Nitrate Determination in Plant Extracts by the Nitrate Electrode

J. L. Paul and R. M. Carlson

The nitrate-specific ion electrode was used to determine nitrate in aqueous extracts of a variety of dried plant tissues. A recommended plant-solution ratio of 1 to 125 gives extract concentrations of 1 to 50 p.p.m. of N for many field-grown crops. Electrode response is linear over this range. The addition of aluminum resin during extraction eliminates bicarbonate interference and reduces organic anion interference. Silver resin is added if chloride exceeds 2% in the tissue and nitrate concentrations are 5 p.p.m. or less. Nitrate determinations by the phenoldisulfonic acid and electrode methods agreed closely enough that for practical purposes the electrode method could be used in place of the phenoldisulfonic acid method. The average relative standard deviation for 16 plant materials for the electrode method was 3.1%.

The nitrate content in petioles of certain dicots or in blades of certain monocots indicates nitrogen deficiency or abundance. A method of nitrate determination, now widely used, consists of aqueous extraction of dried, ground sample, followed by chemical determination of nitrate in the extract with phenoldisulfonic acid. This method is used routinely in many commercial and extension laboratories in California. Details of the procedure are given by Johnson and Ulrich (1950).

In recent months the nitrate electrode became available, and since the range in concentration of plant extracts falls within the range of electrode response, it seemed worthwhile to explore its application to the determination of nitrates in plant material. This paper discusses this application and compares results to those obtained with the phenoldisulfonic acid (PDS) method.

EXPERIMENTAL

Apparatus. Nitrate ion electrode Model 92-07, Orion Research, 11 Blackstone St., Cambridge, Mass.

pH meter with expanded millivolt scale.

Reagents. NITRATE STANDARDS. Prepare at least 100 ml. of 50, 25, 15, 10, 5, 2, and 1 p.p.m. of N from KNO_3 in 0.01*N* KH_2PO_4. More concentrated solutions may be prepared if desired.

ALUMINUM RESIN. The preparation of this reagent is not a critical operation, and, therefore, amounts of resin and details of preparation are left to the operator's needs. The procedure followed in this work was to mix $Al_2(SO_4)_3$ with a slurry of Dowex 50–X8 (50- to 100-mesh) hydrogen-saturated resin, then filter and leach with water to remove salts. The $Al_2(SO_4)_3$ required should exceed the equivalents of resin used by about 25%. Only a slight suction is applied during filtration, so that most of the noncapillary water is removed, but the resin beads remain moist. The resin is best stored in a stoppered cylindrical container. Six-millimeter glass tubing is used to effect resin transfer by pressing an open-end tube into the resin until 1 ml. of resin is collected. It is then dispensed by blowing.

ELECTRODE BEHAVIOR

The nitrate electrode utilizes a liquid ion exchanger separating an internal solution from a sample solution. The electromotive force is a function of the nitrate activity in the sample solution, while at constant ionic strength it is a function of nitrate ion concentration. By using a 1×10^{-2} *M* phosphate buffer in standard solutions, variation in ionic strength of ±50% would introduce an error of only ±2% in accuracy, according to manufacturer's specifications. The salt concentration of most plant extracts estimated in this study fell within these limits, and for this reason a compensating salt was added to the standards.

The electrode responds to anions such as Cl^-, HCO_3^-, and certain organic acid anions such as citrate. All occur in plant extracts. To avoid these interferences, certain procedures must be followed. The addition of Al resin to a major extent eliminates HCO_3^- and organic anion interferences. Its addition lowers aqueous extract pH from ~6 to 3.8 to 4.1, which removes HCO_3^-, and Al forms complexes with organic acid anions. At these low pH values ionization of organic acids is depressed and their interference is decreased.

Extent of Cl^- interference depends on nitrate concentration and the concentration of Cl^- in the sample.

For most field-grown plants, a plant-solution ratio of 1 to 125 gives extract concentrations between 1 and 50

Departments of Environmental Horticulture and Pomology, University of California, Davis, Calif. 95606

p.p.m. of N or 125 to 6250 p.p.m. of NO_3 N in the dried plant tissue, although tissues such as potato petioles may contain 10,000 to 15,000 p.p.m. of NO_3 N. The electrode responds linearly over this concentration range and remains linear far beyond 50 p.p.m. of N. The standard curve is reproducible from day to day.

