

Aluminum Studies. Soil and Plant Analysis of Aluminum by Modification of the Aluminon Method

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The ammonium salt of aurintricarboxylic acid, aluminon, at 0.05% concentration in ammonium acetate buffer is specific and accurate for the determination of aluminum in plant ash extracts or soil extracts, if hydroxylamine hydrochloride is added to prevent iron interference. The color development is made at a set pH, preferably 3.5, and the solution is boiled for 5 minutes to induce maximum absorbance. Interferences such as fluoride or soluble organic matter are evolved with acid. Aluminum hydroxide separation from wet-ashed plant samples prevents interference in the method by high concentration of perchlorate, nitrate, or other salts.

FOR ALUMINUM ANALYSIS OF PLANT AND SOIL SAMPLES a rapid accurate method is needed to study the aluminum content of plants and the available aluminum in the soil.

The colorimetric properties of the dye aluminon were studied by Smith, Sager, and Siewers (8). Alkaline conditions either in preparing stock solutions or in developing the aluminum complex with the dye led to serious errors, causing the dye to fade to its colorless or carbinol form. They suggested using a pH of 4.2 and found that the absorbancy of the dye complex with aluminum gave straight lines in conformity with Beer's law, provided that the solvent cell contained the buffered dye at the same pH and that the photocell response of the colorimeter was also linear. They tested several sources of aluminon at $10^{-3}M$ concentration and 25° C. Sandell (7) discussed aluminon methods and pointed out that close control of pH is necessary for reproducible results and a protective colloid is needed for stability of the aluminon lake.

Ferric iron interference has been removed by extraction with cupferron in chloroform (9), isopropyl ether (5), or precipitation of sesquioxides with ammonium hydroxide followed by sodium hydroxide to separate aluminum from iron (4) or chromatographic separation (7). Reduction of the ferric iron to the ferrous state also has been used to avoid iron interference, using thioglycolic acid in the method proposed by Chenery (3) and Charlot (2) or hydroxylamine hydrochloride in the procedure proposed by Peech and English (6).

Details are given here on the colorimetric properties and the effect of pH variation, heating, and other ions on the absorbancy of the aluminum complex. The recovery of aluminum added to several plant and soil samples is reported.

Reagents

Aluminon-buffer solution. Add 50 ml. of 37% hydrochloric acid to 500 ml. of 2*N* ammonium acetate (pH 7.0) and shake. Add to this solution 100 ml. of 0.5% aqueous ammonium aurintricarboxylate (Eastman Organic Chemicals) and 100 ml. of 2% gum arabic (Will Corp.). Dilute to 1 liter.

Hydroxylamine hydrochloride (Baker), 5 grams in 100 ml. of 1*N* hydrochloric acid.

Aluminum standard, 0.8792 gram $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$ previously kept in a desiccator for a few days in 500 ml. of 0.1*N* hydrochloric acid. Dilute 50 ml. of this solution to 500 ml. to give 10 γ of aluminum per ml.

Procedure

Plant Samples. Oven-dry plant material and grind through a Wiley mill. Dry the sample at 70° C. and weigh out 5 grams. Place the sample in a wide-mouthed Erlenmeyer flask, add 50 ml. of 1 to 5 perchloric-nitric acid mixture and cover with a watch glass. Digest slowly until most of the sample is in solution and then evaporate to dryness. Add 2 ml. of concentrated hydrochloric acid and evaporate to dryness. Add 20 ml. of 2*N* hydrochloric acid and heat until the salts are in solution. Transfer solution and residue to a 100-ml. volumetric flask and dilute to volume. Samples can also be dry-ashed at 600° C. for 4 hours. Dissolve ash in 20 ml. of 2*N* hydrochloric acid and transfer to a 100-ml. volumetric flask.

As the concentration of salts present is likely to cause flocculation of the aluminon-aluminon complex, proceed as follows with separation of the aluminum.

