APPLICATION NOTE

Molecular Spectroscopy

Authors:

Kathryn Lawson-Wood

Ian Robertson

PerkinElmer, Inc.

Seer Green, UK

Water Analysis Using LAMBDA UV-Visible Spectrophotometers: Nitrate-Nitrogen Determination

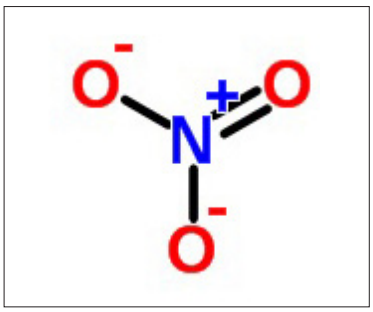

Figure 1. Chemical structure of nitrate. [2]

Introduction

Nitrate (NO_3-), as shown in Figure 1, is more stable than nitrite (NO_2-) and occurs naturally at higher concentrations in water systems. Nitrate is essential to maintain agricultural productivity. However, leaching of excess nitrate from agricultural land into freshwater can cause significant issues such as eutrophication. Eutrophication causes an increase in plant and algal growth, which decreases the dissolved oxygen in the water, often leaving the water uninhabitable to organisms.[1]

In this application, the quantitative analysis of nitrate was performed using the LAMBDA™ 265 UV-Vis spectrophotometer and Merck Spectroquant® cell test. The method used is analogous to DIN 38405-9.

PerkinElmer®
For the Better

Principle

Nitrate ions react with 2,6-dimethyl phenol (DMP) in sulfuric and phosphoric solution to form 2,6-dimethyl-4-nitrophenol (Figure 2) which can be detected spectrophotometrically at 340 nm and is directly proportional to the nitrate-nitrogen concentration. The Merck test kit allows the concentration to be determined without the use of a calibration curve by multiplying the measured absorbance at 340 nm by a known factor.

This method is suitable for the concentration range 0.5 – 25.0 mg/L nitrate-nitrogen (equivalent to 2.2 – 110.7 mg/L nitrate) in a variety of water types including surface, ground and drinking waters. This test kit is not suitable for chloride concentrations exceeding 1000 mg/L, thus cannot be used to determine nitrate-nitrogen concentration in sea water. Any turbid samples must be filtered before analysis and samples with a chemical oxygen demand (COD) above 500 mg/L cannot be analyzed.

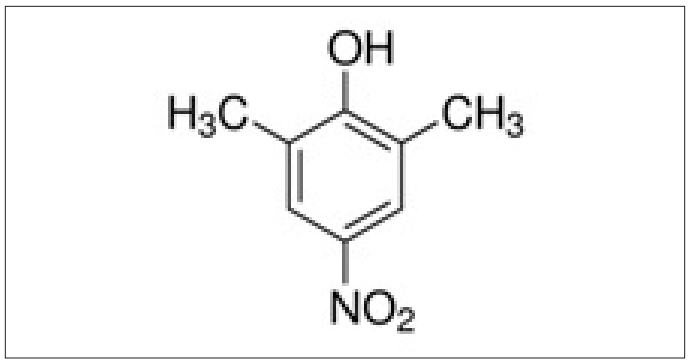

Figure 2. 2,6-dimethyl-4-nitrophenol.[3]

Reagents and Apparatus

1. Merck Spectroquant® nitrate-nitrogen cell test (1.14563.0001) - containing 25 reaction cells, and reagent NO3-1K
2. PerkinElmer LAMBDA 265 PDA UV-Visible Spectrophotometer
3. UV Lab™ software
4. Nitrate nitrogen standard solution (1000 +/- 4 mg/L) supplied by Sigma-Aldrich (53638)
5. Deionised (DI) water
6. Volumetric flasks (100 ml)
7. Micropipettes
8. Cuvettes (10 mm pathlength)

Method

A stock solution of nitrate-nitrogen (1000 mg/L) in water was used to prepare a 15.0 mg/L nitrate-nitrogen solution in a 100 ml volumetric flask by dilution with DI water.

Following preparation of the solution, 1.0 ml of the 15.0 mg/L nitrate-nitrogen solution was added to the reaction cell without mixing the contents. Using a pipette, 1.0 ml of reagent NO_3-1K was placed in the reaction cell, the cell closed, shaken, and left to stand for 10 minutes. The resulting solution color remains stable for 30 minutes after the end of the reaction time. This technique was also carried out for the blank which instead used DI water.

