Technical Assistance
HYDROLOGY PROJECT

World Bank & Government of The Netherlands funded

Training module # WQ - 37

How to measure Oxidised Nitrogen: Cd-reduction and UV Spectrophotometric methods

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CSMRS Building, 4th Floor, Olof Palme Marg, Hauz Khas,
New Delhi – 11 00 16 India
Tel: 68 61 681 / 84 Fax: (+ 91 11) 68 61 685
E-Mail: dhvdelft@del2.vsnl.net.in

DHV Consultants BV & DELFT HYDRAULICS
with
HALCROW, TAHAL, CES, ORG & JPS

Table of contents

1. Module context

This module concerns measurement of oxidised form of nitrogen in water by Cd-reduction and UV spectrophotometric methods. Other related modules are listed below.

While designing a training course, the relationship between this module and the others, would be maintained by keeping them close together in the syllabus and place them in a logical sequence. The actual selection of the topics and the depth of training would, of course, depend on the training needs of the participants, i.e. their knowledge level and skills performance upon the start of the course.

No.	Module title	Code	Objectives
1.	Basic water quality concepts	**WQ - 01**	• Discuss the common water quality parameters • List important water quality issues
2.	Basic chemistry concepts	**WQ - 02**	• Convert units from one to another • Discuss the basic concepts of quantitative chemistry • Report analytical results with the correct number of significant digits.
3.	Advanced aquatic chemistry: solubility equilibria	**WQ – 29**	• Explain the principles of chemical equilibrium • Define solubility product and explain how this relates to water quality assessment • Define the octanol-water partition coefficient and explain how this relates to water quality assessment.
4.	Use of ion selective probes	**WQ - 33**	• Precautions required in use of ion selective probes
5.	Absorption Spectroscopy	**WQ - 34**	• Understand the principles of absorption spectroscopy • Explain the use of absorption spectroscopy for chemical analyses

2. Module profile

Title	:	How to measure Oxidised Nitrogen: Cd-reduction and UV Spectrophotometric methods
Target group	:	HIS function(s): Q2, Q3, Q5, Q6
Duration	:	One lecture session of 30 min., one laboratory session of 120 min. and one concluding session of 30 min.
Objectives	:	After the training the participants will be able to*:* • measure oxidised nitrogen by Cd-reduction and UV spectrophotometric methods • appreciate limitations of the UV method
Key concepts	:	• Cd-reduction Method • UV spectrophotometric method
Training methods	:	Lecture, Laboratory
Training tools required	:	Board, flipchart, OHS, chemical laboratory, spectrophotometer
Handouts	:	As provided in this module
Further reading and references	:	• Chemistry for environmental engineers - C. N. Sawyer, P. L. McCarty & G. F. Parkin, McGraw - Hill, Inc., 1994 • Standard methods for the examination of water and wastewaters, AWWA, 19th edition, 1995

3. Session plan

No	Activities	Time	Tools
1	***Preparations*** • Reagents as in SAPs for measurement of total oxidised nitrogen by Cd-reduction method and measurement of nitrate nitrogen by UV Spectrophotometric method • Samples: A – Tap Water B – Tap water 1L + Stock nitrate (1mL = 100μg NO_3^- - N) 50mL C – Sample B 500 mL + few particles of detergent		
2	***Introduction***: • Describe significance and source of nitrate in water	10 min	OHS
3	***Methods of determination*** • Describe briefly the two methods and the aim of the experiment • Ask participants to read the SAPs	20 min	OHS
4	***Laboratory*** • Divide the class in groups of 2 to 3 persons • Provide stock nitrate solution and ask the participants to prepare atleast 3 standards for each method • While the participants are preparing the standards, demonstrate the working of the spectrophotometer to each group separately • Ask the participants to complete the experiment	120 min	Laboratory
5	***Report and wrap up*** • Ask participants to prepare their report • Discuss results	30 min	

4. Overhead/flipchart master

OHS format guidelines

Type of text	Style	Setting
Headings:	OHS-Title	Arial 30-36, with bottom border line (not: underline)
Text:	OHS-lev1 OHS-lev2	Arial 24-26, maximum two levels
Case:		Sentence case. Avoid full text in UPPERCASE.
Italics:		Use occasionally and in a consistent way
Listings:	OHS-lev1 OHS-lev1-Numbered	Big bullets. Numbers for definite series of steps. Avoid roman numbers and letters.
Colours:		None, as these get lost in photocopying and some colours do not reproduce at all.
Formulas/Equations	OHS-Equation	Use of a table will ease horizontal alignment over more lines (columns) Use equation editor for advanced formatting only

