

Lab - 6

1.1 Nitrogen

Ammonical Nitrogen and TKN

Nitrogen is present in water, wastewater and industrial wastewater/effluent samples in various concentrations and forms. In surface water samples, nitrogen is present mainly as nitrate (NO_3^-), which is the most oxidized form of nitrogen. In groundwater samples nitrogen may be present as both nitrate (NO_3^-) and nitrite (NO_2^-). In raw wastewater samples, and in water contaminated with domestic and industrial wastes, nitrogen is mainly present as ammonical nitrogen ($\text{NH}_4/\text{NH}_3\text{-N}$), and organic nitrogen (Org-N). Two types of nitrogen measurements are performed on wastewater samples, (a). Ammonical Nitrogen ($\text{NH}_4\text{-N}$) and (b). Total Kjeldahl Nitrogen (TKN). The method described here measures ammonia only. However, as we know, wastewater contains both ammonia nitrogen ($\text{NH}_3\text{-N}$), and organic nitrogen (Org-N). Boiling of a sample of wastewater in the presence of H_2SO_4 , K_2SO_4 and CuSO_4 , converts the organic matter in the solution to CO_2 and H_2O . The organic nitrogen (Org-N) present in the sample will be converted to ammonia nitrogen ($\text{NH}_3\text{-N}$). TKN is then defined as:

$$\text{TKN} = (\text{NH}_4\text{-N}) + \text{Org-N, expressed in mg/L NH}_4\text{-N.}$$

Theory:

Ammonia combines with Nessler's Reagent to form a yellowish Complex. This complex absorbs light at 410-440 nm. The extent of light absorbance determines the concentration of ammonia in solution.

Estimation of Nitrogen in Water - Part I

- EDTA Reagent: Dissolve 50 g disodium EDTA dihydride in 60 mL of water containing 10 g NaOH. If necessary apply gentle heat to complete dissolution. Cool to room temperature and dilute to 100 mL. Label as, "**EDTA Reagent for Nesslerization**". *This will be available in the lab.*
- Nessler Reagent: Dissolve 10 g HgI_2 and 7 g KI in a small quantity of water and add this mixture slowly, with stirring, to a cool solution of 16 g NaOH dissolved in 50 mL water. Dilute to 100 mL. Store in dark. Label as, "**Nessler Reagent**". *This will be available in the lab.*
- Stock Ammonium Solution: Make a solution with NH_4Cl such that 1 mL = 10 $\mu\text{g NH}_3\text{-N}$ ". *This will be available in the lab.*
- Mercuric Sulphate Solution: Dissolve 8 g of red mercuric oxide in 100 mL of 6N H_2SO_4 . Label, "**Mercuric Oxide for Nitrogen Digestion**". *This will be available in the lab.*
- **Digestion Solution for TKN determination:** Dissolve 13.4 g of K_2SO_4 in 65mL of water and 20 mL of concentrated H_2SO_4 . Add with stirring, 2.5 mL of mercuric sulphate solution prepared earlier. Dilute the combined

solution to 100 mL with water. Label, "**Digestion Solution for TKN Determination**". *This will be available in the lab.*

Estimation of Nitrogen in Water - Part II

- Take 50 mL of wastewater in a Kjeldahl flask. Add 10 mL of the digestion solution to the flask. Add glass beads.
- Boil briskly, until large amounts of white fumes come out. Continue to boil briskly, until the solution turns transparent. Boil for some more time, and then let the solution cool to room temperature. Total boiling time is approximately 45 minutes or more.
- Transfer the sample to a 100 mL beaker. Rinse the contents of the Kjeldahl flask and transfer into the same beaker, Adjust the pH nearly to 7 using 6N NaOH, 1N NaOH & 0.1N NaOH and then makeup the volume to 50 mL, finally filter it and restore to that beaker. Transfer to a beaker (No. 7). Keep this solution for TKN determination.
- Filter & Dilute the given waste water sample in such a way that the expected concentration lies within the calibration range. Transfer to a beaker (No. 8). Keep this solution for ammonical nitrogen determination.

Preparation of standards for calibration curve

Six aliquots must be prepared containing 0, 1, 2, 3, 4 and 5 mg/L of NH₃-N for the preparation of the calibration curve. These are Samples No. 1 - 6.

