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NIRT: Developing a Nanoscale Sensing Device for Measuring the Supply of Iron to Phytoplankton in Marine Systems

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NIRT: Developing a Nanoscale Sensing Device for Measuring the Supply of Iron to Phytoplankton in Marine Systems

Project Participants

Senior Personnel

Name: Wells, Mark

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Orcutt, Karen

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: King, D. Whitney

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Tripp, Carl

Worked for more than 160 Hours: Yes

Contribution to Project:

Post-doc

Graduate Student

Undergraduate Student

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

Activities and Findings

Research and Education Activities:

Research Focus

Trace metals serve as key co-constituents for numerous enzymes in a wide range of biological systems, and their elevated or impoverished levels in aqueous environments have dramatic consequences at organismal, ecosystem, and human health scales. In most cases, the primary vector by which trace elements enter food webs is via phytoplankton, the single celled phototrophic organisms that form the foundation of aquatic food webs. The environmental consequences of many human activities often result in elevated concentrations of these biologically-required metals (contaminants) in aqueous systems, and the introduction of metals having no biological requirements can interfere in metabolic systems (e.g., Hg and Pb). Contaminant metals are of particular concern in coastal zones and nearshore seawaters, which are experiencing increasing human-related pressures due to shifting global demographics. In other cases, a naturally low supply of required metals (e.g., Fe) restricts phytoplankton productivity enough to have global scale implications for carbon sequestration (see below). In both cases, a paucity of data on metals and their dynamics restricts efforts to obtain predictive insights to environmental impacts resulting from anthropogenic or natural inputs of trace metals to ocean waters.

There are four primary reasons for the sparse datasets of trace metals in coastal and offshore seawaters; bioactive metal concentrations typically are extremely low ($< 5 \times 10^{-9}$ M), the high salt content currently prevents direct (rapid) multi-element measurements (e.g., Bruland et al. 1985; Wells and Bruland 1998; Lohan et al. 2005), the chemical speciation of bioactive metals is complicated in seawater (e.g., Bruland et al. 1991), and sampling is severely restricted on temporal and spatial scales because of expensive, ship-based sampling and the need to return samples to the laboratory for analysis. The first three are analytical challenges that render most analyses time-consuming, and require highly trained personnel and specialized laboratories to ensure accurate determinations. The sampling issue also suffers from the need for high-trained personnel and specialized techniques to avoid contamination of the samples during collection. As a consequence, there is a critical need for sensitive, robust, and analytically reliable sensors for determining metal concentrations in seawater; sensors that can be operated accurately with minimal training, can be used easily from boats and large vessels, and ultimately can be deployed on moorings and drifters to provide high sampling coverage.

The central objectives of this project were to use nanostructure technology to develop sensors capable of measuring both total dissolved Fe and, separately, the fraction of that iron that is biologically available to eukaryotic phytoplankton. Our experiments used two approaches; solid-state detection for total dissolved Fe and biomimetic liposome uptake of reactive iron. The goal was to develop a nanoscale platform amenable for use in complex, high salinity solutions and that can be activated to measure a wide range of metals.

Biologically-Available Fe Sensor:

Liposomes were engineered to sequester siderophore bound iron as a preliminary step to develop an ocean nanosensor specific for biologically available iron. Liposomes were fabricated from phosphatidyl choline and cholesterol. The carrier molecule, lasalocid, was incorporated into the lipid membrane during fabrication of liposomes. Lasalocid coordinated with the siderophore (desferrioxamine, DFB) and uptake of siderophore bound Fe proceeded with the established pH gradient. The liposomes were made by the formation of water droplets, using sonication, and by a double emulsion. This produced liposomes with an acidic core suspended in an aqueous solution (pH 8). The diameter of the liposome nanodevices measured by dynamic light scattering was ~100 nm. The liposomes sequestered Fe radioisotopes but needed to be dialyzed in order to measure the sequestration, which did not allow short-term measurements.