Measurement of Oxidised Nitrogen (1)

- Oxidised Nitrogen present in water in two forms
  - *nitrite*
  - *nitrate*
- High concentrations in water can be problematic
  - *nitrate is thought to be toxic to humans, particularly babies*
  - *oxidised nitrogen is factor in the eutrophication of waters*

Measurement of Oxidised Nitrogen (2)

- Ammonia is released from decomposition of nitrogenous organic matter
- Nitrifying bacteria oxidise ammonia to nitrite and then nitrate
- Nitrite conc. in aquatic environmental samples is usually small.
- Oxidation of ammonia may cause severe depletion of oxygen

Measurement of Oxidised Nitrogen (3)

- Nitrate is lost from water through
  - *uptake by plants*
  - *denitrification*
- Nitrate promotes algal growth
- If phosphate is also present, explosive algal growth can occur (eutrophication)
- Eutrophication can lead to severe water quality problems

Methods of measurement

- Cd reduction method (both NO^-_2 and NO^-_3)
- UV spectrophotometric method (only NO^-_3)
- Ion selective electrode method (only NO^-_3)

Cd-reduction Method:

- Conversion of nitrate to nitrite by reduction
- Measurement of nitrite colorimetrically at 543 nm after developing colour
- Measures both the reduced nitrate and orginally present nitrite
- Only nitrite can be measured without sample reduction
- Nitrate can be calculated by difference
- Interferences
- Range: 0.01 to 1mg NO_3^- - N/L

UV Spectrometric Method

- Nitrate ion and organic matter absorb at 220 nm
- Organic matter only absorbs at 275 nm
- Interferences
  - *Abs. at 275 nm should be < 10% of abs. at 220 nm*
- Simple and less time consuming
- Recommended for unpolluted natural waters

Experiment (1)

- Aim
  - *Determine oxidised nitrogen in different water samples by Cd-reduction and UV methods*
  - *Compare the results obtain by the two methods*

Sample	Source	Probable Concentration
A	Portable water	0 – 2 mg NO_3^- - N/L
B	Surface water	0 – 10 mg NO_3^- - N/L
C	Polluted groundwater	20 - 50 mg NO_3^- - N/L

Experiment (2)

- Report
  - *Applicability of the two methods*
  - *Reason for different results by the two methods*
  - *Comment on water quality*

5. Evaluation sheets

6. Handout

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- Report
  - *Applicability of the two methods*
  - *Reason for different results by the two methods*
  - *Comment on water quality*

Add copy of Main text in chapter 8, for all participants.

7. Additional handout

These handouts are distributed during delivery and contain test questions, answers to questions, special worksheets, optional information, and other matters you would not like to be seen in the regular handouts.

It is a good practice to pre-punch these additional handouts, so the participants can easily insert them in the main handout folder.

8. Main text

Contents

How to measure Oxidised Nitrogen: Cd-reduction and UV Spectrophotometric methods

1. Introduction

Oxidised nitrogen in aquatic environment occurs in two forms i.e. nitrite and nitrate. High concentrations of oxidised nitrogen can cause concern in the aquatic environment for two principal reasons. The first is that oxidised nitrogen is an important factor in the eutrophication of surface waters. The second reason is that they have been associated with methaemoglobinaemia (blue baby disease) in human infants. High nitrate concentrations often make river waters and groundwaters unsuitable for human consumption.

One of the principal sources of oxidised nitrogen in the aquatic environment is organic matter. The nitrogenous part of organic matter (mainly proteins) when decomposed in the aquatic environment by microbes, releases ammonia, which in turn is further oxidised by nitrifying bacteria to nitrite (NO_2^-) and then to nitrate (NO_3^-) in a process known as 'nitrification'. Conversion of ammonia to nitrite is a slower process when compared to conversion of nitrite to nitrate. Therefore in aquatic environment most of the oxidised nitrogen occurs in the form of nitrate nitrogen. This process consumes dissolved oxygen in the water, which can lead to distress or death of aquatic life if minimum dissolved oxygen is not available.