Pipet a suitable aliquot of the supernatant solution containing 10 to 60 γ of aluminum into a 25-ml. centrifuge tube. Add a drop of chlorophenol red indicator.

Neutralize dropwise with concentrated ammonium hydroxide, to the end point. Place the tube in a boiling water bath for 10 minutes. Centrifuge and discard the supernatant liquid. If the salt concentration is lowered sufficiently by further dilution, the aluminum determination may be made directly from the plant digest, provided that the aliquot contains 10 to 60 γ of aluminum. Add 4 ml. of the hydroxylamine hydrochloride reagent to dissolve the aluminum precipitate and transfer this solution to a 50-ml. beaker, using about 25 ml. of distilled water. If the aliquot is pipetted directly into the beaker, add 4 ml. of hydroxylamine hydrochloride reagent and 25 ml. of distilled water. Add 10 ml. of the aluminon-buffer reagent. Adjust the pH to 3.5 using a glass electrode and additions of 2*N* ammonium hydroxide or 2*N* hydrochloric acid as required. Heat on a hot plate and boil gently for 5 minutes. Cool, dilute to a 50-ml. volume, and measure the absorbancy or per cent transmittance at 530 $m\mu$ in a spectrophotometer. Prepare the calibration curve by pipetting 0 to 6 ml. of the standard aluminum solution (10 γ of aluminum per ml.) into a series of 50-ml. beakers proceeding as above with reagents, pH adjustment, heating, and measurement.

For determination of extractable soil aluminum, normal ammonium acetate at a pH of 4.8 can be used with a 10 to 1 extractant to soil ratio and shaking for 30 minutes. If the filtrate is colorless, aliquots can be used directly for aluminum determination as for the standards above. If the filtrate is colored by organic matter, evaporate to dryness, add 10 ml. of 30% hydrogen peroxide, and take to dryness. When fluoride or other aluminon complex-forming soil extractants are used, add 2 ml. of concentrated sulfuric acid to a suitable aliquot and evaporate

to dryness. Wet-ashing of this aliquot is necessary for citrate or for EDTA extractants. With samples having very high ash in proportion to aluminum, proceed as for plant ash in the aluminum determination after destruction of ammonium acetate. Where high sample dilution is needed, proceed directly with transfer of the aliquot to a 50-ml. beaker, add the aluminon and hydroxylamine reagents, dilute to 35 ml., and adjust the pH to 3.5 ± 0.1 . Develop color by boiling for 5 minutes, cool and make to 50 ml. volume and determine absorbancy or per cent transmittance at $530 \text{ m}\mu$ using a Beckman or other suitable spectrophotometer.

Results and Discussion

Wave Length. The absorbancy of the reagent blank and the aluminum complex with aurintricarboxylate was examined at various wave lengths (Figure 1). Minimal absorbance of the reagent blank was found in the range of 550 to $570 \text{ m}\mu$ and it increased steadily at both longer and shorter wave lengths. The absorbance of aluminum complex alone was obtained from the difference between the absorbancy of the mixed ammonium- and aluminum-aluminon lakes and that of the reagent blank. The aluminum lake gave a maximum absorbance over the range 530 to $540 \text{ m}\mu$. A wave length of $530 \text{ m}\mu$ was chosen for this aluminum procedure. Absorbancy and aluminum concentration (0 to 100γ) conformed to Beer's law at this wave length.

