
	Standard Operating Procedure  PRG <small>pollution research group</small>	Effective Date: 20 June 2013	Version: 002
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SOP_Chem_008 Chemical Analysis_ Spectroquant Ammonium Test			Page #: 1 of 4

Standard Operation Procedure – Ammonium Test, Spectroquant Method Cell Test (Cat. No. 1.00683)

1. Scope and Application

- Test measures both ammonium ions and dissolved ammonia in a concentration range of 2 – 150 mg/l NH₄-N.

2. Summary

- Ammonium nitrogen (NH₄-N) occurs partly in the form of ammonium ions and partly as ammonia.
- A pH dependent equilibrium exists between the two forms.
- In strongly alkaline solutions, NH₄-N is present almost entirely as ammonia, which reacts with hypochlorite ions to form monochloramine.
- This in turn reacts with a substituted phenol to form a blue indophenol derivative that is determined photometrically.

3. Apparatus and Glassware

- Spectroquant.
- Pipettes for pipetting volumes of 0.10, 0.20, and 5.0 ml.
- Rectangular cells 10 mm (2 pcs), Cat. No. 114946.

4. Interferences

Concentrations of foreign substances in mg/l or %					
Al ³⁺	1000	Mn ²⁺	100	EDTA	1000
Ca ²⁺	1000	Ni ²⁺	250	Primary Amines	0
Cd ²⁺	1000	NO ₂ ⁻	1000	Secondary Amines	250
CN ⁻	100	Pb ²⁺	1000	Aminophenols	10
Cr ³⁺	100	PO ₄ ²⁻	1000	Aniline	50
Cr ₂ O ₇ ²⁻	1000	S ²⁻	50	Triethanolamine	1000

Cu ²⁺	1000	SiO ₃ ²⁻	1000	Surfactants	1000
F ⁻	1000	Zn ²⁺	500	Na-acetate	10%
Fe ³⁺	25			NaCl	20%
Hg ²⁺	500			NaNO ₃	20%
Mg ²⁺	500			Na ₂ SO ₄	20%

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 NH₄ on well- homogenised samples.
- The pH must be within the range 4 - 13. Adjust, if necessary, with sodium hydroxide or sulfuric acid.
- Filter turbid samples.
- Check the ammonium content with the Merckoquant® Ammonium Test. Samples containing more than 150 mg/l NH₄-N must be diluted with distilled water.

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.
- After the analysis clean the bottles and beakers with distilled water before for drying.
- Dispose any used gloves after completion of analysis.
- Clean hands using antiseptic soap and disinfect with ethanol solution.
- Avoid spillage and contact with skin. In the latter case wash with copious amounts of 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.

- Transfer the 1L solution to a plastic bottle and store at 4 °C.

8. Reagents

- Reagent NH₄-1.
- Reagent NH₄-2 (contains granulate + desiccant capsule).
- Merckoquant® Ammonium Test, Cat. No. 110024.
- Universal indicator strips pH 0 - 14, Cat. No. 109535.
- Sodium hydroxide solution 1 mol/l.
- Sulfuric acid 0.5 mol/l.

9. Calibration

- To calibrate test solutions of 5.0, 10, 50 and 100 mg/l NH₄-N.
- 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. Samples exceeding the highest standard should be diluted and re-analyzed. Report results in mg/L.

10. Procedure

Measuring range of 2.0 – 75.0 mg/l NH₄-N (2.6 – 96.9 mg/l NH₄+):

- Pipette 5.0 ml of reagent NH₄-1, stored between 20°C– 30°C, into a test tube.
- Pipette 0.2 ml of pretreated sample into the test tube and mix.
- Add 1 level blue microspoon of reagent NH₄-2 and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 minutes in a test tube rack, then fill the sample into a 10 mm cell and measure in the photometer.

Measuring range of 5 – 150 mg/l NH₄-N (6 – 193 mg/l NH₄+):

- Pipette 5.0 ml of reagent NH₄-1, stored between 20°C– 30°C, into a test tube.
- Pipette 0.1 ml of pretreated sample into the test tube and mix.
- Add 1 level blue microspoon of reagent NH₄-2 and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 minutes in a test tube rack, then fill the sample into a 10 mm cell and measure in the photometer.

Notes on the measurement:

- Reclose the reagent bottles immediately after use.

- Due to the strong temperature dependence of the colour reaction, the temperature of the reagents should be between 20°C and 30°C.
- Ensure the cells are cleaned, with dry paper towel, for the photometric analysis.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent $\text{NH}_4\text{-2}$.
- The pH of the measurement solution must be within the range 11.5 - 11.8.
- The colour of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.
- In the event of ammonium concentrations exceeding 2500 mg/l, other reaction products are formed and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

11. Chemical Waste Disposal

- Rinse glassware ammonium-free with distilled water; do not use detergent.
- Collect waste in a labelled 2.5L bottle for collection from Waste Tech.

12. Data Quality

Measurement	2 – 75 mg/l $\text{NH}_4\text{-N}$	5 – 150 mg/l $\text{NH}_4\text{-N}$
Standard Deviation (mg/l $\text{NH}_4\text{-N}$)	± 0.49	± 1.0
Confidence Interval (mg/l $\text{NH}_4\text{-N}$)	± 1.2	± 2
Sensitivity (mg/l $\text{NH}_4\text{-N}$)	0.3	1
Accuracy (mg/l $\text{NH}_4\text{-N}$)	± 1.8	± 4.0

13. References

<http://www.merckmillipore.com/ZA/en/products/analytcs-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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