

STSM Report: Flow Injection Analysis - Chemiluminescence to Undertake Iron Analysis in
Seawater

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The primary aim of the short term scientific mission (STSM) undertaken at IFM-GEOMAR was to gain experience in the use of a chemiluminescence flow injection analysis technique with the intention of analysing for iron from within seawater. The technique is used to analyse species that are or can be enhanced to produce a luminescence which is read (in the case of UEA) through a photon multiplier tube (PMT). The technique will then be taken to The University of East Anglia where it will be used to gain insight to the kinetics of redox species and trace metals that interact photochemically within the sea-surface microlayer (sml). The project will then be combined with other sections to produce a n understanding of the biogeochemistry of the sea-surface microlayer. Part of a self imposed restraint was the use of low carbon emission transport in the journey. A series of trains and a ferry with an overnight stay on the outward journey combining to form a 36hr journey. The return with use of a sleeper carriage on the Deutsche Bahn (German Railway) is a 24hr journey.

The report is in three stages: Stage one is the report on the introduction to the technique, its theories, instrumentation, and initial use. Stage two reports on specific use of the method for iron analysis in seawater. Calibration of the system to provide scientifically sound results and a provision for understanding of how the set up of the system affects said results. Each stage has its concluding remarks. The final stage, stage three is a conclusion of the STSM as a whole.

Stage One – Method Introduction

Arrival at IFM-GEOMAR on Monday 14th May commenced with a presentation from fellow Ph.D students Christian Schlosser and Maija Heller (students of Dr. Peter Croot) of the timetable to be undertaken over the course of the next 9 working days. The timetable from Dr Croot was organised so as to introduce a series of flow injection analysis (FIA) protocols through their increasing levels of technical issues and possibility of contamination. Presentation of the timetable was followed by an introduction to the IFM-GEOMAR biogeochemical group. Post introduction a tour of the laboratory was undertaken the tour included a safety briefing. The FIA group then met for an informal discussion.

The initial introduction to the FIA equipment was set around the analysis of hydrogen peroxide (H_2O_2). Hydrogen peroxide reacts with luminol causing the formation of a radical which is the basis of the luminosity. The PMT tube counts the number of photons released as a voltage the peaks of the voltages can then be converted to a concentration through means of a calibration curve. The H_2O_2 FIA demonstration was carried out by Maija Heller. An explanation was provided concerning the set up of the system; the software running the instrument; the set up of the input and output (flow system), more precisely the 10-way port valves. Teflon tubing and other essential equipment such as the peristaltic pump were explained and shown thus aiding in a complete understanding of the system providing a competent working knowledge.

Day two continued with the use of the H_2O_2 FIA. Hydrogen peroxide is one of a series of photochemically produced redox reactive reductants that affect the cycling of trace metals within natural water system. The preparation of reagents for use in the analysis was first demonstrated. A review of the system emphasised the requirement to understand the injection system via the 10-way port valve. A demonstration of the working technique was undertaken to show software and system operation. An independent (non-software controlled) parameter that can be changed is the peristaltic pump (introduces reagents and sample for analysis) speed.

Time was given to gain familiarity with the machine and software. Familiarity was through a series of experiments based on peristaltic pump speeds. Optimum set up for IFM-GEOMAR is at 1ml/min using 1.85mm internal diameter (id) Teflon tubing. Experimenting with how the pumps revolutions per minute (rpm) alter the flow rate and therefore sample

and reagent volumes was undertaken (basic software competency was required). The variable affects the shape of the graph obtained with the PMT tube but the area underneath is broadly similar suggesting concentrations are also similar. Further software capabilities were utilised to demonstrate how the integral of the peaks under observation were similar though form was different.