pH Effects. When gum arabic was used as a protective colloid in the concentration specified, the precipitation of aluminum in the presence of aluminum (0 to 150γ) was prevented upon the addition of mineral acid and neutral salts. The effect of pH was investigated in the range 2.0 to 8.0 . The absorbancy of the lake was determined at $530 \text{ m}\mu$ (Figure 2). The color intensity of the reagent blank increased with the lowering of the solution pH and reached a minimum above pH 5.8 . The absorbancy of the aluminum lake plus excess reagent decreased rapidly below pH 2.5 and above pH 4 , and is maximum at 3.2 . Calculation of the absorbancy of the aluminum lake itself showed a maximum in the pH range 3.2 to 3.8 , with a rapid decrease below pH 3 and above 4.8 . From these curves the errors introduced by improper control of the pH for the color development were obvious. In this procedure the buffer was made so that 10 ml. of the aluminon reagent at pH 4.4 , along with 4 ml. of $1N$ hydrochloric acid, produced a solution pH of 3.5 . Control of the pH by acetate buffer was found adequate for the plant ash samples. However, with soil extracts adjustment of pH after adding the reagents was necessary. Testing and adjusting the pH before color de-

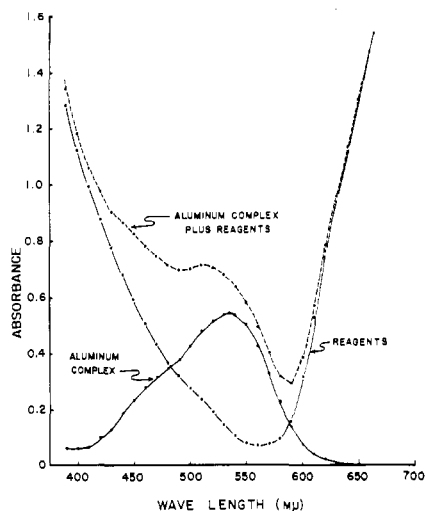


Figure 1. Absorbance characteristics of aluminum complex of aurintricarboxylic acid and of reagents in acetate buffer in range 400 to $650 \text{ m}\mu$

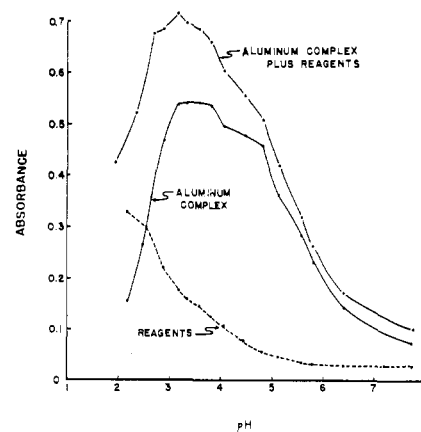


Figure 2. Absorbance characteristics of aluminum complex of aurintricarboxylic acid and of reagents in acetate buffer in pH range 2 to 8

Table I. Effect of Heating on Color Development of Aluminum Aurintricarboxylate in Acetate Buffer

| Treatment | Absorbance ^a at $530 \text{ m}\mu$ | | | |
|------------------------|---|--------------------------------------|---------------------------|--------------------------------|
| | Reagent blank | Solution with $40 \gamma \text{ Al}$ | Soil extract ^b | Plant acid digest ^c |
| Stood for 4 hours | 0.120 ± 0.001 | 0.602 ± 0.010 | 0.383 ± 0.017 | 0.494 ± 0.020 |
| Heated just to boiling | 0.118 ± 0.001 | 0.662 ± 0.028 | 0.426 ± 0.004 | 0.548 ± 0.014 |
| Boiled for 2 min. | 0.118 ± 0.001 | 0.665 ± 0.006 | 0.422 ± 0.010 | 0.553 ± 0.009 |
| Boiled for 5 min. | 0.119 ± 0.001 | 0.681 ± 0.001 | 0.440 ± 0.011 | 0.549 ± 0.004 |

^a Mean absorbance and range found for several determinations.

^b Aliquot of soil extract equivalent to 0.5 gram of Lakeland fine sand leached with normal ammonium acetate at pH 4.8 .

^c Aliquot of dilution from nitric-perchloric acid digestion of shade tobacco leaves equivalent to 0.02 gram of plant material.

velopment increased the accuracy of the determination at slight expense in time.

