

Chapter 1

RESEARCH METHODS

Collection and Analysis of Seawater

Jeffrey Mendez^a, Jess Adkins^b^a Department of Environmental Science and Engineering, California Institute of Technology^b Department of Geological and Planetary Sciences, California Institute of Technology*1. Time Series Sample Collection*

All time series field work was conducted aboard the R/V *Seaworld* UCLA at the Santa Monica Bay Observatory Oceanographic Mooring site. The mooring is anchored at 33° 55.9' N, 118° 42.9' W; the mooring drifts about this point depending on the prevailing surface currents, as shown in figure 1. Seawater was collected using Teflon-coated external spring niskin bottles using Teflon coated messengers (General Oceanics Inc. model 1010X-5L) attached to ¼ inch polyester line. Niskin bottles were rinsed with surface seawater (15 - 40 m) before each day's use and milli-Q (mQ) water after each day of sampling (18.2 MΩ, 2 x 500 mL), and stored wet.

Water was pumped from each niskin bottle through a 0.2 µm cartridge filter (Sartobran cellulose acetate P 150, 0.45 µm prefilter) with a peristaltic pump using C-Flex tubing (acid leached in 10% v/v reagent HCl) into a hepafiltered work space. The filter and tubing were rinsed with at least one liter of seawater before sampling to remove any residual acid and condition the walls to reduce sample iron adsorption (Buck *et al.*, 2007). Samples were collected in 60 or 125 mL low density polyethylene (LDPE) bottles, rinsing each bottle 3 times with the seawater sample before collection. Final samples were acidified with hydrochloric acid (12 M, SeaStar® HCl) at an acid to seawater ratio of 1:1000, ultimately

reaching a pH of 2.0 - 2.3. All sampling and laboratory materials were acid leached using standard trace metal clean techniques.

2. Dust Dissolution Procedures

2.1 Seawater Collection

Four different types of seawater were used in the dust dissolution experiments. Open ocean seawater at two general depths was collected at 30°N 140°W in November 2004 aboard the R/V *Melville* during the Sampling and Analysis of Iron (SAFe) intercomparison cruise (Johnson *et al.*, 2007). Surface seawater was collected with the University of California Santa Cruz (UCSC) trace metal clean surface “sipper” sampler (Bruland Lab), and sub-surface seawater was collected with the University of Hawaii’s 30 L GO-Flo niskin bottles at a depth range of 24-26 m (Measures Lab). There was a 76 m mixed layer during sub-surface sampling; therefore, all relevant chemical and physical properties of the sub-surface water used in these experiments should be identical in the 24-26 m depth range. Sub-surface water was in-line filtered at sea through a 0.2 µm cartridge filter and stored in an acid leached 4 L polycarbonate (PC) bottle unacidified and in the dark. Surface water was in-line filtered at sea through a 0.4 µm cartridge filter and stored in a 25 L high-density polyethylene (HDPE) carboy, also unacidified in the dark.

Seawater from two separate coastal locations was collected and treated in two different ways. The first coastal seawater was collected at 10 m depth while at the Santa Monica Bay Observatory Oceanographic Mooring (33° 55.9’ N, 118° 42.9’ W) aboard the R/V *Seaworld* UCLA in December 2005. Sub-surface sample water was collected using Teflon coated external spring niskin bottles with Teflon coated messengers (General Oceanics Inc. 1010X-5L) on ¼ inch polyester line. Water was pumped from the niskin bottle through a 0.2 µm cartridge filter (Sartobran cellulose acetate P 150) with a peristaltic pump using C-Flex tubing into a hepa-filtered work space. All sampling and laboratory materials were acid leached using trace metal clean techniques. The second coastal seawater was collected

and UV irradiated at UCSC (Bruland Lab) as described in (Donat and Bruland, 1988), but using Biobeads SM-2 and Amberlite XAD-16 resins in lieu of their Sumichelate Q10R resin. Both coastal seawater samples were stored frozen in one or two liter Teflon PFA bottles.

2.2 Dust Collection

Two sources of dust were tested in our dissolution experiments. The Saharan dust was a composite of 12 surface soils that were collected under clean conditions from the Hoggar region (Algeria) (Guieu *et al.*, 2002). The U.S. dust is a composite of 3 superficial deposits collected in natural dust traps in the Nevada desert (South-West of Las Vegas) (courtesy Marith Reheis, USGS). Both Saharan and U.S. dust were hand sieved through successive clean polyethylene meshes of 100 and 20 μm pore diameter. The smallest fraction ($<20 \mu\text{m}$) was collected and stored in a clean glass bottle. The U.S. sample was then autoclaved to destroy any possible bacteria spores, and both samples were stored in a dark cabinet.

2.3 Elemental Dust Analysis

Dust was acid digested inside a Milestone 1200 Mega microwave oven with 1 mL of HF and 3 mL HNO_3 (Suprapur®, Merck). Aluminum (Al), iron (Fe), and manganese (Mn) were determined using calibration curves by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES Ultra traces, Jobin Yvon). Blanks (reagent alone) were below the detection limit. The ratio of measured to recommended concentrations in the BCSS certified reference material ((n=3), National Research Council of Canada; range of weights: 10.7-16.08 mg) was 1.1 ± 0.1 . Grain-size distributions in volume were established for the two dust samples dispersed in ultrapure water with a Mastersizer (Malvern Instruments, UK).

2.4 Dissolution Experiment 1, Dust Variation

Experiment 1 was designed to compare the dissolution effect of different dust types and concentrations over time. Samples were prepared by adding open ocean surface seawater to five 1 L clear Teflon bottles using an acid leached graduated cylinder. Within a 1 L

polyethylene bottle, approximately 10 mg of Saharan dust was added to 1 L of identical seawater. This solution was quickly shaken and proportioned via pipette or graduated cylinder to each of the sample bottles in order to reach the different dust concentrations (0.01, 0.05, 0.5, 1.0, 5.0 mg/L). The sample bottles (including a control bottle which received no dust) were then sealed with parafilm and immersed in a 13°C water bath (temperature of nitracline in the Santa Monica Bay), under a 50% light screen (to mimic the reduced light in the euphotic zone), on the roof of the laboratory. This sequence was repeated for the U.S. dust. The seawater was allowed to mature under the diurnal cycle for 35 days. Samples were removed from this bath on days 1, 2, 4, 7, 14, and 35 at 1:00 PM for about 2 hours to take sub-samples.

Sub-samples were taken to measure the progression of metal dissolution. Once removed from the roof, the bottles' exteriors were cleaned by thoroughly rinsing with mQ water in a hepafiltered flow bench. The parafilm was removed, and the bottles were individually opened for sub-sampling. The filter apparatus was rinsed by pouring 10 mL of the sample seawater through a 0.2 μm filter (Whatman 25 mm polycarbonate membrane). The sub-sample was then taken by pouring another 10 mL of sample through the filter and collecting it in a small high density polyethylene bottle. This sub-sample was immediately split into two and acidified with concentrated hydrochloric acid (12 M, SeaStar® HCl, 10 μL). Following each sample, the filter was exchanged and the filter apparatus was rinsed with diluted nitric acid (~25 mL, SeaStar® HNO₃), followed by clean mQ water (~150 mL).

2.5 Dissolution Experiment 2, Seawater Matrix

The first experiment was designed to compare dust dissolution in different seawater matrices, focusing on the effects of model and natural Fe binding ligands. Open ocean surface seawater, Santa Monica Bay coastal seawater, UV irradiated coastal seawater, and UV irradiated seawater with added organic molecules were used in this experiment. Seven separate “seawaters” were prepared.

1. Santa Monica Bay coastal water, “Coastal Water”
2. Open ocean surface seawater, “Open Ocean Water”
3. UV irradiated coastal seawater (Bruland Lab UVSW), “UV Water”
4. UV irradiated coastal seawater with the addition of citric acid (57 nM, Sigma-Aldrich Cat #25,127-5), “Citrate Water”
5. UV irradiated coastal seawater with the addition of oxalic acid dihydrate (69.5 nM, Sigma-Aldrich Cat # 24,753-7), “Oxalate Water”
6. UV irradiated coastal seawater with the addition of a combination of citric acid and oxalic acid dihydrate (57 nM & 69.5 nM, respectively), “Citrate & Oxalate Water”
7. UV irradiated coastal seawater with the addition of aerobactin (EMC Microcollections) at a concentration of 50.1 nM, “Aerobactin Water”

An initial sub-sample was taken from each bottle to dissolved measure metal concentrations (Mn, Fe) and Fe speciation, including Fe-binding organic ligand concentrations and binding constant. Each sub-sample was taken by directly filtering the sample seawater into a sub-sample bottle, (0.2 μm pore size, 25 mm polycarbonate Whatman). Following each filtration, the filter was exchanged and the filter apparatus rinsed with \sim 150 mL water (18 M Ω) and 5 mL of the next sample. All metal concentration sub-samples were acidified with hydrochloric acid (12 M, SeaStar[®] HCl), and all Fe speciation sub-samples were sealed and frozen.

