QUANTITATIVE ANALYSIS OF NITRATE AND BROMIDE IN AQUATIC SAMPLES USING ION CHROMATOGRAPHY (IC)

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1 Introduction

Nitrates are minor components of seawater, but have major effects on the dynamics of marine life. Nitrates are byproducts of the nitrogen cycle, a naturally occurring process in aquatic environments, and are considered harmless to aquatic life in small doses. At higher levels (typically above 120 ppm), nitrates are detrimental or even fatal to marine life, which is why they must be removed by physical or biological means. Additionally, it has been established that bromide is linearly proportional to salinity, which must be regulated for the general health of aquatic life. Therefore, a need exists for methods to monitor nitrate and bromide levels in aquariums. As of today, there are a limited number of conventional methods available for the quantification of nitrate and bromide in water samples.

In this project, aquarium samples were analyzed using a customized method for Ion Chromatography (IC). IC is a chromatographic separation technique used to identify and quantify the ions in a sample. The preliminary data has shown high sensitivity by producing a broad dynamic range of over three orders of magnitude from <1 to >100 ppm. Within that range, the linearity has produced an R-squared value equal to or greater than 0.999. Using this approach, nitrates are baseline resolved chromatographically from all other interfering ions, producing a method with high chromatographic resolution. Currently, these methods are being used to test water samples from the National Aquarium with speed, accuracy, and precision. **Ultimately, the goal of this research is to create a valid method for quantitative analysis of nitrate and bromide in water samples.**

2 Nitrogen Cycle

All forms of life require nitrogen in some chemical form. Nitrogen is naturally found as a gas in the air with an abundance of about 80%. The nitrogen cycle describes the conversion between the various forms of nitrogen. Aquariums are mainly concerned with nitrate levels, which is produced from ammonia. Ammonia is produced by biological processes such as the decomposition of plant and animal matter and is extremely toxic to aquatic animals. Ammonia must be converted into nitrite by bacteria called *Nitrosomonas*, which is less harmful to aquatic animals. The nitrites are ultimately converted into nitrates by *Nitrobacter* and can accumulate in a tank.

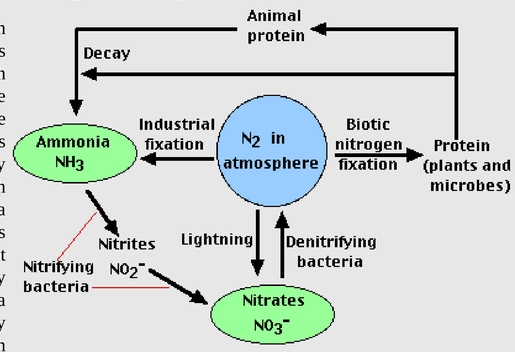


Figure 1: Schematic of the nitrogen cycle, a natural process by which nitrogen is converted into different chemical forms. [1]

3 Ion Chromatography (IC)

Aquarium water samples were analyzed using modified IC methods previously established. IC is a subset of High Performance Liquid Chromatography (HPLC) where samples are separated by charge to identify the ion components of the sample as opposed to separating by size, shape, or mass, which most HPLC techniques use in order to identify and quantify the components of each sample.

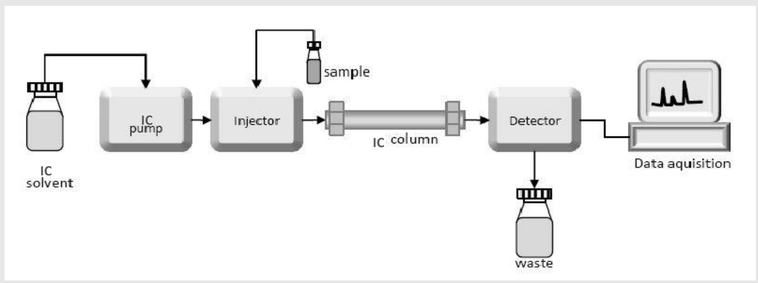


Figure 2. Schematic of how the IC system is arranged and how it operates. [2]

4 Instrumentation

Samples were placed into an autosampler (2) where each injection takes place. Once injected, nitrate levels were analyzed using an IC setup including a Dionex ICS pump (1), a Dionex IonPac AS9-HC column (3) and a UV detector (4) set at 214 nm. A chromatogram for each sample was obtained on the computer (5) in about ten minutes. From each chromatogram, the amount of bromide and nitrate in the sample can be determined by integration of the individual peaks.

