Rapid Direct Polarographic Determination of Zinc in Plant Ash Solutions

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In a rapid, direct polarographic method for the determination of zinc in plant tissue a sample of the dried, ground leaf tissue is dry-ashed. The ash is digested in dilute hydrochloric acid and the solution is made to volume. An aliquot of this solution is mixed with a highly ammoniacal solution of potassium chloride, sodium sulfite, and gelatin. The polarogram is recorded from this solution, the half-wave potential of zinc being —1.402 volts *vs.* S. C. E. at 35° C. The method is satisfactory for the determination of zinc in concentrations between 0.003 and 0.16 mM. There is no interference from manganese(II), nickel(II), cobalt(II), cadmium(II), chromium(III), aluminum(III), or iron(III) in concentrations likely to occur in plant tissue.

Progress in research on zinc in plant nutrition has been handicapped by the difficulties involved in its determination. Several methods of using the polarograph as an aid in analyses have been proposed in recent years (*1*, *6*, *10*, *11*, *13*, *14*). All these methods, however, require preliminary chemical separations which make the methods somewhat objectionable for routine work when large numbers of plant samples are to be analyzed for zinc. The recent method of Hinsvark and others (*5*), though requiring fewer operations than the earlier procedures, still requires time-consuming evaporation and filtration prior to recording the polarogram. The proposed method is a direct polarographic determination on the ash solution without any prior chemical separation. It was developed primarily for determining zinc in tung leaves but has been used successfully with leaf tissue of other plants, such as citrus, apple, cherry, and peach leaves.

Apparatus

The polarograph employed in the development of the procedure was a Sargent-Heyrovský pen-recording Model XXI. Sargent Models III and XII were also satisfactory for routine work. A conventional dropping mercury electrode with a constant of 3.878 $mg.^{2/3}$ $sec.^{-1/2}$ was used, along with a stationary pool of mercury in the bottom of the electrolysis cell which served as an anode. The electrolysis cell and contents were maintained at 35° C. by immersion in a constant-temperature water bath.

All glassware used was Pyrex brand and was thoroughly cleaned by washing with hot 1 to 1 nitric acid, followed by four rinsings with tap water and two with deionized water. Ground-glass stoppers were used for all flasks because of likelihood of contamination from rubber stoppers.

Reagents

All chemicals used in this work were C. P. grade and no further purification was necessary. These included 1*N* hydrochloric acid, potassium chloride, gelatin, anhydrous sodium sulfite, ammonium hydroxide, and 1 to 1 nitric acid.

Zinc-free water was obtained by passing tap water through a column filled with equal volumes of Amberlite IRA-410 and IR-120. The resistance of the effluent, as measured by a Barnstead purity meter, was near 10^6 ohms. All solutions were prepared with deionized water.

Standard zinc solution, 1 mg. of zinc per ml., was prepared by placing 0.25 gram of pure zinc in a 250-ml. volumetric flask. About 50 ml. of water and 1 ml. of sulfuric acid were added and the solution was heated on a steam bath until all the zinc was dissolved. The solution was then diluted to 250 ml. with water.

To prepare a solution containing 10γ of zinc per ml., a 10-ml. aliquot of the stock solution was diluted to 1 liter with 0.2*N* hydrochloric acid.

The electrolyte solution was prepared by dissolving 2.6 grams of gelatin in approximately 100 ml. of hot water. When cool, the solution was diluted to about 400 ml. Then, 25 grams of potassium chloride and 13 grams of sodium sulfite were dissolved in the gelatin solution. The solution was transferred to a 1-liter volumetric flask, 500 ml. of ammonium hydroxide was added, the flask was brought to volume with water, and the solution was mixed.

Procedure

Ashing. Ash 2.0000 grams of ground leaf tissue in a platinum dish, for approximately 6 hours at 450° to 500° C. Digest the ash with about 25 ml. of 1*N* hydrochloric acid at a temperature just below the boiling point. This usually requires about an hour, but digestion of some samples may take longer. When the digestion is complete, cool the solution, transfer to a 50-ml. volumetric flask,

filter if desirable, and make to volume.

