

## DETERMINATION OF NITRATE IN PLANT TISSUE

The nitrate in plant tissue is extracted by shaking with distilled water and an ion exchange resin.

### Equipment Required:

1. EDT directION Model DR359TX Ion Meter or pH Meter with millivolt scale
2. EDT directION Nitrate Combination I.S.E. (3021)

### Reagents Required:

Aluminium resin - Weigh out a known amount of Dowex 50-X8 (50 - 100 mesh) hydrogen-saturated resin and transfer to a beaker. Add 2.2 gm of  $\text{Al}_2(\text{SO}_4)_3$  per 10 gm of resin. Make a slurry by adding a small amount of distilled water. Filter the mixture under low suction or by gravity. Test for excess aluminium salt by rinsing the mixture in the funnel with distilled water. Transfer a sample of the filtrate to a test tube and add 1-2 drops of dilute  $\text{BaCl}_2$ . If precipitate forms, salt is still present in the resin mixture. Repeat the rinsing and testing procedure until no precipitate forms. Store the aluminium resin in a stoppered cylindrical bottle.

Silver resin - If plant tissue samples have a chloride level greater than 2% by weight, and a nitrate level less than 500 ppm  $\text{NO}_3^-$ . The addition of silver resin to the sample will remove chloride levels as high as 6%. The silver resin is prepared in the same way as the aluminium resin, except that 7 gm of  $\text{AgNO}_3$  are added per 10 gm of resin, in place of the  $\text{Al}_2(\text{SO}_4)_3$ . To test for excess silver salts in the mixture, rinse it with distilled water. Add a small amount of  $\text{NaCl}$  to the filtrate. If no precipitate forms, the resin is salt free. If precipitate does form, repeat the rinsing and test procedure until no precipitate forms.

Preservation solution - Dissolve 0.1 gm of phenylmercuric acetate in 20 ml of dioxane. Dilute to 100 ml with distilled water. Add 1 ml of this solution to each litre of distilled water used to prepare all samples and standards.

### Ionic Strength Adjustment Buffer (ISAB)

Dissolve 13.61 gm of  $\text{KH}_2\text{PO}_4$  in distilled water, make up to 1 litre with distilled water. Add 100 ml of ISAB to each 1 litre of distilled water used to make up standards.

### Stock Nitrate solution 1000 ppm

Dissolve 1.631-gm of A.R. grade  $\text{KNO}_3$  in distilled water, make up to 1 litre with the distilled water. Prepare standards of 100, 10 and 1 ppm by serial dilution of the 1000 ppm stock solution in distilled water to which ISAB and Preservation solution have been added.

### Sample Preparation:

Dry a suitable quantity of plant tissue sample in an air-forced furnace as explained in Procedure No. 6.002(a) of the Official Methods of the Association of Official Agricultural Chemists.

Weigh out 0.400 gm of dried, ground plant tissue and transfer to 125 ml Erlenmeyer flask. Add 50 ml of distilled water to the flask. Using 6 mm glass tubing, collect approximately 1-1.5 ml of aluminium resin in the tube by pressing the tube upright into the resin. Dispense the resin into the Erlenmeyer flask by blowing out the tube. (Repeat this collection procedure for the silver resin, if silver resin is also to be added to the sample). Stopper the flask and shake for ten minutes. Filter the suspension through folded filter paper and collect the filtrate in a 100 ml beaker.

### Method:

Immerse the electrode in each of the standards in increasing concentration steps, rinsing the electrodes with distilled water between standards..

Plot a graph on lin/log graph paper of mV response against standard-concentration.

Immerse the electrodes in the sample, record the mV response and plot sample concentration from the graph.

This determination may be carried out directly in concentration units by use of the "Concentration" modes, on EDT directION pH/Ion analysers.

Calculations:

To calculate the nitrate concentration of the original sample multiply the result obtained from the graph or direct from the display (if using an Ion analyser) by 125.

Interferences:

Bicarbonate interference is removed and interference from organic anions such as citrate is reduced by addition of the Aluminium Resin.

Chloride interference is removed by the addition of the silver resin.

Reference

"Nitrate Determination in Plant Extracts by the Nitrate Electrode" Paul, J.L., and Carison, R.M., J. Ag. Food Chem., 1968, 16(5) 766.