APPLICATION NOTE

Molecular Spectroscopy

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Water Analysis Using LAMBDA UV-Visible Spectrophotometers: Nitrite Determination

Introduction

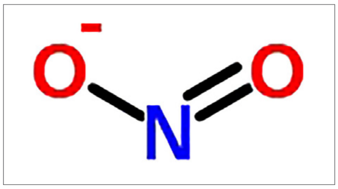

Figure 1 . Chemical structure of nitrite[3].

Nitrite (NO_2^-), as shown in Figure 1, occurs naturally in the environment as an intermediate of the nitrogen cycle through microbial decomposition of organic matter. However, nitrite contains nitrogen in a reasonably unstable oxidation state and readily oxidizes to nitrate (NO_3^-). As a result, nitrite is present at considerably lower concentrations in ground and surface waters than nitrate[1]. Higher concentrations of nitrite are indicative of pollution by industrial wastewater or agricultural run-off. The USEPA established a maximum contaminant level (MCL) of 1 mg/L nitrite-nitrogen in drinking water to help prevent conditions including blue baby syndrome in infants[2].

In this application, the quantitative analysis of nitrite-nitrogen was performed using the LAMBDA™ 265 UV-Vis spectrophotometer and CHEMetrics nitrite test kit.

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Principle

The CHEMetrics nitrite Vacu-vial test kit involves formation of a highly colored azo dye. In an acidic solution, nitrite reacts with a primary aromatic amine in a diazotisation reaction. The product formed is then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) to form a pink-orange azo dye which can be detected spectrophotometrically at 520 nm[4]. The resulting color is directly proportional to the nitrite-nitrogen concentration in the sample. Detection and determination of nitrite (as N) using this method has very little interferences, resulting in a highly selective method. This analysis is suitable for the concentration range of 0 – 1.00 mg/L nitrite-nitrogen in water (equivalent to 0 – 3.30 mg/L nitrite), allowing its concentration to be determined without exposure to hazardous reagents.

Reagents and Apparatus

1. CHEMetrics nitrite-nitrogen Vacu-vials® test kit (K-8503) - containing 30 vials, reference sample, and sample cup
2. PerkinElmer LAMBDA 265 PDA UV-Visible Spectrophotometer
3. UV Lab™ software
4. Nitrite-nitrogen standard solution (1000 +/- 4 mg/L) supplied by Sigma-Aldrich (36427)
5. Deionised (DI) water
6. Volumetric flasks (100 ml)
7. Micropipettes

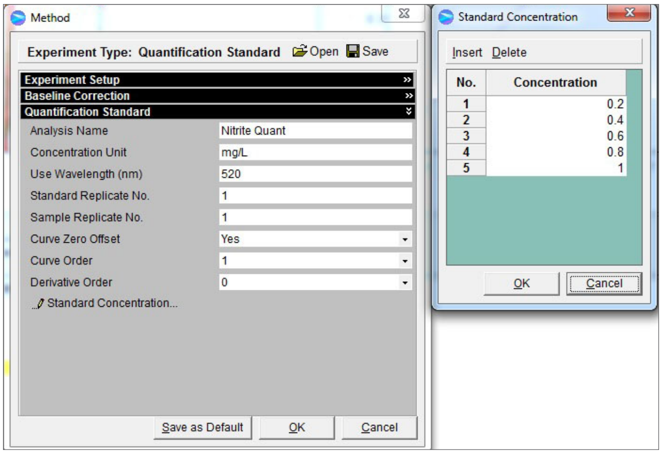
Method

A stock solution of nitrite-nitrogen (1000 mg/L) in water was used to prepare a 50.0 mg/L nitrite-nitrogen working standard in a 100 ml volumetric flask by dilution with DI water. From this working solution, a series of calibration standards within the range 0.2 -1.0 mg/L nitrite-nitrogen (Table 1) in DI water were made up in 100 ml volumetric flasks. A 0.5 mg/L nitrite-nitrogen validation standard was similarly made up.

