a laboratory. Direct sunlight, however, affected the stability of the colored solutions almost immediately. In view of these observations, no special pre-

cautions were taken to exclude light, though it is advisable to make the photometric measurements as soon as possible after completing the extraction. The dithizone reagent will keep satisfactorily for several months, if stored in a dark bottle in a refrigerator.

For the accurate delivery of 10 ml. of dithizone reagent, an all-glass automatic pipet was used. Besides obviating contact with the toxic solvent, the use of this pipet introduced a precise and rapid means of making exact additions to each separatory funnel.

Applicability of Modified Method.

Study of Polarographic Method

MOST OF THE METHODS described in the literature for the polarographic determination of zinc in plants and soils are based on the procedure originally developed by Stout, Levy, and Williams (20), which makes use of the separation of the zinc, and other minor constituents, from the major constituents of the sample by means of an extraction with dithizone in chloroform. This extraction is usually followed by a transfer to an acid solution and subsequent electrolysis in the presence of a suitable electrolyte. Of this type are the procedures of Walkley (22), Takazawa and Sherman (27), Menzel and Jackson (17), Jones (9), Martin (10), and Cholak, Hubbard, and Burkey (7), although authors of the last two references recommend the use of di-2-naphthylthiocarbazon instead of dithizone. As opposed to these, Reed and Cummings (12) remove the iron and aluminum by precipitation with ammonia at pH 4.5.

Although the supporting electrolyte generally used in all these procedures was a solution of potassium thiocyanate buffered to a pH of 4.6, other electrolytes such as ammonia (17) and biphthalate (9) have also been recommended.

These methods have a common disadvantage in that all necessitate the carrying out of a preliminary separation

The photometric method was subjected to rigorous tests on a routine basis. Two laboratory assistants carried out total zinc determinations in triplicate on 30 different soil types and on 95 different leaf samples, taken mainly from citrus and pineapple plants from various regions. In all cases the results were accurate and precise. A feature of these tests was the ease and rapidity with which the assistants mastered the seemingly complex technique of the procedure.

The analyses were carried out most conveniently in batches of six. The most time-consuming factor in the procedure was the shaking period. Unsatisfactory results were obtained by shaking the separatory funnels mechanically, complete extraction of zinc being obtained only when the funnels were shaken vigorously by hand. By putting two assistants on the shaking operation, much of the tediousness was removed while the speed of the analysis was greatly increased. It was found that 24 determinations for zinc could be carried out comfortably in a normal working day. More than 100 leaf samples could be analyzed for zinc in a 5-day week by taking 24 samples into solution every day.

before the actual polarographic determination can be performed. This not only represents a considerable waste of time but is also a possible source of error. A procedure was therefore sought which would eliminate the need for such a separation by making use of complexing agents.

A previous investigation by Eve and Verdier (3) showed that fluoride ions are efficient complexing agents for ferric ions, this being in accordance with the findings of West and Dean (24) and West, Dean, and Breda (25). As indicated above, thiocyanate solutions are particularly well suited to the determination of zinc in the presence of copper, lead, cadmium, nickel, and cobalt (20). A supporting electrolyte containing both these substances was therefore tried and has proved to be a most suitable medium for the direct determination of zinc in plants and soils.

Apparatus

The instrument used was a Leybold Nachfolger polarograph which had been slightly modified to ensure better moving contacts. As it was necessary to use high galvanometer sensitivities in this investigation, a compensator for the charging current was constructed and adapted to the instrument (8).