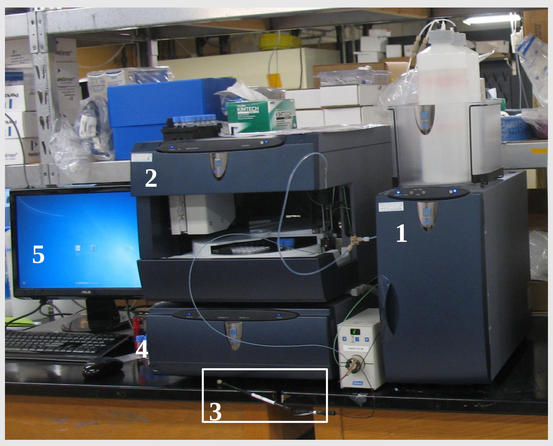


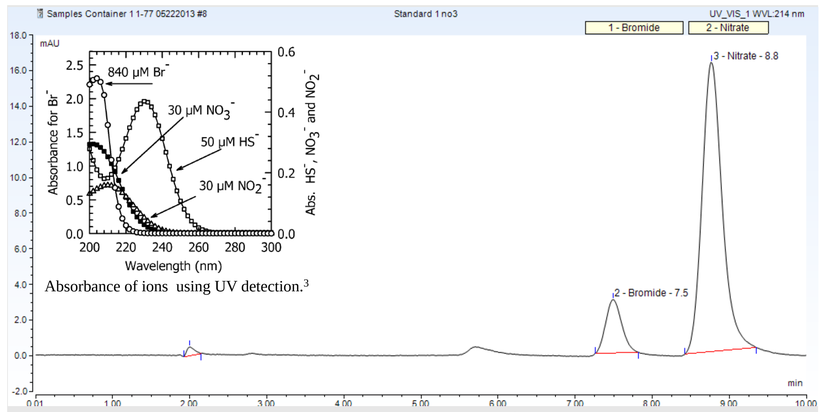
Figure 3. An example of an IC system used in the Molecular Characterization Analysis Complex (MCAC).

5 Current Method

Samples used in these experiments were obtained from the National Aquarium in Baltimore and were each run three times. Before running, samples were diluted by a factor of five. The buffer is a mixture of 17 mM of sodium chloride and 5.0 mM of sodium sulfate. To calibrate the system, five levels of standard with varying concentrations were made from diluting a custom ion standard that contains 6500 mg/L of bromide and 5000 mg/L of nitrate. A flow rate of 0.35 mL/min with a 5.0 µL injection volume was also used.

Table I. Parameters of the method

Column	Dionex IonPac AS9-HC
Flow Rate	0.35 mL/min
Amount Injected	5.0 µL
Wavelength	214 nm
Gradient	Isocratic
Buffer Concentration	5 mM Na_2SO_4 and 17 mM NaCl



Absorbance of ions using UV detection.[3]

Figure 4. A chromatogram of Standard Level 1, which contains 5 mg/mL of nitrate and 6.5 mg/mL of bromide.

