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

Fifth Quarterly Project Report
12/1/2016

Christopher Palmer, Michael DeGrandpre, Steve Amish and Gordon Luikart, PIs
University of Montana, Missoula

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:
We have submitted for purchase an Agilent Cary 60 UV-Vis and Cary Eclipse fluorimeter to quantify analyte binding to synthesized compounds.

Progress towards Milestones:

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

- "Advantages of organic halogen bonding for halide recognition" N. B. Wageling; G. F. Neuhaus; A. M. Rose; D. A. Decato; O. B. Berryman, **2015**, *DOI: 10.1080/10610278.2015.1118101*
- "Protonation and alkylation induced multidentate C-H---Anion binding to Perrhenate" A. M. S. Riel; D. A. Decato; O. B. Berryman, **2015**, *Crystal Growth and Design*, *DOI: 10.1021/acs.cgd.5b01524*
- "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*, 55, 12398-12402. - Front cover artwork, featured by NSF EPSCoR/IDeA Foundation, SciShow, UM press release, Montana Associated Technology Roundtables, Montanan Magazine, UM President's Report, and UM We Are Montana tour.
- "Experimental Evidence of Halogen Bond Hard-Soft Acid-Base Complementarity." A. M. S. Riel; M. J. Jessop; D. A. Decato; C. J. Massena; V. R. Nascimento; O. B. Berryman, **2016**, *Acta Crystallographica Section B*, *in press*. – Invited contribution

B. Synthesis and Crystallography: An initial ligand for arsenate has been synthesized. Various arsenic salts have also been prepared for future quantitative binding studies. Crystallizations have been set up to structurally characterize both halogen bonding and hydrogen bonding complexes.

C. Quantification: Preliminary binding studies have been performed quantifying the strength of the interaction between the ligand and arsenic.

D. 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:

Working with Joe Fanguy, director of technology transfer at UM, a provisional patent application has been filed (application number 62/362,226) based on new technology discovered through this funding.

Total amount of expenditures as of 12/1/16:

Total Budget:	$319,054	
All Expenditures:	$194,167	Salary, benefits, tuition, equipment and supplies
All Encumbrances:	$76,779	Salary, benefits, tuition, equipment and supplies

****

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

Hirings:

Undergraduate student Daniel Olson was hired to assist with code for instrument control and data acquisition. The project continues to support a postdoc (Dr. Jesse Hyslop) 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):

Progress continues to be made on a laboratory CE method for the separation and analysis of anions. Optimization and pre-concentration continues to improve the sensitivity of ion detection with the Limit of Detection becoming twice as good as in the last report. The separation of 6 anions in Figure 1 was attained using a modified capillary. This means that the coating on the inner capillary wall was covalently modified to have a permanent positive charge. Previously the capillary was modified ionically with a positive coating. An ionic coated capillary requires less time to prepare, but has a shorter lifetime, making an autonomous instrument need to periodically re-condition the capillary. A limit of detection of 200 parts per billion was achieved for all 6 anions which now allows the instruments to analyze for low levels of nutrients.

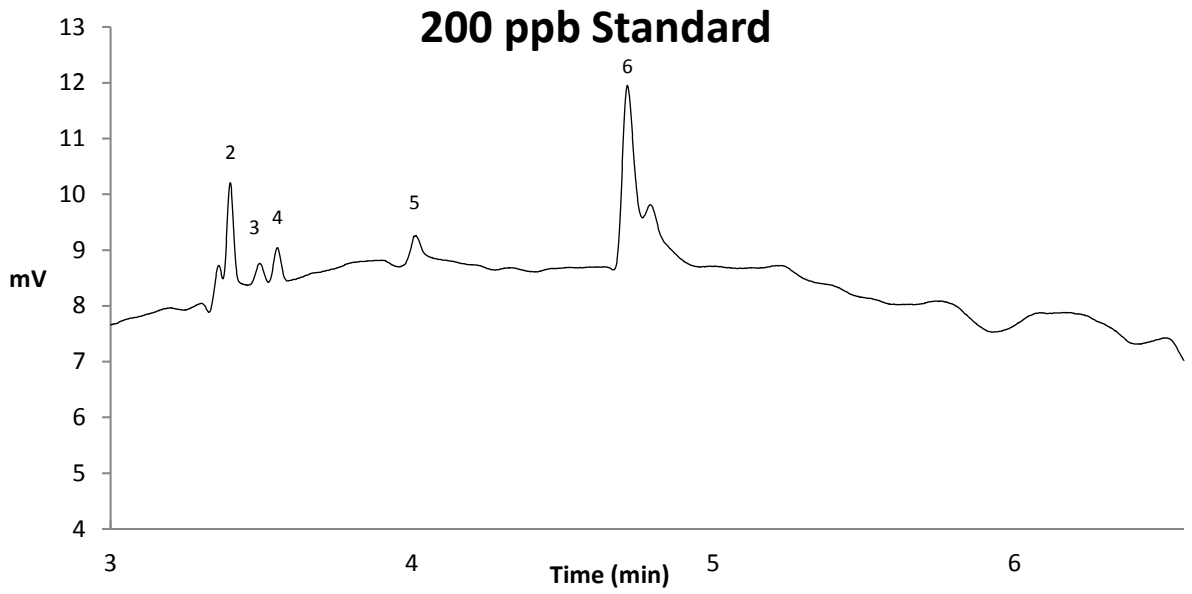

Figure 1 Separation of 6 anions: (1) Bromide, (2) Chloride, (3) Nitrite, (4) Nitrate, (5) Sulfate, (6) Bicarbonate. Separation Parameters: 70mM Borate Buffer at pH 8.2 with a 70cm capillary.

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

Significant development has been put into refining the fluid path through the instrument. The lifter assembly has been redesigned to be lighter and more power efficient (Figure 2). The electronics has been revised to provide better modularity and electronic isolation in collaboration with Vintage Lab Tech, LLC in Missoula MT.

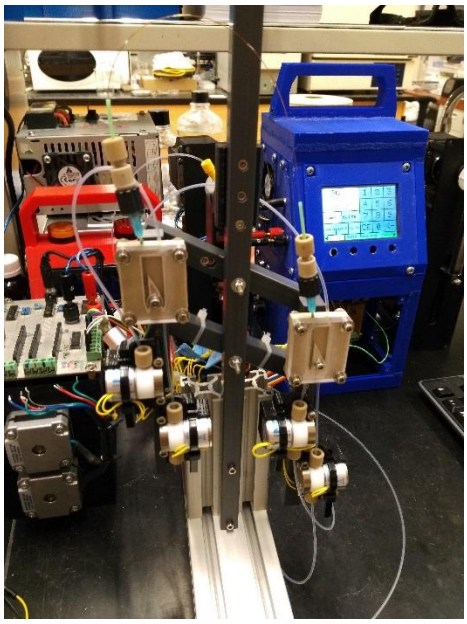

Figure 2 Prototype portable CE instrument components.

C. Analysis of field samples.

Initial analysis of Montana waters using the laboratory method we have developed shows very promising results. Figure 3 is an example electropherogram of the analysis of the Clark Fork River water in Missoula, MT. At the moment the peaks labeled are speculative, and further research has to be done in order to confirm validity of these assignments, as well as their concentrations. This was completed using the covalently modified capillary.

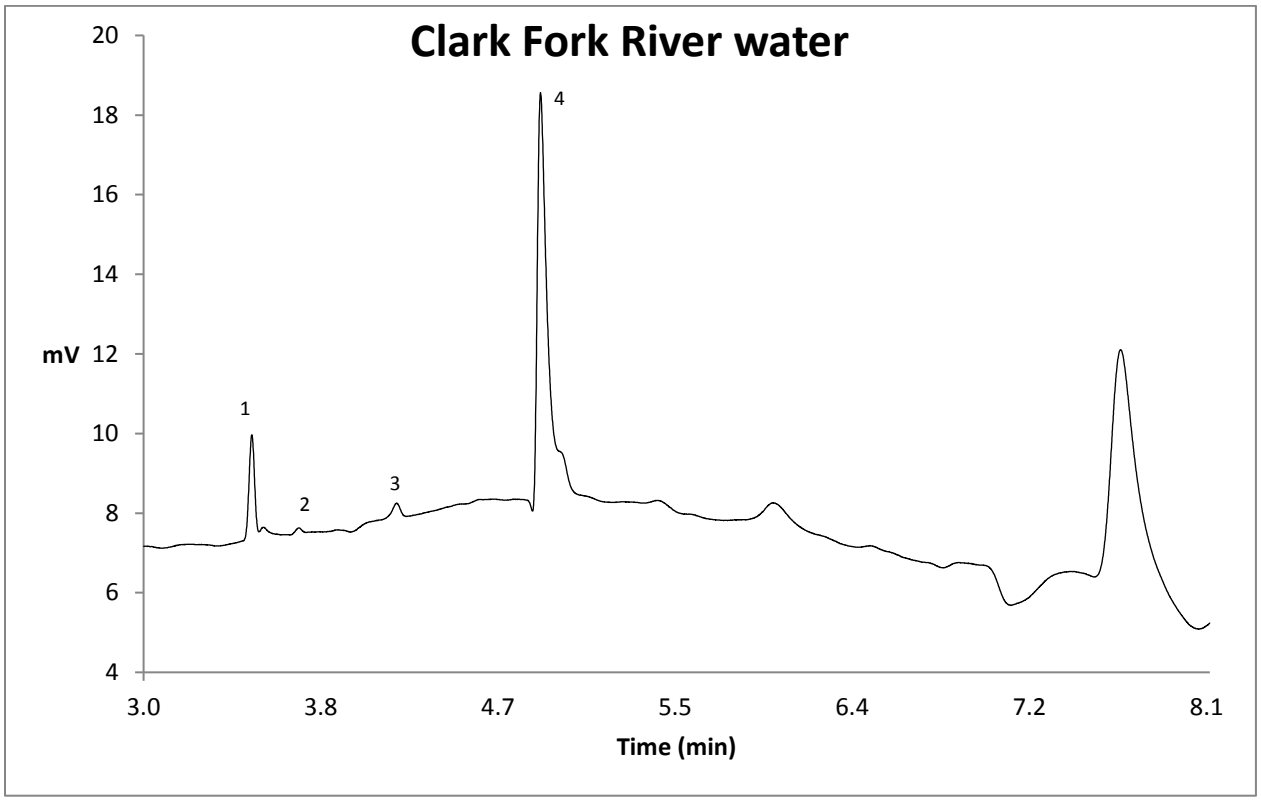

Figure 3 Analysis of Clark Fork River water: (1) Chloride, (2) Nitrate, (3) Sulfate, (4) Bicarbonate. Separation parameters: 70mM Borate Buffer at pH 8.2 with a 70cm capillary.

One publication authored by William Penny and Christopher Palmer, both supported by this project, was accepted for publication:

"Phospholipid bilayer affinities and solvation characteristics by electrokinetic chromatography with a nanodisc pseudostationary phase," *Electrophoresis*, in press,
DOI: 10.1002/elps.201600381

Palmer, Hyslop and McGettrick have met with Joe Fanguy of the UM Office of Research and Sponsored Programs and Montana Enterprise Center to discuss options to protect intellectual property associated with the new instrument design.

Total amount of expenditures as of 12/1/16:

Budget:	$286,350	
All Expenditures:	$160,368	Sal.& ben., tuition, instrumentation and supplies
All Encumbrances:	$ 38,729	Salary & Benefits, Supplies

****

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:
Currently three people are supported by the grant besides the PIs, including Dulaney Miller, Seth Smith, and Rachel Walker. We are currently looking for another molecular biology lab technician to help process additional eDNA samples expected in the Spring and Summer of 2017, and another researcher to lead the eDNA monitoring program.

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.

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 interest.
    a. Collected a second round of controlled experimental samples and field samples for a refined comparison on the sensitivity of existing eDNA sampling methods
    b. Extracted DNA, amplified target DNA, and quantified the amount of target DNA present using qPCR for second round of experimental and field samples.
    c. Designed an emergency eDNA sampling strategy for Flathead Lake after detection of invasive mussel veligers (larvae) in the Missouri River basin became public.
    d. Collected 100 eDNA samples from Flathead Lake with team from the Flathead Lake Biological Station.
2. February 1 – August 1, 2016: Analyze preliminary data. Design autonomous eDNA sampling prototype.
    a. Preliminary design of autonomous sampler prototype completed.
    b. Began researching parts and materials needed to construct prototype.
    c. Produced 3-D models of an eDNA filter capsule prototype to be printed using University of Montana's 3D printer.
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. Continued process of applying for patent on eDNA 'stream sampler' through the University of Montana
    b. Drafted publication reporting large volume samples are more sensitive than small volume using experimental and field data collected using traditional sampling methods.
    c. Attended meetings in Helena with Incident Command Team (ICT) established to respond to the discovery of invasive mussels.
    d. Designed an eDNA sampling strategy and budget for Tiber Reservoir and Canyon Ferry Reservoir to confirm mussel presence for the state of Montana.

e. Attended a Science Panel discussion on invasive mussel detection, containment, and eradication organized by the ICT in Helena.
f. Secured $125k funding from anonymous donors for eDNA research and monitoring.
g. In collaboration with Brian Hand at Flathead Lake Biological station, we submitted eDNA grant proposal to NASA for $1.5M

Total Amount of Expenditures as of 12/1/16:

Total Budget	$396,023.00	
All Expenditures	$105,021.54	Salary, benefits, & supplies
All Encumbrances	$10,715.58	Salary, benefits, & minor equipment

****

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 2 (1st quarter):** The objective during this period is to continue laboratory and field testing of the autonomous alkalinity systems (SAMI-alk) for freshwater applications. These tests have been conducted by post-doctoral researcher Chun-Ze Lai with assistance from Research Associate Cory Beatty. This period included these specific activities:

- The SAMI-alk was used to analyze freshwater for the first time. These laboratory tests showed that the instrument can obtain accuracy and precision comparable to seawater analysis.
- Based on these promising results, we deployed the SAMI-alk in the Clark Fork River for three days in early November. However, due to a programming error the instrument did not collect as many data as planned. The limited data provided evidence of potential application of SAMI-alk in freshwater systems and also identified some problems that were not observed in the lab tests. We have not been able to redeploy the instrument since this initial attempt because of ice formation in the river.
- While we wait for the river ice to dissipate, Chun-Ze has continued characterizing the freshwater response in the lab. These tests have included a more thorough examination of the alkalinity accuracy over a broad range of concentrations and temperatures.
- Reba van Beusekom has continued to test a simplified version of the SAMI-alk that does not require a stirred optical cell.

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

A patent on the technology should be issued in March (this was reported previously but the issue date has been revised):

DeGrandpre, M.D., Martz, T.R. and A.G. Dickson. Tracer monitored titrations, U.S. Provisional Patent Application, Serial No. 60/763,170.

Total amount of expenditures as of 1/9/17:

Total Budget:	$290,971	
All Expenditures:	$178,895	Salary, benefits and supplies
All Encumbrances:	$66,968	Salary, benefits and tuition