Application note

Quantification of Nitrite and Nitrate in Marine Environments through UV-Visible Analysis

Introduction

Nitrogen plays an important role in a variety of natural processes in the atmosphere and in marine environments.[1,2] These environments can include naturally occurring water systems where marine life are commonly found, including freshwater (ponds, lakes) and salt water (seas, oceans) sources. This interplay of various nitrogen-containing compounds is often referred to as the nitrogen cycle and has implications in a wide range of processes. This includes systems with a significant quantity of biomass, meaning materials and organisms which involve nitrogen in the forms of nucleic acids and amino acids.[1,3]

In these marine areas, waste from fish leads to the formation of nitrogen-containing species, among other substances which contribute to the nitrogen cycle as mentioned previously. Some of these compounds, like ammonia, can be toxic to marine life, and therefore are important to quantify for water quality in relatively wild local environments as well as more controlled spaces like aquariums.[3,4] Information regarding the concentration of toxic substances like nitrite can aid in understanding mitigation efforts needed to ensure the water quality improves and/or remains tenable for aquatic life.

There are a variety of methods which can be used to assess the nitrogen content in water environments, including UV-Visible absorption spectroscopy. This spectroscopic technique monitors the response of substances interacting with ultraviolet and visible light. To explain briefly, light of a given wavelength is directed through a sample. If the energy of the photon is sufficient to allow for transitions between the ground and excited state of electrons within a compound, the light is absorbed and is not able to pass through the sample. As the energy gap between ground and excited states is specific to the analyte, the resulting absorption spectrum will also be unique to the substance studied.

This technique is particularly useful for quantification purposes as the absorbance of the source light is proportional to concentration of the substance studied. This correlation is outlined through Beer's law (Equation 1),

$$A_\lambda = \varepsilon_\lambda c l$$

Equation 1.

where A_λ is the measured absorbance at a specified wavelength of light, ε_λ is the molar absorptivity of the compound at the given wavelength, l is the pathlength and c is the concentration of the analyte. However, there are many compounds in which the photon energy needed to induce a transition is too high for UV or visible light to be absorbed. This includes many of the nitrogen-containing compounds in water systems. For these analytes, a colorimetric reaction is often needed in which the analyte of interest interacts with a separate reagent which will either complex or form a product which does absorb in the UV-Visible region. Under these circumstances, the absorbance of the product can be used as an indirect method of quantification.

Herein, samples of water collected from an aquarium were analyzed using a Thermo Scientific™ GENESYS™ 50 UV-Visible Spectrophotometer equipped with the Thermo Scientific™ Water Analysis Software. Using the appropriate colorimetric water analysis kits and corresponding methods, the nitrite and nitrate content were determined. These experiments demonstrate the simple and rapid determination of nitrite and nitrate content afforded through UV-Visible absorption measurements.

Experimental

Sample Preparation

A 100 mg/L standard NO_2-N stock solution was prepared by diluting 1.0 mL of 1000 mg/L NO_2-N solution, used as received, with 1.0 mL DI water. A 1.0 mg/L NO_2-N stock solution was then prepared by diluting 50 µL of 100 mg/L NO_2-N with 4.95 mL DI water. Subsequent standard samples were prepared as outlined in Table 1. A 1000 mg/L KNO_3 stock solution was prepared by dissolving 163.5 mg KNO_3 in 100 mL of water. The concentration of the stock solution was confirmed through UV-Visible absorption measurements, described later. Standard solutions were then prepared by diluting the 1000 mg/L stock solution with DI water (see Table 2). In addition to the standard samples described previously, a water sample from a fresh water aquarium was collected and used as received.

NO_2-Concentration (mg/L)	Volume NO_2-Stock Solution (mL)	Volume DI Water (mL)
0.05	0.25*	4.75
0.10	0.50*	4.500
0.25	1.25*	3.750
0.50	0.025	4.975
0.80	0.040	4.960

*prepared using 1.0 mg/L NO_2-N stock solution. All other samples were made using 100 mg/L NO_3-N stock solution.

Table 1. Sample preparation of nitrate standard samples.

NO_3-Concentration (mg/L)	Volume NO_3-Stock Solution (µL)	Volume DI Water (mL)
5.0	417*	4.583
25.0	125	4.875
70.0	350	4.650

*prepared using 60 mg/L NO_3- stock solution. All other samples were made using 1000 mg/L NO_3- stock solution.

Table 2. Sample preparation of nitrate standard samples.

For nitrite analysis, the 1.14776 Millipore® Spectroquant® nitrite test kit was used to prepare the sample for further analysis. To describe briefly, one "scoop" each, using the scoop included in the analysis kit, of the kit reagent was added to 5 mL of the prepared solutions described in Table 1 as well as the aquarium sample. The solution was allowed to react for 10 minutes before measurements were acquired. This procedure was repeated using DI water in place of the prepared sample to prepare the blank.

