Determination of Copper and Zinc in Soils or Plants

Polarographic Determination in the Same Solution

R. G. MENZEL[1] AND M. L. JACKSON, *University of Wisconsin, Madison, Wis.*

It is frequently advantageous to determine micro amounts of copper and zinc from soils and plants in the same solution; large amounts of other elements must be removed or their interference eliminated. Composition of the base electrolyte, a sodium sulfate–ammonia–gelatin solution, was worked out to achieve sufficient separation of the reduction potentials of copper and zinc for their determination in the same solution by means of the polarograph. The copper and zinc are released from plant tissue by perchloric acid digestion and from soil by hydrofluoric acid, and are separated from the other inorganic constituents by dithizone. The cobalt polarographic wave coincides with that of zinc, but the cobalt content of these materials is usually too small to affect the zinc determination significantly. The range of the method extends down to about 3 micrograms of copper and zinc, with an accuracy within ±2 to 6%. The manganese polarographic wave is satisfactorily separated from that of copper and zinc, but conditions for taking manganese through the dithizone separation were not worked out. Antimony and nickel do not interfere. The procedure is more efficacious than chemical separation of copper from zinc and dithizone determination of each.

COPPER and zinc in soils or plants are determined by a comparatively simple procedure involving extraction as the dithizone complex with final estimation in the same solution by means of a single polarogram.

Dithizone extraction effectively eliminates iron, which in soils and plant materials occurs in relatively higher concentration than copper and zinc. In most practical situations iron must be removed before polarographic analysis, as the cathodic reduction of ferric to ferrous iron takes place at a lower cathode-solution potential difference than that required for the reduction of copper or zinc (*7*). Proposed means of separating iron from other metals to be assayed polarographically are: precipitation of the iron as ferric hydroxide (*4*), separation in ion exchange columns (*3, 8*), and separation into one of the phases of two immiscible liquid solvents (*2, 5, 9, 10*). In solvent extraction methods, dithizone has been particularly helpful in separating certain heavy metals including copper and zinc from iron, manganese, calcium, magnesium, the alkali metals, and phosphates. It has been used for dry-ashed plant materials (*9*), and to separate heavy metals following wet oxidation (*10*). Extraction of copper and zinc from the other salts aids in obtaining a low residual current on the polarogram. Removal of the phosphates also permits the use of an alkaline base electrolyte consisting of ammonia and sodium sulfite, which removes oxygen from the polarographic solution.

The copper and zinc determinations involve three steps:

1. Wet-ashing of tissue with nitric and perchloric acids.
2. Separation of copper and zinc with dithizone.
3. Measurement of copper and zinc concentrations with the polarograph.

The elapsed time required for twelve determinations on plant tissue is approximately 5 hours for step 1, 3 hours for step 2, and 3 hours for step 3. Determinations in soils require evaporation of 100 ml. of solution to dryness, so that step 1 takes about 18 hours of elapsed time. However, the evaporation can be completed overnight.

APPARATUS

Apparatus and reagents used for digestion were adopted from the procedure of Jackson, Chatterjee, Whittig, and Kittrick (*6*). Besides the wet-oxidation apparatus are needed twelve 500-ml. Erlenmeyer flasks, one 1-liter separatory funnel, six 125-ml. separatory funnels, twelve 50-ml. Erlenmeyer flasks, twelve 2 × 7 cm. sample vials, a saturated calomel half-cell, a dropping mercury electrode, and a Model XI Sargent Heyrovský polarograph. For soils, a sand bath, six small platinum crucibles, and twelve 250-ml. beakers are also needed.

[1] Present address, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Md.

REAGENTS

Reagents are listed in order of use.

Commercial reagent grade chemicals were used except where otherwise specified.

Concentrated Nitric Acid.

Ternary Acid Mixture of 10 ml. of concentrated nitric acid, 1 ml. of concentrated sulfuric acid, and 4 ml. of 60% perchloric acid.

For soils concentrated sulfuric acid and 48% hydrofluoric acid are also required.

Ammonium Citrate Buffer, made by adding 50 ml. of 10% citric acid solution to 200 ml. of 4 *N* ammonium hydroxide.

Dithizone, Eastman diphenylthiocarbazone.

Distilled Carbon Tetrachloride. Technical grade or used carbon tetrachloride is purified by washing successively with 20% sulfuric acid, 20% sodium hydroxide, and distilled water. The washed product is then distilled over sodium carbonate in a glass still with ground-glass joints.

Redistilled Water. Distilled water is redistilled in borosilicate glass apparatus.

Hydrochloric Acid, 6 *N*. Constant-boiling hydrochloric acid is distilled in glass apparatus.

Ammonium Hydroxide, 4 *N*. Ammonia from a glass still is trapped in redistilled water cooled in an ice-salt bath.

Base Electrolyte Solution, 2.1 grams of sodium sulfite dissolved in 66 ml. of 0.01 *N* ammonium hydroxide, obtained by dilution of distilled ammonia with redistilled water. This gives enough base electrolyte for one set of twelve determinations. Because ammonia is volatile and sulfite is oxidizable, the solution is freshly prepared each day.

Gelatin, 0.1 gram dissolved with gentle heating in 100 ml. of redistilled water.

Dilute Hydrochloric Acid Washing Solution, about 0.5 *N*. Five hundred milliliters of concentrated hydrochloric acid are added up to 15 liters of distilled water. All glassware used in the determination is cleaned by rinsing twice in this acid, followed by two rinsings in redistilled water.

PROCEDURE

Ashing of Plant Tissue. One to 2 grams of air-dry plant tissue sample, ground in a Wiley mill equipped with a steel screen, are weighed and placed in a 500-ml. Erlenmeyer flask, 10 ml. of concentrated nitric acid are added, and digestion is begun at 100° C. After evolution of dense fumes of nitrogen dioxide, 10 ml. of ternary acid mixture are added and digestion is continued at 190° C. until sulfur trioxide fumes appear. Except for a small amount of silica, the residue in the cooled flask is dissolved by warming in 10 ml. of redistilled water.

Extraction from Soils. One half to 1 gram of air-dry soil sample, finely ground in an agate mortar, is weighed and transferred to a platinum crucible. The soil is moistened with water and 3 drops of concentrated sulfuric acid. Two successive 5-ml. portions of hydrofluoric acid are evaporated from the sample on a sand bath at 180° C. to volatilize the silica. The sample and crucible are placed in a 250-ml. beaker with 10 ml. of concentrated nitric acid and covered with redistilled water. The sample is loosened by heating the solution and washed into the beaker, with redistilled water and a rubber policeman. After evaporation to

dryness, oxidation of organic matter is completed by digestion with 10 ml. of ternary acid mixture at 200° C. No brown color from organic matter must be left if a good dithizone separation is to be obtained.

Dithizone Extraction of Copper and Zinc. Three hundred milliliters of ammonium citrate buffer, 10 ml. of redistilled carbon tetrachloride, and 0.1 gram of dithizone are shaken together vigorously for 1 minute in a 1-liter separatory funnel. The buffer solution becomes red, owing to the solubility of ammonium dithizonate in water. Copper and zinc impurities from the reagents form violet dithizonates soluble in carbon tetrachloride, and are removed by drawing the carbon tetrachloride layer out of the funnel. The buffer solution is extracted once more by shaking for 1 minute with 10 ml. of redistilled carbon tetrachloride, which then has a clear green color, due entirely to the solubility of dithizone in carbon tetrachloride.

