Determination of Aluminum and Iron in Plant Tissue

E. J. RUBINS and G. R. HAGSTROM
Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Conn.

A procedure has been developed for the fluorometric determination of small quantities of aluminum in perchloric acid digests of plant tissue using 8-quinolinol. Iron is first removed and determined by formation of the ferrous complex of 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) at pH 3 to 4, followed by extraction with chloroform containing a little isoamyl alcohol. The fluorescent complex of aluminum with 8-quinolinol is extracted with chloroform at pH 4.7 to 5.1. Quantities of iron up to 20 γ and of aluminum up to 10 γ are determined. Manganese, copper, and zinc in amounts usually encountered in plant tissue do not interfere.

MANY COLORIMETRIC DETERMINATIONS of aluminum in plant tissue have been carried out in this laboratory by the aluminon method of Chenery (*1*) following wet digestion with nitric and perchloric acids. For tissue low in this element, the method lacked sensitivity. A survey of published methods indicated that the fluorometric determination of aluminum as the 8-quinolinate in chloroform had the desired sensitivity and had been the basis of quantitative procedures for aluminum in beer (*10*), phosphate rock (*5*), and steel and bronze (*4*).

Gentry and Sherrington (*2*) reported that aluminum quinolinate is extracted by chloroform from aqueous media at two pH ranges: 4.8 to 6.7 and 8.2 to 11.5. Of the metals that form complexes with 8-quinolinol in the lower of these pH ranges (*2, 3*), only iron, copper, zinc, and manganese commonly occur in dry plant tissue in amounts greater than 5 γ per gram. Iron offers serious interference (*4*), but could be removed and determined by formation of the ferrous-bathophenanthroline complex at pH 3 to 4 in the presence of a little perchlorate ion followed by extraction with chloroform containing a little isoamyl alcohol. Copper is removed as a colorless monocomplex (*8*) by this technique. Possible interference by manganese could be eliminated by extracting aluminum quinolinate below pH 5.8 (*3*). Zinc quinolinate is not retained in the organic phase following chloroform extraction (*2*).

Equipment and Reagents

An Evelyn photoelectric colorimeter with a 540-mμ filter was used for the determination of iron and a Klett fluorimeter Model 2070 with cylindrical cuvettes and Corning No. 5970 primary and No. 3060 secondary filters was used for the determination of aluminum. Separatory funnels of 250-ml. capacity equipped with Teflon valves were used for solvent extractions. A mechanical shaker similar to that described by Holmes and Mullins (*6*) was used for extractions with organic solvents.

All reagents except the concentrated nitric and perchloric acids were stored in polyethylene bottles and the water was prepared by passing distilled water through an Illcoway Research Model cartridge deionizer.

NITRIC ACID, concentrated, reagent grade.

PERCHLORIC ACID, 70 to 72%, reagent grade.

DILUTE PERCHLORIC ACID. Dilute 2 ml. of 70 to 72% perchloric acid to 100 ml.

BUFFER SOLUTION. Dissolve 64 grams of reagent grade ammonium acetate in 160 ml. of water. Add 5 ml. of hydroxylamine hydrochloride and 10 ml. of bathophenanthroline solutions and extract the iron impurity with 20 ml. of isoamyl alcohol. Separate the aqueous phase, add 160 ml. of reagent grade glacial acetic acid, and make up the solution to 400 ml. with water.

HYDROXYLAMINE SOLUTION. Dissolve 50 grams of reagent grade hydroxylamine hydrochloride in 450 ml. of water. Add 10 ml. of bathophenanthroline solution and extract the iron impurity with 20 ml. of isoamyl alcohol. Separate the aqueous phase, make up to 500 ml. with water, and store in a refrigerator.

BATHOPHENANTHROLINE SOLUTION (0.0015*M*). Dissolve 250 mg. of bathophenanthroline (G. Frederick Smith Chemical Co., Columbus, Ohio) in 500 ml. of 95% ethyl alcohol. Store the solution in a refrigerator.

CHLOROFORM. Redistill from an all-glass still.

CHLOROFORM–ISOAMYL ALCOHOL MIXTURE. Dilute 50 ml. of C.P. isoamyl alcohol to 500 ml. with chloroform.

8-QUINOLINOL SOLUTION. Dissolve 10 grams of 8-quinolinol in 500 ml. of water containing 30 ml. of glacial acetic acid.

DILUTE AMMONIUM HYDROXIDE (1.5*M*). Dilute 50 ml. of reagent grade ammonium hydroxide (approximately 15*M*) to 500 ml. with water.

STANDARD IRON SOLUTION. Dissolve 100.0 mg. of pure iron wire in 50 ml. of 4*M* nitric acid. Boil to expel oxides of nitrogen, cool, and dilute to 1 liter. Prepare working standards containing 10 and 1 γ of iron per ml., respectively, by appropriate dilution of the stock standard with 0.2*M* nitric acid.

