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

Sixth Quarterly Project Report
3/1/2017

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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:
We have purchased and received 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 book chapter has been submitted and is in press. In total, three publications related to this work have been published and two are in press.

- "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

- "Aspects of Multi-component Crystals: Synthesis, Concepts and Function." D. A. Decato; O. B. Berryman, **2017**, Tiekink, E. R. T., Eds; De Gruyter Publishers. *In press*.

B. Synthesis and Crystallography: The initial synthetic goals of this project have been accomplished. A ligand for arsenate has been synthesized. Soluble variants of arsenic salts have also been prepared for quantitative binding studies. Crystallizations have been set up to structurally characterize both halogen bonding and hydrogen bonding complexes.

C. Quantification: Binding studies have been performed quantifying the strength of the interaction between the ligand and arsenic in dimethyl sulfoxide. Currently the speciation of the complexes are being determined by JOB plots and computations.

D. Separations: silica particles have been prepared and functionalized with halogen bond donors. Analysis of these materials will be analyzed as new stationary phases for improved anion separation (in collaboration with Palmer group).

Total amount of expenditures as of 3/15/17:

Total Budget:	$319,054	
All Expenditures:	$258,415	Salary, benefits, tuition, equipment and supplies
All Encumbrances:	$22,322	Salary, benefits, tuition, equipment and supplies

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

Hirings:

The project continues to support a postdoc (Dr. Jesse Hyslop) a graduate student (William Penny) and two undergraduate students (Daniel Olson and 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):

Testing on the robustness of the covalently bound capillary coating implemented in the last period has begun with both lot-to-lot consistency and durability over the course of numerous runs being tested.

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 electronics developed in the last period have been implemented and tested. Major improvements to the custom-made operating system have been implemented on the new electronics hardware allowing automated execution of hardware operations and the automated execution of series of operations. This enables the prototype system to run the CE hardware through automated separations methods.
Method storage has been implemented as well, allowing methods to be saved to SD memory cards, and improved serial communications with the pumps has improved efficiency.
The primary CE system has also been transitioned onto battery power. All electronics and most software development was conducted in collaboration with Vintage Lab Tech, LLC in Missoula MT.

Total amount of expenditures as of 3/14/17:

Budget:	$286,350	
All Expenditures:	$183,170	Sal.& ben., tuition, instrumentation and supplies
All Encumbrances:	$ 26,593	Salary & Benefits, Supplies

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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:
None.

Supplies & Equipment purchased:
We purchased and received a freezer for storing eDNA samples. We have purchased and received valves, pvc piping, sensors, and electronic components for prototype construction.

Progress towards Milestones:

1. Analyze preliminary data. Design autonomous eDNA sampling prototype.
    a. Produced two versions of an eDNA filter capsule prototype using University of Montana's 3D printer.
    b. Revised prototype design schematic based on research into new sample preservation techniques.
    c. Contracted electrical engineer Doug McIntire (JDM Enterprises, Inc., Missoula, MT) to develop and test a circuit board for autonomous sampler prototype.
    d. Further refinement of the prototype design schematic based on recommendations from Doug McIntire.
2. Analyze data, prepare intellectual property documents, and prepare research publications. Monitoring work to help protect Montana's waters.
    a. After discovery of invasive mussels in Tiber Resrvoir, we designed and planned collection of eDNA samples from Flathead Lake to determine whether eDNA

from invasive mussels was present. We collected and processed 100 eDNA samples from Flathead Lake with a team from the Flathead Lake Biological Station. No invasive mussel DNA was detected in any of the samples.

b. Begin planning future sampling efforts with both the Confederated Salish & Kootenai tribes and the Blackfeet tribe to use eDNA sampling to protect their waters.
c. Designed and began work on zebra and quagga mussel qPCR assay cross-validation between three independent labs using samples collected across North America. We will identify the most sensitive and specific qPCR assay for use detecting these species in eDNA samples, and demonstrate the repeatability of the results between labs. This work has not been done for any other aquatic invasive species except Asian carp, and is highly valuable for demonstrating the usefulness of the assay.
d. Began testing the effectiveness of digital droplet PCR (ddPCR), a new method testing eDNA samples for presence of a target species, using an assay designed to detect rainbow trout.

Outreach:
Planned and conducted public meetings across the Flathead Basin to promote awareness of the threat posed by invasive mussels, to engage the public in future monitoring efforts, and to report the results of the Flathead Lake sample testing.

Presented MREDI eDNA work at Missoula Interdisciplinary Science League (MISL) launch party aimed at informing kids about Science.

Total Amount of Expenditures as of February 29, 2016:

Total Budget	$314,979.46	
All Expenditures	$155,906.18	Salary, benefits, & supplies
All Encumbrances	$19,956.01	Salary, benefits, & minor equipment

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

Hirings:

None

Equipment Purchased:

No equipment has been purchased.

Progress towards Milestones:

A. **Year 2 (2nd quarter):** The objective during this period has been to continue laboratory and field testing of the autonomous alkalinity systems (SAMI-alk). These tests have been conducted by post-doctoral researcher Chun-Ze Lai with assistance

from Research Associate Cory Beatty. Reba Van Beusekom is also working on a benchtop version of the instrument. This period included these specific activities:

- In past laboratory tests we showed that the instrument can obtain accuracy and precision comparable to seawater analysis. More recently, tests have found good accuracy of alkalinity over a broad concentration range (800 – 3000 µmol/kg) with errors within ± 3 µmol/kg. The long-term stability of the instrument is now under investigation.
- Tests of the SAMI-alk over a broader temperature range (~10-25°C) found a loss of accuracy at temperatures below 15°C. We are still investigating the source of this offset.
- We are prepared to repeat the Clark Fork River deployment conducted in November 2016 but have not been able to do so because of ice and (now) high runoff conditions. We are afraid of losing the instrument due to the high flows and the river is dangerous to work in under these conditions. There might be a short window to deploy in April when flows drop after the low elevation snow melt. If not we will not be able to deploy the instrument until late June at the earliest due to the high flows.
- Reba van Beusekom has continued to test a simplified version of the SAMI-alk that does not require a stirred optical cell. An important aim of this project is to reduce the reagent consumption and complexity of the SAMI-alk used for the studies above. These tests have found that results are more reproducible if larger mixing volumes are used. She is continuing to characterize the performance dependence on different operating parameters.
- We have been assisting the Amish/Luikart team with the design of an autonomous eDNA sampling system, in collaboration with Sunburst Sensors.

B. **Inception – March 1, 2017:** Analyze data, prepare intellectual property documents, prepare research publications.

A patent on the technology has just been issued:

- DeGrandpre, M.D., Martz, T.R. and A.G. Dickson. Tracer monitored titrations, U.S. Patent 2017_02_08_UMT-114X_15/419129.

A related manuscript has been accepted for publication:

- Stets, E.G., Butman, D., McDonald, C.P., Stackpoole, S., DeGrandpre, M.D. and R.G. Striegl (2017). Carbonate buffering and metabolic controls on carbon dioxide in rivers, *Journal of Geophysical Research – Biogeosciences*, accepted. (to be highlighted in the American Geophysical Union weekly newsletter *EOS*)

A related NSF grant has just been funded in collaboration with UM (M. Valett) and MSU (R. Payne, J. D'Andrilli) researchers:

- LTREB: Collaborative Research - River ecosystem responses to floodplain restoration

Total amount of expenditures as of 3/28/17:

Total Budget:	$290,971	
All Expenditures:	$206,479	Salary, benefits and supplies
All Encumbrances:	$46,834	Salary, benefits and tuition