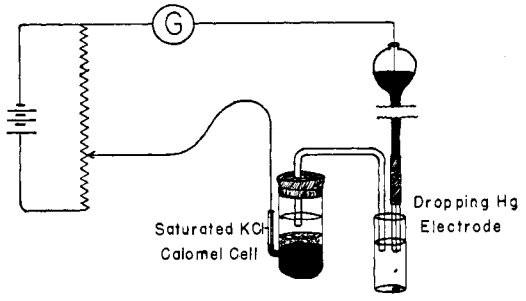

Figure 1. Electrode Assembly for Routine Polarographic Analysis

Twenty-five milliliters of purified buffer solution containing dithizone, and 5 ml. of carbon tetrachloride are placed in a 125-ml. separatory funnel. The plant tissue digest is transferred to the separatory funnel with two washings of the flask with redistilled water. The separatory funnels are shaken for 1 minute to bring most of the copper and zinc into the carbon tetrachloride layer. If the pH of the aqueous phase is not between 9 and 10, as indicated by thymol blue indicator on a spot plate, it is adjusted with distilled hydrochloric acid or distilled ammonium hydroxide. The funnels are again shaken for 1 minute and the carbon tetrachloride phase is withdrawn into a 50-ml. Erlenmeyer flask. None of the silica that collects between the layers must enter the stopcock bore. Two washings, 2 ml. each, of redistilled carbon tetrachloride usually suffice to remove all the copper and zinc, as indicated by the clear green color of the carbon tetrachloride layer in the last washing.

The dithizone in the carbon tetrachloride extract is oxidized by digesting the residue, evaporated to dryness, with 2 ml. of ternary acid mixture at 300° C. on an electric hot plate for 2 hours. Finally the sides of the Erlenmeyer flasks are heated briefly and cautiously above a Meker burner to remove the last traces of sulfuric acid.

Table I. Diffusion Currents (i_d/c) for Copper and Zinc

(In base electrolyte 0.25 *M* in Na_2SO_3, 0.10 *M* in NH_4OH, and containing 0.001% gelatin)

Copper			Zinc		
Concentration, mm./l.	Current, μa.	i_d/c	Concentration, mm./l.	Current, μa.	i_d/c
0.0305	0.0666	2.18	0.115	0.693	6.02
0.0611	0.163	2.67	0.199	1.182	5.95
0.0916	0.224	2.44	0.230	1.344	5.85
..	..	..	0.268	1.577	5.89

Polarographic Determination of Copper and Zinc. After the flasks have cooled to room temperature, exactly 5 ml. of base electrolyte solution and 1 drop of gelatin are added. For samples known to be very low in copper, as little as 2 ml. of base electrolyte may be added. One hour, with occasional gentle swirling of the flask, is allowed for solution of the copper and zinc. The solution is then poured into a dry sample vial.

The mercury column above the end of the capillary is previously adjusted to a height which gives a drop time of about 4 seconds in the base electrolyte with no applied potential. If the capillary is clean, the drop time remains constant for a given height. The sample vial is placed in position with the dropping mercury as one electrode and a potassium chloride bridge leading to a calomel half-cell as the other electrode (Figure 1). Recording of the polarograph is begun with an applied potential of −0.2 volt on the dropping mercury electrode with a galvanometer sensitivity of 2. The half-wave potential for the reduction of copper ($Cu^+ \rightarrow Cu$) in this electrolyte occurs at −0.50 volt. When the potential reaches −0.80 volt the galvanometer sensitivity is changed to 5 to record the zinc wave ($Zn^{++} \rightarrow Zn$), the half-wave potential of which is −1.23 volts. The recording of the polarogram is stopped when the potential reaches −1.50 volts. The temperature of the solution is measured.

After the polarogram (photographic print) is developed and dried, the diffusion current wave height is measured (Figure 2). If the temperature of the polarographic solution was not 25° C., a correction of 2% per degree of difference is made; the correction is added if the temperature was lower and subtracted if it was higher than 25° C.

STANDARDIZATION AND ACCURACY OF DETERMINATIONS

The relationship between diffusion current and concentration of copper and zinc was established with standard samples in the base electrolyte used (Table I). Values for $m^{2/3}t^{1/6}$ for the dropping mercury electrode at the half-wave potentials for copper and zinc were 1.81 and 1.76 $mg.^{2/3}sec.^{-1/2}$, respectively. The diffusion current for zinc shows variation within ±2% from the mean. For copper the variation is ±10% from the mean. At concentrations less than 0.05 millimolar (3 p.p.m.), the copper wave is difficult to measure. The zinc wave is well defined and no concentrations low enough to give difficulty in measuring have been encountered in several hundred analyses of soils and plants.

The reproducibility of copper and zinc determinations was formally tested with corn leaf samples, to which were added known amounts of copper, zinc, antimony, cobalt, and nickel (Table II). Excepting the net result for zinc in sample 2 (obviously contaminated) and that for copper in sample 9, all comparable samples agreed within ±10% of their mean value and were mainly within ±2 to 6%. The probable error of the method has been found in extensive use to be sufficiently low for satisfactory copper and zinc determinations in plant tissue or soils.