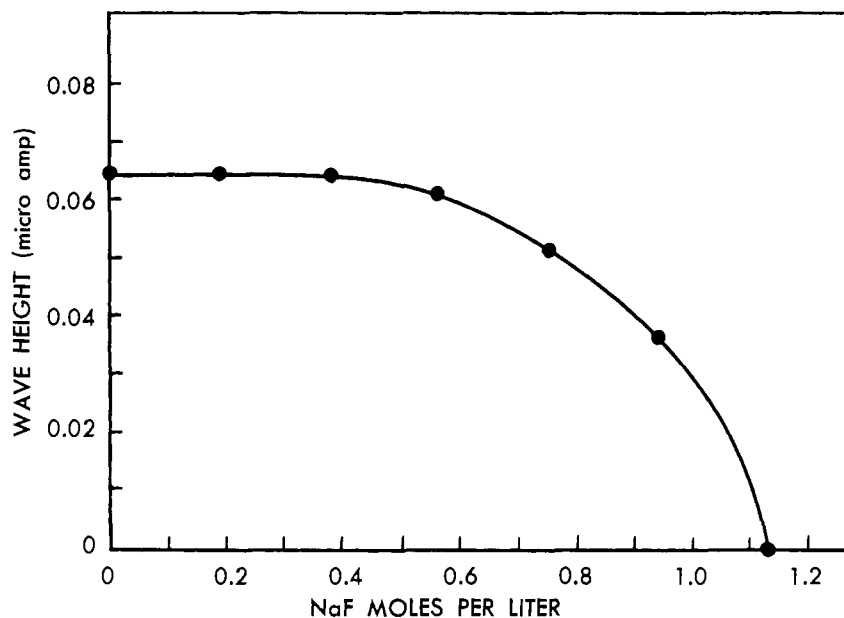


Figure 3. Influence of fluoride concentration on zinc waves

The cells were of internal-pool anode type. When determining half-wave potentials, the potential of the anode was measured against a saturated calomel electrode before and after recording a polarogram. The electrolysis cells were immersed in a thermostat and all readings were taken at $25^{\circ} \pm 0.1^{\circ}$ C. A galvanometer with a sensitivity of 2×10^{-9} ampere per millimeter was used. The drop rate was 3.0 seconds and a mass of 3.0 mg. flowing per second into distilled water on an open circuit at a mercury height of 50.0 cm.

Reagents

Great care was taken in the preparation of solutions and only high-grade reagents were used. Polyethylene bottles were extensively used and proved to be satisfactory for storage. No effect of the type reported by Gatos (4), who found that storage in these bottles resulted in a depression of the polarographic maxima and waves, was noticed in this investigation. The following additional solutions were used:

Potassium thiocyanate, 15%. Prepared from analytical reagent grade potassium thiocyanate and zinc-free water.

Bromothymol blue, 0.1%. Dissolved in the minimum quantity of hot absolute alcohol and then diluted to volume with zinc-free water.

Agar-Agar solution, 0.2%. Dissolved in warm zinc-free water.

Standard zinc solution. As described above.

Procedure

Into a 25-ml. measuring flask, pipet out an aliquot of the plant or soil solu-

tion, prepared as described in Procedure for Plant Material, and containing 10 to 100 γ of zinc. If this solution is very acid, add ammonia to raise the pH to approximately 2. By means of a weighing funnel, add 0.40 gram of sodium fluoride and mix the contents of the flask well. At this stage, the yellow color due to the ferric ions disappears and a fine white precipitate separates out at the same time. Now add 1 ml. of 15% potassium thiocyanate solution, one drop of 0.1% bromothymol blue, and redistilled ammonia dropwise, with shaking, until the solution shows a faint, permanent, blue-green coloration (pH 6 to 7).

Finally add 0.2 ml. of 0.2% agar solution as a maximum suppressor, make up to the mark, and mix thoroughly. Transfer to an electrolysis vessel, remove the oxygen, and electrolyze the solution between 0.8 and 1.2 volts. Under these conditions, zinc gives a well defined wave at -1.03 volts *vs.* the saturated calomel electrode, which is suitable for quantitative determinations.

Experimental Results

Fluoride Concentration. In view of the conflicting statements concerning the formation of a ferric wave in fluoride medium (3, 18, 19, 24, 25) experiments were carried out to see under what conditions complexing was achieved. On the addition of a slightly acid ferric chloride solution to a solution 1M in sodium fluoride and 0.05M in potassium thiocyanate, no wave was obtained for the iron, though zinc, under the same conditions, gave a perfect wave. The formation of the white crystalline pre-

cipitate, mentioned earlier, did not appear to interfere in any way with the determination.

Because subsequent experiments showed that an increase in the fluoride concentration caused a suppression of the cobalt wave formed under the same conditions, its effect on the zinc wave was systematically studied. Figure 3 shows the variation of the height of the zinc wave with the fluoride concentration. These results show that the concentration of the fluoride should not be greater than 0.4M (0.4 gram in 25 ml.).

Influence of pH. In agreement with the results obtained by Shoemaker (18) it was found essential that the fluoride should not be added to ferric solutions which had a pH greater than 2. If the pH is greater, or very much lower than this value, complete complexing of the ferric ions does not take place and subsequent acidification or neutralization fails to bring about the desired effect. It was further found necessary to neutralize the resulting solution immediately after the addition of the fluoride for the following two reasons.

If the solution remains acid (pH less than 5), a rapidly growing wave is formed at about -0.5 volt *vs.* the saturated calomel electrode. This wave occurs even in the absence of iron. It disappears on shaking the electrolysis cell but reappears later.

If the pH is not brought to within a value of 6.0 to 7, another wave starting at about -1.3 volts is formed, which tends to interfere with the determination of zinc. It was assumed, in accordance with the findings of von Stackelberg and von Freyhold (19), that this wave was due to the iron in solution and no attempt was made to verify this. In a recent publication, Rulfs and Stoner (13), on the contrary, consider that the wave at -0.49 volt is due to the iron but the one at -1.36 volts is not. These apparently conflicting views reveal the need for a further complete investigation of the deposition of iron from fluoride solutions.

