

The Hong Kong Polytechnic University  
Department of Civil and Environmental Engineering  
Water and Waste Laboratories

Laboratory Worksheet C16 : **Nitrogen.**

*by KJ THUNG and WS LAM*

**Objective :** To determine the levels of different forms of nitrogen in water and wastewater samples.

**Introduction**

Nitrogen exists in different forms in the nitrogen cycle. They are present in natural waters as well as domestic and industrial wastewater. Organic and ammonia nitrogen exert an oxygen demand in the receiving water. Excessive ammonia and nitrate nitrogen may cause algal bloom in receiving water.

The follow is a summary of different forms of nitrogen in water.

- a. Total Kjeldahl Nitrogen (TKN) is the combination of ammonia and organic nitrogen.
- b. Ammonia nitrogen (from urea and fecal material).
- c. Nitrate nitrogen ( $\text{NO}_3^-$ ), and
- d. Nitrite nitrogen ( $\text{NO}_2^-$ ).

**Detailed Procedures**

1. Determination of Total Kjeldahl Nitrogen, see Laboratory Worksheet C16a.
2. Determination of Ammonia Nitrogen, see Laboratory Worksheet C16b.
3. Determination of Nitrate Nitrogen, see Laboratory Worksheet C16c.
4. Determination of Nitrite Nitrogen, see Laboratory Worksheet C16d.

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Laboratory Worksheet C16a : **Total Kjeldahl Nitrogen (TKN)**.

*by KJ THUNG and WS LAM*

**Objective :** To determine the concentration of Total Kjeldahl Nitrogen in water and wastewater samples.

### **Introduction**

The result of the method is the sum of organic Nitrogen (protein, nucleic acids, urea, and synthetic organic chemicals) and ammonia Nitrogen. The Kjeldahl's method fails to account, completely or in part, for some forms of Nitrogen: azides, azocompounds, hydrazones, hydrazine, hydroxylamine, nitrite and nitrate.

### **Apparatus and Materials**

- Digestion Heating Block
- Digestion Tubes
- $K_2SO_4$  + HgO Tablets
- Sulfuric Acid ( $H_2SO_4$ ) concentrated
- Receiving Indicator Solution
- Standard 0.01M  $H_2SO_4$  ( 1mL = 0.280 mg  $NH_4-N$ )
- Semi-automatic Steam Distilling Unit
- Measuring Cylinders
- Pipette and pipette filler
- Erlenmeyer flasks
- Standard Titration Setup

### **Procedure**

1. **Sample:** 50 mL of water (0.1 – 0.5 mg of Nitrogen in most cases) in a digestion tube.
2. **Reagents for digestion:** to each sample add in the digestion tube add :  
7 g Potassium sulfate anhydrous ( $K_2SO_4$ )  
350 mg Mercuric oxide, red (HgO), or  
two 3.5g  $K_2SO_4$  tablets with mercury oxide and  
10 mL sulfuric acid ( $H_2SO_4$ ) concentrated
3. **Digestion:** stir mix each digestion tube. Heat for 120 minutes at 370 °C.

4. **Cooling:** let the digestion tube cool to 50-60 °C. After cooling, add 75 mL distilled water into each tube before the content crystallizes.
5. **Distillation:** fill an Erlenmeyer flask with 25 mL of Receiving Solution and place it in the receiving position in the steam distilling unit. Place the digestion tube with digested sample in the steaming position in the steam distilling unit. Press the button to start distillation cycle. The distilling unit will automatically add 50 mL of dilution water and 50 mL of 35% sodium hydroxide and perform the distillation process.
6. **Determine:** titrate the content of Erlenmeyer flask by standard H<sub>2</sub>SO<sub>4</sub>. The endpoint is indicated by the colour change from yellowish green to purple gray.

### Calculations

$$\text{conc. of TKN (mg/L)} = \frac{A \times E}{V}$$

where

*A* = vol. of standard acid used

*E* = mass equivalent of NH<sub>4</sub> – N of standard acid

*V* = sample volume

### Question

1. How will the unaccounted for components in TKN determination affect the total nitrogen estimation and what can be done to account for that?

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Laboratory Worksheet C16b : **Ammonia Nitrogen (NH<sub>4</sub>-N)**.

*by KJ THUNG and WS LAM*

**Objective :** To determine the concentration of Ammonia Nitrogen in water and wastewater samples by selective ion electrode.

### **Apparatus and Materials**

- Ion Selective Analyzer
- Ammonia Electrode
- Magnetic Stirrer
- 10M NaOH Solution
- Measuring Cylinders
- Pipette and pipette filler
- Beakers
- Droppers

### **Procedure**

*Calibration (usually done by the technician)*

1. Place 100 mL of 10 ppm and 100 ppm standard into a separate 100 mL plastic beaker. Add 1 mL of 10 M NaOH to each standard.
2. Enter number of standard (1-5)? PRESS **2**.  
Two points calibration and direct measurement with electrode.
3. Place electrode in 10 ppm standard & stir thoroughly with stirring bar.
4. Wait a few minutes until the meter has shown STD 1 = 10.00, CAL AS 10.00?  
Then PRESS **YES**.
5. Rinse electrode, blot dry, and place in the 100 ppm standard. Stir thoroughly.
6. Wait a few minutes until the meter reading has displayed STD 2 = 100.00 AS  
CAL 100.00? Then PRESS **YES**.