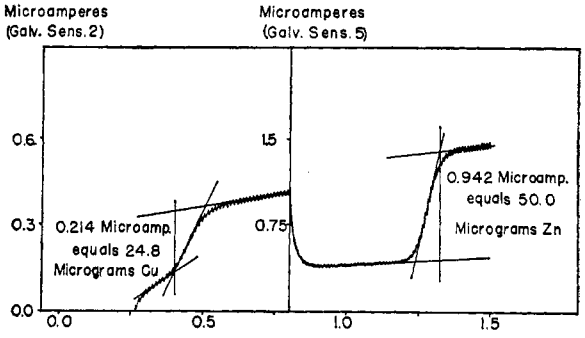

Figure 2. Polarogram Showing Measurement of Copper and Zinc Waves

Blank determinations of copper and zinc in plant analyses were fairly constant at about 1 microgram per sample. In soil analyses, the blank determinations varied up to 5 micrograms of copper or zinc per sample. Possibly the higher blanks were due to difficulties in cleaning the platinum crucibles and rubber policeman.

INTERFERENCE FROM ANTIMONY, COBALT, OR NICKEL

No interference was found from additions of antimony, cobalt, or nickel to corn leaf tissue (Table II). No sign of antimony or cobalt waves could be detected on the polarograms. Subsequent experiments have shown that cobalt is extracted and recorded on the polarograms coincident with the zinc wave by the above procedure. The diffusion current (i_d/c) for cobalt in the sulfite-ammonia base electrolyte was found to be 3.63 microamperes per millimolar concentration. Thus, referring to Table II, the error caused by addition of 4.9 micrograms of cobalt would be only 7.5%

Table II. Reproducibility of Copper and Zinc Determinations

(In five portions of corn tissue from plots with and without minor element fertilizer, recovery of added amounts of copper and zinc, and noninterference of nickel, cobalt, and antimony)

Flask No.	Tissue Weight	Element Added to Flask					Copper Determination				Zinc Determination			
		Cu	Zn	Ni	Co	Sb	Total	Net[a]	In dry tissue	Deviation from mean	Total	Net[b]	In dry tissue	Deviation from mean
	Grams	γ	γ	γ	γ	γ	γ	γ	*P.p.m.*	%	γ	γ	*P.p.m.*	%
							Corn Tissue from Check Plot							
1	1.399	0	0	0	4.9	0	25.3	23.6	16.9	−6.1	43.7	42.5	30.4	−5.9
2	1.429	0	12.5	9.0	0	0	25.7	24.0	16.8	−6.7	129.6	115.9	81.1	[c]
3	Blank	0	0	9.0	0	0	1.7	0	0	[c]	1.2	0	0	[c]
4	1.248	9.7	0	0	0	8.2	35.2	23.8	19.1	+6.1	44.9	43.7	35.0	+8.4
5	1.333	0	0	0	4.9	0	26.5	24.8	18.6	+3.3	43.3	42.1	31.6	−2.2
6	1.227	0	12.5	0	0	0	24.5	22.8	18.6	+3.3	53.3	39.6	32.3	0.0
	Mean	..	..	..	..	..	..	..	18.0	..	..	..	32.3	..
							Corn Tissue from Plots Treated with Minor Element Fertilizer							
7	1.294	9.7	0	0	0	0	38.8	27.4	21.2	+2.7	45.5	44.3	34.2	−9.4
8	1.491	0	12.5	0	4.9	0	33.2	31.5	21.1	+2.2	69.3	55.6	37.3	−1.2
9	1.247	0	0	9.0	0	8.2	23.4	21.7	17.3	[c]	46.8	45.6	36.6	−3.0
10	1.267	9.7	0	0	0	0	37.8	26.4	20.8	+0.7	53.8	52.6	41.5	+10.0
11	1.235	0	0	0	0	8.2	25.8	24.1	19.5	−5.6	49.5	48.3	39.1	+3.8
12	Blank	0	0	0	0	0	0	0	0	[c]	1.2	0	0	[c]
	Mean	..	..	..	..	..	..	..	20.6	..	..	..	37.7	..

