Determination of Nitrate in Aqueous Media

Ebony Arauz Tellez, John Bohannon, Rebecca Mills, Virginia Tam, Isabelle Villarreal, and Melissa Rhoten
Department of Chemistry & Physics, Longwood University, 201 High Street, Farmville, Virginia 23909

Abstract:

Determining the amount of inorganic ions in aqueous media is important in the food industry and environmentally. In the environment high levels of ions like nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}) can lead to unhealthy conditions for aquatic species. Similarly, high levels of nitrate and nitrite can cause health issues in humans. One method for determining nitrate levels in aqueous samples involves reducing the nitrate species to nitrite via a redox reaction with finely divided zinc powder followed by conversion to a diazonium salt and complexation with 1-napthylamine to form a colored complex. In the work described herein, this modified version of the Griess reaction was used to spectrophotometrically determine the amount of nitrate in several environmental and food samples.

Introduction:

Nitrogen moves throughout the environment via the nitrogen cycle. Nitrogen gas from the atmosphere undergoes nitrogen fixation to create ammonium, which bacteria then convert into nitrite and nitrate. Nitrate is necessary for plant growth, and therefore necessary to human and animal life. In excess, nitrate can lead to polluted waterways and eutrophication, which is where a significant increase in aquatic plant growth, such as algae, results in the creation of "dead zones[1]."

Excess nitrate is also harmful to human health, which is found in both water and food. When humans consume nitrate from sources like processed meats, municipal water supplies, or groundwater, a plethora of health complications may result, including but not limited to cancer of the breast, bladder, thyroid, and colorectal areas[2]. The body metabolizes nitrate from these synthetic sources into nitrosamines, which are implicated in the elevated cancer risk[3]. Studies have shown that the risk of health complications increased even at nitrate concentrations below the legal regulatory limit[2]. However, natural sources of nitrate found in spinach, kale, beets, and celery have been shown to be beneficial to human health[3]. Naturally occurring nitrate is coupled with antioxidants to prevent conversion to nitrosamines. Under these conditions nitrate is converted to nitric oxide, a key molecule shown to protect the stomach lining and prevent heart/metabolic disease[3].

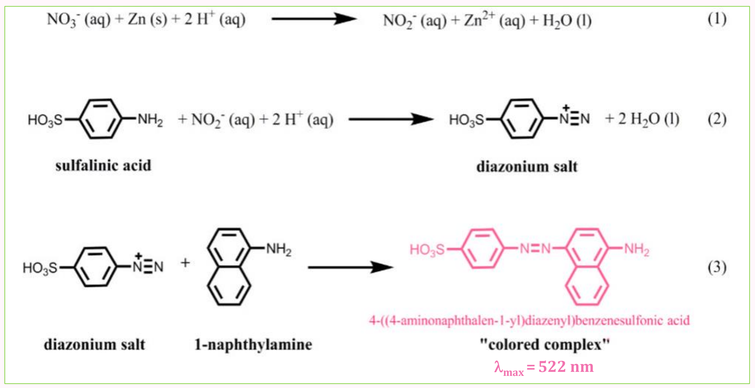
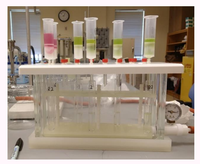


Figure 1. Modified Griess Reaction Scheme[4]

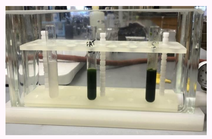
Experimental Procedure:

Collect, wash, dry, and weigh*

Blend & filter*



Solid phase extraction*



Collect extracted liquid*

Griess reaction

- 1.0 mL HCl, 1.0 mL sulfalinic acid, and 2.5 g Zn/NaCl added to each sample followed by stirring for 7 minutes and filtration into a 100 mL volumetric flask.
- 1.0 mL naphthylamine hydrochloride and 1.0 mL sodium acetate added. After 10 minutes, solutions diluted to the mark with DI water.
- Initial volume (water samples) = 50 mL; initial volume (vegetable extracts) = 10 mL.