Preparation of standards: No. 1: 50 mL of distilled water (DW);
No. 2: 5 mL of stock solution (SS) + 45 mL of DW;
No. 3: 10 mL of SS + 40 mL of DW;
No. 4: 15 mL of SS + 35 mL of DW;
No. 5: 20 mL of SS + 30 mL of DW.
No. 6: 25 mL of SS + 25 mL of DW.

You should have prepared such a stock solution where 1ml = 10µg NH₃-N. Thus your calibration range will be 1mg/L – 5mg/L as per the above mentioned calculations for standard preparations.

Measurement of ammonia concentration

- Take Test Tubes to each (Nos. 1-6), add 10 ml of Standard Solutions and (Nos. 7-8), add 10 ml of sample, add 1 drop of EDTA solution and mix well. Add 1 mL of Nessler's reagent to each test tube and mix well.
- Let the colour development proceed for 10 minutes.
- Measure absorbance at 440 nm. For the standards (beaker Nos. 1-6). Then measure the absorbance in beaker Nos. 7 and 8.
- Draw an absorbance versus concentration curve with reading from test tube Nos. 1-6. This is the calibration curve to be used for ammonia and TKN determination in wastewater samples.
- Based on this calibration curve, determine the TKN (No 7) and ammonia (No 8) concentrations in the wastewater sample. Express in mg/L as NH₄-N.

1.2 Phosphorus

Phosphorus occurs in natural waters and in wastewaters are classified as orthophosphates, condensed (pyro-, meta- and other polyphosphates) and organically bound phosphorus. Phosphorus analysis consists of two general steps:

1. Conversion of the phosphorus form of interest to dissolved orthophosphate
2. Colorimetric determination of dissolved orthophosphate.

Estimation of Phosphorus in Water:

Colorimetric Method

➤ Vanadate-Molybdate Reagent :

Solution A: Dissolve **25 g of ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$** in 300 mL of distilled water.

Solution B: Dissolve **1.25 g of ammonium metavanadate, NH_4VO_3** , by heating to boiling in 300 mL distilled water. Cool and add **330 mL of conc. HCl**. Cool solution B to room temperature.

- Pour solution A into solution B, mix and dilute to 1000mL. Label the bottle, "**Vanadate - Molybdate Reagent for Phosphorus Determination**". (*This solution will be made available to you in the laboratory*).
- Make a standard phosphate solution with KH_2PO_4 . Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL such that 1 mL = 50.0 $\mu\text{g PO}_4^{3-}$ - P. (**500 mL**). Store in a plastic bottle. Label the bottle, "**Standard Phosphorus solution, 1 mL = 50.0 $\mu\text{g PO}_4^{3-}$ - P**". *This solution will be made available to you in the laboratory.*

Preparation of wastewater samples for the measurement of ortho, condensed and organic phosphates

Orthophosphates

- Transfer 50 mL of wastewater to a conical flask. This is Sample No 1.

Condensed Phosphates

- Transfer 50 mL of wastewater to a conical flask.
- Add 1 drop of phenolphthalein indicator. If a red colour develops, add 6N HCl drop-wise to discharge colour. Then add 1 mL more of the acid.
- Put the sample in pressure cooker and cook for 30 minutes under pressure.
- Cool and neutralize to a faint pink colour with 6N NaOH solution. Then restore to 50 mL volume, finally filter it and restore to that beaker. This is Sample No 2.

Organic Phosphates

- Transfer 50 mL of the wastewater sample to a Kjeldahl flask.
- Add 1 mL of concentrated sulfuric acid and 5 mL of concentrated nitric acid.
- Digest to a volume of 1-2 mL or further until the solution becomes colourless and white fumes come out to remove nitric acid.
- Cool and add approximately 20 mL of distilled water. Add 1 drop of phenolphthalein indicator and then add 6 N NaOH drop-wise until a pink tinge is seen.
- Make up the final volume to 50 ml, finally filter it and restore to that beaker. This is Sample No 3.

Calibration curve preparation

- Nine aliquots must be prepared containing 0, 5, 10, 15, 20, 25, 30, 40 and 50 mg/L of phosphorus for the preparation of the calibration curve. These are Samples No. 4 - 12.
- The procedure for preparation of aliquots is as follows:

Preparation of standards: No. 1: 50 mL of distilled water (DW);

No. 2: 5 mL of stock solution (SS) + 45 mL of DW;

No. 3: 10 mL of SS + 40 mL of DW;

No. 4: 15 mL of SS + 35 mL of DW;

No. 5: 20 mL of SS + 30 mL of DW.

