Extracting Solution for Potentiometric Determination of Nitrate in Plant Tissue

A. S. Baker and R. Smith

Rapid NO_3^- determinations were made potentiometrically on plant extracts using the Orion NO_3^- selective electrode. A 0.025*M* $Al_2(SO_4)_3$ solution containing 10 μg. per ml. of NO_3^--N was used for extraction. The $Al_2(SO_4)_3$ buffers against changes in the pH of the extract and the activity coefficient of NO_3^-. The pH is buffered close to 3.0 which depresses ionization of weak acids and, thus, interference from their anions. At the calculated 0.375*M* ionic strength of the extracting solution, the small increase in salt level from plant tissue extraction or KNO_3 in the standards causes a negligible decrease in the activity coefficient of NO_3^-. Maintenance of a minimum of 10 μg. per ml. of NO_3^--N in extracts and standards resulted in more stable and rapid meter readings, linearity of the standard curve, and a reduction in the effect of interfering ions.

Development of a NO_3^- selective electrode stimulated interest in its possible use as a simple and rapid means of NO_3^- analysis of plant extracts. This paper describes a procedure for the extraction of NO_3^- from plant tissue and the subsequent potentiometric determination of the NO_3^- concentration in the extract.

EXPERIMENTAL

Reagents. Preservative. Dissolve 0.1 gram of phenylmercuric acetate in 20 ml. of dioxane and add sufficient distilled water to make 100 ml. Both reagents are highly toxic, thus suitable precaution should be taken against ingestion or undue skin contact.

Extracting Solution. Make a 0.025*M* $Al_2(SO_4)_3$ solution which also contains 10 μg. per ml. of NO_3^--N and 1 ml. per liter of preservative.

Standard Solutions. Make a series of standards containing 10 to 100 μg. per ml. of NO_3^--N which are uniformly 0.025*M* with respect to $Al_2(SO_4)_3$ and contain 1 ml. per liter of preservative.

Apparatus. Corning model 12 expanded scale pH meter or equivalent was used. The nitrate electrode was Model No. 92-07, Orion Research Inc., and the calomel reference electrode, Corning No. 476000 fiber type, saturated KCl, or equivalent.

Procedure. Transfer 400 mg. of oven-dried, and ground (to pass at least a 20-mesh screen) plant tissue to a shaking bottle. Add 40 ml. of extracting solution and shake for 15 minutes. Filter through S and S 597 filter paper into a 50-ml. capacity tri-pour beaker (Scientific Products catalog No. B2722-50) and seal with the polypropylene coated closure. The inside bottom surface of these beakers is convex, thus a narrow band of Teflon tubing is placed on each end of a magnetic stirring bar to prevent it from wandering and thus to insure a constant stirring speed. The beakers should be kept sealed until the electrodes are inserted. Determine the potential developed by the NO_3^- electrode-calomel pair in the standards and plant extracts. A calibration curve is prepared on semilog paper placing the potentials on the linear axis and concentrations of NO_3^--N in the standards on the logarithmic axis. Alternatively, the NO_3^--N concentrations can be read directly on the logarithmic scale of the meter using the pH function.

DEVELOPMENT

Extracting Solution. Several extracting procedures were investigated. The accuracy of potentiometric NO_3^- analyses was determined by a comparison of results obtained using a reduction procedure applied directly to the plant material. The latter is similar to the MgO-Devarda alloy method (Black, 1965) but modified for analysis of NO_3^- in plant tissue with conventional macro-Kjeldahl distillation apparatus by Moodie and Cheng (1967). Except for tests of the procedure proposed by Paul and Carlson (1968), the nitrate standards were made in the same type of solution as that used for extraction.

Nitrate levels obtained by distilled water extraction correlated well with those obtained by the reduction procedure but were consistently higher. The latter was probably caused by positive interference of organic anions. With this system, the activity rather than the concentration of NO_3^- must be plotted against the measured potential to obtain linearity. It is also necessary to estimate the ionic strength of the extracts to establish the NO_3^- activity coefficient and then convert to concentration.