STANDARD ALUMINUM SOLUTION. Dissolve 3.1 grams of reagent grade aluminum sulfate ($Al_2(SO_4)_3.18\ H_2O$) in water containing 4 ml. of concentrated nitric acid and dilute to 1 liter to give a solution containing approximately 250 mg. of aluminum. Standardize by thiosulfate-ammonia precipitation (*9*). Prepare working standards containing 5 and 1 γ of aluminum per ml., respectively, by appropriate dilution of the stock standard with 0.05*M* nitric acid.

STANDARD QUININE SULFATE SOLUTION. Dissolve 100.0 mg. of U.S.P. quinine sulfate and dilute to 1 liter with 1*N* sulfuric acid. Dilute 10 ml. of

Table I. Effect of Perchloric Acid Concentration and pH of Aqueous Phase on Iron Recovery

Dilute $HClO_4$, 0.23M, Ml.	pH	Recovery, %, 10 γ Iron
0	3.95	87.4
1	3.90	96.7
2	3.84	99.6
5	3.56	100.0[a]
7	3.25	100.0
10	2.50	88.6

[a] "Standard."

Table II. Effect of Diverse Ions on Recovery of Iron

Ions Added, γ	Bathophenanthroline, Ml.	Iron Recovered, %
20 Fe plus	2	100.0[a]
200 Mn, 5 Cu, 5 Zn	2	97.3
200 Mn, 10 Cu, 5 Zn	2	102.1
200 Mn, 5 Cu, 10 Zn	2	87.6
100 Mn, 10 Cu, 10 Zn	2	82.8
200 Mn, 10 Cu, 10 Zn	2	87.3
200 Mn, 10 Cu, 10 Zn	5	102.1
5 Al, 200 Mn, 10 Cu, 5 Zn	2	98.7[b]
5 Al, 200 Mn, 10 Cu, 10 Zn	5	98.7[b]

[a] "Standard."
[b] Recovery of aluminum 99.5 and 101.9%, respectively.

this solution to 1 liter with 1N sulfuric acid to give a working standard containing 1 γ of quinine sulfate per milliliter.

Procedure

Wet Digestion of Plant Materials. Place a weighed quantity of plant tissue (1 gram or less) in a 125-ml. Vycor flask (Corning 14980). Add 10 ml. of concentrated nitric acid and heat gently on an electric hot plate until the first reaction subsides (30 minutes). Add 2 ml. of 70 to 72% perchloric acid and continue to digest, gradually increasing the heat, until fumes of perchloric acid are given off. Add 2 ml. of concentrated nitric acid and continue the digestion. Again add nitric acid when perchloric acid fumes appear and finally fume at full heat for about 2 minutes. Remove the flask and when cool add 50 ml. of water and heat to boiling. Transfer the digested solution to a 100-ml. volumetric flask, cool, make up to volume, and mix. If silica is not filtered off, allow the solution to stand for several hours after mixing before aliquots of the supernatant liquid are taken for analysis. Run blanks through the digestion procedure.

Separation and Determination of Iron. Transfer an aliquot (not more than 5 ml.) of the plant digestate containing up to 20 γ of iron and 10 γ of aluminum to a 250-ml. separatory funnel. If the aliquot is less than 2 ml., add 2 ml. of dilute perchloric acid. Make up to 120 ml. with water, and add 1 ml. of buffer solution, 2 ml. of hydroxylamine solution, and 2 to 5 ml. of bathophenanthroline solution, mixing after each addition. Two milliliters of bathophenanthroline will usually suffice, but aliquots from solutions containing much copper and zinc may require a larger amount. After 10 minutes, add 10 ml. of chloroform–isoamyl alcohol mixture and shake for 2 minutes. Allow 1 or 2 minutes for the phases to separate and draw off the lower (organic) layer through a small wad of glass wool (Pyrex filtering fiber No. 800) into a 25-ml. volumetric flask. Repeat the extraction with 10 ml. of chloroform. Make up the contents of the flask to volume with chloroform and mix. Determine the transmittance at 540 mμ using chloroform for the 100% setting. Prepare calibration standards by placing aliquots of the working iron standards containing 0 to 20 γ of iron in a 250-ml. separatory funnel, adding 5 ml. of dilute perchloric acid, and proceed as described above. Prepare a calibration curve or calculate the calibration constant, K_{Fe}, from the formula:

$$K_{Fe} = \frac{\log G_0 - \log G_s}{C}$$

Where G_0 is the per cent transmittance of the zero iron blank, G_s is the per cent transmittance of standard amounts of iron, and C is the concentration of iron in micrograms per 25 ml.