Liposomes were made more robust so they could be filtered for short-term Fe uptake

measurements. Liposome devices were constructed using polymerizable diacetylene phospholipid 23:2 diyne in a mixture of natural phosphatidyl choline (PC) and polymerizable diacetylene phospholipids (PDA) 23:2 diyne in a 4:6 molar ratio. This ratio allowed for Fe uptake while 100% diacetylene liposomes did not. The liposomes could be filtered and washed for Fe uptake measurements. Iron uptake by the mixed PC:PDA nanodevices was rapid over the first 15 minutes in maleic acid buffer (pH 7.4) (4% of total iron added [30 nM]) but then did not increase further over the next hour. This cessation of uptake was most likely a diminished pH gradient within the liposome.

Total Dissolved Fe Sensor:

The focus of this aspect of the research was to develop a chip-based biosensor for measuring iron in seawater. The biosensor is based on surface attachment of bacterial siderophore molecule (DFB) to a mesoporous silica film. This approach combines the high selectivity for iron of this biological molecule with the high surface area of the mesoporous scaffolding. Exposure of the chips to seawater results in a solid phase-like extraction of iron to the detector molecule. The novelty of the DFB biosensor is that the measurement occurs while iron is on the chip, rather than eluted to a downstream instrument, thus minimizing opportunities for sample contamination; a major issue for reliable iron determinations at the ultra-low concentrations found in seawater.

As a preliminary test bed, we developed novel DFB functionalized resins to evaluate the reaction rates and pathways for DFB interactions with Fe in seawater. Aside from providing an alternate chemiluminescence approach for quantifying total and biologically available Fe, this effort provided alternate robust quantitative measures of Fe uptake for comparison with both true biological uptake, as well as the optical assessments of iron acquisition by the DFB biosensor.

Our optical detection system is based on the principles of Fourier Transform Infrared Spectroscopy (FTIR). The main advantages of this approach are that it can operate in heterogeneous aqueous environments, will provide fast detection (< 10 min), and can be adapted for high sensitivity /selectivity of trace metals. Forming the cornerstone of the system will be the fabrication of a nanoporous membrane that mimics the performance of solid phase extraction (SPE) in terms of adsorbing and concentrating metals, and that is amenable for direct analysis by infrared spectroscopy. The ability to directly scan these active nanostructures using transmission spectroscopy is a key element as it dramatically simplifies sampling compared to standard SPE protocols by eliminating the need to elute the target compound from the SPE using solvents. Sampling methods were developed that integrate the active nanostructured material on a mesoporous scaffold. The intention is to package these complex nanostructure materials into a simple product that will be mounted in an automated sampling carousel to provide multiple sample collection capabilities during deployment in oceanic waters. Integration of the sampling carousel into a portable Fourier Transform Infrared (FTIR) spectrometer will provide turn-key operation with minimal user intervention. Ultimately, we envision attachment of these active nanostructure sensors onto attenuated total reflectance (ATR) crystals incorporated into a autonomous sensor package for remote sampling and reporting of metal concentrations.

Education Activities

The youth of America is more ethnically and racially diverse than ever before (Statistics 2006), but the recruitment and retention of underrepresented minority groups into scientific fields continues to be a challenge (Culotta and Gibbons 1992; G&ndara and Maxwell-Jolly 1999). Colby College has launched a strategic initiative to increase the diversity of its student body, and specifically to recruit African-American, Latino, Asian American, and Native American (ALANA) students. Recruitment efforts have shown success, as over the past decade ALANA student enrollment rates have increased 220%; ALANA students make up 18.3% of the incoming Colby class. Consistent with reports of

other institutions (2005; Summers and Hrabowski III 2006) many of these minority students declare an interest in science.