The LATER session of day two involved an introduction to the physical set up of a FIA for the study of iron (III), the introduction as carried out by Christian Schlosser. The non-technical term used to explain the tubing system required is 'spaghetti'. The systems in place for IFM-GEOMAR involves two 10-way valves with the second valve port controlling the sample input allowing for 9 analysis to be undertaken concurrently; the first valve controls the sample and load positions . The load/sample valve had a larger sample loop combined with a concentrating column allowing for an introduction of a greater sample quantity. The sample would be run over the concentrating column for 240s. The column is a chemical body that holds iron in place thus increasing the total count. The iron is subsequently washed from the column with ultra clean hydrochloric acid before it is introduced to the reagents to obtain a florescence as counted by the PMT.

The iron (III) demonstration showed how the tubing from the peristaltic pump to the input output valve is combined. The key section is a series of interlinked tubes that add the reagents; luminol, aqueous ammonium and hydrogen peroxide to the eluted sample. The reaction for iron (III) has an optimum temperature of 40oC. The optimum temperature is obtained through the use of a plastic oven to warm the reagents prior to its introduction to the sample following its mixing but prior to its introduction to the flow cell (a perspex spiral coil where the reaction luminosity is obtained through the quantity of photons released). Following the physical set up of the iron (III) analyser Dr Croot provided a set of scientific papers (see below) from which he and his team had established their present analytical set up. The papers were reviewed in the evening and over the following days enabling a distinction of the refinements that the Croot group had developed.

Obata, et al., (1993). Automated Determination of Iron in Seawater by Chelating Resin Concentration and Chemiluminescence Detection

de Jong, et a., (1998). Dissolved Iron at Subnanomolar Levels in the Southern Ocean as Determined by Ship-Board Analysis

Yuan & Shiller. (1999). Determination of Subnanomolar Levels of Hydrogen Peroxide in Seawater by Reagent-Injection Chemiluminescence Detection

Rose & Waite. (2001). Chemiluminescence of Luminol in the Presence of Iron(II) and Oxygen: Oxidation Mechanisms and Implications for Its Analytical Use

Croot & Laan. (2002). Continuous Shipboard Determination of Fe(II) in Polar Waters Using Flow Injection Analysis with Chemiluminescence

Lohan et al., (2005). Determination of Iron and Copper in Seawater at pH 1.7 With a New Commercially Available Chelating Resin, NTA Superflow.

Day three was used to further knowledge of the FIA system through new techniques. A FIA machine is being developed to analyse for superoxide radicals, a short lived oxygen species that is part of the photochemical reactions in natural waters. The analysis for superoxide within the IFM-GEOMAR laboratory is relatively recent so it is undergoing interesting technical and chemical issues. The demonstration by Maija Heller was intended to scan be deduced through the use of a spectrophotometer allowing for the production of a calibration curve which can then be used on the FIA peak area integral therefore providing the concentration therein of the superoxide. The introduction was enlightening as it demonstrated that published methods are not as straight forward or as evident when applied in a laboratory.

The afternoon session considering iron (II) (dissolved iron) analysis was delayed but contingencies had been made. The delay was due to the slow but gradual acceptance of the computers for the new software which controlled the FIA machines (the iron (II) FIA machine was new and had not previously been installed). Instead of the set up for iron (II) as was planned the initial steps for the analysis of iron (III) in seawater was undertaken.

'Day four (calendar time) of the STSM was a German Public Holiday celebrating the ascension of Jesus into heaven so therefore the institute was closed for the day.'

The final day of the first week of the mission was used to show how the Fe (III) FIA system would run calibration standards, undertaken by Christian Schlosser. The system was initiated and cleaned with 1M HCL whilst this was being undertaken for both the load and sample settings the production of standards was completed. A 0,358 μ M iron (II) stock solution was applied to 0.02 μ m cartridge filtered Antarctic seawater (AASW). To each 100ml of AASW 1M ultra clean hydrochloric acid (HCL) was added to prevent the binding of iron with the walls and the formation of colloids, the final pH of the solution must be below pH2 to

make sure all iron is in solution for analysis. The 100ml aliquots of AASW were combined with 20 μ l, 40 μ l, 100 μ l and 200 μ l of the stock solution giving respective concentrations of 0,0716 μ M (0,358/(100/20)), 0,142 μ M (0,358/(100/40)), 0,358 μ M (0,358/(100/100)), 0,716 (0,358/(100/200)) from which to analyse and produce a calibration curve. An ultraclean 2M acetate buffer ~2ml was added to the solutions to increase the pH to approximately pH4.5 - 4.8. The reason for this increase in pH is so that the reaction with luminol at ~pH10.8 - 11.0 does not cover to wide a range. The standards require about an hour to equilibrate. All seawater samples undergo the acidification and then buffering prior to analysis so as to prevent wall/metal interactions.

When loading the standards into the FIA there is a reverse from standard chemistry protocol. Standard protocol would state that the weakest standard is added first and the following standards are added in an increasing concentration. This method helps to prevent any remaining material in the system contaminating a weaker solution so giving that an anomalous result. The Fe FIA method however reverses the protocol and use subsequently weaker standards as a wash to remove material remaining in the system from the higher concentration standards. The washing reduces the risk of contamination in the lower sample concentration. Standards are run in triplicate to allow for an average and a maximum and minima. With time required to concentrate the samples on the column and time for washing off of the iron each standard and hence each sample in triplicate will take approximately 30 minutes to complete with a required volume of about 90ml.

The protocol for the commencement of the analysis is to complete the blank (background iron solution) the standards. If the variance of the standards are statistically similar as given by the co-efficient of determination (r^2) of a calibration curve the analysis can continue. The curve relates peak area to the concentration of the analyte, as the standards are implicitly prepared then the linear regression equation of the best fit line will allow for the calculation of the concentration of the unknown seawater samples.

The later half of the second session involved a theoretical set up of the Fe (II) FIA so as to act as a comparison with the Fe (III) FIA. The tubing is not as varied as with the Fe (III) FIA set up; reagents and sample do not require a pre-warming for optimum temperature, there is also only one reagent for the iron (II) method compared to the 4 involved in the iron (III) method. Further understanding was enhanced within the key region of the 10 way valves especially the load/sample valve. Emphasis was placed on the requirement to understand

how the cycling of the samples, carrier and luminol reagents are administered by the load/sample valve to the luminescence flow cell. The important consideration for iron (II) analysis from within seawater is that with the chemiluminescence technique real time sampling can be undertaken without adding potential contaminants or changing original environmental conditions.

Stage one of the mission: the teaching of clean-room protocols; teaching in the use of FIA machines and software; physically installing machines; studying original analytical methodology; making reagents; FIA problem solving; analysing blanks and standards were all provided to a high standard by the Ph.D students Christian Schlosser and Maija Heller under the directorship of Dr. Peter Croot.

Stage Two – Iron Analysis

The second week of the mission aimed to establish a competent working knowledge in the use of the FIA systems for iron analysis. The initial step was through the plumbing of the 10-port valve for iron (II) analysis. The system was set up for sample injection mode to enable an analysis later as a comparison to reagent injection mode. A constant flow of carrier fluid (MilliQ - ultra purified water) is passed into the light cell where it mixes with luminol reagent at a constant rate giving a background count. Sample is loaded over time (about 40 seconds) into a tube referred to as loop. The sample is then injected into the luminol through the carrier fluid, this is as inject mode when the 10-way port valve then reverts to its original load mode.

Teething problems with the systems factory settings instigated a fact finding mission on the maker's website and a problem solving exercise with relation to the physical set up. The exercise produced an excellent demonstration of system knowledge that had been provided over the previous sessions. Using initially MilliQ water to obtain the movement of fluid through the system it was obvious that the load and inject modes were reversed compared to normal factory settings. Upon solving the problem a gentle introduction of reagents and artificial iron solutions were used to gather information to input the correct analysis settings into the software. Peaks that formed due to the luminol and iron solution were off varying shapes. Physical manipulation of the FIA settings produced more acceptable peak shapes.