[a] Blank and added copper subtracted from total.
[b] Blank and added zinc subtracted from total.
[c] Not included in mean.

of the diffusion current due to 40 micrograms of zinc. As the cobalt content of plant tissue rarely exceeds 1 p.p.m. (*1*) compared to 20 to 40 p.p.m. of zinc, its influence on the zinc determination would rarely exceed 2% and can be neglected, except with plants with low zinc content.

A wave for nickel was found at −0.90 volt, but it was well separated from the copper and zinc waves. Many alfalfa samples analyzed have shown a measurable wave corresponding with that for nickel. However, an accuracy of better than ±10% for nickel determination would be difficult to attain consistently, as nickel often occurs in plants in lesser concentrations than copper. A more accurate determination of nickel would require modification of the procedure to separate it from copper.

LITERATURE CITED

(1) Beeson, K. C., U. S. Dept. Agr., *Misc. Pub.* 369 (1941).
(2) Cholak, J., and Hubbard, D. M., IND. ENG. CHEM., ANAL. ED., **16**, 333 (1944).
(3) Cranston, H. A., and Thompson, J. E., *Ibid.*, **18**, 323 (1946).
(4) Cummings, R. W., and Reed, J. F., *Soil Sci. Soc. Am. Proc.*, **5**, 167 (1941).
(5) Heller, K., Kuhla, G., and Machek, F., *Mikrochemie*, **23**, 78 (1937).
(6) Jackson, M. L., Chatterjee, B., Whittig, L. D., and Kittrick, J. A., unpublished manuscript.
(7) Kolthoff, I. M., and Lingane, J. J., "Polarography," New York, Interscience Publishers, 1941.
(8) Riches, J. P. R., *Nature*, **158**, 96 (1946).
(9) Stout, P. R., Levy, J., and Williams, L. C., *Collection Czechoslov. Chem. Communs.*, **10**, 129 (1938).
(10) Walkley, A., *Australian J. Exptl. Biol. Med. Sci.*, **20**, 139 (1942).

RECEIVED January 23, 1951. A portion of a thesis submitted by R. G. Menzel in partial fulfillment of the requirements for the degree of doctor of philosophy, Department of Soils, University of Wisconsin, June 1950. The work was supported in part by a fellowship grant to the Wisconsin Agricultural Experiment Station by the Tennessee Corp., Atlanta, Ga.

Photometric Determination of Traces of Silver

E. B. SANDELL AND J. J. NEUMAYER
School of Chemistry, University of Minnesota, Minneapolis, Minn.

Trace quantities of silver can be isolated by precipitation with stannous chloride from hydrochloric acid medium when tellurium is used as collector, and determined photometrically with *p*-diethylaminobenzylidenerhodanine as reagent. As little as 0.5 p.p.m. of silver can be determined when a 1-gram sample is taken. The method is intended for the determination of minute amounts of silver in sulfides, meteoritic iron, plant ashes, etc.

SMALL amounts of silver in complex inorganic materials are usually determined by cupellation with final weighing. Optical spectrography has also been applied in the final determination after isolation of silver in a lead button. In one such procedure (*1*), the limit of detectability is reported as 0.1 p.p.m. of silver.

The photometric procedure described is a rather general one, which can be applied in the presence of much iron, lead, copper, nickel, cobalt, etc., as well as amounts of the noble metals likely to be encountered in natural materials. Silver is isolated by precipitation with stannous chloride from hydrochloric acid solution with tellurium as collector; it is doubtless present in the precipitate as telluride. The colorimetric or photometric determination is carried out with *p*-diethylaminobenzylidenerhodanine as reagent. This compound (actually the methyl analog) was first used as a qualitative reagent for silver by Feigl, and has since been applied to some extent in the determination of silver (*3*). The rhodanine reagent has the advantage of great sensitivity and good selectivity. The red-colored product formed with silver is insoluble, so that the determination is based on the photometry of a colloidal suspension.

In an indirect method described by Ringbom (*2*) silver is precipitated by *p*-dimethylaminobenzylidenerhodanine, the washed precipitate is dissolved in potassium cyanide solution, and its