When in use the electrode should be calibrated with standards at intervals to check drift. If the electrode is behaving normally, drift in the standard curve should not be more than a few per cent, but if contaminants such as HCO_3^- are present in the standards, our experience indicates that electrode drift can be particularly troublesome. It is imperative to use distilled water of good quality in the preparation of standards.

The time required for the electrode to give steady readings depends on nitrate concentration. Steady readings are obtained within seconds for concentrations greater than 15 p.p.m., but several minutes may be required below 2 p.p.m. If response time is limiting at very low values, it may be necessary to adjust the plant-solution ratio so that the extract concentration is greater. Samples must be stirred at constant speed while reading. Expanded scale readings are particularly susceptible to noise; stirring must, therefore, be constant and stirring bars should spin uniformly.

PROCEDURE

Weigh 0.4 gram of dried, ground, plant material into a 125-ml. Erlenmeyer flask, add 50 ml. of water and 1 ml. of Al resin; stopper and shake for 10 to 15 minutes; filter through Whatman No. 2 folded filter paper. Transfer the filtrate to a 100-ml. beaker and read standards and unknowns under constant stirring conditions. Report results as parts per million of N on the dry weight basis and use the symbol p.p.m. of NO_3 N.

This procedure was used to determine nitrates reported here. It is adaptable to routine work. The filtration step may be eliminated by reading the suspension directly. Several determinations have been carried out on samples before and after filtration. No differences in readings were observed, but it was suspected that fouling of the electrode might result from plant colloids after extended use in suspensions. This requires further evaluation.

RESULTS

Nitrate Recovery. To assess the efficiency of nitrate recovery nitrates were added to tissue high in nitrate and tissue which was nitrate-free (Table I). These very accurate recoveries have been repeated with other species and other levels of addition.

Chloride Interference. Chloride may be present in plant tissue at trace concentrations to 10% of the dry weight, but normal levels are 0.5 to 2%. Since the nitrate electrode responds to chloride, it causes increased nitrate values. The extent of Cl^- interference was examined under a wide range of NO_3^- and Cl^- concentrations. Within the context of the procedure used here Cl^- concentration in the tissue of 2% or greater caused a positive error in NO_3^- reading of 10% or greater at NO_3 N levels of 500 p.p.m. in the tissue or less. To obtain accurate results Cl^- should be removed when tissues contain

Table I. Nitrate Recovery from High-Nitrate and Nitrate-Free Tissue

Plant	P.P.M. of NO_3 N in Tissue		
	Added	Total	Found
Tomato	0	2792	2792
	564	3356	3330
	1128	3920	3950
	1692	4484	4510
Grape petiole	282	282	287
	564	564	570
	1128	1128	1100

500 p.p.m. of NO_3 N or less and 2% or more of Cl^-. Above 10 p.p.m. of N in the extract (875 p.p.m. of NO_3 N in tissue) interference from Cl^- is small even at 5% tissue Cl^- or greater, and its removal is not necessary.

Cl^- may be removed from low nitrate tissue through the addition of a silver salt, but several complications may be introduced if this is done. Unless stoichiometric Ag^+ is added, excess salt causes a depression in activity coefficients, and if silver sulfate is used, sulfate interferes. A third complication arises from an excess of Ag^+ in the extract, which apparently interacts with the calomel electrode, causing drift in readings accompanied by significantly low results.

The following procedure is recommended to remove chloride. Silver is added as silver resin. The advantage of adding silver in this form is that the anion (resin) is retained in the residue after filtration. This resin is prepared in the same way as Al resin by using $AgNO_3$ in place of aluminum sulfate. One and one-half milliliters of Ag resin is added at the same time that Al resin is added; this amount of resin will effectively remove 5 to 6% Cl if the above plant-solution ratio is used.