Further insights into software were provided through the iron (II) analysis. Concentrations which extend the capabilities of the software scale of measurement present a peak which has peak to negative numbers; the peak thus forms a trough within the centre of the peak. Other information about tuning pump speed to the appropriate rpm and loop size so as to introduce correct sample volume were all discussed. References were also made to iron (III) analysis where a shoulder was noted during the formation of the peak. The solution was probably an advanced iron/HCL solution passing through the concentrating column which had dried and was unevenly packed over transit time from cruise to laboratory. Handouts were provided to show diagrammatically the set up of the 10-way valves for both iron (II) analysis and H2O2.

The second day of the competency part continued analysis on the Fe (II) FIA. Following on from previous work the system was initialised and analysis was undertaken on Atlantic seawater spiked to provide a 1 μ M iron solution. The system was set and tuned through steps learnt from previous work. The primary aim was to establish a decay curve for iron (II) as it reduced within the seawater. The spiked solution was initially too great to establish a working curve therefore a second solution was produced of concentration 2.5nM. The half life of iron (II) can then be calculate and appeared to be approximately 40s for the ambient room temperature. The reduction decay of iron (II) however was slower then expected when reduction occurred to approximately 1/3 concentration; the primary reason was that the pH of the seawater was lower then average so preventing a faster decay rate at the lower iron concentrations.

The second half of the experiment continued with a second seawater solution of concentration 1nM. The decay of this solution was inhibited thus indicating that the pH was too low to invoke further reduction of iron. Slight changes were noted in the solution concentration. Reasons for these changes could have been cloud cover that when decreased increased photochemical cycling of iron and increased the iron (II) concentrations. The system was run for a few hours to help in later calibration. Discussion followed about iron (II) and iron (II) FIA systems. The protocol was supplied for iron (III) analysis and instructions were given for the following day to independently (though observed) produce the standards and run them through the system.

Production of standards and analysis on the Fe (III) FIA was undertaken professionally. Obtaining a final calibration r-squares co-efficient of 0.977 (0.999 with four points). The day work though taken slowly was a success. The previous training and advice enabled a full use of software and machine. Standards and reagents were produced, the system was cleaned and prepared and there was little difficulty in software use.

The penultimate day of the mission was a use of the Fe (II) FIA. The machine software and tuning were mildly different to the Fe (III) FIA but with aid the software and machine 'spoke'. Two experiments were run in tandem on the machine. A comparison of sample and reagent injection modes combined with a change in pump speed leading thus to changes in load time. Sample injection is when reagent is ran constantly through the flow cell with sample injected into the reagent stream, compared to reagent injection which injects the reagent into the sample flow stream. Differences occurred with the baseline, with no reagent

and therefore no luminol in the system the base line for reagent injection was low with noise (scatter in the photon counts) seemingly decreased. Peak areas when considered from the different baselines under visual consideration were similar. The different injection modes seem both to work for a slower produced radical from the luminol iron (II) reaction but with a faster produced radical as with the super-oxide with a fast decay rate then the sample injection mode is preferable.

The final day of the STSM was used for question and answer sessions based on the CL-FIA technique. As well as the q. and a. held throughout the day when required training was provided in the use of an older software version as will be used at UEA to run the photon multiplier tubes and the FIA system.

Stage two of the STSM had successfully fulfilled its requirements. The stage provided the understanding in following through samples from initial preparation to final measurement; this was well instructed and supervised when undertaken independently.

Stage Three – Concluding Remarks

Leaving from IFM-GEOMAR is a strong knowledge of the methodology behind a major instrument in the analysis of seawater. The training for this method was given to a high degree and covered all sections of the method. Instrumentation, theory, chemical and physical parameters were all introduced with full explanations. The mission had been a success with providence providing a coupling of the correct timing for the mission, funding and the Croot group obtaining a new FIA system.

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