Nitrate is lost from water when plants convert nitrate to organic forms of nitrogen which are taken up by animals, including humans, when they feed on vegetation. It may also be lost through denitrification process under anoxic condition when it is converted to N_2. Because nitrate is an essential plant nutrient it promotes the growth of algae and other aquatic plants in surface waters. If excess concentrations of nitrate and phosphate are present, there is sometimes an unnatural, explosive growth of algae in water bodies; a process known as 'eutrophication'. In eutrophicated waters, the availability of dissolved oxygen to the aquatic life is affected. The algal mass when die, is decomposed by bacteria which leads to reduction in the dissolved oxygen in the water.

It can be seen from the above that nitrate plays an important part in water quality chemistry and hence its determination is vital to the understanding of many water quality processes.

2. Determination Methods

The following three methods are recommended for the measurement of oxidised nitrogen:

a. Cadmium reduction and spectrophotometric determination of ($NO_2^- + NO_3^-$)-N
b. UV spectrophotometric method for NO_3 - N
c. Ion selective electrode method for NO_3 - N

In this module the first two methods will be studied.

Cadmium Reduction Method

In this method the NO_3^- in the sample is first reduced to nitrite quantitavely in the presence of Cd granules treated with $CuSO_4$. The nitrite thus produced and any nitrite present in the sample is reacted with sulphanilmide and N - (1- napthyl) - ethylenedeamine to form a coloured dye which is measured spectrophotometrically at 543 nm.

The method can also be sued to measure only NO_2^- - N directly and determine NO_3 - N by difference.

The applicable range of the method is 0.01 to 1 mg NO_3^- - N/L

Suspended matter, iron, copper and other metals and oil and grease in the sample may cause interference, which can be removed by suitably treating the sample.

UV Spectrophotometric Method:

This method relies on the fact that the nitrate ion absorbs UV radiation at 220 nm. Because dissolved organic matter may also absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement made at 275 nm is used to correct the NO_3^- value. The extent of this correction is related to the nature and concentration of the organic matter. If the correction is more than 10%, this method should not be used.

The method is simple and less time consuming as compared to the first method. However, it is recommended only for uncontaminated natural waters and potable water supplies.

3. Aim

To determine the concentration of NO_3^- - N in different samples of water by Cadmium reduction and UV Spectrophotometric methods and compare the results obtained from the two methods

4. Method

a. Collect a sample from each of the buckets marked A, B and C.

Sample	Source	Probable concentration
A	Potable water	0 - 2 mg NO_3 - N/L
B	Surface water	0 - 10 mg NO_3 - N/L
C	Polluted groundwater	20 - 50 mg NO_3 - N/L

b. Study the SAP for the two methods which you will use for NO_3^- determination.
c. Determine NO_3^- - N by UV Spectrophotometric method first.
d. Using the information from c above, determine NO_3^- - N by Cd reduction method.

Note: You may have to dilute the samples to keep the NO_3^- concentration within the calibration range of each method.

5. Observation and calculation

UV Spectrophotometric Method

a. Fill in the following table as you proceed with the test.

Sample	Absorbance at 220 nm (R)	Absorbance at 275 nm (S)	T = 2S	U = R-T
0.2 mg NO_3^- - N/L standard				
0.4 mg NO_3^- - N/L standard				
0.8 mg NO_3^- - N/L standard				
1.4 mg NO_3^- - N/L standard				
2.0 mg NO_3^- - N/L standard				
3.0 mg NO_3^- - N/L standard				
5.0 mg NO_3^- - N/L standard				
7.0 mg NO_3^- - N/L standard				
Sample A				
Sample B				
Sample C				

b. Use the values of the standard solutions (U) in the table to plot calibration curve of nitrate vs absorbance.
c. Read the nitrate concentration of the three samples from the standard curve for the U values for each sample

Cadmium Reduction Method

a. Fill in the following table as you proceed with the test.

Sample	**Absorbance at 543 nm**
0.05 mg NO_3^- - N/L	
0.2 mg NO_3^- - N/L	
0.4 mg NO_3^- - N/L	
0.6 mg NO_3^- - N/L	
0.8 mg NO_3^- - N/L	
1.0 mg NO_3^- - N/L	
Sample A	
Sample B	
Sample C	

b. Use the values of standard solutions in the table to plot calibration curve of nitrate vs. absorbance.
c. Read the nitrate + nitrite concentration in the samples from the calibration curve.

6. Report

When writing your report the following aspects should be addressed.

- the aim of the investigation
- the results that you have produced
- applicability of the two methods
- reasons for the difference in results if any obtained from the two methods
- the nitrate concentration of the samples and what this could mean in terms of water quality