

84-021 AMMONIUM AND NITRATE extracted by 2N KCl**1. Application**

1.1 This method involves an equilibrium extraction of the soil sample with 2N KCl and determination of ammonium and nitrate by an auto analyser system. It appears very suitable as a standard procedure for the determination of exchangeable ammonium, because it is simple and convenient and yields highly reproducible results. Also this extraction procedure is satisfactory for the determination of nitrate and nitrite and it yields extracts which can be safely stored for a short period of time before analysis. A possible defect of this method is that some loss of ammonium by volatilization may occur when 2N KCl is used for analysis of alkaline soils. Note the following points:

- a) Since this extraction is done on a sample at field moisture content it is important to do the analysis immediately after sampling to avoid erroneous results due to rapid changes through ammonification, nitrification, and other microbial processes.
- b) It is difficult to avoid some delay in analysis, therefore transport the samples to the laboratory and store in air tight plastic bags at a cool temperature. However, airtight and cool will give anaerobic conditions and reduction of NO_3 within a few days (1-2) for some samples.

2. Apparatus

- 2.1 Reciprocating shaker (160 vibrations per minute).
- 2.2 Repipet dispensing bottle (accuracy $\pm 1\%$, reproducibility $.1\%$).
- 2.3 125 mL Erlenmeyer flasks.
- 2.4 Filter funnels.
- 2.5 Whatman #2 filter paper or 2V folded.
- 2.6 Drying cans.
- 2.7 Auto-analyser, proper manifold and distillation apparatus for automatic colorimetric determination (Technicon). Colorimeters have flow cells 50 mm for ammonia and 15 mm for nitrate.

3. Reagents

- 3.1 2N Potassium Chloride (KCl): Dilute 149.2 g of potassium chloride in a liter volumetric flask with distilled water.

- 3.2 Lithium acetate buffer: Dissolve 335.7 g of lithium hydroxide ($\text{LiOH}\cdot\text{H}_2\text{O}$) slowly with stirring in 545 mL of glacial acetic acid. Dilute with distilled water until volume is near 2 liters. Allow to cool and check the pH (it should read between 5.0-5.5) adjust if necessary and make to final volume of 2 liters. Filter this solution to remove fine particles.
- 3.3 Ninhydrin: Dissolve 16 g of ninhydrin in 600 mL of dimethyl sulfoxide (DMSO). Add 160 mL lithium acetate buffer. Add 36 mL of glacial acetic acid to a pH of 8.0. Use more acetic acid if necessary. Make up to 1 liter with distilled water. Store in an amber bottle open to air. The reagent is stable for months at room temperature.
- 3.4 Hydrazine Sulfate solution ($\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{SO}_4$): Dilute 0.52 g of hydrazine sulfate in a liter volumetric flask with distilled water. Add 2-3 drops of concentrated sulfuric acid. Store in an acid washed borosilicate glass bottle.
- 3.5 Titanous chloride solution, 20%: Dilute 15 mL of 20% titanous chloride stock solution to 100 mL in volumetric flask with distilled water. This is made up fresh each day.
- 3.6 0.5N Sodium Hydroxide (NaOH): Dilute 20 g of sodium hydroxide in a liter volumetric flask with distilled water.
- 3.7 0.1N Hydrochloric Acid (HCl): Dilute 8.9 mL of concentrated hydrochloric in a liter volumetric flask with distilled water. This acid is used to convert NH_3 gas from the distillation apparatus back to solution.
- 3.8 Levor IV wetting agent.
- 3.9 Standard (ammonium and nitrate - N) solution: Dissolve 2.3596 g of ammonium sulfate (NH_4)₂SO₄ and 3.611 g of potassium nitrate KNO₃ in water, dilute the solution to a volume of 500 mL in a volumetric flask, and mix the solution thoroughly. If pure, dry reagents are used, this solution contains 1000 $\mu\text{g}/\text{mL}$ of ammonium - N and 1000 $\mu\text{g}/\text{mL}$ of nitrate - N. Store this solution in a refrigerator.

