UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 20 June 2013	**Version:** ***003***
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SOP_Chem_004 Chemical Analysis_ Spectroquant Total Nitrogen			Page #: ***1 of 4***

Standard Operation Procedure – Total Nitrogen, Spectroquant Method, Cell Test (Cat. No. 1.14763)

1. Scope and Application

- Total nitrogen is the sum of total kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate-nitrite. It can be derived by monitoring for organic nitrogen compounds, free-ammonia, and nitrate-nitrite individually and adding the components together.

2. Summary

- Organic and inorganic nitrogen compounds are transformed into nitrate according to Koroleff's method by treatment with an oxidizing agent in a thermoreactor. In a solution acidified with sulfuric and phosphoric acid, this nitrate reacts with 2,6-dimethylphenol (DMP) to form 4-nitro-2,6-dimethylphenol that is determined photometrically.
- Test measures the total nitrogen, in a concentration range of 10 – 150 mg/l N, of solutions with a maximum of 2% sodium chloride.

3. Apparatus and Glassware

- Spectroquant
- Reaction cells
- Thermoreactor
- Pipettes

4. Interferences

Concentrations of foreign substances in mg/l or %					
Al^{3+}	1000	Hg^{2+}	1000	Surfactants	500
Ca^{2+}	1000	Mg^{2+}	1000	CSB (K-Hydrogen phthalate)	3500
Cd^{2+}	1000	Mn^{2+}	1000		

Cl^-	10000	Ni^{2+}	1000	Na-acetate	10 %
Cr^{3+}	100	Pb^{2+}	1000	NaCl	2 %
$Cr_2O_7^{2-}$	100	PO_4^{3-}	1000	Na_2SO_4	10 %
Cu^{2+}	1000	SiO_3^{2-}	1000		
F^-	1000	Sn^{2+}	1000		
Fe^{3+}	1000	Zn^{2+}	1000		

When the quantity of reagent N-1K is doubled, the tolerable COD increases to 7000 mg/l. In the event of higher COD values, false-low results are obtained.

5. Collection, Preservation and Storage

- Collect faecal samples in 1L plastic buckets.
- Preferably, analyse samples immediately after sampling.
- Store samples at 4 °C or freeze dry samples.
- Preserve wastewater samples by acidifying with concentrated sulphuric acid to pH 2 and faecal samples by freeze drying or freezing.
- Determine TN on well- homogenised samples.
- Check, where necessary, the COD with the Spectroquant® COD Cell Test. In the event of COD values of more than 7000 mg/l, the sample must be diluted with distilled water.
- Reclose the reagent bottles immediately after use.

6. Safety Precautions

- Handle concentrated 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 clear water keep it for drying.
- Dispose the used gloves after completion of analysis.
- Clean the hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

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

- Reagent N-1K
- Reagent N-2K
- Reagent N-3K

9. Calibration

- To check the photometric measurement system (test reagent, measurement device, and handling) and the mode of working, nitrogen (total) solutions, 10.0 mg/l N, and 100 mg/l N can be used.
- Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solutions. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor. Report only those values that fall between the lowest and the highest calibration standards. Dilute and reanalyse samples exceeding the highest standard. Report results in mg/L.

10. Procedure

- Pipette 1 ml of pre-treated sample into an empty cell.
- Add 9 ml of distilled water into cell and mix.
- Add 1 level blue micro spoon of reagent N-1K and mix.
- Add 6 drops of reagent N-2K, close cell and mix.
- Heat the cell at 120˚C in the preheated thermoreactor for 1 hour. Shake the cell briefly after 10 minutes.
- Pipette 1 ml of the digested solution into a reaction cell. Do not mix.
- Pipette 1 ml of reagent N-3K the reaction cell, close the cell and mix. Wear eye protection and hold the cell only at the top.
- Leave the hot reaction to stand for 10 min (reaction time). Do not cool with water.
- Measure in the photometer.

Notes on the measurement:

- Analyse immediately after sampling.
- Reclose the reagent bottles immediately after use.
- For photometric measurement, the cells must be clean. Wipe, if necessary, with a dry paper towel.

- The colour of the measurement solution remains stable for 30 min after the end of the reaction time stated above. (After 60 min, the measurement value would have increased by 5 %).

11. Waste Disposal

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

12. Data Quality

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

13. References

http://www.merckmillipore.com/ZA/en/products/analytics-sample-prep/test-kits-and-photometric-methods/instrumental-test-systems-for-quantitative-analyses/photometric-measurements-spectroquant-system/spectroquant-tests/

APPROVAL OF STANDARD OPERATING PROCEDURE

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