Heating Effect. Heating of the aluminon-acetate solution hastened the color development. Four different heat treatments on pure chemical, plant digest, and soil extract were investigated (Table I). After cooling in a water bath to room temperature and making to 50-ml. volume, the color intensity was measured at $530 \text{ m}\mu$. No difference was found between treatments for the reagent blank. However, the formation of the aluminum lake with aluminon did not reach the maximum even after 4 hours. Heating the solution increased the absorbance and reduced the variability. The maximum absorbance was obtained by boiling gently for 5 minutes. Longer boiling did not increase the absorbance further, and excessive evaporation caused some deposit of the aluminon lake on the sides of the beaker. There was no precipitation of the lake, if a concentration of 100γ of aluminum or less was present.

Table II. Effect of Hydroxylamine Hydrochloride Concentrations on Color Development of Aluminum Aurintricarboxylate Lake

| $\text{NH}_2\text{OH}\cdot\text{HCl}$ Added, Gram | Absorbance | |
|---|-------------------------|-------------------------|
| | Final solution pH 4.8 | Final solution pH 3.5 |
| 0 | 0.478 | 0.649 |
| 0.1 | ... | 0.649 |
| 0.2 | 0.423 | ... |
| 0.4 | ... | 0.648 |
| 0.6 | 0.409 | 0.651 |
| 0.8 | 0.424 | ... |

The color was stable for several hours in open beakers and at least 24 hours in 50-ml. volumetric flasks in the operating range (0 to 60γ aluminum). Therefore, the suggested procedure of boiling was preferable to the more common practice of letting the color develop at room temperature.

Table III. Ions Tested for Interference in Aluminum Determination with Aurintricarboxylate by Modified Procedure

| Ion | Aluminum | | Aluminum Found, γ | Difference, γ |
|---------------------------------------|--------------------------|--------------------------|--------------------------|----------------------|
| | Amount Present, γ | Amount Present, γ | | |
| Mn ⁺² | 50 | 40 | 40.1 | +0.1 |
| Co ⁺² | 50 | 40 | 39.9 | -0.1 |
| Ni ⁺² | 50 | 40 | 40.1 | +0.1 |
| Pb ⁺² | 50 | 40 | 39.8 | -0.2 |
| Zn ⁺² | 50 | 40 | 40.0 | 0.0 |
| Cu ⁺² | 50 | 40 | 39.9 | -0.1 |
| Ca ⁺² | 100 | 40 | 39.9 | -0.1 |
| Ca ⁺² | 500 | 40 | 40.3 | +0.3 |
| Mg ⁺² | 100 | 40 | 40.1 | +0.1 |
| Mg ⁺² | 250 | 40 | 40.1 | +0.1 |
| Fe ⁺³ | 50 | 30 | 29.8 | -0.2 |
| Fe ⁺³ | 100 | 30 | 29.7 | -0.3 |
| Fe ⁺³ | 150 | 30 | 30.1 | +0.1 |
| Fe ⁺³ | 200 | 30 | 31.0 | +1.0 |
| Fe ⁺² | 500 | 30 | 31.7 | +1.7 |
| Fe ⁺³ | 1000 | 30 | 40.6 | +10.6 |
| Sn ⁺⁴ | 50 | 40 | 39.7 | -0.3 |
| Cr ⁺⁶ | 50 | 40 | 50.8 | +10.8 |
| P (in PO ₄ ⁻³) | 25 | 40 | 39.6 | -0.4 |
| P (PO ₄ ⁻³) | 50 | 40 | 40.8 | +0.8 |
| P (PO ₄ ⁻³) | 100 | 40 | 40.8 | +0.8 |

Hydroxylamine Concentration. The effect of hydroxylamine hydrochloride on the aluminum color development was studied (Table II). At pH 4.8 the addition of hydroxylamine hydrochloride crystals apparently reduced the absorbance. However, at pH 3.5 no difference in color intensity was found at the several concentrations tested. The difference in absorbance at pH 4.8 and 3.5 agreed with the data found in studying the effect of pH.