Table 1. NO_3-N calibration standards.

	NO_3-N concentration (mg/L)
Standard 1	0.2
Standard 2	0.4
Standard 3	0.6
Standard 4	0.8
Standard 5	1.0

Using the UV Lab™ software, the LAMBDA 265 instrument parameters were set, as shown in Figure 2, to measure the absorbance at 520 nm for each of the calibration standards. An alternative method, as stated by the test kit, simply requires the absorbance at 520 nm of the sample of interest to be measured and an equation set up to calculate the nitrite-nitrogen concentration as shown in Equation 1. Although this method avoids producing a calibration curve, it may introduce a large degree of error in results obtained.



Experiment Type: Quantification Standard

Analysis Name: Nitrite Quant
Concentration Unit: mg/L
Use Wavelength (nm): 520
Standard Replicate No.: 1
Sample Replicate No.: 1
Curve Zero Offset: Yes
Curve Order: 1
Derivative Order: 0
Standard Concentration...

No.	Concentration
1	0.2
2	0.4
3	0.6
4	0.8
5	1

Figure 2 . Instrument parameter and method set up.

Equation 1.

Nitrite-nitrogen concentration (mg/L)

$$= -0.06 * (A_{520})^2 + 0.722 * (A_{520}) + 0.009$$

Following preparation of the standards, the sample cup was filled with the lowest nitrite-nitrogen standard up to the 25 mL mark. The tip of the Vacu-vial ampule was placed in the sample cup, snapped, and the ampule then inverted several times to promote mixing. The ampule was dried, left to stand for 10 minutes, and the absorbance measured in the spectrophotometer after performing background correction using the reference sample supplied in the test kit. This method was applied to all standards and a calibration curve produced using the absorbance at 520 nm. The same test procedure was carried out for the 0.5 mg/L nitrite-nitrogen validation standard, with five replicate absorbance measurements.

Results

Spectra and calibration curve of the nitrite-nitrogen standards are shown in Figures 3 and 4 respectively. The linear regression coefficient (R^2) obtained from the calibration curve, displayed in Figure 4, is greater than 0.999 indicating a high level of data correlation.

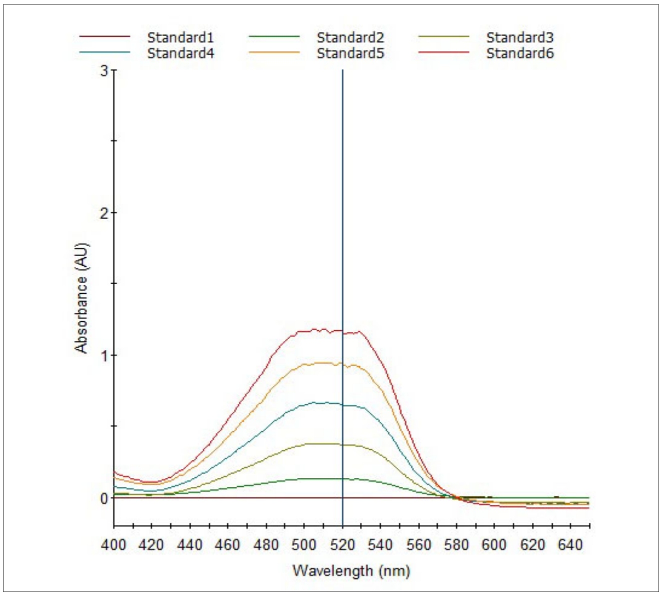


Figure 3 . Nitrite-nitrogen calibration spectra.

