Water Analysis using LAMBDA Spectrophotometer: Nitrate Nitrogen (NO$_3$-N), Brucine Method

Introduction
In this application note, the quantitative analysis of Nitrate nitrogen (NO$_3$-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 NO$_3$-N/mL)  
2. Unknown sample  
3. 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$_2$SO$_4$) : D.I water.  
5. Brucine-Sulfanilic acid solution  
   - Dissolve 1 g brucine dihydrate (C$_23$H$_{26}$N$_2$O$_4$ • 2H$_2$O) and 0.1 g sulfanilic acid (H$_2$NC$_6$H$_4$SO$_3$H) 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 mg/L) 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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Concentration (mg/L)</th>
<th>AU (410.00 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard 1</td>
<td>0.00</td>
<td>0.0005</td>
</tr>
<tr>
<td>2</td>
<td>Standard 2</td>
<td>0.04</td>
<td>0.0158</td>
</tr>
<tr>
<td>3</td>
<td>Standard 3</td>
<td>0.08</td>
<td>0.0383</td>
</tr>
<tr>
<td>4</td>
<td>Standard 4</td>
<td>0.20</td>
<td>0.105</td>
</tr>
<tr>
<td>5</td>
<td>Standard 5</td>
<td>0.40</td>
<td>0.2061</td>
</tr>
</tbody>
</table>

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 mg/L (see Table 2).

<table>
<thead>
<tr>
<th>Name</th>
<th>Concentration (mg/L)</th>
<th>Dilution Factor</th>
<th>AU (410.00 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.23</td>
<td>1.0</td>
<td>0.1155</td>
</tr>
</tbody>
</table>
Conclusion
Using the LAMBDA 465 and UV Lab Software, quantitative analysis of nitrate nitrogen (NO$_3$-N) in water was performed. Rapid acquisition of spectra and good sensitivity were obtained with LAMBDA 465, generating a good calibration with an $R^2$ value of 0.9991. UV Lab Software was used effectively for quantitative analysis and to process the data efficiently.

Figure 2. The spectra of NO$_3$-N standards by brucine method.

Figure 3. The calibration curve of NO$_3$-N standards.