Development and Commercialization of Autonomous Chemical and Biological Instrumentation for Water Quality Monitoring

Fourth Quarterly Project Report
9/1/2016

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Objective 1: Small organic sensor for arsenate: Orion B. Berryman

Hirings:
No new hires were made during this period. Currently four people are supported by the grant including Christopher Grubb (undergraduate student), Evan McManigal (undergraduate student), Asia Riel (graduate student) and Daniel Decato (graduate student).

Equipment Purchased:
No additional major equipment was purchased during this period.

Progress towards Milestones:

A. Publications: Since the last report one publication related to this work is in press. In total, two publications citing this funding source have been published and one is nearing publication.

- "A Halogen Bond Induced Triple Helicate Encapsulates Iodide." C. J. Massena; N. B. Wageling; D. A. Decato; E. M. Rodriguez; O. B. Berryman* **2016**, *Angewandte Chemie International Edition*, *in press*.

B. Synthesis and Crystallography: Initial ligand has been synthesized. Various arsenic salts have also been prepared for future quantitative binding studies.

C. Separations: Additive compound has been identified and tested to improve separation of halides in capillary electrophoresis (in collaboration with Palmer group).

The following activities have been undertaken under this objective:

A provisional patent application has is being evaluated based on new technology discovered through this funding.

Total amount of expenditures as of 9/1/16:

Total Budget:	$319,054	
All Expenditures:	$125,242	Salary, benefits, tuition, equipment and supplies
All Encumbrances:	$16,543	Salary, benefits, tuition, equipment and supplies

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Objective 2: Field capable capillary electrophoresis methods and instrument
Christopher Palmer

Hirings:

Dr. Jesse Hyslop was hired as a postdoctoral research associate. Dr. Hyslop will oversea day-to-day operations of the project. The project continues to support a graduate student (William Penny) and an undergraduate student (Tristan McGettrick)..

Equipment Purchased:

No additional major equipment was purchased during this period.

Progress towards Milestones:

A. Robust laboratory CE method for analysis of anions (nutrients, fracking return) and cations (fracking return):

Continued progress was made on a laboratory CE method for the separation and analysis of anions bromide, chloride, nitrate, nitrite, sulfate, fluoride, bicarbonate and phosphate. Optimization and pre-concentration work has greatly improved the sensitivity of ion detection with the Limit of Detection in ppb. The separation of 6 anions in Figure 1 was achieved using an ionically modified capillary. An ionic coating will temporarily give the inner capillary wall a positive charge allowing for the separation to take place. It is also easily applied to capillary so there is little prep time. While the 500 parts per billion limit of detection achieved is not the final limit of detection desired, it is a major improvement.

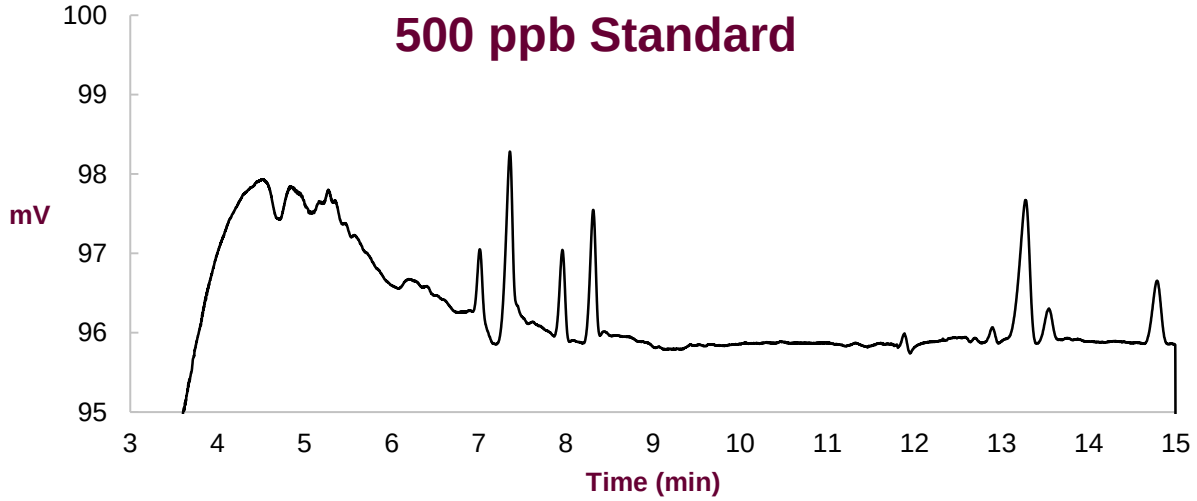

Figure 1 Separation of 6 anions: (1) Bromide, (2) Chloride, (3) Nitrite, (4) Nitrate, (5) Bicarbonate, (6) Phosphate. Separation Parameters: 70mM Borate Buffer at pH 8.2 with a 70cm capillary.

B. Working field-able CE instrument technology addressing power source, detection, sample introduction, and data collection and analysis. Adaptation of methods from bench-top CE to field-able technology

The prototype instrument has been redesigned from commercial and 3D-printed parts and components (Figure 2) into a smaller and more robust configuration. Improved custom built electronics and control modules have been developed, manufactured, and tested in collaboration with Vintage Lab Tech, LLC in Missoula MT. A prototype purpose-built operating system has also been developed and implemented to provide electronic control of all instrument functions.