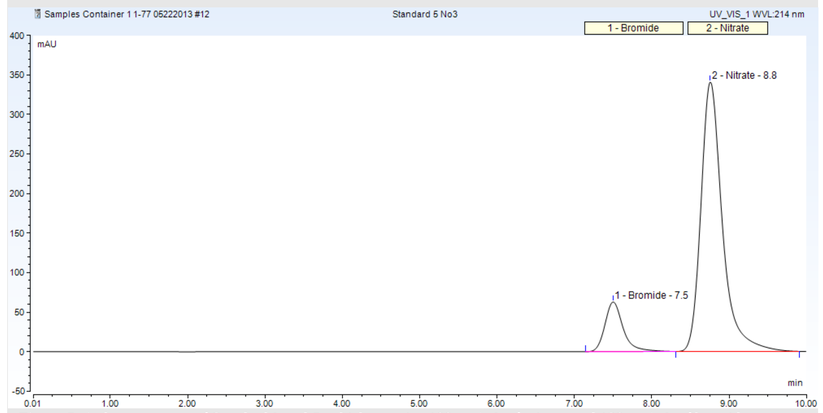


Figure 5. A chromatogram of Standard Level 5, which contains 100 mg/mL of nitrate and 130 mg/mL of bromide.

6 Calibration Curve

The five levels of standard were run three times and the resulting values for each standard were averaged. These values represent the area under a peak from the chromatogram and are proportional to the concentration of bromide and nitrate. Therefore, the averaged values for area are plotted against the concentration of each standard for bromide and nitrate to obtain a linear equation.

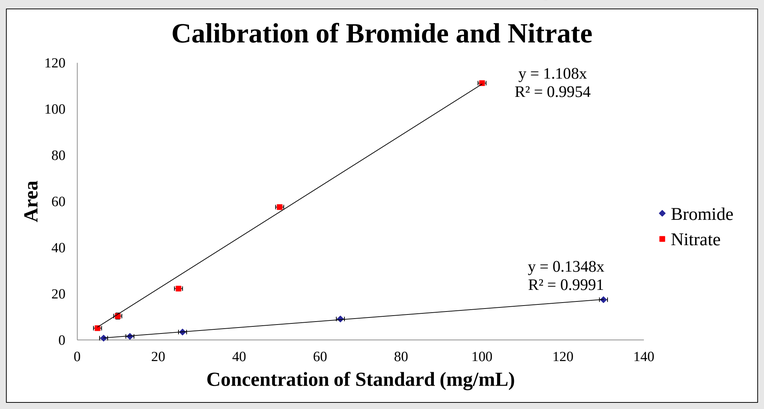


Figure 6. The resulting calibration curve after running each of the five level of standards as a triplicate.

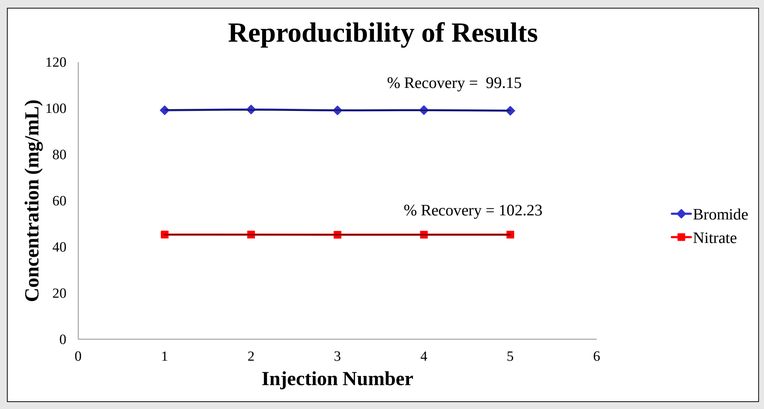


Figure 7. A standard was run five times and its concentration was calculated in order to ensure reproducibility.

7 Water Sample Analysis

Using a calibration curve for reference, the concentration of nitrates for each sample was determined. Since each sample was run three times, the resulting values for area were averaged. The average area was then plugged into the equation determined from a calibration curve to solve for concentration. Finally, since each sample was diluted by a factor of five before they were run, each calculated concentration was multiplied by five to get the actual concentration.