After sub-sampling ($t=0$), the initial seven seawater samples were each split into two 1 L Teflon bottles, for a total of fourteen bottles. One bottle from each water type was sealed as a control, while the other bottle was saved for the dust addition. A mixture of 8.45 mg of dust and 52 mL of seawater was quickly shaken and then proportioned via pipette to each of the seven dust addition sample bottles in order to establish a dust concentration of 1.2 mg/L. This concentration is representative of typical dust deposition over ocean water (Duce and Tindale, 1991). Immediately following the addition of the dust, a sub-sample

($t=30$ min to 2 hr 40 min) was taken from each bottle to measure the dissolved metal concentrations (Mn, Fe) and Fe speciation.

The sample bottles (including the control bottles) were sealed with parafilm, placed in clear zipper bags, and immersed in a 13°C water bath on the roof of the laboratory under a 50% light screen. The seawater samples were allowed to mature under the diurnal cycle for 28 days. Samples were removed from this bath on days 0.25, 0.5, 1, 2, 4, 7, 14, and 28 for ~2 hours to take sub-samples.

2.6 Dissolution Experiment 3, Light Exposure

Experiment 3 was designed to compare the dust dissolution effects of light on seawater, with and without amendments of the siderophore aerobactin, in order to elucidate the mechanism of siderophore-promoted dissolution. Two bottles of open ocean sub-surface seawater (SAFe; see Seawater Collection) were used in this experiment. One bottle was left unaltered, “Seawater”; while aerobactin was added to the second, “Aerobactin Water.”

The Aerobactin Water was prepared by dissolving 1.088 mg of solid aerobactin in 1 mL of seawater. 111 μ L of this solution was transferred to the seawater bottle via pipette to establish an aerobactin concentration of 51.1 nM. All work with solid aerobactin was conducted in an Ar filled glove bag in order to reduce any thermal oxidation and decomposition. Sub-samples of Seawater and Aerobactin Water were then taken to measure initial metal concentrations (Mn, Fe) and dissolved Fe speciation. The filter apparatus was rinsed by pouring 5 mL of the sample seawater through a 0.2 μ m filter (Whatman 47 mm polycarbonate membrane). The rinse water was then used to rinse each container. The sub-sample was then taken by pouring another 5 mL of sample seawater through the filter collecting it in a small high density polyethylene bottle. This was repeated for a duplicate sample, followed by a sub-sample for Fe speciation analysis which required approximately 350 mL. The metal sub-samples were acidified with hydrochloric acid (12M, SeaStar® HCl, 10 μ L) while the Fe speciation sub-sample was sealed and

frozen. Following each sample, the filter and the filter apparatus were rinsed with clean mQ water.

A portion of both seawater types was poured into two different types of Teflon bottles. One bottle was translucent Teflon (the “Light” bottle) and the other was an identical bottle wrapped in black electrical tape to prevent light exposure (the “Dark” bottle). These four new samples became the “no dust” controls. A concentrated solution of dust in seawater ($0.87 \text{ g}_{\text{dust}}/\text{L}$) was added via pipette to the remaining Aerobactin Water and Seawater samples to reach a dust concentration of 1.1 mg/L . Immediately following this addition, a sub-sample was taken to measure initial Fe speciation. The samples were again partitioned into “Light” and “Dark” bottles, resulting in 8 total samples bottles: Seawater: light-no dust, dark-no dust, light-dust, dark-dust ; and Aerobactin: light-no dust, dark-no dust, light-dust, dark-dust.

The Light bottles were sealed with parafilm and placed in a clear zipper bag, while the Dark bottles were sealed and placed in three brown bags to further reduce light exposure. All samples were immersed in a 13°C water bath on the roof of the laboratory under a 50% light screen. The samples were allowed to mature under the diurnal cycle for 18 days. Samples were removed from this bath on days 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, and 18 for ~ 2 hours to take sub-samples.

3. Isotope Dilution

The iron (Fe) concentration was determined through isotope dilution (Wu and Boyle, 1998) on an inductively coupled plasma mass spectrometer (ICP-MS, Finnigan Element 1). In isotope dilution, an enriched isotope of the analyte is added to the sample (referred to as a spike). This spike sets the ratio of the common to the enriched isotope. Once set, only the isotope ratios of the natural sample, the spike, and the resulting (or mixed) sample (which is measured on the ICP-MS), as well as the quantities of sample and spike used need to be known in order to calculate the original elemental concentration. Because the

isotopes will react in the same manner during a chemical reaction, numerous nonquantitative laboratory steps can be used to enrich or purify the sample without fear of changing the sample's set isotope ratio. Any changes will occur equally to both isotopes, preserving the initial ratio set by the spike. The analyte concentration is calculated with the isotope dilution (eq. 1), where C is the concentration, R is the isotope ratio, V is volume, % is the percentage of isotope, and the subscripts sa , sp , and m represent sample, spike, and mixed respectively.

$$C_{sa} = \frac{(R_{sp} - R_m) V_{sp} \%_{sp}}{(R_m - R_{sa}) V_{sa} \%_{sa}} C_{sp} \quad (\text{eq. 1})$$

3.1 Limitations

In equation 1, the calculated concentration of the analyte is dependent on the natural isotope ratio, the spike isotope ratio, the concentration of the spike, and the volumes of sample and spike added to the mixture. Any errors from these quantities' true values will result in errors in the final calculated concentration.

A mixed ratio which is similar to the natural or the spike ratio will result in large errors. Focusing on equation 1, when R_m approaches either R_{sa} or R_{sp} , the values of $(R_m - R_{sa})$ or $(R_{sp} - R_m)$ approach zero and the error associated with R_m propagated through equation 1 grows asymptotically, leading to low precision in the final calculated analyte concentration. R_m at the geometric mean of the spike and the sample's isotope ratio will center the ratio between these asymptotes and minimize this error as seen in figure 2.

A drawback of isotope dilution is that to reach the geometric mean of these two ratios, the concentration of the analyte must already be known. To get around this predicament we conduct our analysis with high purity spikes which have isotope ratios many orders of magnitude different from our natural samples. In figure 2, the right hand asymptote is set by the sample's ratio, while the left hand asymptote is set by the spike's ratio. By using a

high purity spike, we can shift the left hand asymptote in figure 2 further to the left, creating an error curve with a low error region which spans many orders of magnitude. In this situation, even if the resulting mixed isotope ratio is greatly different from the geometric mean, there should be a broad enough region in the error curve where the propagated error is acceptably low. Using high purity spikes allows us to add the same quantity of spike to every sample, even if they have different concentrations, and they will all most likely fall within this acceptable R_m region. For all of our measurements it was assumed that the isotope ratio ($^{56}\text{Fe}/^{57}\text{Fe}$) of the sample was equal to the known crustal value of 43.3006 (natural iron isotope variation is -4‰ to $+1\text{‰}$ $\delta^{56}\text{Fe}/^{54}\text{Fe}$ (Johnson and Beard, 2005); therefore, we believe that although our assumption is not completely accurate, the natural variation from this value is small.) Our spike is 93.547% ^{57}Fe with a ratio of 0.0673 (determined by Oak Ridge National Laboratories). These ratios (along with the spike concentration) lead to an measurable Fe concentrations range of 23.7 nM to 0.04 nM, which is well within oceanic values. Acceptable concentrations have been defined as those which have propagated errors less than 1.5% of the Fe concentration.

3.2 Fe Spike

The concentration of the spike solution was chosen so the range of measurable sample concentrations (23.7 nM – 0.04 nM) reasonably matched the range of oceanic values, and to maximize the accuracy despite uncertainty in the spike ratio. If the spike is contaminated with natural iron, inaccuracies in the calculated sample concentration will occur from the change in overall spike concentration and the spike ratio. This inaccuracy will depend on the amount of contamination, and vary with the quantity of spike used. We can minimize these errors by making the spike solution an ideal concentration. To determine the ideal spike concentration, we created a model which simulated changes in the spike ratio and concentration due to contamination from natural atmospheric Fe. For the ideal spike model described below, we measure the spike quantity in a relative manner by

keeping track of the ratio of the spike volume to sample volume, the volume ratio (V_{sp}/V_{sa} in eq. 1).