Determination. Transfer to a 25-ml. volumetric flask an aliquot of the ash solution containing 0.01 to 0.12 mg. of zinc. If more than a 10-ml. aliquot is required, ash a larger sample. Bring the flask to volume with the electrolyte solution. Stopper and shake the flask and let it stand for about 15 minutes. Longer standing is not necessary, but does no harm. Shake the flask thoroughly immediately before transferring a portion of the solution to the polarographic cell. Immerse the polarographic cell in a constant-temperature water bath maintained at 35° C. and allow it to reach equilibrium. Since the solution contains sulfite ions, further oxygen removal is unnecessary. Select a sensitivity that will give a curve height of 10 to 60 mm. and, using a dropping mercury cathode, record the polarogram between −1.2 and −1.6 volts applied. The half-wave potential of zinc occurs at −1.402 *vs.* the saturated calomel electrode. Measure the diffusion current through the half-wave but use the peak currents instead of average currents as suggested by Schulman (*12*). Calculate the concentration of zinc from the diffusion currents of known concentrations of zinc.

Calibration Curve. For preparation of the calibration curve, eight composite samples of tung leaves, ranging from 0.078 to 1.22 meq. per 100 grams of dry leaf tissue (25.2 to 394 p.p.m.), were analyzed for zinc by the standard method of Cowling and Miller (*3*). The average of six determinations for each sample was used to calculate the zinc content. Each sample was run polarographically 8 to 10 times and the average diffusion current calculated. A curve was then constructed by plotting diffusion current against concentration. The curve obtained in this manner was checked for accuracy by adding known increasing amounts of the zinc standards to the ash solutions. The concentration of zinc in these samples was determined from the polarographic calibration curve. The results were well within experimental error. It is felt that this method of calibration is more accurate than one employing only standard zinc solutions, because one standard is checked against another.

If zinc salts are to be used for the standards, they should be prepared in 0.2*N* hydrochloric acid.

Accuracy and Precision

As a test of the precision of the method, six of the standard samples were run polarographically, 10 to 13 times each. The diffusion current quotients, $i_{d/c}$, and the standard deviation of the individual determinations for each sample were calculated. The data in Table I show that the precision of the proposed procedure is well within the accepted standards for routine determination of zinc in plant material.

Table I. Precision of Method

Concn. of Zinc, mM	Diffusion Current Quotients, $i_{d/c}$[a]	Standard Deviations of $i_{d/c}$
0.00615	11.05	1.278
0.00784	13.00	1.111
0.02599	11.46	1.127
0.03375	12.95	0.8711
0.04245	13.00	0.9116
0.08167	14.89	0.7554

[a] Mean of 10 to 13 determinations.

The accuracy of the method was checked as follows. Three sets of seven solutions with known increasing amounts of added zinc were prepared. The first set, A, contained only the zinc that was added. In the second set, B, the zinc was added to an aliquot of one of the standard leaf-ash solutions which was 0.00493 m*M* with respect to zinc. In the third set, C, zinc was added to an aliquot of a standard leaf-ash solution which was 0.04134 m*M* with respect to zinc. Zinc in each solution was determined polarographically (Table II). The average from all 21 samples gives 101.18% recovery with a standard deviation of 6.98. This is well within experimental error.

Although there are several sources of errors in recording the polarograms, two of the most important precautions necessary to avoid major difficulties are: maintenance of an atmosphere of low humidity necessary for optimum performance of the polarograph, and acid cleaning of the outside as well as the inside of the polarographic cells when a water bath is to be used. Otherwise, the water bath apparently acts as a large condenser, which distorts the polarographic waves.

Table II. Recovery of Zinc Added to Sample

Concn. of Zinc Added, mM	% of Total Zinc Recovered		
	A	B	C
0.00302	118.54	97.99	98.74
0.00612	103.27	95.66	97.83
0.01224	100.00	98.14	94.36
0.01836	102.61	94.89	98.41
0.03021	98.94	93.88	103.00
0.06122	97.14	96.40	109.37
0.12243	115.65	104.99	104.90
Average	105.16	97.42	101.02
Standard deviation	7.40	3.68	5.12

A. Added to blank solution.
B. Added to sample of leaf ash solution which had zinc concentration of 0.00493 m*M*.
C. Added to sample of leaf ash solution which had zinc concentration of 0.04134 m*M*.