4. Procedure

4.1 Moisture Content

- 4.1.1 Weigh 10.0 g of wet soil in a preweighed can.
- 4.1.2 Dry in an oven at 105°C overnight.
- 4.1.3 Cool, weigh and record the weights of the oven dry soil.

4.2 Extraction Procedure

- 4.2.1 Weigh 12.5 g of moist sample into a 125 mL Erlenmeyer flask.
- 4.2.2 Use a repipet dispensing bottle and add 50 mL of 2 N potassium chloride solution and stopper.
- 4.2.3 Shake for 30 minutes at 160 oscillations per minute.
- 4.2.4 Filter gravimetrically through #2 filter paper into a scintillation vial. Store the extracts in a refrigerator until analysed.
- 4.2.5 The extracts can be analysed directly on a Technicon auto-analyser. Dilutions are made when necessary.

4.3 Determination of NH_4 and NO_3 by autoanalyser.

- 4.3.1 Check that the correct filters and aperture are in the colorimeter: 570 m μ and No. 5 aperture (reference slide).
- 4.3.2 Turn on colorimeter lamp and allow at least 30 minutes to warm up before running baseline as in 4.3.10 below.
- 4.3.3 Allow the oil bath to reach 115°C before starting the proportioning pump. This is done because the glass to teflon parts have to be hot to seal the distillation apparatus joints.
- 4.3.4 Place proper manifold on pump and attach all connections.
 - a) manifold to sampler
 - b) manifold to sampler wash receptacle
 - c) manifold, colorimeter, and wash receptacle overflow to waste sink.
 - d) connect manifold to heating bath (115°C) and distillation system
 - e) connect manifold to delay coil in heating bath 95°C
 - f) connect heating bath to jacketed mixer to colorimeter
 - g) connect cooling bath (72°C) to jacketed mixer.
- 4.3.5 Place all reagent lines in distilled water.
- 4.3.6 Stretch manifold pump tubes, lower pump rollers and start pump. Run for at least 5 minutes with lines in water.
- 4.3.7 Arrange standards and samples on sampler, starting at position No. 1 on the inner rim. Insert U-shaped pin in appropriately numbered hole on the outer rim to stop sampler at the last sample.
- 4.3.8 Insert reagent lines in full reagent bottles. Check with diagram for correct connections. Distilled water is used as sample wash.

- 4.3.9 Turn on pen drive of recorder (bottom switch). Top switch must never be turned off.
- 4.3.10 Check zero adjustments with zero aperture on sample side of colorimeter. Adjust pen to 0% transmittance (T) by means of the zero control on the colorimeter. Remove the zero aperture.
- 4.3.11 With reagents running and sample line in distilled water establish a baseline at 100% transmittance (T) by means of the 100% T control on the colorimeter.
- 4.3.12 Check the gain on the recorder and adjust it if necessary.
- 4.3.13 When reagent baseline is satisfactory, switch on sampler.
- 4.3.14 Shut off the sampler after the last sample has been taken, stopping the probe in the wash receptacle (or transfer the probe to a container of distilled water).
- 4.3.15 When the last sample has been recorded, shut off the pen drive (bottom switch) and, if work has been completed the colorimeter lamp.
- 4.3.16 Place sample and reagent lines in detergent solution and run for 15 minutes. Rinse sample and reagent lines with 10% HCl for 15 minutes. Remove sample and reagent lines from acid rinse, place in distilled water and rinse for 15 minutes
- 4.3.17 Stop pump and raise roller assembly.
- 4.3.18 Release tension of pump tubes on manifold.
- 4.3.19 Cap all reagent bottles and return to shelf. Remove all samples from sampler and clean up any spillage.
- 4.3.20 Prepare standard curve from recorded readings of standards and calculate $\text{NO}_3 - \text{N}$ and $\text{NH}_4 - \text{N}$ in samples.