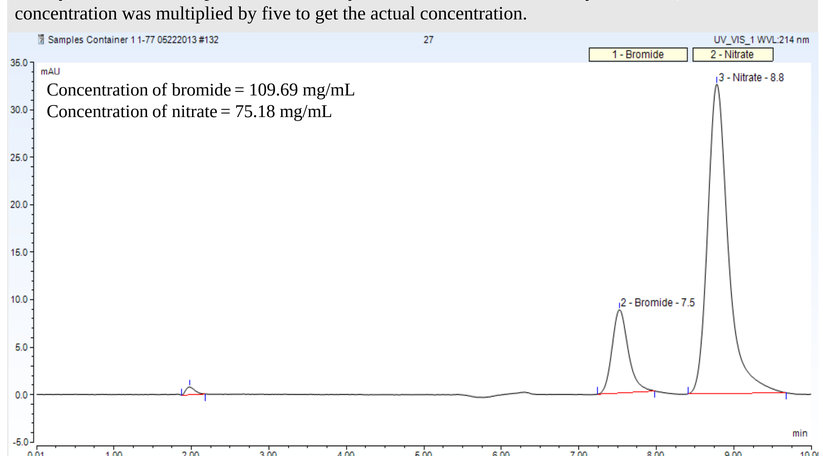


Figure 8. A chromatogram of a sample .

8 Further Method Development

Once the current method was implemented, further method development was done in order to improve the method's efficiency. Different concentrations of buffer were used in order to examine the effects on retention time while keeping all the other conditions constant. By increasing buffer concentration, the retention time is decreased, allowing for faster sample analysis.

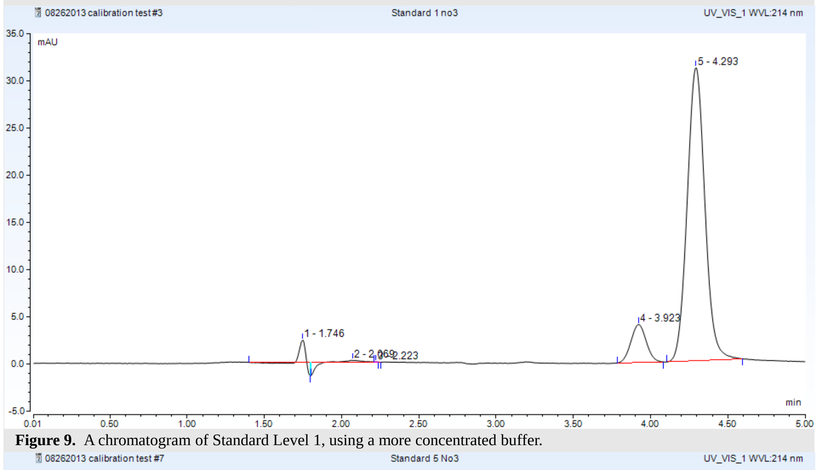


Figure 9. A chromatogram of Standard Level 1, using a more concentrated buffer.

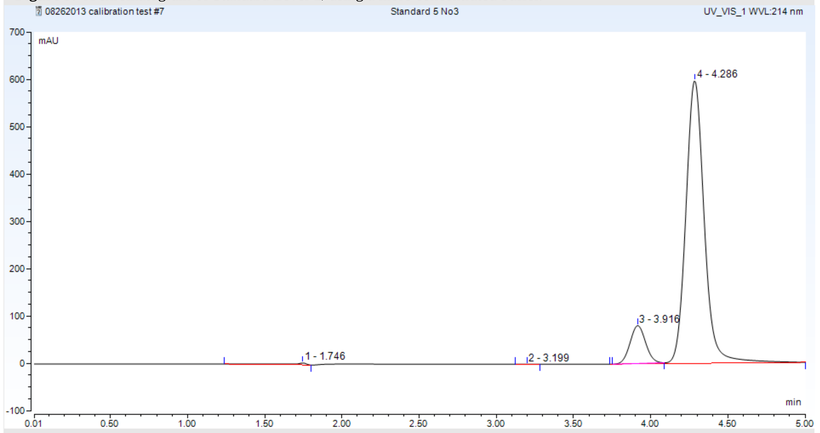


Figure 10. A chromatogram of Standard Level 5, using a more concentrated buffer.