Discussion

Electrolyte. Several electrolytes suggested by others (*7*, *9*, *11*, *15*) were

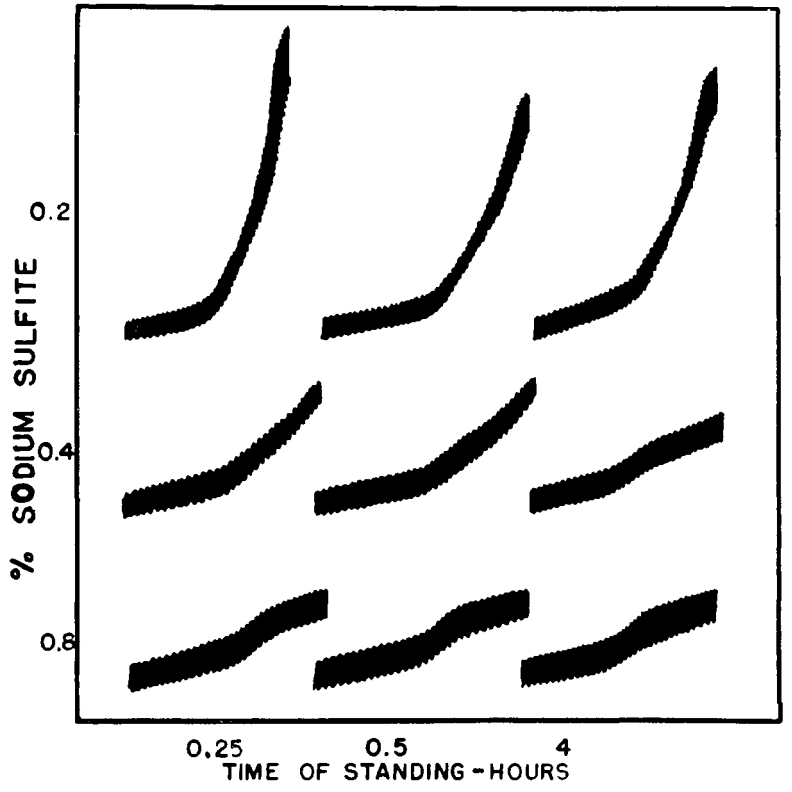

Figure 1. Effect of sodium sulfite concentration and length of time of standing on polarographic wave. Zinc concentration 0.007844 mM

tried but for various reasons were unsatisfactory for a direct method. The electrolyte used by Halbrook (*4*) for determination of zinc in fertilizer appeared most promising. However, several points required further investigation.

Halbrook (*4*) reported that a small lump of sodium sulfite was added to each sample for oxygen removal. As this statement seemed to indicate that the concentration of sulfite was not critical, after the first few runs, the sulfite added was not weighed, but the amount was approximated. Shortly afterward, it was found that the curves and the results could not be duplicated. When the estimated amounts of sodium sulfite were weighed, they were considerably different from the estimated weight of the original quantity. As a result, a test was run to determine the amount of sodium sulfite and time of standing required at two levels of zinc, 0.007844 and 0.09674 m*M*. Three concentrations of sodium sulfite—0.2, 0.4, and 0.8%—were used.

It was found that the concentration of sodium sulfite was critical at the low but not at the high concentration of zinc. When the high level of sodium sulfite was used at the low level of zinc, the curves were much easier to measure than when low levels of sulfite were used (Figure 1). Another advantage of the high concentration of sulfite was the increase in curve height at the low concentration of zinc. This permitted the determination of smaller concentrations of zinc with greater accuracy. For the times tested—0.25, 0.5, and 4 hours—the length of time of standing was not important.

In the first runs, the constituents of the electrolyte were added individually, as is customary. However, considerable time is required for the preparation of samples by this method. If one solution could be added to the aliquot of the leaf-ash solution, the time for preparation could be shortened considerably. Such a solution, in which the final concentrations of the ingredients were equal to the concentrations of those ingredients when added individually, was prepared and tested (Table III). When the ingredients were mixed and added as one solution, there was less deviation than when the constituents were added individually. The diffusion current quotients were significantly smaller when the single solution was added than when the ingredients were added individually, but no explanation is offered for this. The addition of the electrolyte as one solution was adopted because of the convenience and greater precision.