As ALANA enrollment at Colby has increased, proportionate numbers of minority students are enrolling in freshman-level gateway science courses. General Chemistry is one of the largest gateway courses in the sciences at Colby. However, particularly for African-American, Latino, and Native American students, the numbers remain grim. Based on concerns over the lack of retention of minority students in the sciences, in 2003 co-PI King and a group of Colby science colleagues formed the Support in the Sciences (SIS) group to explore the performance of underrepresented groups in the science programs at Colby. In particular, SIS members were concerned by the attrition of minority students in gateway courses. With full support from the administration, the SIS group commissioned the Director of Institutional Research and Assessment to study academic performance of all students in the sciences and the results showed general minority underperformance in General Chemistry. In response to this problem, co-PI King created a new course, Turbo Chemistry in 2005. This two-semester invitation-only course, taken concurrently with General Chemistry, was designed to improve student's grades in General Chemistry through directed group study, application of chemistry to interesting campus issues, extra challenging homework, practice exams, and detailed discussions of laboratory exercises. We have specifically targeted ALANA students for invitation into Turbo Chemistry. Rather than a remedial course, the goal of Turbo Chemistry is to take students beyond the traditional general chemistry experience, and in the process establish their confidence in doing science.

References

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Bruland KW, Coale KH, Mart L (1985) Analysis of seawater for dissolved Cd, Cu and Pb: an intercomparison of voltammetric and atomic absorption methods. *Marine Chemistry* 17: 285

Bruland KW, Donat JR, Hutchins DA (1991) Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnology and Oceanography* 36: 1555-1577

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Summers MF, Hrabowski III FA (2006) Preparing Minority Scientists and Engineers *Science* 311: 1870-1871

Wells ML, Bruland KW (1998) An improved method for rapid preconcentration and determination of bioactive trace metals in seawater using solid phase extraction and high resolution inductively coupled plasma mass spectrometry. *Marine Chemistry* 63: 145-153

Findings: (See PDF version submitted by PI at the end of the report)**Training and Development:**

Training and Development

Over the duration of the grant, six Colby undergraduate students have been actively involved in this research. Over eighty percent of these students are currently attending graduate programs in oceanography, chemistry, medicine, or environmental engineering.

Table II. Colby Students involved with this work.

Name Year Destination

Eric Roy 2004 University of Maine, Ph.D. Oceanography

Ken Pitter 2004, 2005 Cornell University, M.D./Ph.D Medicine

Mike Walsh 2005 Cornell University, Ph.D. Environmental Engineering

Hillary Easter 2005, 2006 Colorado School of Mines, M.S. Environmental Chemistry

Karen Prisby 2006, 2007 Chemical Industry

Lauren Brown 2007 University of Maine, M,S. Environmental Engineering

In addition, the project has led to the research training of Dr. Karen Orcutt, a Postdoctoral Scholar who was instrumental in doing the preliminary work that led to this project. The expertise she developed in the project was a key factor in her successfully obtaining a faculty position at the University of Southern Mississippi in the final year of the project.

Outreach Activities:

Outreach Activities

Outreach activities to communicate our research efforts, and the field of nanotechnology in general, have been both local and field-based. Local activities include the incorporation of nanotechnology principles in undergraduate and graduate curricula (see above) as well as general public outreach efforts. Dr. Wells has participated each year in the local chapter of the international Lego-league program as a Oceanography and nanotechnology expert. These programs focus on providing elementary and middle school students opportunities to investigate, plan, and implement programmed robotic missions that emphasize nanotechnology and other engineering and environmental knowledge bases. In addition, Dr. Wells successfully obtained funding to bring a Maine High School Teacher on a research cruise to the central North Pacific where the chip-based sensor was evaluated. In addition to maintaining a live link with science classes in his school, where students interacted through questions and discussion, a public science blog was initiated during the cruise that hosted >700 hits from the public. Dr. Wells also has devoted time to reviewing web-based educational sites on behalf of the Maine COSEE office.

Dr. Orcutt taught a graduate seminar course in her new position at USM, Dept. of Marine Science titled "Nanoparticles in Marine Science" that covered topics from sensors to biogeochemistry. The overall theme of the course was the environmental implications of nanotechnology.

