IMPROVED METHODOLGY FOR ION PROFILING IN AQUARIUM WATER

Margaret E. LaCourse, Ian W. Shaffer, Joshua A. Wilhide, William R. LaCourse

Molecular Characterization and Analysis Complex, UMBC, 1000 Hilltop Circle, Baltimore MD 21250

1 Introduction

Aquariums are an important part of modern society, providing both a way to explore the wonders of the ocean and a source of entertainment as well as an avenue for researching aquatic life. Assaying artificial seawater is essential for the proper maintenance of aquariums in order to ensure that its composition is similar to that of natural seawater, providing aquatic life with the healthy environment it needs to thrive. Therefore, it is of critical importance to determine the profile of cations and anions present in the water from various dissolved salts. Current methods typically employ ion chromatography, but these methods are lacking, requiring long run times and/or providing insufficient data. **The goal of this project is to improve, optimize, and validate an ion chromatographic method for the determination of ions in aquarium water.**

Table 1: Concentrations of the ions of interest in natural seawater.[1]

Concentrations of Ions of Interest in Natural Seawater			
Cation		**Anion**	
Lithium	0.17 ppm	Fluoride	1.2 ppm
Sodium	10,500 ppm	Bromide	65 ppm
Potassium	380 ppm	Phosphate	0.07 ppm
Magnesium	1,350 ppm	Nitrate/Nitrite	15 ppm
Calcium	400 ppm	Chloride	19,000 ppm
Strontium	8 ppm	Sulfate	2,700 ppm

2 Ion Chromatography

Ion Chromatography works by separating various charged components in solution using an ion-exchange column followed by electrochemical detection.
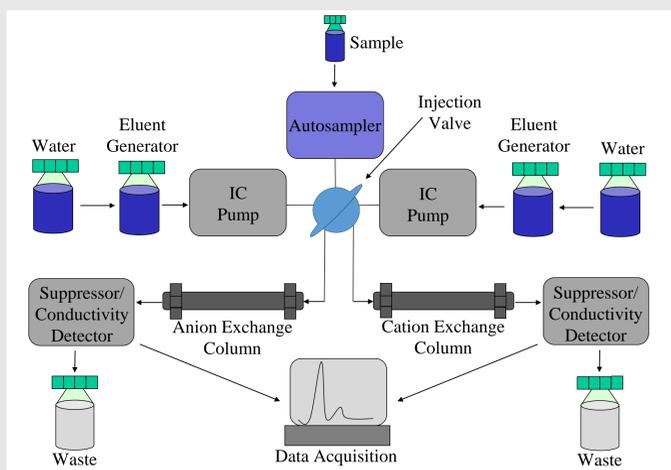

Figure 1. Block diagram of the current anion-cation ion chromatography instrumentation.

3 Instrumentation Setup

The instrumentation set up consists of **2 Dionex ICS-2100 RFIC systems** (one cation, one anion) and a **Dionex ICS Series AS-AP autosampler** with a switch valve to ensure equal injection amounts to each pump. The ion-exchange columns used are: **Anion Column: AS9-HC; Cation Column: CS16**.
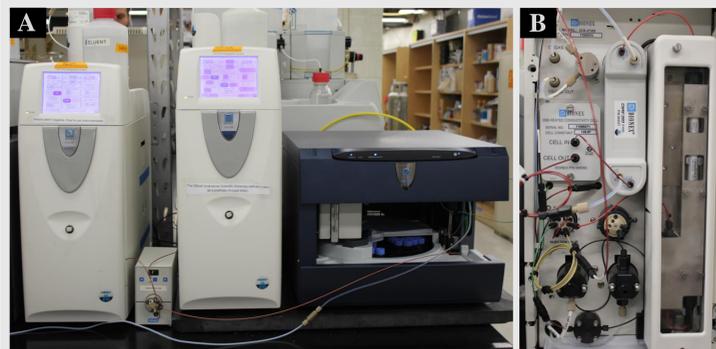

Figure 2. A) Overall view of the anion-cation instrumentation setup. **B)** View of the inside of the cation module.

4 Conditions

Each aquarium water sample is prepared by a 1:5 (v/v) dilution in ACS grade water. The eluent for the cation pump is methanesulfonic acid (MSA) and the eluent for the anion pump is potassium carbonate (K_2CO_3).

Table 2: Conditions for the original and current anion-cation method.

Condition	Cation		Anion
	Original Value	Improved Value	Current Value
Eluent Molarity (mM) (Isocratic)	32	46	12
Flow Rate (ml/min)*	0.40	0.40	0.25
Injection Volume (μL)	1.5	5	5
Column Size (mm)	3 x 250	3 x 250	2 x 250
Column Temperature (ºC)	60	35	35
Suppressor Current (mA)*	38	38	15
Cell Temperature (ºC)	55	35	35

* These parameters are still undergoing optimization

5 Cation Optimization

The cation method needed improvement to shorten the run time and decrease the column temperature in order to extend the life of the column. Additionally, peak characteristics needed improvement in order to produce more symmetrical peaks and linear calibration curves.
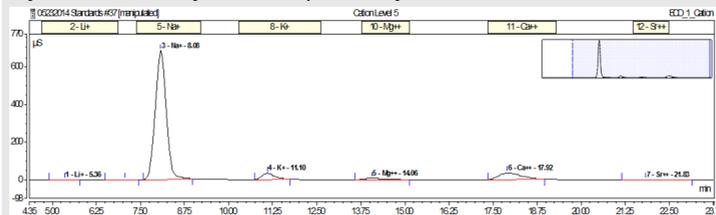

Figure 3. Typical separation of standard with cations in the ratio of natural seawater using the original method.

Factors Affecting Retention Time

For the plots below, 100ppm solutions of each ion were injected to determine their retention times.
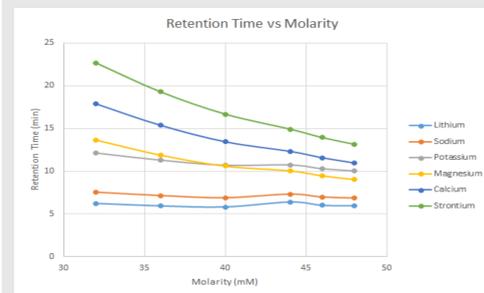
Eluent Generator Molarity

- Changing the eluent generator molarity changes the retention time of each ion
- Changes in molarity affect the retention times of the divalent cations much more than the retention times of the monovalent cations
- Increasing the molarity from 32mM to 46mM shortens the method and creates sharper peaks

Figure 4. Graph representing the change in retention time of each ion in minutes as correlated to the molarity of the eluent (mM).

Column and Cell Temperature

- Changing the temperature of the column and the cell changes the retention time of each ion
- Decreasing the cell temperature from 55ºC and the column temperature from 60ºC down to 35ºC creates a better separation and extends the life of the column
- According to the manufacturer, the CS16 column was not designed to be run above 35ºC and doing so drastically shortens column life
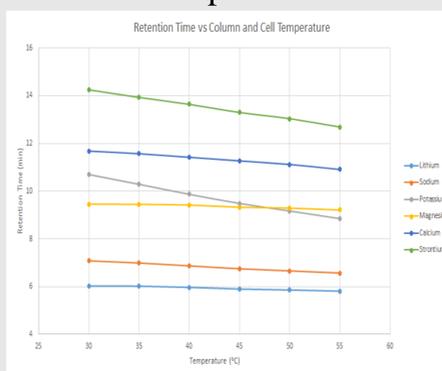

Figure 5. Graph representing the change in retention time of each ion in minutes as correlated to the temperature of the cell and column.

Improved Method Results

- The original cation method was improved to become more efficient and effective and the column temperature was lowered to improve column lifetime
- Retention time decreases with increased mobile phase molarity, but the rate of change varies based on specific ions
- By controlling the variables of column and cell temperature and the molarity of the eluent, a shorter separation with better peak shapes is attained
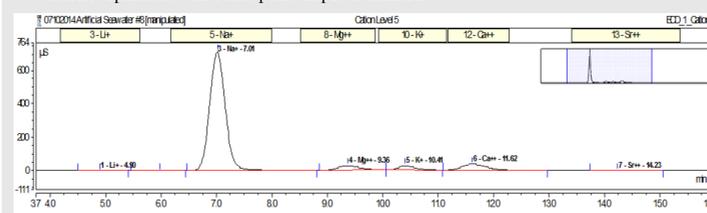

Figure 6. Typical separation of standard with cations in the ratio of natural seawater using the improved method.

Factors Affecting Peak Characteristics

- Changing injection volumes of each sample by varying the injection loop volume changes the peak characteristics such as asymmetry and peak area
- Finding the optimal injection volume allows for the best peak shape and most linear calibration curve
- Testing various volumes while maintaining a uniform tubing length allows for the testing of an individual volume
- All tests were performed using a calibration curve consisting of 5 levels of standards with ions in the ratio of natural seawater
- Examining a plot of peak area versus the mass injected of each ion allows for the determination of the optimal loop size by the linearity of each curve
- The 5uL injection volume proved to be the optimal loop size

Table 3: Conditions for the variable injections volumes.

Experimental Injection Volumes		
Peek Tubing ID (in)	Tubing Length (cm)	Loop Volume (uL)
0.02	10	20.3
0.015	10	11.4
0.01	10	5.1
0.007	10	2.5
0.005	10	1.3

Injection Volume Comparison

Experimenting with various injection volumes allowed for the determination of the optimal volume of sample. Plotting the peak area versus the mass of the ion injected for each cation and comparing the curves allowed for selection. Five cation standards in the ratio of natural seawater were used. Below are select plots of the data. For a better visualization of the curves, they were offset from each other by adding a known amount to each peak area value. The mass of the ion injected was determined by multiplying the concentration of the ion in the standard injected by the loop size used.