No. 6: 25 mL of SS + 25 mL of DW.

No. 7: 30 mL of SS + 20 mL of DW.

No. 8: 40 mL of SS + 10 mL of DW.

No. 9: 50 mL of SS.

- Take Test Tubes to each (Nos. 1-9), add 8 ml of Standard Solutions and (Nos. 10-12), add 8 ml of sample, add 2 ml of the vanadate-molybdate reagent and mix well.
- Allow 10 minutes for colour development. Measure absorbance at 470 nm.
- Prepare concentration versus absorbance curve.

Measurement of ortho, condensed and organic phosphates:

- Determine the phosphate concentration in Sample No 1. This is the orthophosphate concentration (A) in wastewater.
- Determine the phosphate concentration in Sample No 2. This is the sum of orthophosphate and condensed phosphate concentration (B). Thus the condensed phosphate concentration is given by (B-A).
- Determine the phosphate concentration in Sample No 3. This is the total phosphorus concentration (C) in water, which is the sum of orthophosphate, condensed phosphate and organic phosphate concentration. Thus organic phosphate concentration is given by (C-B).

Lab Exercise – 6

6A Understanding About Spectrophotometry

1. What is spectroscopy? What is its importance? **(5 Marks)**
2. The absorbance of a 2.31×10^{-5} M solution of a compound is 0.822 at a wavelength of 266 nm in a 1.00 cm cell. Calculate the molar absorptivity at 266 nm. **(10 Marks)**
3. A compound with a molecular mass of 292.16 was dissolved in a 5 mL volumetric flask. A 1.00 mL aliquot was withdrawn, placed in a 10 mL volumetric flask, and diluted to the mark. The absorbance at 340 nm was 0.427 in a 1.00 cm cuvette. The molar absorptivity for this compound at 340 nm is $6130 \text{ M}^{-1} \text{ cm}^{-1}$. Calculate the concentration of the compound in the cuvette. What was the concentration of compound in the 5 mL flask? How many milligrams of compound were used to make the 5 mL solution? **(15 Marks)**

6B Understanding About Nitrogen in Water

1. How does the concentrations of various forms of Nitrogen (e.g. Organic N, Ammonical N, Nitrite N & Nitrate N) present in polluted water change under aerobic conditions with time? Draw a graph. **(10 Marks)**

6C Estimation of Nitrogen in Water

1. 0.200 g of a urea (FW = 60, $(\text{NH}_2)_2\text{CO}$) sample is analysed by the Kjeldahl method. The ammonia is collected in a 50 mL of 0.05 M H_2SO_4 . The excess acid required 3.4 mL of 0.05 M NaOH. Find the percentage by weight of the urea in the sample. **(15 Marks)**
2. A sample of 0.50 gm of an organic compound was treated according to Kjeldahl's method. The ammonia evolved was absorbed in 50 ml of 0.5 M H_2SO_4 . The residual acid required 60 ml of 0.5 M solution of NaOH for neutralization. Find the percentage of nitrogen in the compound. **(15 Marks)**

6D Understanding About Phosphorus in Water

1. What is the difference between orthophosphate, polyphosphates and organic phosphorous? In which form must the phosphorus be for colorimetric analysis? **(10 Marks)**

6E Estimation of Phosphorus in Water

1. How is the analysis for phosphorus conducted to differentiate between the three forms of phosphorus? **(10 Marks)**
2. Measurement of total phosphorus in water can be achieved by acid digestion of the water sample followed by formation of a reduced phosphomolybdate species called molybdenum blue which is intensely coloured. The concentration is determined by measurement of

absorption at 470nm. The following calibration data were used in a phosphate determination in lake water samples:

mg/L	Absorbance at 470 nm
0.000	0.001
0.100	0.122
0.250	0.304
0.500	0.612
1.000	1.216

A 10.00 mL sample of lake water and a 10.00 mL aliquot of MilliQ water (digestion blank) were separately acid digested and each made up to 25.00 ml. The phosphorus in each digest was then treated to form molybdenum blue and absorbance measured. For the lake water sample digest an absorbance of 0.228 was measured, and for the blank digest an absorbance of 0.025 was measured. Calculate the concentration of phosphorus in original lake sample in mg/L.

(10 Marks)