9 Calibration Curve

Using the new buffer, the same five levels of standard were run and the resulting values for each standard were averaged. These averaged values representing area are plotted against the concentration of each standard for bromide and nitrate to obtain a linear equation. As the percent recovery indicates, using the new buffer does not optimize the method.

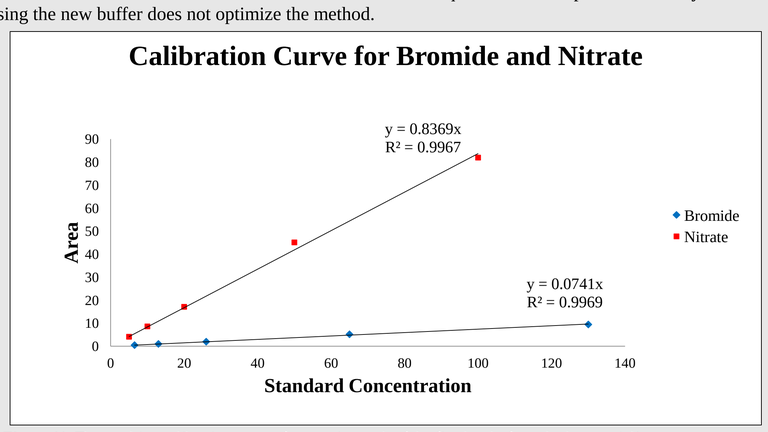


Figure 11. The resulting calibration curve after running each of the five level of standards as a triplicate.

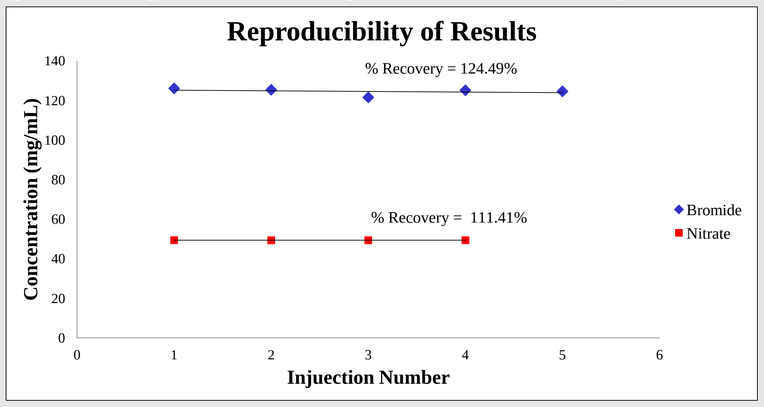


Figure 12. A standard was run five times and its concentration was calculated in order to ensure reproducibility.

10 Conclusion

The IC method developed for this project is a viable method for analyzing and quantifying nitrate and bromide levels in water samples, specifically aquarium water samples. By using this method, nitrate and bromide ions were isolated from other ions chromatographically, validating the method's ability to produce high baseline chromatographic resolution. Additionally, each sample has a run time of about ten minutes, meaning the water samples are run in an efficient, timely manner. The use of calibration curves integrated throughout each sample run ensure that the samples are run with high accuracy and precision as a linearity with an R-squared value of about 0.999 is produced. While further method development has produced a faster method, the viability of the new method is unknown as the effects of the higher buffer concentration do not optimize the method and may be detrimental the column. Therefore, other means of reducing retention time must be explored.

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Sources:
1. *http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NitrogenCycle.html*
2. *http://www.intechopen.com/books/column-chromatography/chromatography-in-bioactivity-analysis-of compounds*
3. *http://www.mbari.org/chemsensor/papers/isus.pdf*