Journal Publications

- C. Jiang, H. Li and C. P. Tripp., "An infrared method for in situ studies of polymer/surfactant adsorption on silica powders from aqueous solution", *Applied Spectroscopy*, p. 1419, vol. 57, (2003). Published,
- C. Jiang, A. Gamarnik and C. P. Tripp., "Tripp Identification of Lipid Aggregate Structures on TiO₂ Surface Using Headgroup IR Bands", *J. Phys. Chem.*, p. 4539, vol. 109, (2005). Published,
- C. Jiang, C. Chen and C. P. Tripp., "Molecular and Dynamic Study of the Interaction of Anionic Surfactants with Liposomes", *Journal of Colloid and Interface Science*, p. , vol. , (2007). Submitted,
- E. G. Roy, M. L. Wells and D. W. King, "Persistence of iron(II) in surface waters of the western subarctic Pacific", *Limnology and Oceanography*, p. , vol. , (2007). Accepted,
- E. G. Roy, C. Jiang, M. L. Wells and C. P. Tripp, "A chip-based biosensor for dissolved iron determinations in seawater", *Analytical Chemistry*, p. , vol. , (2007). Submitted,
- K.M. Orcutt and M. L. Wells, "A liposome based nanodevice for sequestering siderophore bound Iron", *Journal of Membrane Science*, p. 247, vol. 288, (2007). Published,

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

Measuring the spatial and temporal variability in dissolved Fe and biologically available Fe is one of the central issues facing marine biogeochemistry today. The main limitation here has been the intense sampling and analytical hurdles for measuring Fe down to the picomolar levels occurring in surface seawater. The work here has produced a functioning biomimetic sensor for Fe determinations that has been verified and tested in offshore, Fe deplete waters. This advance, though still short of a fully deployable sensor, provides the strong foundation for reaching this goal.

Contributions to Other Disciplines:

This project provides the foundation for developing functioning Fe sensors for deploying on remote vehicles and moorings. This system, once created, will provide a comprehensive dataset on Fe distributions and seasonal patterns. Marine biogeochemical and climate modellers have identified these data as a key factor needed to improve climate forecasting.

Contributions to Human Resource Development:

This project supported Dr. Karen Orcutt in her postdoctoral studies, and was a key factor in helping her obtain her position as Assist Professor at Southern Mississippi University.

The project also supported and encouraged several undergraduate students at both Colby College and the University of Maine to work in marine science and nanotechnology.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Organizational Partners

Any Book

Any Web/Internet Site

Any Product

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering

Quantification of bioavailable Fe using biomimetic nanoscale devices: Function in seawater.

Liposome-based nanodevices were successfully engineered for Fe uptake and made more robust by incorporating polymerizable lipids. The rate of uptake was rapid and the concentrations of iron transported by these DFB-specific nanodevices is on the same order as total dissolved iron concentrations in coastal and offshore seawaters (~0.1- 1.0 nM). This work also may provide the conceptual scaffolding for developing other nanosensors for related oceanographic applications.

Quantification of bioavailable iron in marine ecosystems: Reduction and detection of siderophore complexed iron using a photoactive oxalate ternary complex.

A method was developed to detect the desferrioxamine B-Fe(III) complex at nanomolar concentrations in aqueous samples. This complex is photochemically stable and resists reduction by standard reductants and ligand exchange. Consistent with the work of Kunkely and Vogler (16), formation of a new complex was observed with the upon addition of the bidentate oxalate ligand to DFB-Fe(III) solutions. This oxalate containing complex was found to be photoactive upon ultraviolet radiation, resulting in the reduction of Fe(III) to Fe(II). The reduced iron was then quantified using the colorimetric reagent ferrozine, generating an analytical signal that is easily quantified at 562 nm using absorbance measurements with a long pathlength flow cell, in excellent agreement with expected values. This method provided an reporting methodology for quantification of Fe(III)-DFB transported into liposome vesicles.

Desferrioxamine B Oxalate Ligand Exchange by UV-Vis Spectroscopy: The next step in developing a quantitative analytical method for measuring bioavailable iron in marine ecosystems.

The specific goal of this study was develop a robust analytical technique for quantification of Fe(III)-DFB complexes at nanomolar concentrations. Past work showed that oxalate (Ox) promotes photodissociation of Fe(III)-DFB to Fe(II), and we are specifically interested in the mechanism of this process. A model was developed for ligand exchange between Fe(III)-DFB and Fe(III)-tris oxalate using known thermodynamic constants for Fe(III)-DFB and Fe(III)-trisoxalato complexes and adjusting for ionic strength. This simple ligand exchange model model was confirmed by monitoring the UV-VIS absorbance of the system at a variety of oxalate concentrations and pH. Contrary to the work of Kunkely and Vogler (16), the model did not include ternary complexes. Next, the rate of Fe(II) production during UV irradiation was examined. The results showed that the rate of Fe(II) production was due entirely to the $[\text{Fe}(\text{Ox})_3]^{3-}$ species, and that reoxidation of Fe(II) occurred via reactive oxygen intermediates. This reoxidation could be avoided by either decreasing the oxygen concentration or by adding a Fe(II) stabilizing reagent, such as ferrozine. Further studies are need to extend these results to sub nanomolar concentrations, but this work clearly shows that oxalate at moderate concentrations and pH 3.5 is capable of extracting Fe(III) from the siderophore DFB. This work is being prepared for submission to Environmental Science and Technology.

IR spectroscopic studies of DFB transport across liposomes

While the focus of our IR spectroscopic work was in the area of solid-state detection for total dissolved Fe, it was also utilized to provide a molecular understanding of the uptake of reactive iron by liposomes. However, direct measurement of liposomes in aqueous solutions by IR spectroscopy was not possible owing to the strong absorption of infrared light by water. The first step to overcome this problem was the development of a general infrared spectroscopic method that enables the monitoring of the adsorption of

surfactants, polyelectrolytes and liposomes on high surface area oxide particles from aqueous solution. (tripp ref 1) The method is based on the use of a ZnSe IRE coated with a layer of titania particles. The titania surface could be converted to silica using atomic layer deposition with sequential addition of an amine catalyst, gaseous SiCl_4 and water vapor. It is shown that bare titania and silica coated titania particles on the ZnSe crystals are stable to flowing aqueous solutions below $\text{pH} = 8$ and can be used to study the dynamics of polymer/surfactant adsorption on silica.

This method was then extended to study the nature of the dipalmitoylphosphatidylcholine (DPPC) aggregated structures adsorbed on TiO_2 . (tripp ref 2) DPPC molecules were assembled on TiO_2 using Langmuir Blodgett (LB) deposition methods or by directly flowing the DPPC liposome solution across the TiO_2 coated ATR crystal. It is found that there is a direct correlation between the intensity and frequency position of the zwitterionic headgroup IR bands with the nature of LB films. Specifically, it is shown that the bands due to PO_2 modes are sensitive to changes in the degree of hydration of the LB films, and that the symmetric deformation vibrational mode ($\delta_s +\text{N CH}_3$) is sensitive to interaction with oppositely charged surface sites. Using this information it is found that the liposomes adsorbed on TiO_2 remain intact as vesicles, and that the vesicles are stable and not removed to flowing water. It is also shown that the antisymmetric deformation vibrational ($\delta_{as} +\text{N CH}_3$) modes are sensitive to changes in lateral lateral DPPC interactions. This information was used to show that there is a lateral interaction between each positively charged $+\text{N}(\text{CH}_3)_3$ headgroup and negatively charged PO_2 headgroup of the adjacent DPPC molecule in the adsorbed vesicles and LB films. This study provides a framework for the use of this IR technique in studies of adsorption and transport of molecules across membrane interfaces. (tripp ref 3)

Development of a chemical model for Fe(III)-DFB ligand exchange with oxalate based on our findings.

Relevant thermodynamic data are listed in Table 1.

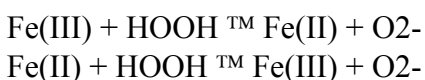
	log K	Ref	K _n	ε ₄₂₆	Ref
Fe(HDFB) ⁺	30.6	Smith (1977)	K ₆	2460	Monzyk (1982)
Fe(Ox) ₃ ³⁻	9.4	Faust (1993)	K ₉	72	-----
Fe(Ox) ₂ ⁻	16.2	Faust (1993)	K ₈		
Fe(Ox) ⁺	20.4	Faust (1993)	K ₇		
	pKa				
H ₄ DFB ⁺	8.39	Smith (1977)	K ₃		
H ₃ DFB	9.03	Smith (1977)	K ₄		
H ₂ DFB ⁻	9.7	Smith (1977)	K ₅		
H ₂ DFB ²⁻	---	-----	---		
H ₂ Ox	1.25	Smith (1977)	K ₁		
Hox ⁻	4.266	Smith (1977)	K ₂		
Ox ²⁻	----	-----			