The recently published procedure of Paul and Carlson (1968), which appeared while this work was in progress, was investigated. They propose extracting the plant tissue, in the presence of an Al-saturated cation exchange resin, with distilled water. Nitrate standards are made up in a 0.01*M* KH_2PO_4 solution. With this system the activity coefficient (γ) of NO_3^- in the standards varies slightly ($\gamma \simeq 0.900$ at 1 μg. per ml. NO_3^--N, $\gamma \simeq 0.875$ at 100 μg. per ml. NO_3^--N) so that the standard curve is not linear when plotted against NO_3^- concentration. Further, the activity coefficient of NO_3^- in plant extracts is estimated to vary between 0.865 and 0.925 because of variations in ionic strengths. Paul and Carlson

Western Washington Research and Extension Center, Puyallup, Wash. 98371

estimate that salt levels in their extracts varied between 0.005 and 0.02*N*. Their idea of using Al^{+3} to lower the pH of the extract which, in turn, depresses the ionization of weak acids whose dissociated anions may interfere, appears sound. They also state that Al^{+3} complexes many organic acids.

It was reasoned that a practical extracting solution would be one that buffered the pH at a low enough level to depress the ionization of weak acids and have a sufficiently high ionic strength that the small increases caused by solutes in plant tissue and standards would be negligible. Several buffers were investigated. A 0.15*M* KH_2PO_4, 0.12*M* H_3PO_4 buffer (pH 2.2) worked as well as the proposed 0.025*M* $Al_2(SO_4)_3$ buffer except that an erroneously high NO_3^- level was measured on an alfalfa sample. The calculated ionic strength of 0.025*M* $Al_2(SO_4)_3$ is 0.375*M*. The ionic strength of 10 and 50 μg. per ml. NO_3^--N standards were varied from 0.375 to 0.405*M* with Al_2SO_4. No change in the activity of NO_3^- could be detected with the Orion electrode. Greater than a 0.03*M* increase in ionic strength was not attempted because the authors have found that plant tissue samples rarely result in as much as a 0.015*M* increase in ionic strength in the extracts obtained at the proposed extraction ratio. Increases of 0.03*M* might be obtained from some plants grown on soils high in soluble salts. A plot of potential *vs.* NO_3^- concentration was linear between 10 and 100 μg. per ml. of NO_3^--N when standards were made up in 0.025*M* $Al_2(SO_4)_3$ indicating that variation in the KNO_3 concentrations had no measurable effect on the activity coefficients. The pH of the proposed extracting solution was 2.95 and plant tissue extracts were 0.05 to 0.13 pH units higher. It is difficult to make a precise concentration of $Al_2(SO_4)_3$ from reagent grade $Al_2(SO_4)_3 \cdot 18\ H_2O$. According to the J. T. Baker Chemical Catalog 660, the $Al_2(SO_4)_3 \cdot 18\ H_2O$ assay may vary between 99 and 105%. The $Al_2(SO_4)_3$ concentration need not be precise, but it is important that the concentration be the same in the extracting solution and NO_3^- standards. Each of these solutions should be made by dilution of aliquots of the same, more concentrated $Al_2(SO_4)_3$ solution.

It is convenient to maintain a 10 μg. per ml. concentration of NO_3^--N in the extracting solution for three reasons. The response is linear above this concentration of N making it possible to read the NO_3^- concentration directly on the logarithmic scale of the pH meter using the expanded pH function. At NO_3^--N concentrations as low as 1 to 3 μg. per ml., long periods of equilibration are required to obtain a stable reading. Often the drift in the potential reading appears indeterminate. At a concentration as low as 5 μg. per ml., only 10 to 40 seconds are required to obtain a stable reading. The longer period is required when the solution read previously deviates markedly in the NO_3^- concentration. As discussed below, the effect of interfering anions is depressed at the higher NO_3^- concentrations.

At 10 μg. per ml. of NO_3^--N a 1 mv. decrease in potential equals 0.4 μg. per ml. (or 0.004% NO_3^--N in the plant tissue at the proposed extraction ratio). This is adequate sensitivity for most purposes. During normal operations a precision of ±0.4 mv. can be expected. If the pH function is used, even greater precision can be expected as the meter can be adjusted for drift. At lower NO_3^- concentrations, greater sensitivity is obtained.