The ideal spike model tested the effects of atmospheric Fe contamination in the spike on the accuracy of the calculated sample Fe concentration. Within the model, we combined hypothetical spikes of varying C_{sp} , V_{sp} , and R_{sp} , (R_{sp} varied about the Oak Ridge value) with hypothetical samples of varying V_{sa} , and fixed C_{sa} and R_{sa} ($C_{sa}=0.1$ nM, R_{sa} = crustal values 43.3006). We calculated the R_m of each hypothetical mixture using equation 2:

$$R_m = \frac{C_{sa} {}^{56}Fe_{sa} V_{sa} + C_{sp} {}^{56}Fe_{sp} V_{sp}}{C_{sa} {}^{57}Fe_{sa} V_{sa} + C_{sp} {}^{57}Fe_{sp} V_{sp}}, \quad (\text{eq. 2})$$

where ${}^{56}Fe_{sa}$ is the percentage of ${}^{56}Fe$ in the hypothetical sample, ${}^{56}Fe_{sp}$ the percentage of ${}^{56}Fe$ in the spike, ${}^{57}Fe_{sa}$ is the percentage of ${}^{57}Fe$ in the sample, ${}^{57}Fe_{sp}$ the percentage of ${}^{57}Fe$ in the spike. Using these R_m values as if they were measured on the mass spectrometer, we calculated the sample concentrations (eq. 1) as using the Oak Ridge value for R_{sp} (rather than the variable R_{sp}). This calculated concentration was different than the hypothetical concentration. We compared the calculated sample concentrations to the hypothetical concentration, and plotted the accuracy as percent change in concentrations versus the relative spike volume, V_{sp}/V_{sa} (Fig. 3).

In figure 3 we see that at low volume ratios (10^{-2} to 10^{-3}) differences in the R_{sp} of $\pm 3.6\%$ result in inaccuracies in the calculated sample concentration of less than 0.5%. These relatively low inaccuracies stay low at small volume ratios; however, as the volume ratio increases with larger spike volumes, the inaccuracy in the calculated sample concentration grows asymptotically. In order to make sure that we can accurately measure our sample's concentration even with a slight error in our spike ratio, we must choose a volume ratio for our method which lies to the left of these asymptotes. These curves are specific to the spike concentration chosen for the model, and the asymptotes will shift to higher or lower

volume ratios depending on the chosen spike concentration. By reducing the spike concentration, the asymptotes are shifted towards higher V_{sp}/V_{sa} ratios, making larger volume ratios usable; however, lower spike concentrations require more spike to reach the geometric mean of the isotope ratios and, thus, a larger V_{sp}/V_{sa} . These curves are also dependent on the sample's concentration, where lower sample concentrations shift the asymptotes to smaller V_{sp}/V_{sa} and larger concentrations shift them to higher V_{sp}/V_{sa} . In order to determine the best concentration for a spike in combination with the best volume ratio, we used a combination of the propagated error plot from figure 2 and data obtained from the volume ratio model demonstrated in figure 3.

Figure 4 is data from the ideal spike model (right hand y-axis) superimposed onto figure 2 (left hand y-axis). We began by calculating the R_m of the hypothetical mixtures from the ideal spike model, where $C_{sa} = 0.1$ nM, $C_{sp} = 5$ nM, and V_{sp}/V_{sa} vary (the correct isotope ratios: R_{sa} , R_{sp} , were used in each case). These R_m values were plotted against V_{sp}/V_{sa} on top of figure 2. We then repeated the process for hypothetical samples within our concentration range. This gave us a series of curved lines, representing our sample concentration range, which span the R_m space between R_{sa} and R_{sp} (Fig. 4).

By adjusting the spike's concentration, we shift these curves in order to make all the sample concentration curves fit within the R_m range, which resulted in a low propagated error at a volume ratio less than the asymptotes in figure 2. We determined that a V_{sp}/V_{sa} of 0.1 and a spike concentration of 5 nM would result in propagated errors less than 1.5% for all sample concentrations of interest. We diluted the ^{57}Fe spike such that its working concentration was 5.22 nM Fe.

The iron spike was made from solid ferric oxide (Fe_2O_3) synthesized at the Oak Ridge National Laboratory to have a ^{57}Fe isotopic abundance of 93.547% and $^{56}\text{Fe}/^{57}\text{Fe}$ ratio of 0.0673. This solid (0.926 mg) was added to a solution of nitric acid (10% v/v SeaStar® HNO_3 , 100 mL) and hydrochloric acid (12M, SeaStar® HCl , 18.4 g) to form a solution of

10% HNO₃ and 10% HCl, and heated it overnight. This resulted in our primary iron spike (1° ⁵⁷Fe spike). A secondary spike (2° ⁵⁷Fe) was created by adding 266.62 mg of the 1° ⁵⁷Fe spike to 125.709 g of nitric acid (2% v/v SeaStar® HNO₃) by pipette. The secondary spike is 0.214 μM Fe and can be used for samples within the 100 nM – 1 μM range. The tertiary spike (3° ⁵⁷Fe spike) was made by adding 10.088 g of the 2° ⁵⁷Fe spike to 414.2 g of nitric acid (2% v/v SeaStar® HNO₃) by pipette. The tertiary spike is 5.22 nM Fe and is the working spike used for all the seawater samples.

4. Laboratory Procedures

4.1 The MagIC Method

All sample preparations are conducted within a Class 100 laminar flow bench using standard trace metal clean techniques. Seawater samples are processed through a modified Isotope Dilution MagIC (Magnesium Induced Co-precipitation) method (Wu and Boyle, 1997; Wu and Boyle, 1998), concentrating the metals by a factor of 20 while removing a majority of the ions in the sample. In a 1.7 mL polypropylene centrifuge tube (Globe Scientific Inc. Cat #111712 and 111672C) 100 μL of an iron spike (3° ⁵⁷Fe) and 1 mL of the sample were combined. A small amount of ammonium hydroxide (18-60 μL, 18.1 M SeaStar®) was added to the sample, increasing the pH and precipitating a small percentage (approximately 2-5%) of the magnesium (Mg) in the form of magnesium oxy-hydroxides, MgO_x(OH)_y. All surface reactive species (including Fe and Mn) adsorb (or co-precipitate) to the magnesium solids. The amount of ammonium hydroxide needed to precipitate the Mg was determined by trial and error on each type of sample during method development (approximate volumes of base are listed in appendix II). Differences in the volume of base required for precipitation result from varying amounts of acidification of the samples or strength of base, which changes with age of solution. Ammonium hydroxide is labile; therefore, we preserved its concentration and strength by double bagging the bottle and storing it in a low evaporation environment, only opening it to remove daily supplies (which lose their strength over the course of about 12 hours). After ammonia addition, the

samples were allowed to develop $\text{MgO}_x(\text{OH})_y$ solids for 3 minutes. They were centrifuged for two minutes at 8000 rpm, separating the precipitate. The supernatant was poured out, the precipitate was re-centrifuged, and the remaining liquid was shaken out. Ideal precipitates should be gel-like, a translucent whitish color, and about 1 mm in diameter. Increasing the volume of base and the time of development will allow the magnesium to form more crystalline precipitates. Crystalline precipitates can be lost during the pouring steps, as they are easily mixed into the liquid, gel-like or amorphous precipitates stick to the walls of the vial and are more easily retained. Following these isolation steps, the precipitate was dissolved in nitric acid (5% v/v, 50 mL, SeaStar®).

4.2 Manganese Analysis

Manganese (Mn) cannot be directly determined through this isotope dilution process because it is monoisotopic. Mn concentrations were measured using both an internal ^{57}Fe isotope spike and an external standard calibration line between ^{55}Mn and the ^{57}Fe spike. The internal ^{57}Fe spike set the initial elemental ratio between Fe and Mn and reduced the effects of sensitivity fluctuations during analysis. The external standardization provided a calibration for reactivity differences between Fe and Mn during the chemical processing and analysis. The ^{57}Fe spike added to samples for the iron measurement was used for the Mn measurement as well. The external Mn standards (ranging from 0.6 nM to 4.9 nM Mn) were prepared by adding increasing additions of a MnNO_3 solution (24.5 nM Mn; 25, 50, 100, 250 μL) to 1 mL of low manganese seawater. The 3° ^{57}Fe spike equivalent to the samples was added to these standards and taken through the modified MagIC procedure described above.

