UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 20 June 2013	**Version:** ***002***
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SOP_Chem_006 Chemical Analysis_ Nitrate Electrode Method			Page #: ***1 of 4***

Standard Operation Procedure – Nitrate Electrode Method

1. Scope and Application

- Total oxidised nitrogen is the nitrate plus nitrite nitrogen. Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia. Determination of nitrate is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques. Application range for nitrate electrode method includes 0.14 to 1400mg NO_3^- -N/L.

2. Summary

- The NO3- ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger. The electrode responds to NO3- ion activity between 10-5 and 10-1 M (0.14 to 1400 mg NO3- -N/L). The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

3. Apparatus and Glassware

- pH meter, expanded scale or digital, capable of 0.1 mV resolution.
- Double junction reference electrode (Orion model 90-02 or equivalent) Fill outer chamber with (NH4)2SO4 solution.
- Nitrate ion electrode (Orion model 93-07, Corning model 476134 or equivalent).
- Carefully follow manufactures instructions regarding care and storage.
- Magnetic stirrer: TFE- coated stirring bar.

4. Interferences

- Chloride and bicarbonate ions interfere when their weight ratios to NO_3^- -N are >10 or >5, respectively. Ions that are potential interferences but do not normally occur at significant levels in portable waters are NO^{2-}, CN^-, S^{2-}, Br^-, I^-, ClO^{3-}, and ClO^{4-}.

- Although the electrodes function satisfactory in buffers over the range of pH 3 to 9, erratic responses have been noted where pH is not held constant.
- Because the electrode responds to NO_3^- activity rather than concentration, ionic strength must be constant in all samples and standards.
- Minimize this problem by using a buffer solution containing Ag_2SO_4 to remove Cl^-, S^{2-}, Br^-, I^-, and CN, sulfamic acid to remove NO_2^-, a buffer at pH 3 to eliminate HCO_3^- and to maintain a constant pH and ionic strength, and $Al_2(SO4)_3$ to complex organic acids.

5. Collection, Preservation and Storage

- Start nitrate determinations promptly after sampling.
- If storage is necessary, store up to 2 days at 4°C, disinfected samples are stable for much longer without acid preservation.
- For longer storage of unchlorinated samples, preserve with 2ml concentrated H2SO4/L and store at 4°C.
- When sample is preserved with acid nitrite and nitrate cannot be determined as individual species.

6. Safety Precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.
- After the analysis, clean bottles and beakers with water then dry.
- Dispose used gloves after completion of analysis.
- Clean hands using antiseptic soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.
- Prepare and keep colour reagent in fume hood.

7. Sample Preparation –Faecal Sludge

1. Weigh out 2.0000g of well-mixed faecal sludge sample.
2. Blend the weighed sample with 500ml of distilled water in a 1L blender for 30 seconds on the highest speed.
3. Add 250ml distilled water and blend on highest speed until the sample is homogenised (this could range from 30 to 60 seconds).
4. Transfer the blended mixture into a 1L volumetric flask.
5. Add 200ml of blender washings into the flask and top up to 1L with distilled water.
6. Transfer the 1L solution to a plastic bottle and store at 4 °C.

8. Reagents

Stock Nitrate Solution

- Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 hours.
- Dissolve 0.7218g in water and dilute to 1000ml, 1.00ml = 100µg NO3- -N.
- Preserve with 2ml CHCl3/L.
- This solution is stable for at least 6 months.

Standard Nitrate Solution

- Dilute 1.0, 10 and 50ml stock nitrate solution to 100ml with water to obtain standard solutions of 1.0, 10, and 50 mg of NO3- -N/L respectively.

Buffer solution

- Dissolve 17.32g Al2(SO4)3.18 H2O, 3.43g Ag2SO4, 1.28g H3BO3 and 2.52g sulfamic acid (H2NSO3H) in about 800ml water.
- Adjust to pH 3 by slowly adding 1N NaOH.
- Dilute to 1000ml and store in a dark bottle.

Sodium Hydroxide

- NaOH 1N.

Reference Electrode filling solution

- Dissolve 0.53g $(NH4)_2SO_4$ in water and dilute to 100ml.

9. Calibration

- Transfer 10ml of 1mg NO3- -N/L standard to a 50 ml beaker, add 10 ml buffer and stir with a
- magnetic stirrer.
- Immerse tips of electrodes and record millivolt reading when stable (after 1 min).
- Remove electrodes, rinse and blot dry. Repeat for 10-mg NO_3- -N/L and 50 mg NO_3- -N/L standards.
- Plot potential measurements (X axis- in millivolts) against NO_3- -N concentration (Y).
- A straight line with a slope of +57 ±3 mV/decade at 25°C should result.
- Recalibrate electrodes several times daily by checking potential readings of the 10mg NO3- -N standard and adjusting the calibration control until the reading plotted on the calibration curve is displayed again.

10. Procedure

- Transfer 10ml sample to a 50ml beaker, add 10ml buffer solution and stir (1min) with a magnetic stirrer.
- Measure standards and samples at about the same temperature.

- Immerse electrode tips in sample and record potential readings when stable (1min).

11. Waste Disposal

- Collect waste in a 2.5L bottle for Waste Tech collection.

12. Calculation and Data Analysis

- Prepare a standard curve by plotting potential measurements (X axis- in millivolts) against NO3- -N concentration (Y). Compute sample concentration directly from curve.

13. Data Quality

Measurement	10 – 150 mg/l NO_3
Standard Deviation (mg/l COD)	± 1.1
Confidence Interval (mg/l COD)	± 3
Sensitivity (mg/l COD)	2
Accuracy (mg/l COD)	± 5

14. References

- Standards Methods for the Examination of Water and Wastewater, 18th Edition, p. 4-124, Methods 4500 NO3 D. Nitrate Electrode Method (1992)

APPROVAL OF STANDARD OPERATING PROCEDURE

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