Preservative. This solution prevents biological changes in NO_3^- concentrations, making it possible to prepare large volumes of the standards which can be stored at room temperature. Tests indicate that the preservative in the extracting solution prevents changes in the NO_3^- concentrations of plant extracts stored for 64 hours at room temperature. Longer storage was not attempted. No interference could be detected from twice the recommended concentration of preservative.

Temperature Control. It is important that extracts and standards attain room temperature in the sealed beakers because the measured potential is a function of the solution temperature. Not only does evaporation from an open beaker cause concentration of the NO_3^- but the solution temperature drops below that of the ambient air. An insulating material should be placed on the surface of the magnetic stirrer if it becomes warm with continuous operation.

Electrode Behavior. The slope of the calibration curves was close to the theoretical Nernst slope. The slope did not change as the electrode aged, but the electrode constant became more positive, increasing as much as 20 mv. in 5 months. The latter may be caused by changes in the concentration of salts in the aqueous internal solution of the NO_3^- electrode. When not in use, the electrode tip is immersed in a NO_3^- solution (the proposed extracting solution) as suggested by the manufacturer. It is necessary to calibrate with standards daily because of the variation in the electrode constant and ambient air temperature.

A fiber type saturated KCl, calomel electrode is used as the reference electrode in preference to the sleeve-type Ag, AgCl electrode (Orion Research Inc. Model No. 90-01) because there is considerably more Cl^- contamination of sample solutions from the latter. This occurs even when the KCl concentration in the sleeve-type electrode is one half that of the fiber type. The type of reference electrode used does not affect the slope of the calibration curve but, of course, they have different electrode constants.

Interfering Anions. The manufacturer's instruction manual lists common interfering anions and their selectivity constants. The selectivity constant for NO_3^- is arbitrarily set at unity and the others are relative to it. There are a few anions with selectivity constants approaching or exceeding that of NO_3^-. Of these only I^-, with a selectivity constant of 20, might be present in acidified aqueous extracts of plant tissue in amounts sufficient to interfere. Miller (1958) lists the I_2 concentrations found in common grains and forages. The highest level found was 320 μg. per 454 grams of corn grain. If all of this were extracted as I^- at the proposed extraction ratio, the I^- concentration would be $5.5 \times 10^{-8}M$. In the presence of 10 μg. per ml. of NO_3^--N, this should cause an apparent increase in NO_3^--N of 0.015 μg. per ml., an amount too small to measure.

In a similar manner, it can be calculated that $H_2PO_4^-$ and SO_4^{-2} in plant extracts should not interfere. Although their concentrations are apt to be much higher than I^-, their selectivity constants are very low. When the proposed procedure is used, the SO_4^{-2} level in the extracting solution and standards is high enough to interfere but it is uniform at any one concentration of NO_3^-.

Chloride is possibly the most serious interfering ion. Although the selectivity constant is only 6×10^{-3} (the value given in the instruction manual), it is generally the most abundant inorganic anion extracted from plant tissue. Paul and Carlson (1968) state that normal plant samples contain between 0.5 and 2.0% Cl^- and that above 10 μg. per ml. of NO_3^--N in the extract interference from Cl^- is small even at levels of 5% tissue Cl^- or greater, and that its removal is not necessary. They propose the use of Ag-saturated resin to eliminate Cl^- interference on samples that are low in NO_3^-. Silver resin depressed the apparent NO_3^- concentration below that obtained in the absence of Cl^-