### *Measurement*

1. Transfer 100 mL of sample into a 100 mL beaker. Add 1 mL 10 M NaOH to it.
2. Rinse electrode, blot dry, and place it into the prepared sample.
3. Stir the sample thoroughly with stirring bar.
4. Wait for a few minutes until the meter reading has shown “**READY**”, take the reading of the sample concentration in mg/L.
5. Recalibrate every 1 or 2 hours with the standard solutions if necessary.

### **Calculations**

Concentration of ammonia nitrogen can be directly read from the meter.

### **Question**

1. How can the amount of organic nitrogen be determined by using ammonia nitrogen value indirectly?
2. Is there other way to measure ammonia nitrogen or organic nitrogen?

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Laboratory Worksheet C16c : **Nitrate Nitrogen ( $\text{NO}_3^-$ -N)**.

by WS LAM

**Objective :** To determine the concentration of Nitrate Nitrogen in water and wastewater samples by Cadmium reduction method.

**Apparatus and Materials**

- HACH DR/820 Colorimeter
- HACH NitraVer 5 Pillow
- Sample Cells
- Measuring Cylinders
- Beakers

**Procedure for using the DR/820 colorimeter**

1. Turn on the colorimeter.
2. Press the “PRGM” key and then enter 51 followed by “ENTER”.
3. Fill a sample cell with 10 mL of sample.
4. Add the contents of one NitraVer 5 Pillow to the sample cell and cap the cell.



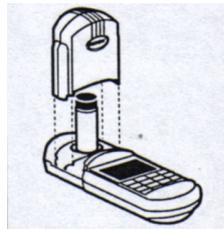
Step 3



Step 4

5. Press “TIMER” then “ENTER”. A one-minute reaction period will begin. Shake the sample cell *vigorously* until the timer beeps.

6. After the timer beeps, the display will show: **5:00 TIMER 2**. Press “ENTER”. A five-minute reaction period will begin. Let the sample cell to stand until the timer beeps.
7. Fill another cell with another 10 mL of the same sample as blank. Wipe off any fingerprints or liquid.
8. Place the blank into the cell holder. Tightly cover the cell with the instrument cap.



Step 8

9. Press “ZERO” to zero the colorimeter.
10. Place the reacted sample into the cell holder. Tightly cover the cell with the instrument cap.
11. Press “READ” and record the nitrate concentration reading of the sample in mg/L.

## Reference

Method 8039, DR/820 Datalogging Colorimeter Handbook, HACH.

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Laboratory Worksheet C16d : **Nitrite Nitrogen ( $\text{NO}_2^-$ -N)**.

*by WS LAM*

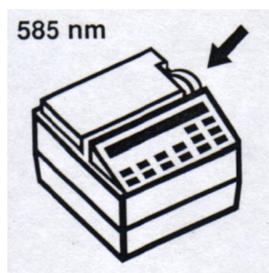
**Objective :** To determine the concentration of Nitrite Nitrogen in water and wastewater samples by Ferrous Sulfate method.

**Apparatus and Materials**

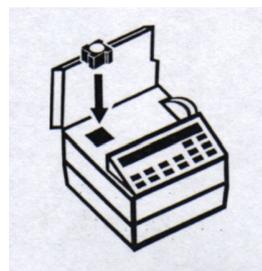
- HACH DR/2010 Spectrophotometer
- HACH NitriVer 2 Pillow
- Sample Cells
- Measuring Cylinders
- Beakers

**Procedure for using the DR/2010 Spectrophotometer**

1. Turn on the spectrophotometer.
2. Press 373 followed by “ENTER”. The display will show: **Dial nm to 585**.
3. Insert the 10 mL Cell Riser into the cell compartment.

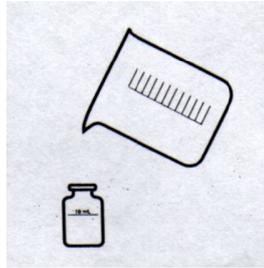


Step 2



Step 3

4. Fill a sample cell with 10 mL of sample.
5. Add the contents of one NitriVer 2 Pillow to the sample cell. Stopper and shake to dissolve.

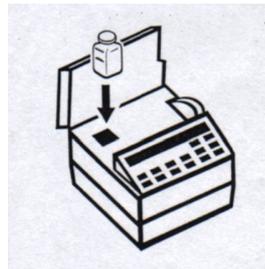


Step 4



Step 5

6. Press “SHIFT” then “TIMER”. A ten-minute reaction period will begin. Let the sample stand and don't disturbed until the timer beeps.
7. Fill another cell with another 10 mL of the same sample as blank. Wipe off any fingerprints or liquid.
8. Place the blank into the cell holder. Cover the cell with the light shield.



Step 8

9. Press “ZERO” to zero the spectrophotometer.
10. Gently invert the prepared sample twice. Remove the stopper. Place the reacted sample into the cell holder. Close the light shield.
11. Press “READ” and record the nitrite concentration reading of the sample in mg/L.

## Reference

Method 8153, DR/2010 Spectrophotometer Handbook, HACH.