Using the UV Lab software, the LAMBDA 265 instrument parameters were set, as shown in Figure 3, to measure the absorbance at 340 nm, and an equation set up to calculate the nitrate-nitrogen concentration as shown in Equation 1. The factor stated in this equation is specific for a 10 mm pathlength cuvette. Following measurement of the blank, five repeat absorbance readings of the known nitrate-nitrogen solution were recorded after transferring to a 10 mm cuvette.

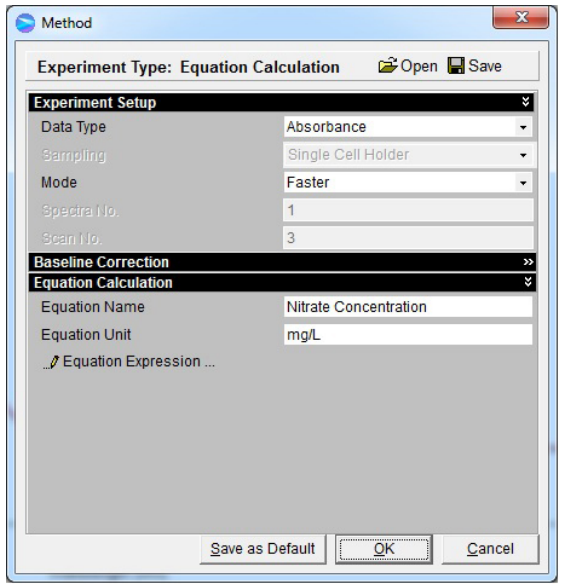

Figure 3. Instrument parameters and method setup.

Equation 1.

Nitrate-nitrogen concentration (mg/L) = A_{340} * 19.9

Results

Figure 4 shows spectra from five repeat runs of the 15.0 mg/L nitrate-nitrogen solution, with the results shown in Table 1. The mean absorbance at 340 nm was determined to be 0.801, correlating to a mean calculated concentration of 15.9 mg/L nitrate-nitrogen using Equation 1. The results obtained had a reasonable degree of accuracy and high level of repeatability, with a relative standard deviation of 0.08%.

Note: If nitrate concentration is required rather than nitrate-nitrogen, multiply the equation result by 4.43.

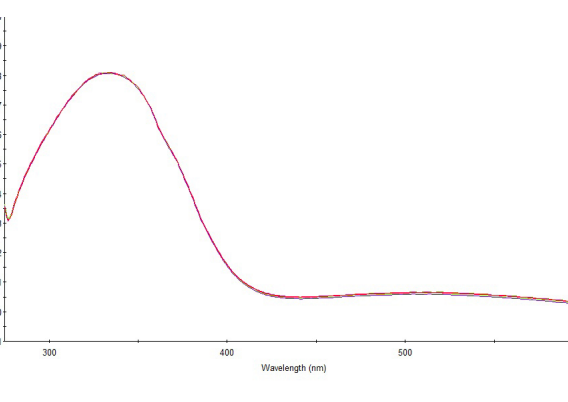

Figure 4. Overlaid UV-Vis spectra of repeat measurements of nitrate solution.

Table 1. Results for repeat measurements.

Nitrate-nitrogen Solution	Absorbance at 340 nm	Nitrate-nitrogen Concentration (mg/L)
Repeat 1	0.800	15.9
Repeat 2	0.801	15.9
Repeat 3	0.801	15.9
Repeat 4	0.800	15.9
Repeat 5	0.801	15.9

Conclusion

Quantitative analysis of nitrate-nitrogen was achieved with a high level of repeatability using the LAMBDA 265 and UV Lab™ software with rapid acquisition of spectra and results. The CHEMetrics nitrate-nitrogen test kit allows reasonably accurate determination of nitrate-nitrogen in water samples within the range 0.5 - 25.0 mg/L without time consuming sample preparation or exposure to hazardous chemicals.

References

1. Wang, L. et al., (2015). *The changing trend in nitrate concentrations in major aquifers due to historical nitrate loading from agricultural land across England and Wales from 1925 to 2150*. Science of The Total Environment
2. http://www.chemspider.com/Chemical-Structure.918.html?rid=40b85dda-b68c-4eb5-a366-07f169b67c93 Chemical Structure of Nitrate
3. http://www.sigmaaldrich.com/catalog/product/aldrich/132713?lang=en&region=US&_sm_au_=iVVT0MD1JNNFvMDH Chemical Structure of 2,6-dimethyl-4-nitrophenol

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

012834_01 PKI