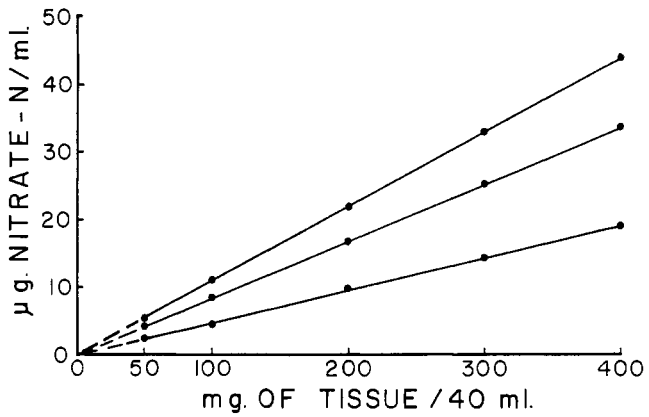

Figure 1. Effect of dilution on concentration of NO_3^- determined in extracts of three plant samples minus 10 μg. per ml. of nitrate-N initially present in extracting solution

Table I. Effect of Cl^- Concentration on Apparent Increase in NO_3^- Concentration[a]

	Apparent Increase in NO_3^--N, μg./ml.			
	Distilled Water[b]		0.025*M* $Al_2(SO_4)_3$[b]	
	Cl^-, μg./ml.		Cl^-, μg./ml.	
NO_3^--N, μg./ml.	100	500	100	500
3	0.8	2.2	0.9	2.4
5	0.6	2.4	0.9	2.5
10	0.5	1.6	0.7	2.2
20	0.4	1.2	0.7	1.8
30	0.3	0.8	0.6	1.3

[a] KCl and KNO_3 were used to make the NO_3^- and Cl^- standards At the proposed extraction ratio, any of the values for NO_3^--N or Cl^- concentration can be interpreted in terms of % in the plant tissue by multiplying by 0.01. [b] The solutions were made in both distilled water and in 0.025*M* $Al_2(SO_4)_3$.

interference. Thus they suggest that Ag^+ removes interference other than Cl^-. When testing their procedure on plant tissue, these writers found that Ag-resin depressed the apparent NO_3^- level more at high NO_3^- concentrations than at low ones. This is opposite to the expected behavior of interfering anions. It was also noted that the activity of NO_3^- appeared lower in standards made with $AgNO_3$ than those made with KNO_3 and the disparity increased proportionally with the NO_3^- concentration. It thus does not appear expedient to attempt to use Ag to eliminate halide interference.

Tests with standard solutions (Table I) indicate that Cl^- interference is inversely proportional to the NO_3^- concentration, but it does not become negligible at 10 μg. per ml. of NO_3^--N as predicted by Paul and Carlson (1968). It is possible that they neglected to correct for the change in the activity coefficient that accompanies the higher salt level required to attain various Cl^- levels. Indeed, in the distilled H_2O system, the apparent activity of NO_3^- decreased with added Cl^- at 20 and 30 μg. per ml. of NO_3^--N. The amount of interference per unit of Cl^- decreased as the Cl^- concentration increased. Chloride interference was slightly but consistently greater in the proposed $Al_2(SO_4)_3$ solution than in distilled H_2O. Similar data obtained with an electrode in which the filling solution, membrane, and liquid ion exchanger had not been changed for 5 months indicated that Cl^- interference was about twice as high as that shown in Table I. The manufacturer suggests that the above components be changed every 2 months. The data in Table I were obtained with an electrode in which these components were a week old.

RESULTS AND DISCUSSION

Plant tissue NO_3^- obtained with the proposed procedure was compared (Table II) to that obtained by the above mentioned reduction procedure and the colorimetric brucine method B (Baker, 1967). Tests indicate that recovery of added NO_3^- tend to be slightly more complete with the reduction and potentiometric procedures than with the brucine procedure. The poorest recovery of added NO_3^- (89% recovery) was obtained on sample 11 by the brucine procedure. Samples 1 through 9 (Table II) are orchardgrass samples from a N fertilizer study. Samples 1 and 3 contained about 30% volunteer white clover and weeds. Sample 10 is alfalfa and 11 is a mixture of many grass species, clover, and weeds. Agreement is good for samples 4 through 11 except on sample 11 where the brucine NO_3^- level is low. The NO_3^- levels obtained by the potentiometric procedure on samples 1, 2, and 3 display large percentage errors but only small positive errors in the actual amount of NO_3^-. This error is probably due mostly to Cl^- interference. Samples 1 through 3 contained 0.78 to 0.81% extractable Cl^- while sample 4 had 0.36%. Low NO_3^- samples displayed higher apparent NO_3^- levels as the NO_3^- concentration in the extracting solution was decreased. This would be predicted from the Cl^- interference data in Table I. The four separate determinations made on extracts containing an initial 10 μg. per ml. of NO_3^--N indicate that the precision of the procedure is good. The data in Figure 1 also attest to the ruggedness