Thiocyanate Concentration. A study of the shape and separation of the nickel, zinc, cobalt, and manganese waves in thiocyanate-fluoride medium, showed that best results were obtained when the thiocyanate concentration was 0.062M. At this concentration, zinc, nickel, and manganese give well defined waves which are all suitable for the quantitative determination of these elements. The cobalt wave, however, is not well developed and tends to show a type of "prewave" with the consequent drawing out of the whole wave.

Maximum Suppressor and pH Indicator. The use of gelatin was found to shift the cobalt wave by approximately 0.05 volt towards more positive potentials, thus diminishing the separation

between the zinc and cobalt waves. Other ordinary suppressors, which are reduced at the dropping mercury cathode are not suitable because of the interference they caused at the high galvanometer sensitivities used. Eventually, agar-agar was found to be satisfactory. Bromothymol blue, which is added to the solution to serve as an indicator, was found to be reduced also at about -0.85 volt but at the concentrations required, even at the maximum galvanometer sensitivity, the wave produced was negligible. Measurements carried out with a pH meter showed that this indicator was suitable for use in these solutions as, in all cases, they had a pH between 6 and 7 when adjusted to the correct color.

Applicability of Procedure. Values of the half-wave potentials in sodium fluoride and in potassium thiocyanate are given for comparison (Table II). The above values for potassium thiocyanate alone refer to unbuffered solutions of this substance. Stout and associates (20) found that better separation is achieved in solutions buffered to a pH between 4 and 5, but gave no actual numerical values for the half waves. Moreover, these values show that with the above mixed indifferent electrolyte, good separation between the zinc and cobalt waves is achieved while the iron, which is usually present in much larger quantities than the other metals, will not interfere with the determination of any of the elements mentioned in Table II.

Other elements such as aluminum and molybdenum do not give waves in fluoride solutions, while calcium, magnesium, sodium, and potassium are reduced only at more negative values and therefore would not interfere.

In order to test the accuracy of the method for the determination of zinc under conditions somewhat similar to those likely to be encountered in plant and soil extracts, the following experiment was carried out.

A series of standards containing between 20 and 120 γ of zinc was analyzed polarographically: first, in the absence of any foreign ions, second, in the presence of 0.08 gram of iron, and third, in the presence of 0.08 gram of iron and 120 γ of copper, 60 γ of cobalt, 80 γ of nickel, and 2 mg. of manganese. This final solution was equivalent to a solution from 2 grams of a soil containing 1000 p.p.m. of manganese, 10 to 60 p.p.m. of zinc, 60 p.p.m. of copper, 30 p.p.m. of cobalt, and 40 p.p.m. of nickel, but corresponds to a larger excess of iron than would normally be met with in practice. The results obtained showed a straight-line relationship between the concentration of the zinc ions and their diffusion current, and further, that the above ions do not interfere with the determination of zinc even under con-

Table II. Half-Wave Potentials of Trace Elements in Sodium Fluoride and Potassium Thiocyanate

Trace Elements	0.5M NaF (25)		0.4M NaF and 0.062M KCNS, V. at pH 6-7	1M KCNS (26) V.
	V.	pH		
Cu	-0.003	5.1	-0.0	-0.0
	One wave only		-0.45	
Ni	-1.12	4.3-6.4	-0.68	-0.70
Zn	-1.14	4.2-6.4	-1.03	-1.06
Mn	-1.55	2.4-6.8	-1.55	-1.55
Co	-1.38	2.9-6.0	-1.3	-1.03

ditions where their concentration is relatively larger than normally is to be expected.

The method could further lend itself to the simultaneous determination of copper, nickel, manganese, and even possibly cobalt, though the presence of phosphate might interfere with the determination of manganese.

Statistical Comparison of the Two Methods

The accuracy and precision of the two methods were tested by comparing the results obtained on a soil solution, prepared by an exhaustive perchloric acid extraction technique. This ensured the presence of a large excess of foreign ions. The photometric measurements were carried out on three different instruments—a Hilger Spekker Absorptiometer fitted with a green filter (500 to 550 $m\mu$), a Cenco-Sheard Spectrophotometer set at first at 535 $m\mu$ and then at 520 $m\mu$, and a Beckman Model DU spectrophotometer set at 520 $m\mu$. The results are shown in Table III.