5. Calculations

- 5.1 Moisture factor = $\frac{\text{moist soil}}{\text{dry soil}}$
- 5.2 $\mu\text{g/g NH}_4 \text{ \& NO}_3 = \frac{\mu\text{g/mL in sol'n} \times \text{extractant (mL)} \times \text{moisture factor} \times \text{dil.}}{\text{wt. of soil (g)}}$
- 5.3 $\mu\text{g/g NH}_4 = \frac{\mu\text{g/mL in sol'n} \times \text{extractant (mL)} \times \text{moisture factor} \times \text{dil.}}{\text{wt. of soil (g)}}$

5.4 NH_4 & $\text{NO}_3 - \text{NH}_4 = \mu\text{g/g NO}_3$

6. Precision

6.1 Insufficient data available

7. References

- 7.1 J.R. Quinn, J.G.A. Boisvert, and I. Wood 1973. Semi-automated Ninhydrin assay of Kjeldahl Nitrogen. Analytical Biochemistry 58, 609-614.
- 7.2 Bremner, J.M. 1965. Inorganic forms of nitrogen In Methods of Soil Analysis. C.A. Black, ed. Agron. No. 9, Part 2 pp. 1179-1237.

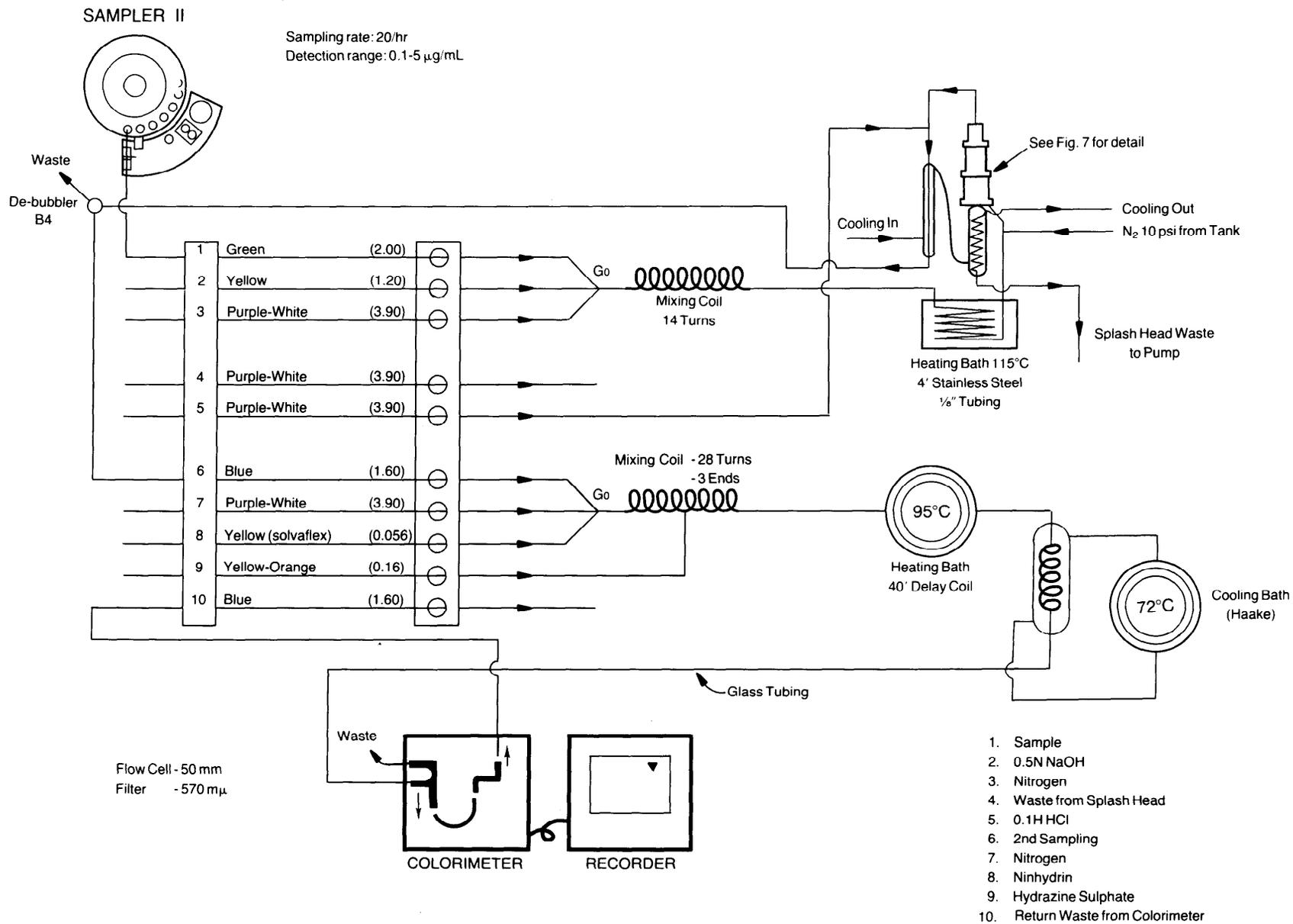


Fig. 5 Flow diagram for the determination of ammonium in 2N potassium chloride extracts by an autoanalyser.