4.3 Blanks and Standards

Metal contamination associated with chemical handling was determined by processing 50 μL of “blank” seawater through MagIC. “Blank” seawater has been determined to have 0.65 nM Mn and 0.07 nM Fe. Using 20 times less sample allowed us to precipitate

MgOx(OH)_y from the sample without taking a large quantity of analyte with it. It was then assumed that all analyte found in the sample was added as a contaminate during the sample preparation steps. The MagIC procedures for these blanks are similar to the samples described above, although the addition of 200 µL of base was necessary because the smaller quantity of initial Mg was more difficult to precipitate. In the MagIC method for samples, magnesium oxy-hydroxides are only allowed to develop for 3 minutes, resulting in a 5% precipitation of the Mg. We attempt to precipitate all the Mg in our blanks by adding 5-6 times more base and allowing the precipitate to develop for over 30 minutes. As described above, this made the blank precipitate more crystalline, and thus greater care is required to preserve the sample during the separation process.

Mass fractionation in the sample uptake and delivery system of the mass spectrometer was corrected by running spiked gravimetric standards (SGS), which have a known isotopic ratio near the value of our spiked samples. SGS are measured using the same mass spectrometer method, and the measured (mass fractionated) SGS isotope ratio is corrected back to the known isotope ratio. That correction factor can then be used on all samples run during that analysis. SGS samples are prepared by adding 25 µL of a concentrated SGS solution to 1 mL of the “blank” seawater and then taking the mixture through the MagIC chemistry. This results in a SGS sample with the same solution matrix as our samples.

5. Sample Analysis

5.1 Matrix Matching

It is important to run all standards and blanks in a solution with a matrix similar to the samples; this process is called matrix matching. In our method we attempt to match the high magnesium matrix resulting from the MagIC method by adding our standard solutions to seawater with relatively low initial analyte (0.65 nM Mn and 0.07 nM Fe) and treating them in the same manner as a sample. This process yields standards in an equally high

magnesium matrix as well as allowing us to use the same blank subtraction and mass fractionation analysis on every sample and standard.

5.2 Analysis Mechanics

All samples, standards, and chemical blanks were analyzed in an identical manner on a Finnigan Element I magnetic sector inductively coupled plasma mass spectrometer (ICP-MS). Analysis of Fe and Mn was conducted in medium resolution to separate the natural isotopes from argon molecules (^{55}Mn and $^{40}\text{Ar}^{15}\text{N}$, ^{56}Fe and $^{40}\text{Ar}^{16}\text{O}$, ^{57}Fe , and $^{40}\text{Ar}^{16}\text{O}^1\text{H}$). The Finnigan Element I is a single detector mass spectrometer with an Ar plasma ionization source. In a single detector mass spectrometer, isotopes are measured one at a time, jumping from isotope to isotope starting at the low masses and moving towards the higher masses (called a scan), and then repeating in order to gain precision in the measurement. As the spectrometer is isotope jumping, the ionization plasma can fluctuate in intensity, producing an uneven stream of ionized analyte. It is therefore important to minimize the time spent on each isotope in order to minimize the effect of the plasma fluctuation in between isotopes during the same scan. Mn and Fe were analyzed by scanning the center 10% of each isotope peak for 0.6 seconds and repeating 32 times. Each scan yielded a measurement of counts (atoms hitting the detector) for each isotope. We divided the counts of ^{55}Mn and ^{56}Fe by the counts of ^{57}Fe for each scan and averaged the ratios from every scan, giving us an average R_m and standard error, σ , that we can use in equation 1 and equation 3 (see below).

Machine background noise (instrumental blank) was measured by analyzing dilute HNO_3 (5% v/v SeaStar® HNO_3) through the same analysis method. This noise was then subtracted from the samples' and standards' intensities before we evaluate the ratio of natural to spike isotope. Machine blank is less than 300 counts per second (cps) at the beginning of each days' analysis and decreases with time as Fe and Mn wash out of the system and the walls of the instrument are coated with Mg. Blank intensity is typically

0.2% of the Mn and Fe signal. After blank subtraction, the measured SGS ratios are divided by their true ratio (description of SGS analysis given below) to yield a correction factor. Standard and sample ratios were then divided by the SGS correction factor (changes varied by $28.5 \pm 14.6 \text{ \%}/\Delta\text{amu}$ per run) to yield final ratios.

5.3 Calculations

Each sample's Fe concentration is determined by using the isotope dilution equation (eq. 1). As described above, R_m is the average measured ratio from the mass spectrometer after an instrumental blank subtraction and SGS correction. Each sample's Fe concentration is then calculated using equation 1. Every sample run is subject to a chemical handling blank subtraction. The blank samples (described above) are calculated as if they were regular samples ($V_{sa} = 1.0 \text{ mL}$, not $50 \text{ }\mu\text{L}$), and all blanks are averaged (0.1 nM Fe and 0.06 nM Mn , $n=3-4$). The average value is subtracted from all samples and relative standards to yield a final Fe concentration.

The Mn concentration is calculated using the elemental ratio of Mn to ^{57}Fe , using equation 3 to give a preliminary concentration.

$$Mn_{sa} = \left(\frac{^{55}\text{Mn}}{^{57}\text{Fe}} \right)_m \cdot (V_{sp} \text{ } ^{57}\text{Fe}_{sp} C_{sp} + V_{sa} \text{ } ^{57}\text{Fe}_{sa} C_{sa}) \quad (\text{eq. 3})$$

These preliminary Mn concentrations are then corrected for elemental differences in efficiency during co-precipitation and uptake into the mass spectrometer, using the Mn standards described above. The preliminary concentrations of these standards are plotted against the Mn concentrations predicted given the known concentration of the MnNO_3 standard and volumes of each addition. The slope of this line represents the difference in the efficiency between iron and manganese, and each sample's concentration is divided by the slope of the standard calibration line to correct for this difference. This accounted for a 10-20% decrease in Mn concentration depending on the day of processing and analysis.

Blanks are also corrected in this manner, and are then subtracted from sample's concentration to yield the final Mn concentration measurement.

5.4 Sample Blanks and Standards

Contamination which occurred during sample handling was corrected by blanks run with each set of samples. Chemical blanks (prepared as described in section 4.3) had an average Fe concentration of $0.09 \text{ nM} \pm 0.11 \text{ nM}$ (2σ) and Mn concentration of $0.06 \text{ nM} \pm 0.02 \text{ nM}$ (2σ). The contamination of Fe and Mn in our blanks over time are plotted in figure 5. In general, Mn blank concentrations were very consistent, while the Fe blank concentration were more variable. Fe blanks were higher and more variable in our early work, and became more consistent with time.

A laboratory internal standard was run during sample analysis in order to determine day-to-day consistency and monitor any possible drift within any particular analysis. The low Fe and Mn “blank” seawater was used as the first consistency standard and had an Fe concentration of $0.05 \text{ nM} \pm 0.03 \text{ nM}$ and Mn concentration of $0.66 \text{ nM} \pm 0.06 \text{ nM}$, later replacement standards (consistency standard 2 and 3) had concentrations of $1.36 \pm 0.07 \text{ nM Fe}$, $5.36 \pm 0.41 \text{ nM Mn}$; and $0.24 \pm 0.06 \text{ nM Fe}$, $1.54 \pm 0.13 \text{ nM Mn}$. The consistency standards over time are plotted in figure 6. The set of consistency standards with increasing Fe concentrations ($[\text{Fe}] = 2.25 - 5 \text{ nM}$) was a mixture of the blank seawater with $25 \mu\text{L}$ of an Fe standard. Each day a small amount ($1.5 - 2 \text{ mL}$) of the Fe standard was poured into a Teflon beaker. This “daily supply” was the source of the $25 \mu\text{L}$ Fe addition. This was designed to give us a consistency standard with more Fe than our blank seawater; however, because the volume of the “daily supply” was small and because it was not fully replaced, but simply added to each day, evaporation within the laminar flow bench increased the concentration over time. We therefore switched to a seawater standard with a set Fe concentration. Consistency standards 2 and 3 were surface seawater samples without any alterations. Our consistency can be measured by the first low Fe / Mn “blank”

seawater for low Fe concentrations and by consistency standards 2 and 3 for seawater samples with Fe concentrations above 1 nM.

A SGS solution was measured before and after each set of samples to determine mass fractionation and its drift over the course of each run. The $^{56}\text{Fe} / ^{57}\text{Fe}$ ratio of the SGS solution was determined by alternating measurements of the SGS and a solution with a known Fe isotope ratio (IRMM-014.0006). The $^{56}\text{Fe} / ^{57}\text{Fe}$ ratio of our SGS was 1.570 ± 0.014 with a variability of $25.9 \pm 13.7 \text{ } \%/ \Delta \text{amu}$ per day, with no overall drift in the ratio over time. The measured SGS ratios over time are plotted in figure 7.

FIGURES

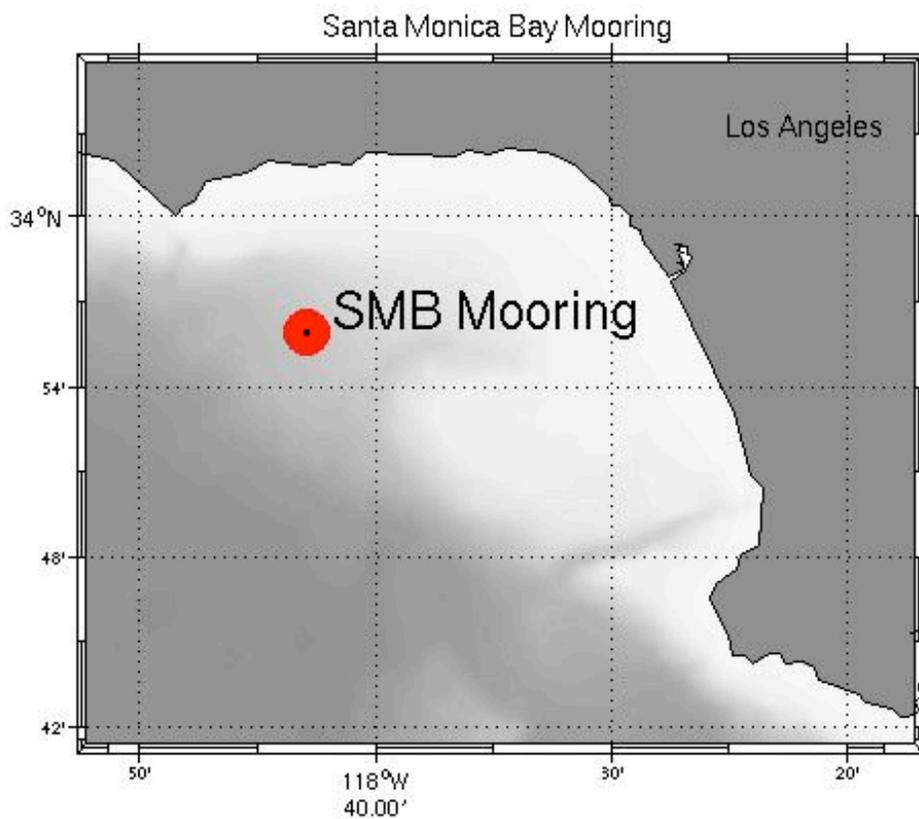


Figure 1: The Santa Monica Bay Observatory Oceanographic Mooring site is anchored at $33^{\circ} 55.9' N$, $118^{\circ} 42.9' W$. Due to surface and sub-surface currents, the mooring rotates on its chain about this point. Time series samples were collected as close to this mooring site as possible.

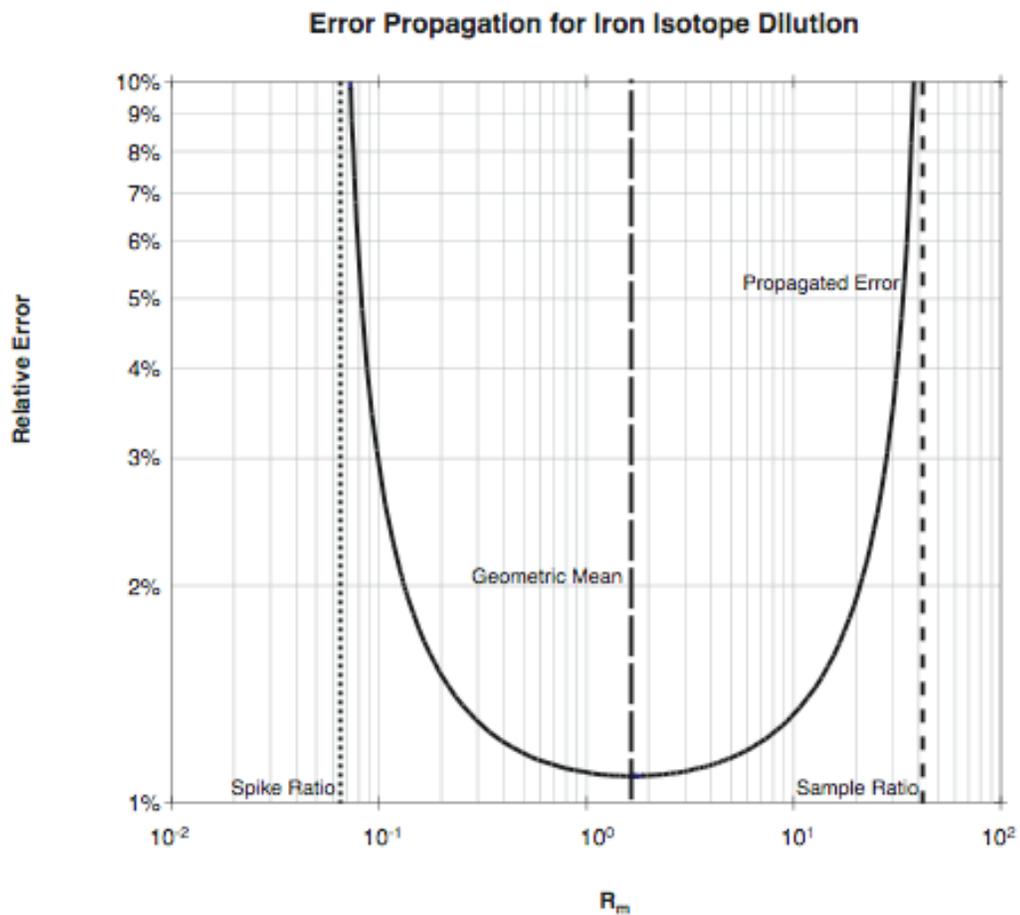


Figure 2: Error in sample concentration resulting from propagating the error in R_m through the isotope dilution equation. Using a σ of 1% for all R_m . Errors will rapidly increase as R_m approaches R_{sa} or R_{sp} .

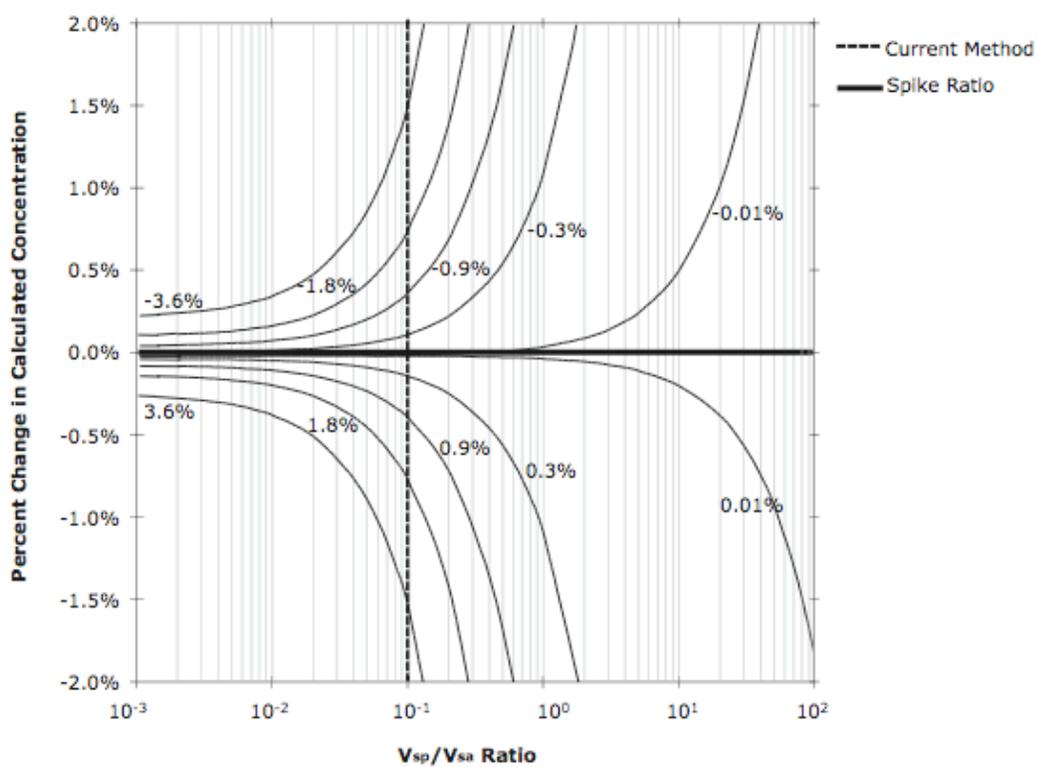


Figure 3: Contour plot of errors resulting from the incorrect spike ratios in the isotope dilution equation. This example uses $C_{sp} = 5.22$ nM, $C_{sa} = 0.1$ nM, and $R_{sa} = 43.3006$. The heavy black line represents the correct spike ratio, the light black lines are spike ratios which deviate from the correct ratio by the labeled amount, and the dotted black line is the V_{sp} / V_{sa} ratio used in our method (1000 μ L sample : 100 μ L spike).

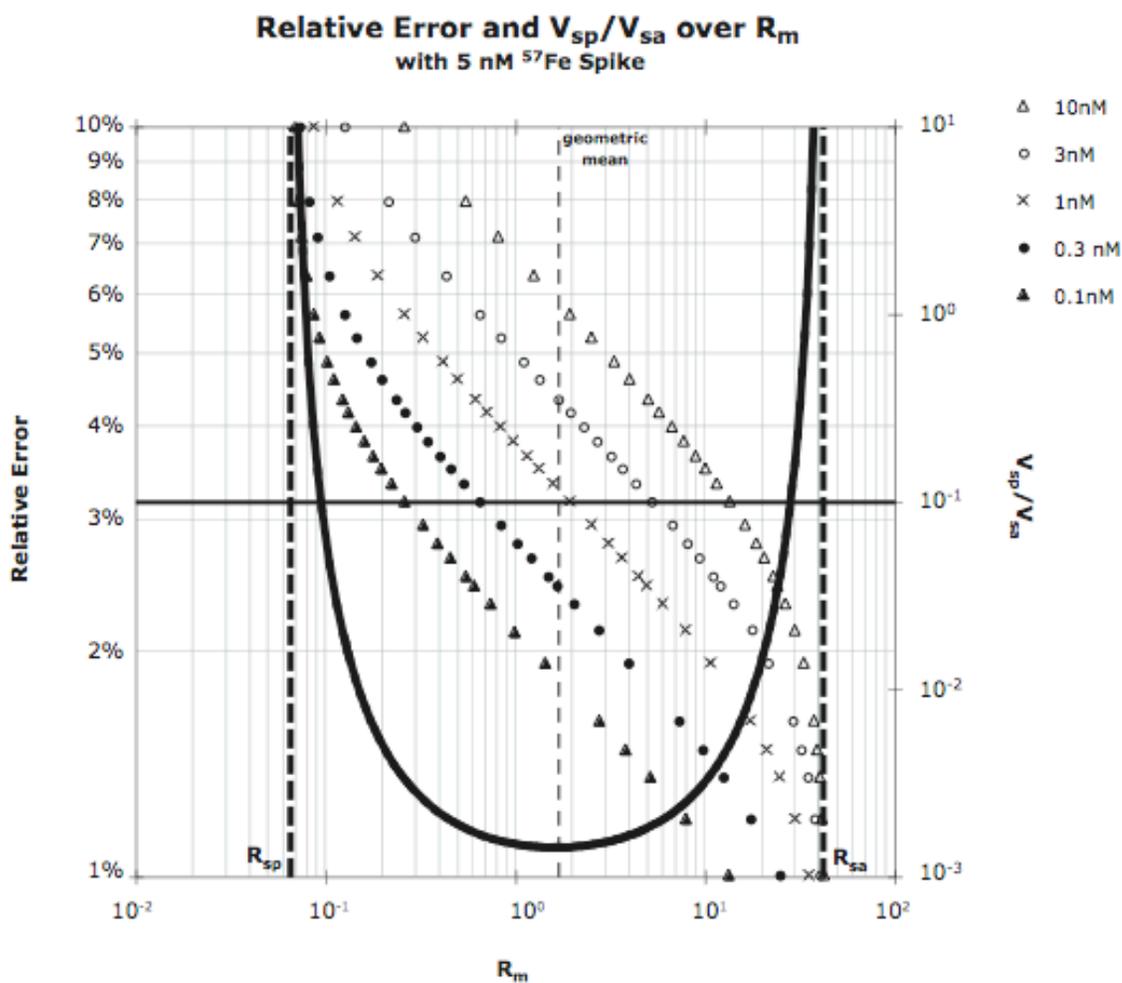


Figure 4: Calculated R_m associated with V_{sp}/V_{sa} ratios for a 5 nM ^{57}Fe spike with sample concentrations of 10 nM - 0.1 nM, plotted on the error propagation graph from figure 2. By constructing this plot we were able to determine the most appropriate spike concentration based on both error associated with non-ideal R_m and V_{sp}/V_{sa} ratios. The propagated error associated with a V_{sp}/V_{sa} ratio of 0.1 for 10, 3, 1, 0.3, and 0.1 nM samples was 1.45, 1.15, 1.08, 1.13, and 1.36% respectively.

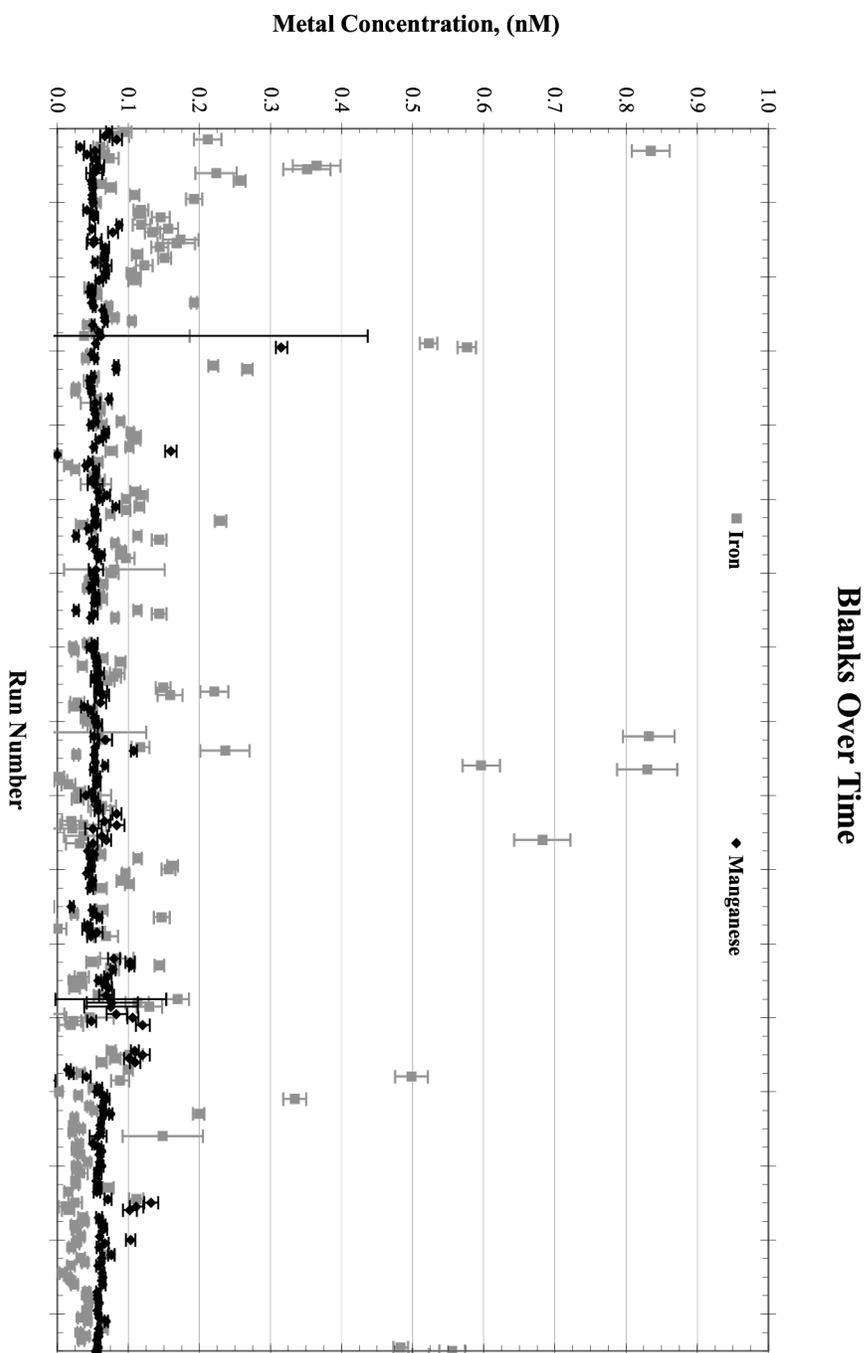


Figure 5: Contamination levels in blank samples over time. Iron (gray squares) were variable, ranging from 0.01 – 0.8 nM, while manganese (blank diamonds) were consistently about 0.06 nM. Error bars are 2 σ of the standard error of the measured ratio processed through the isotope dilution equation (eq. 1).