Spectrophotometry

*Vegetable samples only

Results & Discussion:

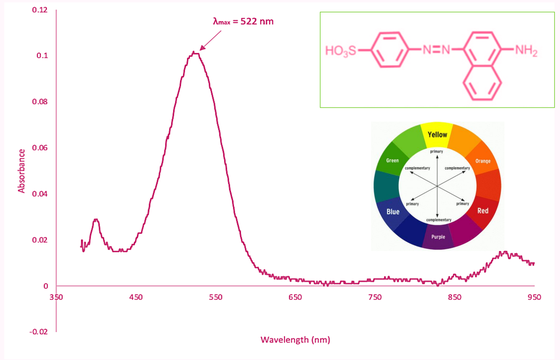


Figure 2. Absorbance spectrum of the Griess "colored complex" using a Vernier SpectroVis spectrophotometer with a pathlength of 1 cm

- Calibration curves are used to understand how an instrument responds to an analyte and to predict the concentration in an unknown sample.
- Calibration standards were prepared according to Table 1.
- All calibration solution were taken through the Griess reaction to generate the "colored complex".
- A calibration curve (Figure 3) was constructed using the data collected from the calibration standards.
- Linear regression was applied to generate the equation of best fit, which was used to calculate the nitrate content of the water and vegetable samples.

Table 1. Dilutions and concentrations of calibration standards

Calibration Solution	Volume of KNO_3 Stock Solution (mL)	Concentration of Standard (M)	Absorbance at 522 nm
1	1.00	3.32×10^{-5}	0.024
2	5.00	1.66×10^{-4}	0.102
3	10.00	3.32×10^{-4}	0.188
4	15.00	4.98×10^{-4}	0.242
5	20.00	6.64×10^{-4}	0.376

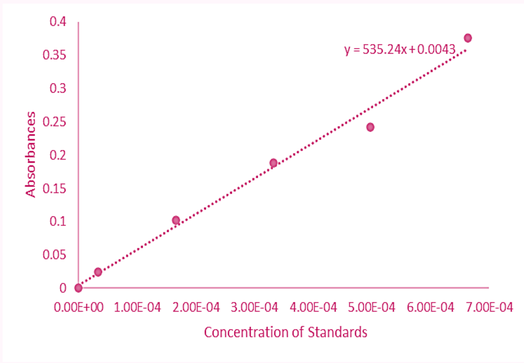


Figure 3. Calibration curve for standard solutions at 522 nm

Table 2. Nitrate concentrations of water samples

Sample	$[NO_3^-]$ (M)	$[NO_3^-]$ (ppm)
Well Water #1 (Powhatan Co.)	$2 \times 10^{-5} \pm 1 \times 10^{-5}$	0.002 ± 0.001
Well Water #2 (Prince Edward Co.)	$6 \times 10^{-5} \pm 1 \times 10^{-5}$	0.007 ± 0.002
Perrier^{tm} Water	$1 \times 10^{-4} \pm 6 \times 10^{-5}$	0.02 ± 0.007

Table 3. Nitrate concentrations of vegetables

Sample	$[NO_3^-]$ (M)	$[NO_3^-]$ (mg/100 g plant material)
Celery	$0.001 \pm 2 \times 10^{-4}$	25 ± 4
Radishes	$0.001 \pm 9 \times 10^{-4}$	37 ± 30
Bok Choy	$0.002 \pm 7 \times 10^{-4}$	82 ± 30

- Two additional water samples (Powhatan Pond and Farmville Tap) were tested and revealed no detectable nitrate.
- The lack of nitrate in the pond water sample was surprising given that geese inhabit the area and their feces could be a source of nitrate pollution.
- Two additional vegetables (spinach and mustard greens) were analyzed. Nitrate was not detectable in these samples due to interference from naturally occurring pigments in the plants (likely xanthophylls, carotene, or pheophytin).
- The allowable limit for nitrate in bottled water (established by the EPA) is 45 ppm[5].
- Estimates of nitrate in Bok choy fall between 103 to 309 mg per 100 g of plant material, depending on growing conditions[6].

Conclusions:

This experiment was successful in quantifying nitrate concentrations in both water and vegetable samples. Future studies will focus on finding a more efficient method for removing the pigments from leafy greens in order to eliminate interferences.

References: