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OCN 633 Lab

Dissolved Zn^{2+} by μSI-LOV

We will use a slightly modified version of the method of Grand *et al*. (2011) to determine dissolved Zn in the 4 samples from the field trip. Since the method was originally designed for open ocean work (Zn^{2+}=0.05-10nM), we will have to dilute some of the field trip samples.

We will use the following solutions for the analysis (already prepared):

- **Carrier:** 0.5M CH_3COONH_4, pH 8.4. This solution is trace metal clean.
- **Reagent:** 10μM FluoZin-3 prepared in 0.5M CH_3COONH_4, pH 8.4.
- **Samples:** collected during the field trip, vacuum filtered through a 0.2 μm MilliPore membrane filter. The filtered samples were then acidified with 0.5mL of 6N HCl and kept refrigerated until analysis.

To calibrate the instrument, we will perform a 4 point calibration using filtered acidified (with 24mM HCL) surface seawater of low Zn content collected using trace metal clean protocols in the South Pacific Ocean.

Sample and Standards Preparation

Good Lab Practice*: Wear gloves at all times, work in the laminar flow bench and try to stay TM clean! Do not touch anything outside the flow bench with your clean gloves, do not touch anything inside the flow bench with your bare hands.*

Prior to using a pipette tip: Rinse it 3 times in acidified DI water followed by 3 rinses in DI water. This ensures that your pipette tip is trace metal clean.

1. Dilute the samples labeled "Channel" and "Channel 1" by a factor of 2 using low Zn surface seawater. Prepare your diluted samples in 30mL LDPE bottles. Use the 10mL pipette and a clean pipette tip to perform the dilution.

2. Dilute the sample labeled "TB" by a factor of 10 using low Zn surface seawater. Prepare your diluted samples in 30mL LDPE bottles. Use the 10mL pipette and a clean pipette tip to perform the dilution.

Sample Name	Dilution Factor	Final Volume (mL)	Volume of Sample added (mL)	Volume of low Zn seawater added (mL)
Open Ocean	0	30	30	0
Channel	2	30		
Channel 1	2	30		
TB	10	30		

3. Prepare the working standards. Using a clean graduated cylinder, measure 100mL of low Zn surface seawater and carefully pour it in the bottles labeled +1, +2 and +3.

4. Spike the bottle labeled +1, which contains 100mL of low Zn surface seawater, with 35μL of the 15μM Zn stock standard. Calculate the concentration of Zn in the +1 standard.

5. Spike the bottle labeled +2, which contains 100mL of low Zn surface seawater, with 100μL of the 15μM Zn stock standard. Calculate the concentration of Zn in the +2 standard.

6. Spike the bottle labeled +3, which contains 100mL of low Zn surface seawater, with 200μL of the 15μM Zn stock standard. Calculate the concentration of Zn in the +3 standard.

7. Pour about 100mL of low Zn surface seawater in the +0 bottle (no need to measure the exact volume with the graduated cylinder since we will not spike it with Zn).

Working Standard Bottle Label	Volume of 15μM Zn stock std solution added to 100mL of low Zn seawater	Zn Concentration in working standard in nM
+0	0	
+1	35μL	
+2	100μL	
+3	200μL	

Manifold and Experimental Protocol

The instrument is a μSI-LOV (FIALab Instruments, Bellevue, USA). We will use a 470nm LED (blue) for excitation and a PMT fitted with a 525nm bandpass filter to monitor the fluorescent product. The PMT scans at 8Hz with a 125ms integration time. Below are the steps of the analysis (you do not need to include any of this in your report):

1. Fill the holding coil with carrier solution and flush the flow cell
2. Aspirate 50μL of reagent into the holding coil at 25μL/s
3. Aspirate 75μL of reagent into the holding coil at 25μL/s
4. Start the PMT to get a good baseline
5. Dispense 475μL of the reagent/sample mixture into the flow cell at 15μL/s. As this happens, you will see a peak appear on the computer screen in about 40s. Its height is proportional to the concentration of Zn in the sample.

Analysis

1. Run the calibration curve. Start with the +0 standard and then analyze the +1, +2 and +3 standards in triplicate. This will be done automatically by the instrument and software.

2. Run the SAFE-S reference sample. SAFE-S is a reference sample used by trace metal geochemists around the world to validate their work. It is a sample of surface seawater from the North Pacific, which contains ~0.07nM Zn (consensus value). This concentration is well below the detection limit of the μSI-LOV method. As such, we will assume that the mean peak height of the SAFE-S sample is equal to the procedural blank. We will not use the intercept of the calibration curve to calculate the blank since it will overestimate the true blank.

3. Run the diluted samples from the field trip in this order: Open Ocean, Channel, Channel 1 and TB.

Calculations

I will email you the raw data this weekend (i.e., the peak heights of all standard and samples). Calculate the concentration of Zn in your samples as follows:

1. Use data from the +0, +1, +2 and +3 standards to produce a calibration curve. Calculate the slope and intercept. Make sure you include the graph and equation of the calibration curve in your report.

2. Calculate the concentration of Zn in your samples using the following equation.

$$[Zn] = \frac{PeakHeight}{Slope}$$

This [Zn] is the ***uncorrected*** concentration of Zn in your samples. We still need to correct these data for the procedural blank.

3. Calculate the value of the procedural blank and subtract it from the uncorrected [Zn] values calculated above. To do this, calculate the mean concentration of Zn in the SAFE-S reference sample and subtract it from the uncorrected Zn concentration

calculated above. You have now corrected the data for the procedural blank. **But remember that we diluted the samples in step 1 of the *Sample and Standards Preparation*!** You need to account for this to produce your final Zn numbers.

Sample	**Dilution Factor**	**Uncorrected [Zn]***	**Blank Corrected [Zn]****	**Final [Zn] (accounting for dilution)**
Open Ocean	0			
Channel	2			
Channel 1	2			
TB	10			

* peak height/slope
**Blank Corrected Zn = Uncorrected [Zn]- Zn concentration of SAFE-S

3. Calculate the Limit of Detection (LOD). We define it as 3 times the standard deviation of a sample with the lowest possible Zn content. In our case, the sample with the lowest Zn concentration is the SAFE-S reference sample. Calculate the LOD by multiplying the standard deviation of the SAFES-S replicates (in nM) by 3. LOD=3*σ.

4. Calculate the precision of the method at 5, 15 and 30 nM Zn. To do this, calculate the Relative Standard Deviation (RSD) of the +1, +2 and +3 standards. Report the precision of the method at 5nM in your report. [e.g., the precision of the method was 2.3% at 5nM Zn].

Your report

No more than 3 pages with figs and refs. If you need less than 3 pages to make your case, please do so!
You report should include at least:

- a brief description of the method, solutions and protocol used to perform the analysis
- a calibration curve in graphical format and its equation.
- the LOD and precision of the method
- a brief description and interpretation of the trends seen along the transect. I you see an interesting gradient in concentrations along the transect, you may want to back it up use of appropriate statistics.
- a brief comparison of your data with that of other estuarine, coastal and open ocean environments.