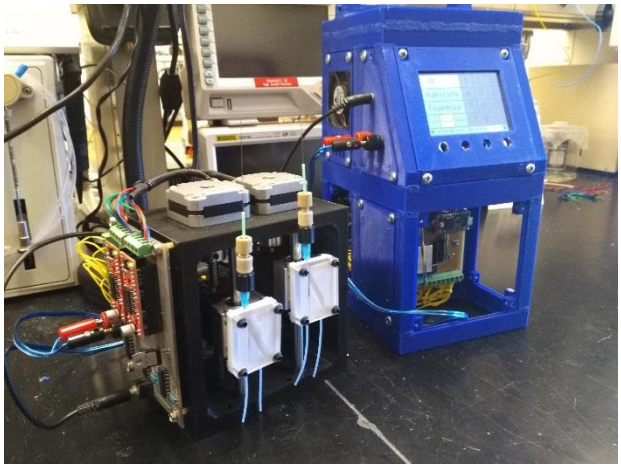

Figure 2 Prototype portable CE instrument components.

C. Analysis of field samples.

No changes in this criteria during the reporting period.

Total amount of expenditures as of 9/1/16:

Budget:	$286,350	
All Expenditures:	$ 111,064	Sal.& ben., tuition, instrumentation and supplies
All Encumbrances:	$ 57,412	Salary & Benefits, Supplies
Total:	$168,476	

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Objective 3: Testing and optimization of large-volume water sampling and filtration techniques for the autonomous collection of eDNA samples using DNA tests for multiple invasive and rare/threatened species along with related environmental data (water temperature, flow, and turbidity)

Hirings:

No new hirings. Currently three people are supported by the grant besides the PIs, including Dulaney Miller, Seth Smith, and Rachel Walker.

Supplies & Equipment purchased:

Purchased additional qPCR assays needed for testing samples. Purchased additional reagents and lab consumables needed for extracting DNA from samples and testing them. Purchased some tools necessary to make 15 eDNA stream samplers for testing.

Progress towards Milestones:

1. August 1, 2015 – February 1, 2016: Collect preliminary data on sensitivity of existing eDNA sampling methods. Develop qPCR assays for detection of species of invasive and native species of interest.
    a. Completed sequence database of existing native and invasive species for designing qPCR assays.
    b. Tested design modifications to the eDNA sampler ('stream sampler') field protocol and collection bucket.
    c. Collected a second round of 150 controlled experimental samples and 150 field samples for a refined comparison on the sensitivity of existing eDNA sampling methods
2. February 1 – August 1, 2016: Analyze preliminary data. Design autonomous eDNA sampling prototype.
    a. Conducted paired sample comparison between the two extraction protocols on 30 samples to determine the quantity and quality of DNA being extracted using the two methods.
    b. Researched existing commercially available filters for evaluation of their potential use in an autonomous eDNA sampler prototype.
    c. Continued work on the preliminary design of autonomous sampler prototype.
    d. Began researching parts and materials needed to construct first prototype.
3. August 1, 2016 – February 1, 2017: Field-test autonomous eDNA sampling prototype.
    a. Not yet started
4. February 1 – July 31, 2017: Analyze data, prepare intellectual property documents, and prepare research publications.
    a. Began process of applying for patent on eDNA 'stream sampler' through the University of Montana.

Total Amount of Expenditures as of 9/1/16:

Total Budget	
All Expenditures	Salary, benefits, & supplies
All Encumbrances	Salary & benefits

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Objective 4: Lab testing of a combined pH and alkalinity system for in situ freshwater measurements: Mike DeGrandpre

Hirings:

There were no new hirings during this period. Currently, three people are supported on the grant – Graduate student Reba Van Beusekom, Research Associate Cory Beatty, and Post-doctoral associate Chunze Lai.

Equipment Purchased:

No equipment has been purchased.

Progress towards Milestones:

A. **Year 1 (4th quarter):** The milestone during this period was to complete the laboratory testing of the autonomous alkalinity systems (named SAMI-alk). During the last quarter we built three autonomous alkalinity systems in collaboration with Sunburst Sensors (www.sunburstsensors.com). The goal of the laboratory testing was to gain a better understanding of the parameters that control the instrument performance, i.e. precision, accuracy and long-term reproducibility. Post-doctoral researcher Chun-Ze Lai and graduate student Adam Prody, who is funded on a separate NSF grant, undertook these studies with assistance from Research Associate Cory Beatty. This period included these specific activities:

   - Adam Prody and Cory Beatty conducted a study in August 2016 of the instrument performance using Scripps Institution of Oceanography's seawater test tank. Three SAMI-alk systems were placed in the tank along with other sensors for CO_2, pH, temperature and salinity. These tests provided very valuable data for understanding the performance of the autonomous alkalinity systems.
   - During this time Chunze-Lai rewrote the data processing software for the SAMI-alk so that it could be used for the analysis of freshwater. She began laboratory analysis of freshwater. This is the first time the SAMI-alk has been used for freshwater analysis.
   - Reba van Beusekom has continued to test a simplified version of the SAMI-alk that does not require a stirred optical cell. Her work has shown that performance improves as mixing of the sample and titrant increases.

B. **Inception – July 31, 2017:** Analyze data, prepare intellectual property documents, prepare research publications

This grant has supported publication of a manuscript closely related to the grant objectives, cited here:

Lai, C-Z., DeGrandpre, M.D., Wasser, B., Brandon, T., Clucas, D., Jaqueth, E.J., Benson, Z., Beatty, C. M. and R.S. Spaulding **(2016)**. Spectrophotometric measurement of freshwater pH with purified meta-cresol purple and phenol red, *Limnol. Oceanogr. Methods,* doi: 10.1002/lom3.10137.

Total amount of expenditures as of 9/1/16:

Total Budget:	$290,971	
All Expenditures:	$150,000	Salary, benefits and supplies
All Encumbrances:	$33,000	Salary, benefits and tuition