Interferences. The interferences and reactions of ions in other aluminum methods have been studied in some detail (4, 7, 8). For the present procedure several ions were tested for their effect on the absorbance. Among the ions tested, only high iron concentration and chromium interfered (Table III). Addition of more hydroxylamine eliminated the iron interference. Because chromium levels in plant and soil extracts are very low, no interference was anticipated and it was avoided where aluminum was separated previously as the hydroxide. Phosphate did not affect the color development at the above levels or where the phosphorus-aluminum ratio was 20 to 1 in the method of Strafford and Wyatt (9) or Chenery (3). Separation of the aluminum as the hydroxide and the phosphate at pH 6.4 was found satisfactory, because the phosphorus to aluminum ratio is reduced greatly. In the ammonium acetate soil

Table IV. Recovery of Added Aluminum in Plant Digests and Soil Extracts

| Sample | Aluminum Added, γ | Aluminum Found, γ | Recovery, % | Aluminum Concn., P.P.M. |
|---------------------------|--------------------------|--------------------------|-------------|-------------------------|
| Apple leaves | 0 | 20.4 | | 204 |
| | 4.0 | 24.4 | 100.0 | |
| Cherry leaves | 0 | 40.2 | | 322 |
| | 20.0 | 60.9 | 103.5 | |
| Citrus leaves | 0 | 32.1 | | 247 |
| | 20.0 | 52.5 | 100.5 | |
| Peach leaves | 0 | 21.0 | | 168 |
| | 20.0 | 39.5 | 92.5 | |
| Oat leaves | 0 | 28.9 | | 58 |
| | 20.0 | 49.3 | 102.0 | |
| Tomato fruits | 0 | 39.7 | | 0.75 ^a |
| | 20.0 | 58.5 | 104.0 | |
| Norfolk lfs. ^b | 0 | 86 | | 172 |
| | 30 | 115 | 96.7 | |
| Red Bay lfs. ^b | 0 | 54 | | 220 |
| | 30 | 85 | 103.1 | |
| Red Bay lfs. ^c | 0 | 63 | | 1890 |
| | 30 | 93 | 100.0 | |
| Klej fs. ^c | 0 | 31 | | 890 |
| | 20 | 62 | 103.1 | |

^a Fresh weight basis.

^b Extracted with 1N NH₄Ac at pH 4.8.

^c Extracted with 0.3N NH₄F in 0.1N HCl.

extracts the phosphorus-aluminum ratio in the filtrate was much less than unity, so that the phosphate was not an interference. No color development occurred in the presence of the complex forming reagents, fluoride, oxalate, citrate, or EDTA; hence their removal by acid digestion is necessary. Hydrogen peroxide must also be destroyed for the hydroxylamine hydrochloride to be effective in preventing ferric ion interference.

Calibration Curve. A linear function exists between the absorbance or the logarithm of the per cent transmittance and aluminum concentration. The calibration curve should be prepared with each batch of samples using the same reagents as for the sample. Small variations in the color of the reagent blank can be measured against water and are compensated for by setting the reagent blank as zero absorbance or 100% transmittance.

Recovery Studies. To test the applicability of the procedure, known amounts of aluminum were added to the aliquots of various plant digests and soil extracts (Table IV). Satisfactory recovery of the aluminum added was obtained from both plant and soil extracts. The duplicate results showed very close agreement.

This procedure for aluminum has been used in this laboratory on a wide variety of plant samples and soil extracts. Where unusually low or high aluminum

levels were encountered, selection of the dilution needed was the major difficulty encountered. For instance, where soil was extracted with 0.03N ammonium fluoride in 0.1N hydrochloric acid solution, 2 ml. of 100 to 1 extractant to soil dilution was used. Where neutral salt solutions were used to extract light sands, an aliquot equivalent to 5 to 10 grams of soil sample was necessary. With most plant samples an aliquot equivalent to 0.5 to 1.0 gram of dry weight was found satisfactory.

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