Consistency Standard Over Time

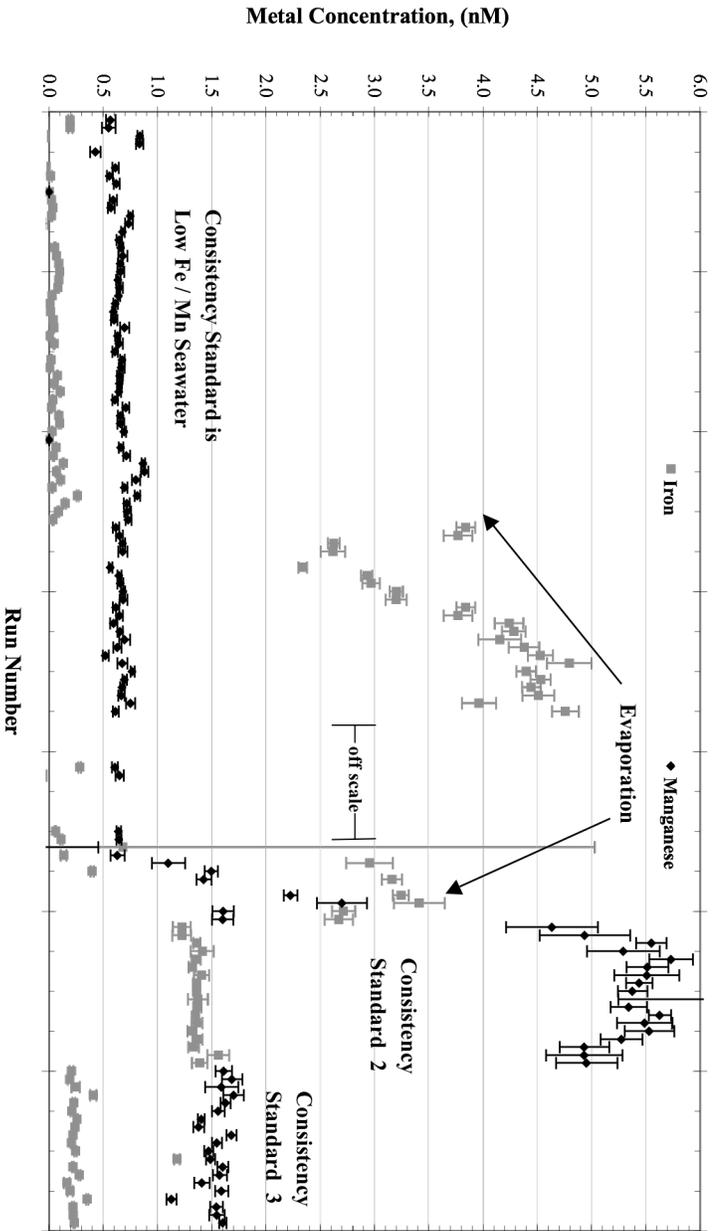


Figure 6 : Consistency levels in the laboratory internal standards over time. Iron (gray squares) concentration in the original standard was 0.05 nM, while manganese (black diamonds) were 0.66 nM. A 25 μ L addition of an Fe standard was added to each standard to increase the concentration; however, we found evaporation to become a source of variation as can be seen in the increasing Fe concentrations. Later replacement standards had concentrations of 1.36 ± 0.07 nM Fe, 5.36 ± 0.41 nM Mn; and 0.24 ± 0.06 nM Fe, 1.54 ± 0.13 nM Mn. Error bars are 2σ of the standard error of the measured ratio processed through the isotope dilution equation (eq. 1).

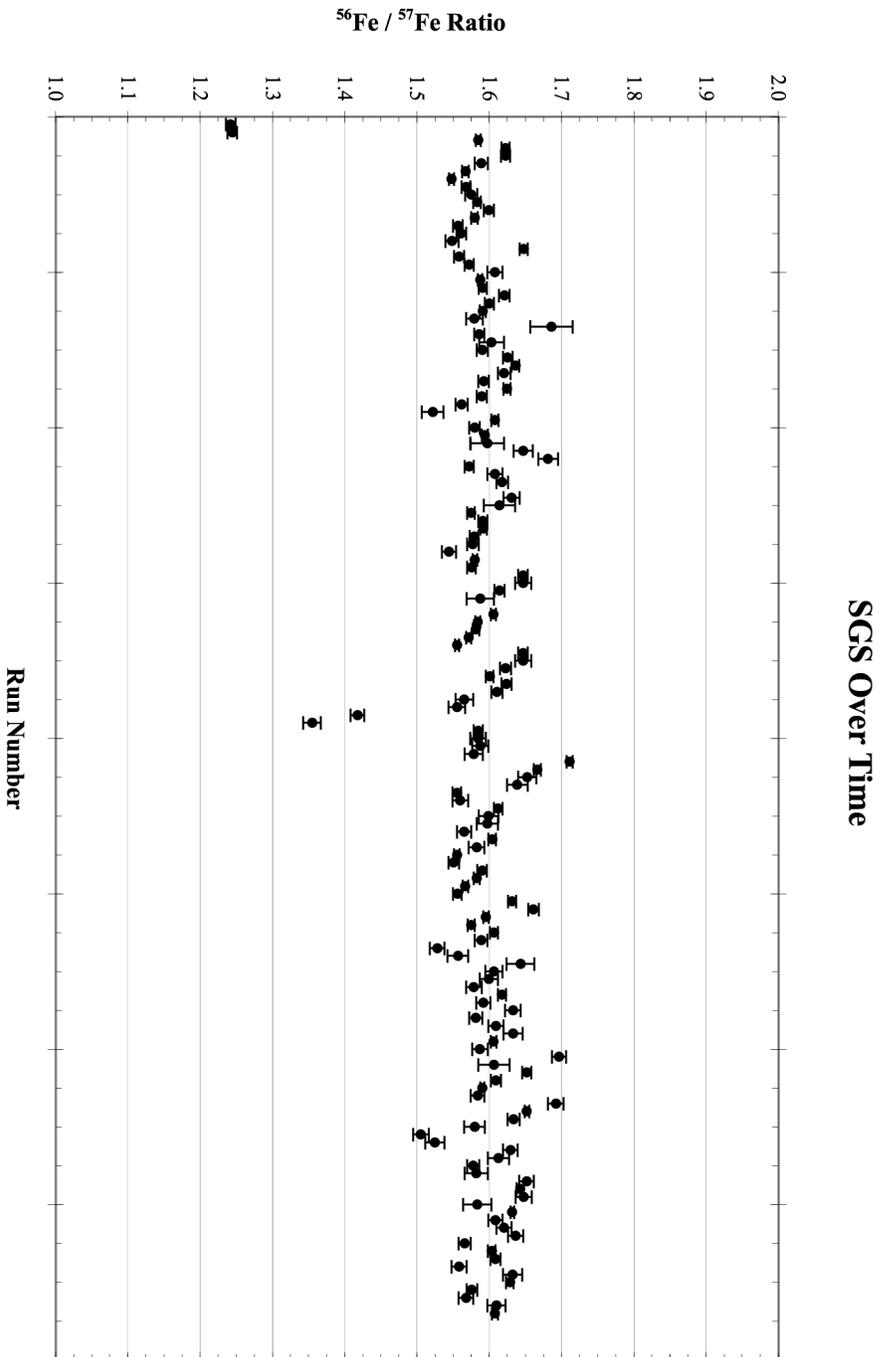


Figure 7: The measured SGS ratios over time. The measured $^{56}\text{Fe} / ^{57}\text{Fe}$ ratio of our SGS was 1.570 ± 0.014 . SGS variability was $25.9 \pm 13.7\text{‰}$ amu per day and $26.0 \pm 29.9\text{‰}$ amu for all SGS samples.

APPENDIX I

Step by step process of sample preparation

This process can be done over the course of a few days, but once a step is started it must be completed. The end of each step is a natural stopping place, and the process can be picked up at a later date with no detrimental effects to the samples. It is advisable to multitask during step 2 in order to speed up the process, but the times required for precipitate development must not be exceeded. Do not move onto step 3 more than 24 hours before analysis.