In Figure 1 Cl^- was added to a tissue having 0.27% Cl^- initially. Addition of 1.5 ml. of Dowex 50-X8 in Ag form reduced the NO_3 N values from 465 p.p.m. at 5% Cl^- to

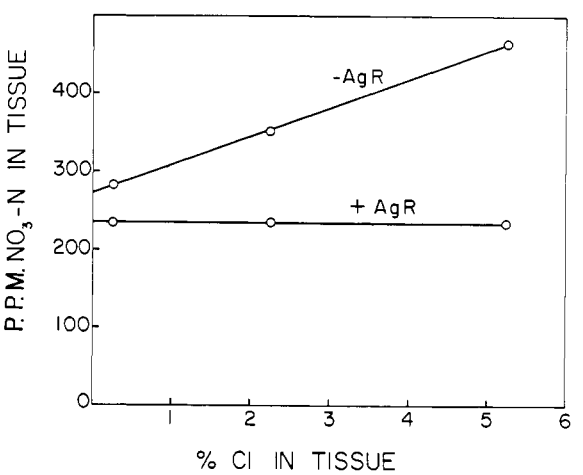

Figure 1. Influence of increasing Cl^- on NO_3 N values in grape petiole tissue determined by nitrate electrode in presence and absence of silver resin (AgR)

226 p.p.m. for all Cl^- levels. The curves do not intersect at 0% Cl, while in standard NO_3^- solutions to which Cl^- was added the two curves intersect at 0% Cl. In the presence of plant tissue the silver is apparently removing an interference other than Cl^-. For many tissues this interference is usually in the order of 30 to 50 p.p.m. of NO_3 N.

Comparison of Electrode and PDS Methods. Nitrate nitrogen values in different plant species determined by both methods are listed in Table II. For grape petioles each PDS value is the mean of 10 determinations. For grass, cotton, sugar beets, tomatoes, and mixed tissue, PDS values are means of four determinations. Values determined by the electrode method are means of four determinations, and a measure of variability of this method is indicated. The low standard deviation is probably due to the large sample size and the inherent precision of electrochemical measurements.

DISCUSSION

Results obtained by the electrode method agree reasonably well with those obtained by the PDS method and suggest that the electrode method may be substituted for the PDS method. Nutritionists have established critical tissue concentrations of NO_3 N as measured by the PDS method. As these critical levels are generally accepted criteria in many laboratories, it is important to preserve them. These critical concentrations should not be altered if the electrode method is used. Speed of measurement recommends the electrode method for routine work.

The electrode method can be used to determine plant nitrates in buffered extracts. When concentrated phosphate and citrate buffers are used, results are the same as with the water–Al resin extract procedure. If a buffer is preferred, pH should be kept at ≤ 4 and standards made up in the buffer. Interference from citrate buffers is significant below solution concentrations of 5 p.p.m. of N, where marked deviation from Nernst response is observed. This does not, however, diminish the usefulness of a citrate buffer.

The plant-extract ratio used here was found to contain salt levels between 0.005 and 0.02*N* as estimated by electrical conductance. These salts are mainly organic (50 to 80%), the remainder being Cl^- and NO_3^-. Since these organic anions are usually not determined, the extent of their interference cannot be anticipated. The interference was not serious at the extract concentrations encountered in this study, but if smaller plant-solution ratios are used, interference may be significant at low nitrate concentrations. Another consequence of smaller plant-solution ratios is increased salt concentration in the extract. This lowers the activity coefficient, which leads to lower results. Widening the plant-solution ratio should have an opposite effect, owing to a decrease in organic anion concentration and ionic strength. Widening the plant-solution ratio above 1 to 125 for several tissues listed in Table II had little influence upon the tissue nitrate level found, but influence of dilution on highly saline tissue might cause changes in the determination of tissue nitrate levels.

The determination of nitrate in plant extracts by the nitrate electrode is affected by salt concentration, chloride, bicarbonate, and organic acid anions. The analyst should take precautions to eliminate or make compensation for these influences. The procedure followed in this study should eliminate most of these difficulties, at least for the type of tissues listed in Table II.

Table II. NO_3 N Values Determined by Phenoldisulfonic Acid (PDS) and Nitrate Electrode Methods

Tissue	P.P.M. of NO_3 N in Tissue	
	PDS	Electrodes[a]
Grape petioles		
1	254	291
2	397	443
3	660	666
4	750	848
5	1167	1166
6	1548	1548
7	2148	2006
8	3129	3136
9	4656	5110
10	850	882
Grass		
1	299	326
2	2496	2394
Tomato	2820	2670
Sugar beet	2200	2390
Cotton	2480	2428
Mixed tissue	1910	2053

[a] Average relative standard deviation for means of electrode method = 3.1%.

LITERATURE CITED

Johnson, C. M., Ulrich, Albert, *Anal. Chem.* **22,** 1526–9 (1950).

Received for review March 11, 1968. Accepted June 7, 1968.