The 1.14773 Spectroquant® kit was used to prepare samples for the nitrate analysis experiments. For these analyses, 1 "scoop" of the included powder reagent was dissolved in 5 mL of the included reagent solution. This was repeated to prepare enough solutions for each standard and sample needed. 1.5 mL each of the prepared standard samples (Table 2) as well as the aquarium sample were then added to the individually prepared reagent solutions. A blank sample was made following the same steps as described previously using DI water instead of a prepared sample.

Instrument Parameters

The concentration of the prepared NO_3- stock solution was checked using a Thermo Scientific™ Evolution™ One Plus Spectrophotometer. A scan measurement between 200 nm and 400 nm of the stock solution, used as prepared, was acquired, using DI water as a blank/background sample. For this measurement, the bandwidth was set to 1.0 nm, a 1.0 nm data interval was used, and the integration time was set to 0.25 s. The sample was held in a 1.0 cm quartz cuvette.

For the nitrite and nitrate analyses, the Thermo Scientific GENESYS 50 UV-Visible Spectrophotometer equipped with the Thermo Scientific Water Analysis Software was used to determine the nitrite and nitrate samples, respectively. The nitrite samples were tested using the 14776H10 method while the 14773H10 method was used for the nitrate samples. In both experiment sets, the samples were held in a 1.0 cm quartz cuvette and measured in triplicate. As described previously, the blank measurement was collected using a DI water sample treated with the appropriate reagents per the kit's instructions.

Results/Discussion

Table 3 outlines the results for standard nitrite solutions collected using the GENESYS Water Analysis software. To determine the validity of the pre-set curve, the percent difference between the anticipated nitrite concentration and the reported concentration according to the Water Analysis software was determined. The percent difference was calculated according to Equation 2,

$$\%Diff = \frac{|C_{calc} - C_{prep}|}{\frac{C_{calc} + C_{prep}}{2}} \times 100\%$$

Equation 2.

where C_{calc} is the concentration calculated from the software and C_{prep} is the prepared concentration. As shown in Table 3, the percent difference for all samples measured is below 5%, indicating the pre-built method is providing accurate results. The aquarium samples analyzed resulted in a NO_2-N concentration of 0.187 ± 0.002 mg/L (0.618 ± 0.007 mg/L NO_2-). This value is below the accepted safe levels for NO_2-N in marine environments,[4] indicating the aquarium this sample was collected from is not in danger of stress or toxicity for the fish as a result of nitrite levels.

Prepared NO_2-N Concentration (mg/L)	$A_{525\ nm}$ (A.U.)	Calculated Concentration NO_2-N (mg/L)	Calculated Concentration NO_2-(mg/L)	Percent Difference (%)
0.05	0.119 ± 0.002	0.004 ± 0.001	0.157 ± 0.002	4.8
0.10	0.254 ± 0.002	0.102 ± 0	0.336 ± 0	2.0
0.25	0.629 ± 0.004	0.252 ± 0.002	0.831 ± 0.006	0.8
0.50	1.292 ± 0.002	0.519 ± 0.001	1.711 ± 0.002	3.7
0.80	2.094 ± 0.004	0.840 ± 0.002	2.771 ± 0.005	4.9

Table 3. Measured absorbance at 525 nm and calculated concentrations of nitrite standards. Percent difference calculations are based off the prepared nitrite concentrations.

Prepared NO_3- Concentration (mg/L)	$A_{525\ nm}$ (A.U.)	Calculated Concentration NO_3-N (mg/L)	Calculated Concentration NO_3-(mg/L)	Percent Difference (%)
5.0	0.098 ± 0.002	1.22 ± 0.03	5.4 ± 0.1	7.7
25.0	0.462 ± 0.005	5.80 ± 0.06	25.7 ± 0.3	2.8
70.0	1.30 ± 0.02	16.2 ± 0.3	72 ± 1	1.4

Table 4. Measured absorbance at 525 nm and calculated concentrations of nitrate standards. Percent difference calculations are based off the prepared nitrate concentrations.

Similar to the nitrite analysis, standard nitrate samples of known concentration were measured using the 14773H10 UV-Visible method. The resulting absorbances and calculated concentrations (NO_3-N and NO_3-) are included in Table 4. Percent difference calculations are also included and indicate that the calculated results are <10% different from the expected results, with the greatest percent difference of 7.7%. While this is a greater percent difference than the nitrite analysis described earlier, it is still low enough to ensure a good deal of accuracy for this analysis. When the aquarium sample was measured, the NO_3-N content was found to be 14.27 ± 0.03 mg/L (63.2 ± 0.1 mg/L NO_3-). According to literature, safe nitrate levels are below 50 mg/L.[5] The results obtained here suggest that the aquarium quality is slightly lower than is ideal, indicating additional efforts may be needed to remove the excess nitrate from solution.

Conclusions

Because nitrate and nitrite are key players in the nitrogen cycle, it is evident these compounds will be found in marine environments like aquariums. Consequently, it is imperative the concentrations of these components, among many others, is known, so adverse effects for fish and other aquatic organisms can be avoided. As demonstrated in the results included herein, the use of UV-Visible absorption spectroscopy allows for a quick and accurate determination of compounds like nitrate and nitrite, including in water samples pertinent to aquatic life.

References

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