Table III. Effect of Method of Adding Electrolyte Constituents on Diffusion Current Quotients

Concn. of Zinc, mM	Diffusion Current Quotients ($i_{d/c}$), Constituents Added: Individually	In one solution
0.00784	15.30	8.53
0.00859	16.42	12.81
0.01542	11.41	11.54
0.01741	13.32	11.37
0.02015	13.80	10.27
0.02599	12.81	10.16
0.03375	13.54	12.21
0.03451	13.27	11.91
0.03824	20.66	11.85
0.04094	12.75	11.63
0.04245	13.78	12.53
0.04818	12.18	12.52
0.05613	12.28	12.29
0.08167	14.77	13.57
Average	14.02	11.66
Standard deviation	2.32	1.28

t found, 3.57; *t* required at 0.01, 3.01.
Electrolyte is 0.1*N* potassium chloride, 6*N* ammonia, and contains 0.78% sodium sulfite and 0.15% gelatin.

Table IV. Stability of Electrolyte Solution

Concn. of Zinc, mM	Diffusion Current Quotients, $i_{d/c}$: Fresh solution	Week-old solution
0.01086	12.98	13.63
0.01637	12.95	13.80
0.02000	13.55	13.20
0.01673	13.21	13.15
0.01564	12.85	13.17
0.01443	12.34	13.17
0.01290	12.25	13.49
0.01127	12.78	13.31
0.00877	13.45	12.54
0.00794	13.10	13.30
Average	12.95	13.28
Standard deviation	0.42	0.34

t found, 1.64; *t* required at 0.01, 3.25.

The maximum benefit from preparing a larger volume of electrolyte solution would be obtained only if it were stable for at least several days. To determine the effects of aging, a fresh solution of electrolyte was prepared and used to run seven samples. One week later, the same seven samples were again run with the same electrolyte solution. The data (Table IV) showed that there was no significant difference in the results obtained with fresh solution and week-old solution. Thus, a volume of solution sufficient for at least a week can be prepared.

Effect of Precipitate. In the study of the various factors affecting the accuracy and precision of the method, the effect of the precipitate formed upon the addition of the electrolyte was considered. When the solutions were transferred from the flasks to the cells, every effort was made at first to pour off only the supernatant liquid. Without filtering or centrifuging, it was impossible to prevent the transfer of small quantities of the precipitate. As it was not expedient to remove the precipitate, it was decided to mix it thoroughly with the solution before the transfer. For several determinations the flasks were shaken immediately before transfer of the solution to the cells. This resulted in a completely homogeneous mixture being added to the cell. The results of this test were outstanding.

Seven to 10 determinations were made on each of seven samples in order to compare the effect of shaking and no shaking. A standard deviation was calculated for each sample. In all cases, the standard deviations of the shaken samples were much smaller than those of samples not shaken (Table V).

Table V. Effect of Shaking Sample Prior to Transferring to Cell

Conc. of Zinc, mM	Standard deviation of $i_{d/c}$: Shaken	Not shaken
0.00615	1.1996	1.8224
0.00784	0.7522	2.5344
0.02015	0.8770	2.0543
0.02599	1.0892	2.5519
0.03375	0.8216	1.5484
0.04245	0.5866	1.4740
0.08167	0.6831	1.8551
Pooled standard deviation	0.0298	0.0758

F for difference in variability of two methods, 6.45.
F required at 0.001, 3.36.

There are two possible explanations for the greater variability in the samples not shaken. The precipitate, largely aluminum and iron hydroxides, may be "fixing" some of the zinc. This could be accomplished as direct surface adsorption, through coprecipitation, or by both. Appreciable amounts of the zincate ion are coprecipitated with cadmium hydroxide when the ratio of cadmium to zinc is large (*8*). If the zinc trapped in this precipitate was not always included in the solution that was polarized, there would be considerable variation in the results. Another explanation involves the possible effect of the hydroxide on the viscosity of the solution.