Table II. Tissue Nitrate Levels Determined by the Potentiometric, the Brucine, and a Reduction Procedure

	Per Cent NO_3^--N in Plant Tissue							
			Potentiometric Procedure[a]					
Sample No.	Brucine	Reduction	1	2	3	4	5	6
1	0.003	0.005	0.012	0.011	0.012	0.011	0.018	0.021
2	0.006	0.006	0.013	0.013	0.013	0.011	0.017	0.021
3	0.005	0.010	0.015	0.015	0.017	0.013	0.018	0.020
4	0.011	0.013	0.014	0.015	0.016	0.015	0.018	0.018
5	0.022	0.025	0.022	0.022	0.024	0.023	0.026	0.027
6	0.059	0.061	0.060	0.059	0.060	0.063	0.062	0.062
7	0.184	0.185	0.187	0.188	0.192	0.192	0.186	0.187
8	0.329	0.337	0.332	0.333	0.338	0.341	0.332	0.332
9	0.433	0.434	0.433	0.432	0.442	0.435	0.427	0.432
10	0.023	0.025	0.022	0.023	0.024	0.022	0.025	0.026
11	0.048	0.056	0.055	0.056	—	—	0.057	0.057

[a] Numbers represent separate determinations. In determinations 1, 2, 3, and 4 the extracting solution contained 10 μg./ml. of NO_3^--N; in 5, 5 μg./ml. and in 6, 3 μg./ml. of NO_3^--N.

of the procedure. Fifty to 400 mg. of the three orchardgrass samples (7, 8, and 9 in Table II) were extracted with 40 ml. of the proposed extracting solution. Only a few points deviated slightly from a straight line when the concentration of NO_3^--N in the extracts minus the 10 μg. per ml. initially present was plotted against the mg. of plant tissue extracted. Interpolation of lines indicate that they all intersect at the origin.

The procedure has been used for NO_3^- analysis on a large number of grass, field corn, corn silage, and strawberry petiole samples. Analyses made by the reduction procedure on selected samples of each of these materials indicate agreement comparable to that in Table II. The simplicity, rapidity, precision, and apparent accuracy should commend its use for routine analyses. The positive error inherent in the procedure for samples that are very low in NO_3^- or very high in Cl^- should be considered. This should not be a problem when one is interested in toxic levels of NO_3^- in animal feeds. The proposed procedure should also be practical for determining critical response levels of NO_3^- on many crops. Usually plant samples for this purpose are taken at an early stage of growth when the critical NO_3^- level is highest. Soluble Cl^- generally accumulates as plants age.

ACKNOWLEDGMENT

Martin S. Frant, Orion Research Inc., 11 Blackstone St., Cambridge, Mass. 02139, is credited with suggesting the preservative solution.

LITERATURE CITED

Baker, A. S., J. AGR. FOOD CHEM. **15,** 802 (1967).
Black, C. A., "Methods of Soil Analysis," part 2, pp. 1195–98, Am. Soc. Agron., Madison, Wis., 1965.
Miller, D. F., Publication 585, Natl. Acad. Sci. Natl. Res. Council, Wash., D. C. (1958).
Moodie, C. D., Cheng, H. H., Dept. of Agronomy, Washington State University, Pullman, personal communication, 1967.
Paul, J. L., Carlson, R. M., J. AGR. FOOD CHEM. **16,** 766 (1968).

Received for review February 20, 1969. Accepted June 27, 1969. Scientific Paper No. 3232, College of Agriculture, Washington State University, Pullman, Wash. Work conducted under Project 1425.