- Potassium shows a clear separation of the effectiveness of each loop size. The 1uL loop was too small, while the 20uL loop was too large.
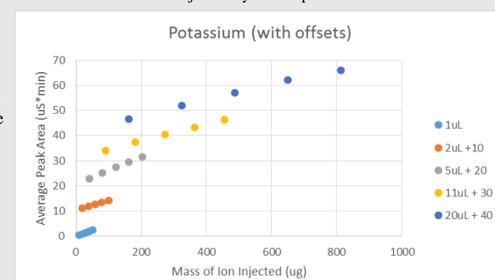- The 5uL loop gave optimal separation as evidenced by the linear trend over a concise mass range.

Figure 7. Plot of average peak area versus the mass of potassium injected for each injection volume using a set of 5 standards.
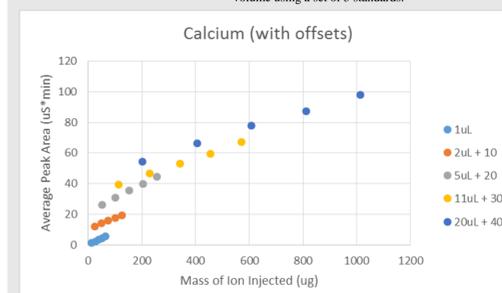
- Calcium also shows a clear separation of the effectiveness of each loop size. The 1uL loop was too small, while the 20uL loop was too large.
- The 5uL loop gave optimal separation as evidenced by the linear trend over a concise mass range.
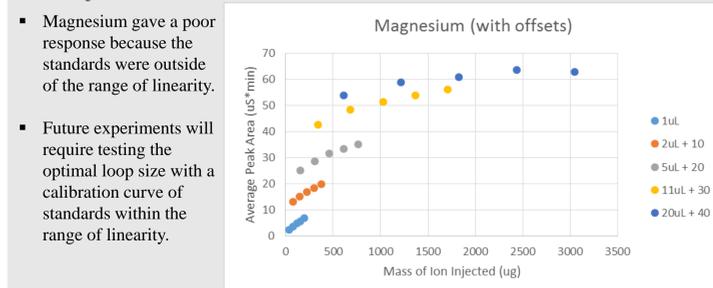

Figure 8. Plot of average peak area versus the mass of calcium injected for each injection volume using a set of 5 standards.

- Magnesium gave a poor response because the standards were outside of the range of linearity.
- Future experiments will require testing the optimal loop size with a calibration curve of standards within the range of linearity.

Figure 9. Plot of average peak area versus the mass of magnesium injected for each injection volume using a set of 5 standards.

6 Additional Cations

- Additional significant cations in seawater include ammonium and rubidium
- Initial analysis of these ions at concentration of 100 ppm proved that they were detectable using the current cation analysis method
- Future research will allow for the incorporation of the detection of these ions into this cation method to allow for a more robust analysis
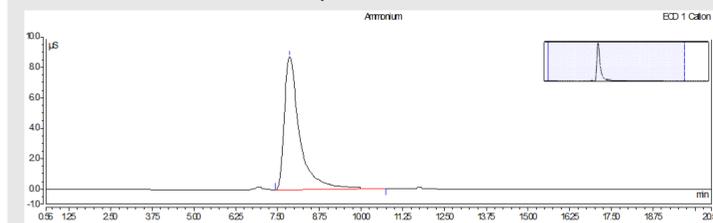

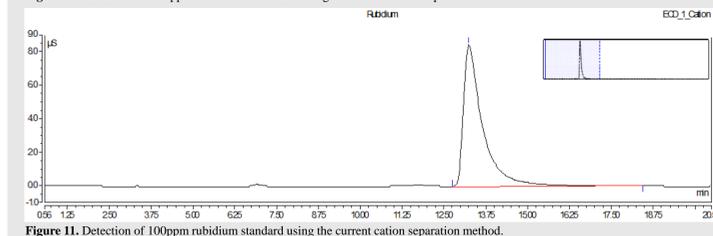**Figure 10.** Detection of 100 ppm ammonium standard using the current cation separation method.

Figure 11. Detection of 100ppm rubidium standard using the current cation separation method.

7 Conclusion

The previous anion-cation separation method required improvement because the run time was lengthy and the high temperature shortened the life of the column. Experimentation showed that retention time decreases with increased mobile phase molarity, and the rate of change varies based on specific ions. Additionally, retention time decreases with increased cell and column temperature, and the rate of change varies based on specific ions. The separation of ions in seawater can be optimized through control of these variables. Also, peak characteristics such as peak symmetry and peak area can be optimized by choosing the best injection volume. Future experiments include further improving the cation method by optimizing variables such as suppressor voltages, flow rate, dilution factors, and sample storage. The magnesium separation still needs optimization to create a standard curve because of its limited range of linearity. Additional steps also include validating the improved cation method. Overall, the initial anion-cation method for the determination of ions in aquarium water was improved and optimized.

Reference: [1] All values in the table provided by the National Aquarium in Baltimore.