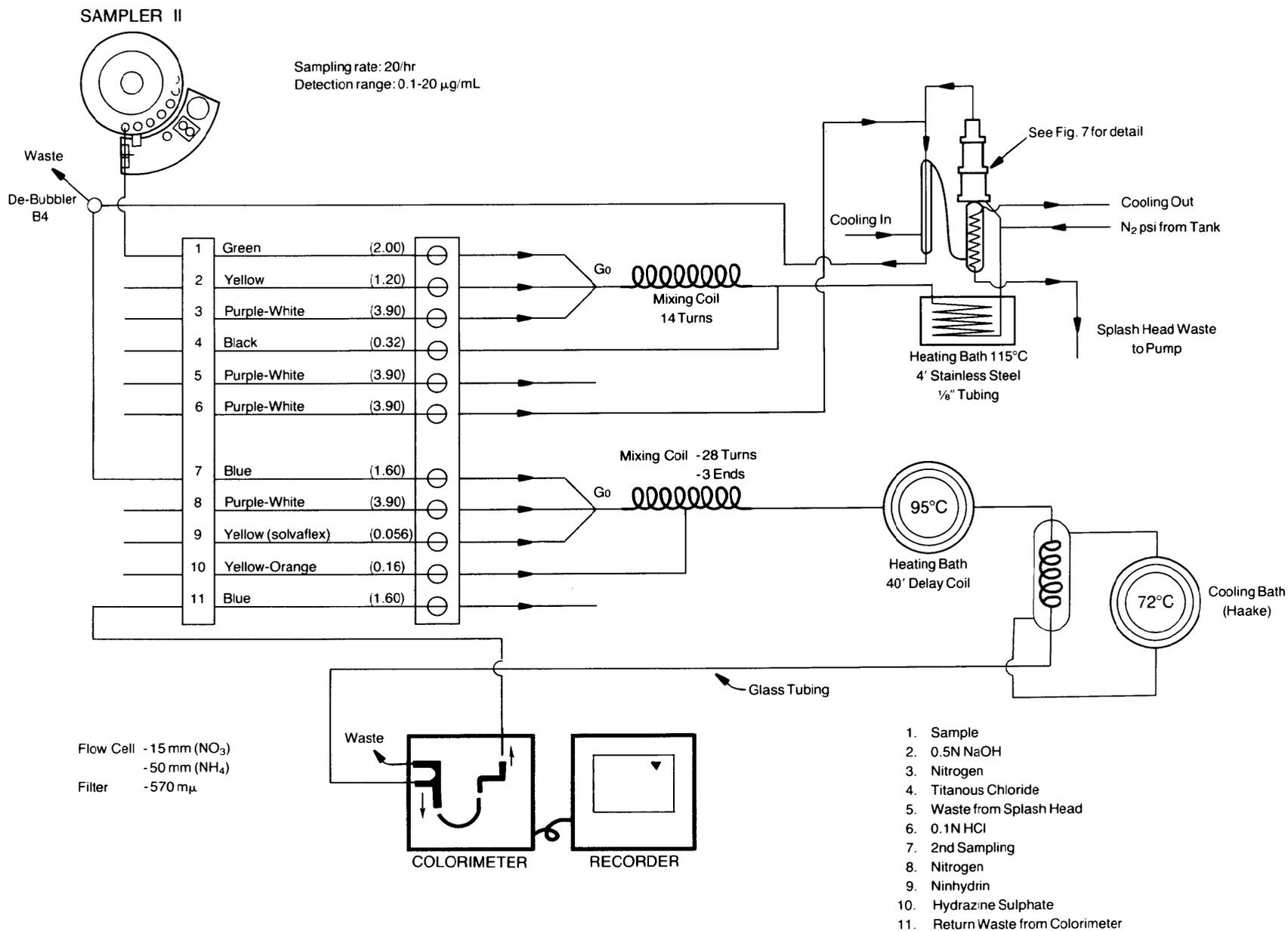


Fig. 6 Flow diagram for the determination of ammonium plus nitrate in 2N potassium chloride extracts by an autoanalyser.