Determination of Aluminum. To the aqueous phase remaining from the extraction of iron, add 2 ml. of 8-quinolinol solution and then 5 ml. of dilute ammonium hydroxide solution, mixing after each addition. After 10 minutes, add 15 ml. of chloroform and shake solution for 2 minutes. Filter the organic layer through Whatman No. 42, 9-cm. filter paper into a 50-ml. volumetric flask. Repeat the extraction with another 15-ml. portion of chloroform. Make the contents of the flask to volume by adding chloroform and mix. Set the potentiometer scale of the Klett fluorimeter at 200, insert the tube containing 1 p.p.m. of quinine sulfate solution, and obtain a null galvanometer setting with the slit adjustment knob. Using this null setting, obtain potentiometer scale readings for the standards, the unknowns, and the digestion blanks. Prepare standards by placing aliquots of the working aluminum standards containing 0 to 10 γ of aluminum in 250-ml. separatory funnels, add 5 ml. of dilute perchloric acid, and carry through the entire procedure, including the extraction of iron. Remove amounts of iron larger than 20 γ prior to the determination of aluminum by increasing the volume of bathophenanthroline. Prepare a calibration curve or calculate the calibration constant, K_{Al}, from the formula:

$$K_{Al} = \frac{P_s - P_0}{C}$$

Where P_s is the fluorimeter potentiometer scale reading for standard amounts of aluminum, P_0 is the scale reading of the zero aluminum blank, and C is the concentration of aluminum in micrograms per 50 ml.

Results and Discussion

Determination of Iron. There was a linear relation between log per cent transmittance and iron concentration up to 20 γ per 25 ml. in the presence of 2 to 7 ml. dilute (0.23M) perchloric acid. Increasing the amount of 0.0015M bathophenanthroline from 2 ml. to 5 ml. did not change K_{Fe}.

The effect of different amounts of dilute perchloric acid upon the pH of the system and upon the recovery of iron is shown in Table I. The low recovery of iron when 10 ml. of dilute perchloric acid were added, is attributed to incomplete formation of the colored complex at pH 2.50. The low recovery, in the presence of less than 2 ml. of dilute perchloric acid, is attributed to the failure of the colored complex to enter completely into the organic phase. The complex can be removed completely in the absence of perchlorate by substituting isoamyl or n-hexylalcohol for the chloroform–isoamyl alcohol mixture, but this requires the transfer of the aqueous phase to another separatory funnel for removal of aluminum. The separation of phases using the chloroform-isoamyl alcohol mixture is much more rapid than when isoamyl or n-hexyl alcohol alone is used (7).

The recovery of iron from synthetic mixtures of manganese, copper, and zinc indicated that when these approached the maximum levels likely to be encountered in aliquots of plant digestate, 2 ml. of 0.0015M bathophenanthroline was not sufficient to assure complete recovery of 20 γ of iron. Representative data are given in Table II. Five milliliters of 0.0015M bathophenanthroline gave complete recovery in the presence of 200 γ of manganese and 10 γ each of copper and zinc. Two milliliters were sufficient with 5 γ each of copper and zinc in the presence of 200 γ of manganese.

Table III. Effect of Bathophenanthroline Treatment on Eliminating Iron Interference in Aluminum Determination

Iron, γ	0.0015M Bathophenanthroline, Ml.	K_{Al} 5 γ Al	K_{Al} 10 γ Al
0	2	22.7	22.8
10	2	22.7	
20	2	22.4	22.8
50	2	20.5	
100	2	15.0	
100	5	22.2	

Table IV. Effect of Perchloric Acid Concentration on Calibration Constant for Aluminum

Dilute $HClO_4$, 0.23M, Ml.	K_{Al}
0	22.3
1	22.4
2	21.9
5	22.8
10	22.7

Table V. Recovery of Iron and Aluminum Added to Samples of Plant Tissue

Sample	Iron Found, γ/gram	Iron Added, γ	Iron Recovered, %	Aluminum Found, γ/gram	Aluminum Added, γ	Aluminum Recovered, %
Alfalfa	64.5	50.0	101.4	47.2	53.6	96.9
		100.0	99.6		107.1	96.7
	59.2	50.0	98.6	58.7	53.6	96.0
		100.0	99.9		107.1	96.3
Red clover	88.0	50.0	97.0	38.4	53.6	100.4
		100.0	97.0		107.1	104.5
Quack grass	45.8	50.0	99.7	11.5	53.6	96.1
		100.0	99.4		107.1	95.5
Timothy	29.9	50.0	101.5	10.9	53.6	98.5
		100.0	99.8		107.1	96.6
Tobacco	230.7[a]	100.0	102.6	244.6[a]	107.1	99.2

[a] γ per 0.200 gram.