Effect of Interfering Ions. The main advantage of the proposed procedure is that interfering ions from the leaf-ash solution need not be separated prior to the recording of the polarogram. As a check on the effect of ions likely to interfere, a solution containing manganese (II), nickel(II), cobalt(II), cadmium (II), chromium(III), aluminum(III), and iron(III) was prepared. These ions were selected because they have half-wave potentials near that of zinc. Aliquots of this solution were added to leaf-ash solutions, so that the concentration of these ions was equivalent to the highest amount of each that would be likely to occur in leaf tissue. These concentra-

Table VI. Diffusion Current Quotients as Affected by Interfering Ions

Concn. of Zinc, mM	Diffusion Current Quotient ($i_{d/c}$), Solution with Interfering Ion	
	Not added	Added
0.00615	11.38	11.06
0.03375	12.00	12.32
0.04245	12.93	12.63
0.19348	11.16	11.01
Average	11.87	11.76

t found, 1.57; t required at 0.01, 5 84.

Concentration of Added Ions

Ion, mM		Equivalent P.P.M. in Leaf
Mn^{++}	1.457	5000
Ni^{++}	0.00136	5
Co^{++}	0.00136	5
Cr^{+++}	0.00154	5
Fe^{+++}	0.0573	200
Al^{+++}	2.965	5000
Cd^{++}	0.000712	5

tions (Table VI) were calculated on the basis of the sample weight used in the zinc determination. Over a wide range of zinc concentrations the presence of these added ions had no effect. The t value was 1.57 when 5.84 was needed for significance at the 1% level.

The effect of the individual ions was tested at about 10 times the maximum concentration likely to occur in leaf solution. Even at this high concentration, only cobalt and aluminum had an effect. Cobalt tended to increase the wave height, while aluminum tended to decrease it.

In a subsequent test, it was found that cobalt did not interfere if its concentration in the plant tissue was below 20 p.p.m., and aluminum, if its concentration was below 50,000 p.p.m. Plant material very rarely contains concentrations as large as these (*2*).

Although manganese did not interfere with the zinc wave, it gave a well-defined wave at −1.69 volts *vs.* S. C. E. Preliminary tests indicated that manganese and zinc could be determined in the same solution.

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MODE OF ACTION OF PESTICIDES

Mechanism of Reaction of Di-*n*-propyl-2,2-dichlorovinyl Phosphate (DDP) with Esterases

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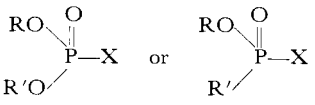
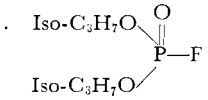
Di-*n*-propyl-2,2-dichlorovinyl phosphate (DDP) is an active antiesterase; its rate of reaction with various esterases is approximately that of diisopropyl phosphorofluoridate (DFP), tetraethyl pyrophosphate (TEPP), and isopropylmethyl phosphonofluoridate (sarin). In contrast with the latter antiesterases, DDP is very stable toward hydrolysis and does not appear to react with catechol and a hydroxamic acid. The reaction of DDP with chymotrypsin is stoichiometric and is accompanied by introduction of phosphorus into the protein. During the reaction chlorine is released in organic alkali-labile form which has been identified as dichloroacetaldehyde. The mechanism of these reactions is discussed with reference to the reactions of other irreversible antiesterases with susceptible enzymes.

ESTERASE INHIBITORS OF THE ORGANOPHOSPHORUS TYPE may be generally divided into two classes.

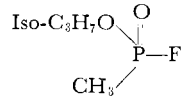
Class I with the general structure



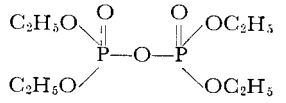
contains a linkage (P—X) which is relatively readily broken by spontaneous hydrolysis, enzymatically catalyzed hydrolysis (*24, 28*) as well as during reactions with various esterases. Representative members of this class include phosphofluoridates, alkyl pyrophosphates, and alkyl-*p*-nitrophenyl phosphates. Chemi-

1. 

Diisopropyl phosphorofluoridate, DFP

2. 

Isopropyl methyl phosphonofluoridate, sarin

3. 

Tetraethyl pyrophosphate, TEPP