Using the model it is possible to calculate the distribution plot for Fe(III) species as a function of pH for iron in the presence of DFB and oxalate (figure 4). These results suggested that DFB covalently bonded to resin solid phase would result in an analytical column for Fe(III) that would have a high affinity for Fe(III) at a pH above 5.0 and could be eluted with oxalate at a pH below 4. The Tripp group successfully synthesized the DFB resin and we have used this material as part of a flow injection analysis instrument capable of nM detection of bioavailable iron.

Critical steps in the instrument development included quantification of the resin loading capacity, determination of optimal loading and elution steps, and development of a chemiluminescent method for Fe(III)-tris oxalate determination.

The loading and elution steps used for Fe(III) analysis in seawater is shown below. The DFB column is first rinsed with oxalate to remove and residual iron. Oxalate will precipitate the Mg(II) in seawater so the oxalate rinse is followed by a dilute HCl rinse to remove oxalate from the column. Next, 20 ml of seawater is passed through the column with bioavailable Fe(III) being retained. Again, the column is rinsed with dilute HCl. This step removes the sea salts from the column but does not elute the iron from the DFB complex. Finally the column is eluted with 0.1 M oxalate at pH 3.6.

We used a modification of the Obata method to measure Fe(III)-tris oxalate eluted from the column. The Fe(III)-trisoxalate eluent was mixed with 3% hydrogen peroxide and then mixed with an alkaline luminol solution to initiate chemiluminescence. Under alkaline conditions hydrogen peroxide will react with Fe(III) to produce superoxide.



Superoxide in the presence of excess hydrogen peroxide will oxidize luminol in a two-step mechanism to produce the chemiluminescence product 3-aminophthalate. Current detection limits for Fe(III) added to dilute acetate buffers (pH 6) or seawater are 1 nM. As expected, addition of DFB to our Fe(III) standards eliminates the analytical signal since solution phase Fe(III)-DFB complexes are not retained on the column.

The analytical system was used to measure “bioavailable” iron in Darmascotta estuary water (8 nM). Parallel to these experiments, Karen Orcutt used radiolabeled iron to determine iron uptake rates in native plankton cultures to determine the actual bioavailable pool of Fe(III) (7-17 nM). There was close correspondence between both measures, indicating that the chemiluminescent analyses indeed are measuring a biologically relevant fraction of Fe in seawater.

Refinement to the chemiluminescent analytical system focusing on optimal loading and elution times.

Significant work was also performed on procedures to clean the oxalate reagent. A Fe(III) profile was taken from the Gulf of Maine up the Kennebec River to the 10 o/oo salinity boundary. Analysis of the particle rich river water demonstrated that particle loading onto the DFB resin interferes with this method. The resin cartridge effectively retains particles and these solid phases are solubilized by the oxalate eluent. This interference can be avoided by prefiltering the samples.

Development of an IR based method for detecting total Fe

The information derived from DFB columns were critical to the development of a solid phase support that extracts Fe(III) from seawater and that is amenable to IR spectroscopic analysis. This led to a simple chip-based method to measure dissolved iron in natural seawater. (tripp ref 4) The device comprises an iron-specific chelating biomolecule, desferrioxamine B (DFB), covalently immobilized on a mesoporous silica film. Changes in infrared spectral signatures of the immobilized DFB upon complexation provides a precise measure of iron on the surface of a chip exposed to seawater. The biosensor has a detection limit of 48 pM for 1 L sample and was used to accurately measure iron in subarctic Pacific waters without interference from other elements in seawater. This technology offers a significant step towards obtaining accurate iron measurements on autonomous research platforms.

Future Publications

In addition to the papers published and currently in review, there are a suite of up to 8 other publications currently in the works that describe other aspects of the project. We anticipate having the bulk of these submitted within 2008.