APPLICATION NOTE

ALTE. EL UV/Visible Spectroscopy



Water Analysis using LAMBDA Spectrophotometer: Nitrate Nitrogen (NO₃-N), **Brucine Method**

Introduction

In this application note, the quantitative analysis of Nitrate nitrogen (NO₃-N) was performed by Brucine

method. Data was rapidly acquired using the LAMBDA[™] 465 UV/Vis Spectrophotometer and processed using the UV Lab[™] Software.

Principle

When a water sample containing nitrate ions is treated with brucine in sulfuric acid condition, a yellow compound is created. The quantity of nitrate nitrogen can be determined by measuring the absorbance of the yellow compound at 410 nm.



Reagents and Apparatus

- 1 Nitrate ion standard solution (0.001 mg NO₃-N/mL)
- 2. Unknown sample
- Sodium chloride solution (30 W/V%)
 Dissolve 30 g NaCl in 100 mL D.I water.
- 4. Sulfuric acid solution (4 + 1)- 4 : 1 = Sulfuric acid (H_2SO_4) : D.I water.
- 5. Brucine-Sulfanilic acid solution
 - Dissolve 1 g brucine dihydrate (C₂₃H₂₆N₂O₄ \cdot 2H₂O) and 0.1 g sulfanilic acid (H₂NC₆H₄SO₃H) in 3 mL HCl, dilute to be 100 mL with D.I water.
- 6. LAMBDA 465 (PDA UV/Vis Spectrophotometer)
- 7. UV Lab Software
- 8. Cuvette (10 mm pathlength)

Procedure

- 1. Prepare serial volume (1~10 mL) of nitrogen ion standard solution (0.001 mg NO_3 -N/mL) in 25 mL nessler tubes for standards, dilute to 10 mL with D.I water. Then perform the experiment as following procedure, and prepare calibration curve in a range appropriate for the concentration of the sample.
- 2. Prepare suitable volume of unknown sample in a 25 mL nessler tube.
- 3. Dilute to 10 mL with D.W.
- 4. Add 2 mL sodium chloride solution (30 W/V%).
- 5. Add 10 mL sulfuric acid solution (4 + 1).
- 6. Shake strongly and cool in flowing water.
- 7. Add 0.5 mL brucine-sulfanilic acid solution.
- 8. Mix by shaking, heat 20 min in double boiler.
- 9. Cool with flowing water, dilute to volume with D.I water.
- 10. In Quantification Standard mode, measure the absorbance of the standards with reference to standard 1 (0 ppm) at 410 nm.
- 11. In Quantification Sample mode, measure the absorbance of the unknown sample and calculate its concentration.

Instrument Parameters

The instrument parameters of the LAMBDA 465 are as follows: Figure 1 shows experimental setup.

Experiment Setup

Data type:AbsorbanceSampling:Single cellMode:(Spectra no.: 1/Scan no.: 30/ Integration no.:1/Gain no.: 1)

Experiment Method

Use wavelength: 410 nm Curve dimension: 1

Experiment Setup	
Data Type	Absorbance
	Single Cell Holder
Mode	Fast
	30
	1
Baseline Correction	
Quantification Standard	NO2 N
Analysis Name	NO3_N
Concentration Unit	ppm
Use Wavelength (nm)	410
Standard Replicate No.	1
Sample Replicate No.	1
Curve Zero Offset	Yes
Curve Order	1
Derivative Order	0
Standard Concentration	1

Figure 1. Experimental setup for NO₃-N analysis.

Result

1. Calibration curve

Figure 2 shows spectra of the NO_3 -N standards. Table 1 and Figure 3 show data and the calibration curve for the five standards. The correlation coefficient R^2 is 0.9991.

Table 1. Calibration data of NO₃-N standards.

No.	Name	Concentration (ppm)	AU (410.00 nm)	
1	Standard 1	0.00	0.0005	
2	Standard 2	0.04	0.0158	
3	Standard 3	0.08	0.0383	
4	Standard 4	0.20	0.105	
5	Standard 5	0.40	0.2061	

 $R^2 = 0.99912$

Function : Y = 0.2089 X + 0.0021

2. Unknown sample

The concentration of the unknown sample was determined using the calibration curve of Figure 3. The concentration of the unknown sample is 0.23 ppm (see Table 2).

Table 2. Concentration of unknown sample.

Name	Concentration	Dilution	AU
	(ppm)	Factor	(410.00 nm)
Sample 1	0.23	1.0	0.1155



Conclusion

Using the LAMBDA 465 and UV Lab Software, quantitative

analysis of nitrate nitrogen (NO₃-N) in water was performed.

with LAMBDA 465, generating a good calibration with an R²

quantitative analysis and to process the data efficiently.

Rapid acquirement of spectra and good sensitivity were obtained

with a value of 0.9991. UV Lab Software was used effectively for

Figure 2. The spectra of NO₃-N standards by brucine method.



Figure 3. The calibration curve of NO_3 -N standards.

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