

## Quantitative Analysis of Nitrate and Nitrite in Water Samples using UV-1900i

Zhen Hao Lee, Qi An Tan, Ai Ming Chua  
Centre for Application Solutions, Shimadzu (Asia Pacific) Pte Ltd, Singapore

### User Benefits

- ◆ UV-1900i with CHEMetrics test kit enables easy and accurate measurement of nitrate-nitrogen and nitrite-nitrogen with simple sample preparation.
- ◆ Nitrate-nitrogen and nitrite-nitrogen concentration can be calculated directly using “Advanced Formula” of photometric function in LabSolutions™ UV-Vis.

### Introduction

Nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) are two water soluble nitrogen compounds that occur naturally in the environment. Nitrate is used mainly in inorganic fertilizers as it is essential for plant growth. Nitrite is used for food preservation, such as meat curing due to its antimicrobial effect against pathogenic bacteria [1].

The concentration of nitrate and nitrite in environmental water such as ground water and surface water are usually low. However, accumulation of nitrate and nitrite may occur due to several reasons, such as agricultural run-off and contamination from human or animal wastes. This can lead to potential health risk and negatively affect the environment. For example, excessive nitrate in drinking water could lead to a temporary blood disorder in infants known as blue baby syndrome [2]. Thus, it is important to monitor the level of nitrate and nitrite in various water sources. The U.S. Environmental Protection Agency (US EPA) has fixed a maximum contaminant level (MCL) of 10 mg/L nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) and 1 mg/L nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ) in drinking water.

There are different analytical methods which can be used to measure nitrate and nitrite in water, and one of them is colorimetric method using UV-VIS spectrophotometer. Although it is a simple and convenient method, it involves tedious reagent preparations which is time consuming. This can be overcome using pre-formulated reagent pack or kit. This application news introduces the quantitative analysis of  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  in various water samples using Shimadzu's UV-1900i with CHEMetrics Vacu-vials kit (Fig. 1), based on American Public Health Association (APHA) Standard Method [3, 4] respectively.



Fig. 1 CHEMetrics Vacu-vials Kit

### Experimental

Three types of water samples were used in this analysis: commercially available bottled water, tap water and lake water. The lake water sample was diluted 10 times with

Type E-1 ultra-pure water (Milli-Q Millipore system, Germany) prior to measurement, while the bottled and tap water were measured without any sample pretreatment.

The 1000 ppm  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  standards from Merck, Germany were used to prepare 0.5 ppm  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  standard respectively for validation measurement.

The UV-1900i UV-VIS spectrophotometer (Fig. 2) was used for photometric measurement. Table 1 lists the measurement conditions.



Fig. 2 UV-1900i UV-VIS Spectrophotometer

Table 1 Measurement conditions

Instrument	: UV-1900i
Measurement Mode	: Absorbance
Measurement Wavelength	: 520 nm
Slit Width	: 1.0 nm
Accumulation Time	: 0.1 s

For  $\text{NO}_3\text{-N}$  analysis, a cadmium foil pack from the nitrate test kit was added to a reaction tube containing 15 mL of sample. The mixture was shaken vigorously for 3 minutes, followed by allowing it to sit undistributed for 2 minutes. The treated solution was then poured into a sample cup without transferring any cadmium particles. Subsequently, the tip of the Vacu-vial ampoule was immersed into the sample cup and snapped by pressing against the sample cup. The ampoule was inverted several times to ensure homogenous mixing, followed by wiping it dry. It was then left to stand for 10 minutes before measurement was taken. A background correction was performed using the sealed blank ampoule provided with the test kit before sample measurement.

For  $\text{NO}_2\text{-N}$  analysis, the sample cup was first filled with 25 mL of sample. This is followed by immersing the tip of the Vacu-vial ampoule into the sample cup and snapping the tip. The ampoule was then inverted several times and wiped dry. It was left to stand for 10 minutes before measurement was performed.

To obtain the concentration of NO<sub>3</sub>-N and NO<sub>2</sub>-N, equations (1) and (2) from the test kit was used for calculation as shown below:

$$\text{NO}_3\text{-N (ppm)} = 0.02 + 1.66 (A_{520}) - 0.39 (A_{520})^2 \quad (1)$$

$$\text{NO}_2\text{-N (ppm)} = 1.17 (A_{520}) - 0.67 (A_{520})^2 + 0.24 (A_{520})^3 \quad (2)$$

where A<sub>520</sub> is the absorbance of the solution measured at 520 nm.