Discussion

Both the modified photometric method and the new polarographic method yielded accurate results, as there was no significant difference between the mean zinc values obtained by these two totally independent methods. The precision of the photometric procedure, with a standard deviation of only 0.52

p.p.m. in 30 p.p.m. of zinc, was very good. The difference in precision obtained on the Cenco when set at the two different wave lengths, justified the decision to carry out the measurements at 520 rather than at 535 $m\mu$, as recommended by other workers. The increased precision obtained on the Beckman was partly due to the fact that it was possible to use an effective band width of only 1 $m\mu$ on this instrument. However, reasonably accurate and precise results can be obtained by the use of cheaper instruments like the Cenco and Spekker.

Although the accuracy of the results given by the polarographic method is much less than that of the photometric procedure, it has several advantages. The method is shorter and less tedious, fewer reagents are needed, and it could be used for the simultaneous determination of other metals present in the material analyzed.

Acknowledgment

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Table III. Statistical Comparison of Methods on a Standard Soil Solution

Factor	Photometer				Polarograph
	Spekker Green filter 500-550 $m\mu$	Cenco 535 $m\mu$	Cenco 520 $m\mu$ P.p.M.	Beckman 520 $m\mu$	
Mean Zn (30 repl.)	30.7	30.8	30.9	31.0	31.5
Standard deviation	0.73	0.67	0.59	0.52	1.26
Stand. deviation, %	2.38	2.18	1.91	1.68	4.00
Standard error				0.095	0.222
Standard error of difference between means					0.70
Difference between means (not significant)					0.5

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YEAST ANALYSIS

Spectrophotometric Semimicrodetermination of Ergosterol in Yeast

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In a method for spectrophotometric determination of ergosterol in yeast samples containing 5 to 100 mg. of yeast solids, the yeast is digested with alcoholic alkali and ergosterol is extracted in a single extraction with a measured volume of pure *n*-heptane. Absorbances at 281.5 and 230 $m\mu$ are determined on an aliquot of the heptane diluted in absolute ethyl alcohol. Amount of "ergosterols" are determined from the 281.5- $m\mu$ measurement, and amount of 24(28)-dehydroergosterol is determined from the 230- $m\mu$ measurement. The difference in these values gives the ergosterol content.

A RAPID METHOD OF ERGOSTEROL DETERMINATION, applicable to small samples, was needed in a study of the formation of ergosterol by yeast cultures under a variety of propagational conditions. It was desirable that the method give results corresponding to the amount of purified ergosterol that could be isolated from the yeast.

Methods for the determination of ergosterol in yeast (7, 2, 5-10, 12, 17) usually employ saponification of the yeast with strong aqueous or alcoholic alkali, extraction of the unsaponifiable matter with an immiscible solvent, and measurement of ergosterol by colorimetric or spectrophotometric means, either with or without digitonin precipitation.

In this laboratory the method of Castille and Ruppel (5), except for use of stronger alkali in the saponification and omission of the digitonin precipitation,

was used as a reference method. It required 2 to 5 grams of compressed yeast, and an operator could analyze 6 to 12 samples per day. In attempts to simplify the method, it was found that *n*-heptane, which could be obtained in a spectroscopically pure grade, extracted the yeast sterols from the yeast digested with alcoholic alkali cleanly and quantitatively in a single extraction. Dilution of the *n*-heptane with absolute ethyl alcohol and determination of absorbances at 281.5 and 230 $m\mu$ gave values closely agreeing with the reference method. A determination could be made on as little as 5 mg. of yeast solids, and an operator could carry out 24 or more analyses per day.

Ergosterol is generally considered to be the primary sterol of yeast and is by far the most readily isolated. A number of other yeast sterols which have been reported are zymosterol (17), " α -dihydroergosterol" (4), since shown to be 5-dihydroergosterol (13), ascosterol (15), fecosterol (15), episterol (14), hyposterol (14), and 24(28)-dehydroergosterol (3). Under the usual conditions

of yeast growth, such as used in baker's yeast manufacture, the sterol produced is predominantly ergosterol (about 80%). Except for 24(28)-dehydroergosterol, the other sterols can be considered to be minor constituents. It has been found in this laboratory that, although there are variations with yeast strains, any of a wide variety of yeast strains is capable of making as much 24(28)-dehydroergosterol as ergosterol.

Because of the complexity of the yeast sterols, colorimetric methods of measurement cannot be expected to be satisfactory for determination of ergosterol content. Work in this laboratory on the fractionation of yeast unsaponifiable matter showed nearly all the spectral absorption in the 220- to 300- $m\mu$ region to be due to ergosterol and 24(28)-dehydroergosterol. As the latter had an *E* (1%, 1 cm.) value at 281.5 $m\mu$ almost equal to that of ergosterol, and showed an intense spectral absorption band at 230 $m\mu$ where that of ergosterol was low, it was possible to calculate percentage of "ergosterols" from the 281.5- $m\mu$ measurement, and

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