Determination of Aluminum. The effectiveness of 2 ml. of 0.0015*M* bathophenanthroline in preventing iron interference in the determination of aluminum is shown by the values for K_{Al} (Table III). The value for K_{Al} was sensibly constant when up to 20 γ of iron were originally present, but there was a lowering of K_{Al} at higher amounts, indicating that all the iron had not been removed. The use of 5 ml. of 0.0015*M* bathophenanthroline with 100 γ of iron restored K_{Al} to values approaching those obtained with 2 ml. of bathophenanthroline and iron not in excess of 20 γ.

The effect of various amounts of 0.23*M* perchloric acid upon K_{Al} was studied in a series of 54 determinations in which aluminum was varied from 1 to 10 γ and iron from 0 to 20 γ. The pH of the aqueous phase following extraction of aluminum ranged from 5.1 to 4.7 and the values of K_{Al} were sensibly constant from 1 to 10 γ of aluminum. Iron, originally present in amounts up to 20 γ, did not lower K_{Al} even when the amount of dilute perchloric acid was outside the recommended range of 2 to 5 ml. The data are summarized for various acid concentrations in Table IV. K_{Al} for the entire series of 54 determinations was 22.6 with a coefficient of variation of 2.53%.

The recovery of aluminum from synthetic mixtures of manganese, copper, and zinc approaching or exceeding the maximum amounts likely to be encountered in aliquots of plant digestate is given in a footnote to Table II. Pretreatment with 2 ml. of bathophenanthroline gave good recovery from systems containing 20, 200, 10, and 5 γ of iron, manganese, copper, and zinc, respectively. Further studies indicated that 10 ml. of bathophenanthroline were needed for complete aluminum recovery with 100 γ of iron in the presence of 10 γ each of copper and zinc.

Recovery of Iron and Aluminum. Various amounts of iron and aluminum were added to plant tissue prior to digestion with nitric and perchloric acids. Tissue content of iron and aluminum and percentage recoveries are given in Table V. Coefficients of variation for a minimum of three replicate digestions of the six plant samples averaged 3.42% with a maximum of 6.75% for iron and 2.69% with a maximum of 4.92% for aluminum. Recoveries of added iron ranged from 97.0 to 102.6%. Recoveries of added aluminum ranged from 95.5 to 104.5%.

Miscellaneous Comments on Method. Although colorimetric readings for iron were made shortly after extraction, the great stability of the ferrous-bathophenanthroline complex under ordinary laboratory conditions has been noted (*8*). Goon and coworkers (*4*) noted that the fluorescence of aluminum quinolinate reaches a maximum immediately and remains constant for at least 24 hours. These observations were confirmed in the course of the present study. The precaution of Goon *et al.* of not allowing the sample under observation to be exposed to ultraviolet light for undue lengths of time was followed.

Blank values for iron and aluminum were low and there was little or no difference between values of calibration blanks and digestion blanks for either element. Separatory funnels with Teflon valves are recommended, because a few types of stopcock grease contain material that fluoresces in chloroform solution. Some lots of reagent grade chloroform contained impurities that gave in the presence of 8-quinolinol a yellowish green fluorescence apparently identical with that of aluminum quinolinate. Redistilled chloroform was satisfactory and it was found that chloroform reclaimed by distillation from aluminum quinolinate wastes was also satisfactory. No attempt was made to reclaim chloroform from the iron determinations, because of the blue fluorescence of the isoamyl alcohol–chloroform mixtures. Whether this is a property of isoamyl alcohol itself or of some impurity was not investigated.

All glassware was washed by merely rinsing with tap water and then with deionized water with the exception of the 96% silica glass digestion flasks and the separatory funnels. They were rinsed with 1 to 1 nitric acid before being put through the regular washing procedure.

Literature Cited

(1) Chenery, E. M., *Analyst* **73,** 501–2 (1948).
(2) Gentry, C. H. R., Sherrington, L. G., *Ibid.*, **71,** 432–8 (1946).
(3) *Ibid.*, **75,** 17–21 (1950).
(4) Goon, E., Petley, J. E., McMullen, W. H., Wiberley, S. E., *Anal. Chem.* **25,** 608–10 (1953).
(5) Grimaldi, F. S., Levine, H., U. S. Geological Survey, Trace Elements Investigations, Rept. **60,** (1950).
(6) Holmes, R. S., Mullins, J. F., *Soil Sci.* **69,** 233–7 (1950).
(7) Johnson, C. M., Arkley, T. H., *Anal. Chem.* **26,** 572–3 (1954).
(8) Smith, G. F., McCurdy, W. H., Diehl, H., *Analyst* **77,** 418–22 (1952).
(9) Treadwell, F. P., Hall, W. T., "Analytical Chemistry, II. Quantitative Analysis," 7th ed., pp. 91–2, Wiley, New York, 1930.
(10) Tullo, J. W., Stringer, W. J., Harrison, G. A. F., *Analyst* **74,** 296–9 (1949).

Received for review February 5, 1959. Accepted July 1, 1959.