In the photometric function of LabSolutions UV-Vis, these calculations based on equation (1) and (2) can be performed directly from measured absorbance values by setting the calculation formula in the Formula window. Fig. 3 shows an example of the formula setting for the calculation of NO<sub>3</sub>-N concentration. The calculated results with the set formula can be displayed automatically.

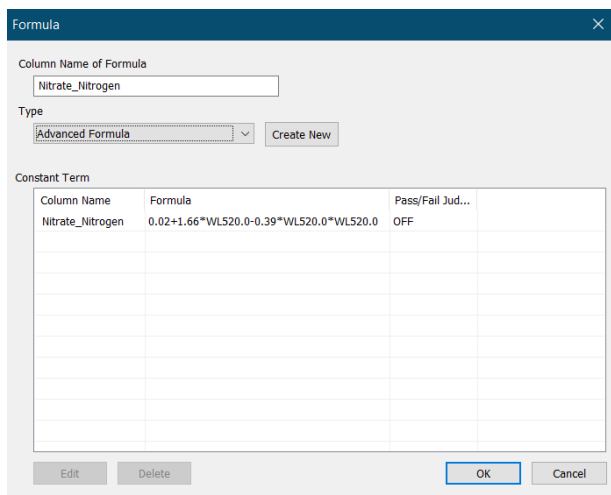


Fig. 3 Formula settings for NO<sub>3</sub>-N calculation

## Results and Discussion

To check accuracy of equations (1) and (2), two standards of 0.5 ppm for NO<sub>3</sub>-N and NO<sub>2</sub>-N were prepared, and 5 replicate measurements were carried out. Table 2 shows the average result of the 5 replicate measurements. A good relative standard deviation (RSD) of less than 1 % was obtained for both NO<sub>3</sub>-N and NO<sub>2</sub>-N, which shows good repeatability. The results were accurate as the recovery percentage was within 5 %.

Table 2 Average 5 replicate measurements of 0.5 ppm NO<sub>3</sub>-N and NO<sub>2</sub>-N standards

Testing Parameter	NO <sub>3</sub> -N	NO <sub>2</sub> -N
Average Abs at 520 nm (A <sub>520</sub> )	0.316	0.585
Average concentration (ppm)	0.505	0.503
RSD (%)	0.998	0.251
Recovery (%)	101.1	100.6

Table 3 shows the average measurement results of 5 replicates for the 3 water samples. In all 3 water samples, the measured concentration of NO<sub>3</sub>-N is much higher than that of NO<sub>2</sub>-N. This is because nitrate is a more stable ion form compared to nitrite. Most nitrogen containing compound in natural water, including nitrite tend to be converted to the nitrate form. Thus, nitrite level are generally low or negligible [5].

Table 3 Measurement results of 3 water samples (5 replicates)

Sample	NO <sub>3</sub> -N (ppm)	NO <sub>2</sub> -N (ppm)
Bottled water	0.718	0.029
Tap water	1.141	0.044
Lake water	1.776	0.318

## Conclusion

The quantitative analysis of NO<sub>3</sub>-N and NO<sub>2</sub>-N in various water samples has been demonstrated in this application news using Shimadzu UV-1900i and CHEMetrics Vacu-vials kit. The analysis of standards with known concentration showed the measurement can be performed with good accuracy and precision. It is also possible to determine the concentration of NO<sub>3</sub>-N and NO<sub>2</sub>-N directly based on the absorbance value using a pre-set calculation formula in photometric function of LabSolutions UV-Vis.

## Reference

- McKnight, G., Duncan, C., Leifert, C., and Golden, M. (1999). Dietary nitrate in man: Friend or foe? *British Journal of Nutrition* 81(5): 349-358.
- Greer, F. R., and Shannon, M. (2005): Infant methemoglobinemia: the role of dietary nitrate in food and water. *Pediatrics* 116 (3): 784-786.
- APHA Standard Methods for the Examination of Water and Wastewater (23<sup>rd</sup> ed) (2017). Method 4500 - NO<sub>3</sub><sup>-</sup>E.
- APHA Standard Methods for the Examination of Water and Wastewater (23<sup>rd</sup> ed) (2017). Method 4500 - NO<sub>2</sub><sup>-</sup>B.
- National Academy of Sciences (1981). The health effects of nitrate, nitrite and N-nitroso compounds. National Academy Press, Washington, DC, USA.

LabSolutions is a trademark of Shimadzu Corporation in Japan and/or other countries.