Step 0. Set-up

1. Remove all needed centrifuge tubes and caps from 0.1% acid (HCl) container.
2. With blue cap tool, screw caps on tubes with 0.1% HCl filling the tubes
3. Rinse the outside of the tubes thoroughly with mQ water
4. Dry the caps with clean lint-free cloth
5. Label all caps with profile date or experiment name as well as sample identity

Step 1. Fill Tubes

1. With 1000 μL fixed pipette, clean new pipette tip
 - a. If original sample volume is scarce, use 500 μL pipette to reduce waste during rinses
2. Open one tube and empty the acid out, close tube
3. Add 1000 μL of sample to tube and screw cap on
4. Invert and thoroughly mix sample in tube
5. Open tube and pour sample out, close tube
6. Add 1000 μL of sample to tube and screw cap on
7. Invert and thoroughly mix sample in tube
8. Open and vigorously shake out contents (less than 3 μL should remain), close tube
9. Add 1 mL of sample and screw cap closed
10. Label side of tube identical to cap
11. Expel pipette tip
12. Repeat Step 1.2 – 1.11 for every sample
13. Fill “daily supplies” with new supply, if needed
 - a. consistency standard
 - b. low Fe and Mn seawater
 - c. Mn standard

- d. SGS primary
 - e. ^{57}Fe 3° spike
14. With 1000 μL fixed pipette, clean new pipette tip (may use for steps 1.15 – 1.17)
 15. For consistency standards repeat steps 1.2 – 1.10 with consistency standard seawater
 16. For all SGS (specific gravimetric standard), repeat steps 1.2 – 1.10 with low Fe and Mn seawater
 17. With 25 μL fixed pipette, clean new pipette tip
 18. For all SGS, add 25 mL of SGS standard (2°)
 19. Expel tip
 20. For all Mn standards, repeat steps 1.2 – 1.10 with low Fe and Mn seawater
 21. With 25 μL fixed pipette, clean new pipette tip
 - a. For Mn 1 standard, add 25 μL (1 addition) of 3° Mn standard
 - b. For Mn 2 standard, add 50 μL (2 additions) of 3° Mn standard
 - c. For Mn 3 standard, add 100 μL (4 additions) of 3° Mn standard
 - d. For Mn 4 standard, add 250 μL (10 additions) of 3° Mn standard
 22. For all chemical blanks, repeat steps 1.2 – 1.5 with low Fe and Mn seawater
 23. With 50 μL fixed pipette, clean new pipette tip
 24. For all blanks, add 50 μL of low Fe and Mn seawater
 25. Label all blank samples
 26. With 100 μL fixed pipette, clean new pipette tip
 27. Add 100 μL of 3° ^{57}Fe spike to samples, consistency standards, Mn standards, and blanks, close tubes
 - a. DO NOT add spike to the SGS tubes !!
 28. Vigorously shake all tubes to thoroughly mix sample and spike

Step 2. Precipitation

1. With 200 μL adjustable pipette, clean new pipette tip up to 200 μL
2. Fill daily supply of NH_4OH with fresh NH_4OH from SeaStar® bottle
3. Add 200 μL of NH_4OH to blanks, close tubes
4. Shake, and let sit for at ≥ 30 minutes
5. Add 32 μL of NH_4OH to samples, close tubes
6. Quickly mix base with samples and let sit for 2-3 minutes
7. Centrifuge for 2 minutes at 8000 rpm
 - a. Pay close attention to balancing the centrifuge
8. Remove all tubes from centrifuge, inverting them and setting them on flow bench counter
 - a. Liquid should separate from solid
9. Open tube and gently pour out liquid without disturbing solid, close tube

- a. Ideal solid will be a small translucent clear-to-whitish smear extending from the tip of the centrifuge tube upward no more than 3 mm. Too much solid will be difficult to dissolve later and can alter the eventual mass spectrometer analysis. Minimize the solid by reducing the NH_4OH used and time before centrifugation.
10. Again, centrifuge for 2 minutes at 8000 rpm
11. Remove from centrifuge, and with one or two powerful motions force the liquid to the top of the tube and into the cap
 - a. If the solid moves with the liquid down the side of the tube or completely into the cap, re-centrifuge
12. Open the tube and shake the liquid out of the cap, close tube
13. Repeat steps 2.5 – 2.12 for every sample and standard
 - a. The quantity of NH_4OH added to standards will vary with the strength of the primary NH_4OH , and acidity of final standard; a list of typical additions can be found in appendix II
14. Centrifuge the blanks, repeat steps 2.7 – 2.12
 - a. Use more caution and less powerful motions
 - b. The solids appearing at the bottom of the blank tubes will be fine white crystals and will be more concentrated towards the tip of the tube, this solid is less sticky and can be removed from the tube with the liquid if caution is not taken

Step 3. Dissolution

1. No more than 24 hours before analysis add 50 μL of 5% HNO_3 to every tube
2. Shake vigorously to dissolve solid
3. If solid remains, the pellet was too big and must be redone
4. Centrifuge for 10 – 30 seconds at 8000 rpm to push all liquid to bottom of tube
5. With blue tool remove the cap of tube and place in analysis tray, remove cap from tool
6. Spot one is indicated by a black dot on the tray
7. Repeat for every tube

Step 4. Ready for analysis

1. Measure isotope ratio on ICP-MS

APPENDIX II

Typical quantities of NH_4OH added to samples, standards, and chemical blanks for precipitation step of MagIC method

Samples

1:1000 conc. HCl acidified samples: 32 mL

1:500 conc. HCl acidified samples: 42 mL

Consistency Standards

Unacidified: 28 mL

Acidified: Follows Sample

SGS

25 mL addition: 18 mL

Blanks

50 mL Unacidified seawater with spike: 200 mL

1000 mL Unacidified Seawater without spike: 15 mL

Mn Standards

Mn (25 mL addition): 25 mL

Mn (50 mL addition): 35 mL

Mn (100 mL addition): 55 mL

Mn (250 mL addition): 100 mL

APPENDIX III

Method for Fe and Mn Analysis on Element I - Saved As FeMR.met

Low + Med + High

Runs/Passes (Meas.): 0*0 + 32*1 + 0*0

Runs/Passes (Eval.): 0*0 + 32*1 + 0*0

Time per Pass [min:sec:ms] [h:min:sec]

Low 00:00:000 00:00:00

Med. 00:02:102 00:01:08

High 00:00:000 00:00:00

Total Time 00:01:08

Locked	Isotope	Accurate Mass	Mass Offset	Mass Window	Mass Range	Magnet Mass	Settling Time	Sample Time	Samples per Peak	Search Window	Integration Window	Detection Mode
No	⁵⁵ Mn	54.9375	changes	10 %	54.937-54.938	54.938	0.300	0.1000	60	50	60	Counting
No	⁵⁶ Fe	55.9344	changes	10 %	55.933-55.935	54.938	0.001	0.1000	60	50	60	Counting
No	⁵⁷ Fe	56.9349	changes	10 %	56.934-56.936	54.938	0.001	0.1000	60	50	60	Counting

BIBLIOGRAPHY

- Buck, K.N., Lohan, M.C., Berger, C.J.M. and Bruland, K.W., 2007. Dissolved iron speciation in two distinct river plumes and an estuary: Implications for riverine iron supply. *Limnology and Oceanography*, 52(2): 843-855.
- Donat, J.R. and Bruland, K.W., 1988. Direct Determination of Dissolved Cobalt and Nickel in Seawater by Differential Pulse Cathodic Stripping Voltammetry Preceded by Adsorptive Collection of Cyclohexane-1,2-Dione Dioxime Complexes. *Analytical Chemistry*, 60(3): 240-244.
- Duce, R.A. and Tindale, N.W., 1991. Atmospheric transport of iron and its deposition in the ocean. *Limnology and Oceanography*, 36(8): 1715-1726.
- Guieu, C., Loye-Pilot, M.D., Ridame, C. and Thomas, C., 2002. Chemical characterization of the Saharan dust end-member: Some biogeochemical implications for the western Mediterranean Sea. *Journal of Geophysical Research-Atmospheres*, 107(D15).
- Johnson, C.M. and Beard, B.L., 2005. Biogeochemical cycling of iron isotopes. *Science*, 309(5737): 1025-1027.
- Johnson, K.S. *et al.*, 2007. Developing Standards for Dissolved Iron in Seawater. *EOS*, 88(11): 131-132.
- Wu, J. and Boyle, E.A., 1997. Low Blank Preconcentration Technique for the Determination of Lead, Copper, and Cadmium in Small-Volume Seawater Samples by Isotope Dilution ICPMS. *Analytical Chemistry*, 69: 2464-2470.
- Wu, J. and Boyle, E.A., 1998. Determination of iron in seawater by high-resolution isotope dilution inductively coupled plasma mass spectrometry after $\text{Mg}(\text{OH})_2$ coprecipitation. *Analytica Chimica Acta*, 367: 183-191.