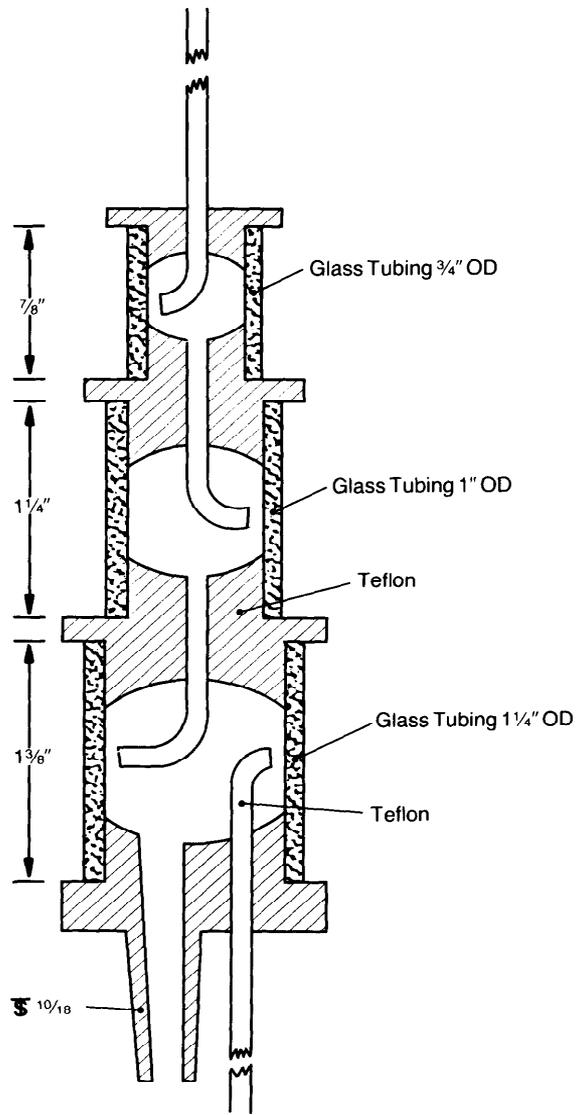


Fig. 7 Distillation apparatus used in the determination of ammonia and nitrate.