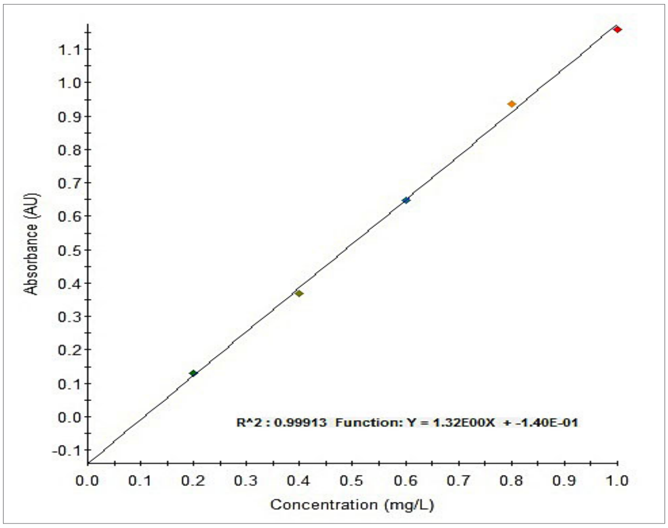


Figure 4. Nitrite-nitrogen calibration curve with displayed R^2 value (0.999) and straight line equation (y= 1.32 x – 0.14)

Figure 5 shows spectra from five repeat runs of the 0.50 mg/L nitrite-nitrogen validation sample, with the results shown in Table 1. The mean absorbance at 520 nm was determined to be 0.511, correlating to a mean calculated concentration of 0.49 mg/L nitrite-nitrogen using the equation of the straight line. The results obtained had a high level of accuracy and repeatability with a relative standard deviation of 0.23 %.

Note: If nitrite concentration is required rather than nitrite-nitrogen, multiply the calculated nitrite-nitrogen concentration by 3.3.

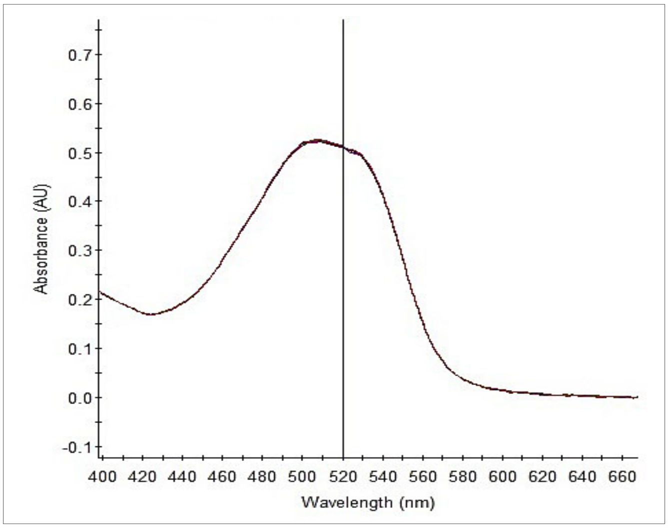


Figure 5. Overlaid UV-Vis spectra of repeat measurements of 0.5 mg/L nitrite-nitrogen validation standard.

Table 2. Results for repeat measurements.

Nitrite-nitrogen Solution	Absorbance at 520 nm	Nitrite-nitrogen Concentration (mg/L)
Repeat 1	0.509	0.49
Repeat 2	0.510	0.49
Repeat 3	0.512	0.50
Repeat 4	0.510	0.49
Repeat 5	0.513	0.50

Conclusion

Quantitative analysis of nitrite-nitrogen was achieved with a high degree of accuracy and repeatability using the LAMBDA 265 and UV Lab™ software with rapid acquisition of spectra and results. The CHEMetrics nitrite-nitrogen test kit allows determination of nitrite-nitrogen in water samples within the range 0 - 1.0 mg/L without time consuming sample preparation or exposure to hazardous chemicals.

References

1. http://www.who.int/water_sanitation_health/dwq/chemicals/nitratenitrite2ndadd.pdf.
2. Yaron, B., Dror, I. and Berkowitz, B. (2012) *Soil-Subsurface Change: Chemical Pollutant Impacts.* Berlin: Springer.
3. http://www.chemspider.com/Chemical-Structure.921.html?rid=308a2d4a-638f-4db8-94ff-4897a52a0cb2.
4. American Public Health Association, *Standard Methods for Examination of Water and Wastewater,* 18th ed., 1992, Method 4500-NO_2^- B.

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