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

Seventh Quarterly Project Report
6/1/2017

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

Hirings:
One new hire was made during this period. Ben Palmer has been hired for the summer facilitate transferring laboratory technology into instrumentation development. Currently five 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 received and are operating 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 is in press and another journal article is published. In total, four publications related to this work have been published and one is 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, **2017**, *Acta Crystallographica Section B*, *B73*, 203-209. – 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. Quantification: Binding studies have been performed quantifying the strength of the interaction between the ligand and arsenic in dimethyl sulfoxide. The speciation of the complexes has been determined by JOB plot and the data suggests a 2:1 host:guest ration.

C. Calculations: Density Functional Theory calculations have been performed to understand the unusual binding stoichiometry for arsenate.

D. Separations: silica particles have been prepared and functionalized with halogen bond donors. Initial analysis of these materials as new stationary phases for improved anion separation is underway (in collaboration with Palmer group). Modification and testing of materials will continue.

Total amount of expenditures as of 6/1/17:

Total Budget:	$319,054	
All Expenditures:	$305,517	Salary, benefits, tuition, equipment and supplies
All Encumbrances:	$2,641	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):

A final method for anions using a non-covalently bound polymer coating for the capillary walls has been developed. This method allows separation of chloride, nitrate, nitrite, carbonate, phosphate and an internal standard (benzene sulfonic acid). The method has been transferred to the in-house built field-able CE system.

A method for separation of cations, which does not require capillary coating, was also developed.

Publications:

"Phospholipid Bilayer Affinities and Solvation Characteristics by Electrokinetic Chromatography with a Nanodisc Pseudostationary Phase," William M. Penny, Harmen B. Steele, J.B. Alexander Ross, Christopher P. Palmer, (2017) Electrophoresis, 38, 738-746.

"Evaluation of Poly([2-(Acryloyloxy)ethyl]trimethylammonium Chloride) Cationic Polymer Capillary Coating for CE and Electrokinetic Chromatography Separations," Julie R. McGettrick and Christopher P. Palmer, (2017) Journal of Separation Science, submitted.

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

A fully-functional prototype portable CE system has been built and demonstrated. The system is able to run the laboratory-based methods with minimal manual operation, and has been used for the analysis of Clark Fork River water.

A custom-made operating system and newly designed electronics hardware allow automated execution of series of operations to allow the instrument to run through automated separations methods that include capillary preconditioning, sample introduction, separation, detection, and data logging. Method storage has been implemented as well, allowing methods to be saved to SD memory cards. The primary CE system operates on battery power. To be made fully portable, the commercial detector system still needs to be converted to 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 6/14/17:

Budget:	$286,350	
All Expenditures:	$238,425	Sal.& ben., tuition, instrumentation and supplies
All Encumbrances:	$ 44,349	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)

Steve Amish and Gordon Luikart

Hirings:
Doug McIntire, Electrical engineer, JDM Enterprises, Inc.

Supplies & Equipment purchased:
We purchased and received a new qPCR machine increasing the number of eDNA samples that can be tested and the sensitivity of the tests. We also received many components needed for assembly of three autonomous eDNA sampler prototypes including: solenoids, circuit boards & processors, vacuum sensors, flow sensors, filter housings, and pumps.

Progress towards Milestones:
1. Analyze preliminary data. Design autonomous eDNA sampling prototype.

a. Designed and built an LCD text display and selector switches to setup sampling programs.
b. Built a mock-up consisting of the LCD text display and selector switches, a DC latching solenoid valve, a manual valve, a flow meter, a turbidity sensor, a vacuum sensor and a pump connected to a 12-volt, 33 amp-hour battery to test and refine the motor drive circuit.
c. Improved the motor drive circuit to run over a wider speed range and operate as cool and quiet as possible.
d. Coded and refined the motor control software so a specific flow rate and volume can be specified for sampling.
e. Designed software so turbidity and vacuum are monitored before and during sample collection with programmable thresholds to avoid damage to the instrument.
f. Designed, ordered, and received modular driver boards that will operate 8 solenoid valves each.
g. Designed, ordered, and received the main control boards with non-volatile memory to store all data associated with a sample (volume, collection time, sample time, filter number, and external water quality sensor reading) from internal or external sensors (via a RS-232 serial data port).

2. Analyze data, prepare intellectual property documents, and prepare research publications. Monitoring work to help protect Montana's waters.
    a. We processed, analyzed, and wrote reports for over 150 eDNA samples from Flathead Lake, the Whitefish Lake Institute, the Blackfeet Tribe, and the Confederated Salish and Kootenai Tribe, the Swan River, and Glacier National Park. No invasive mussel eDNA was detected in any of the samples.
    b. Hosted a visiting USGS scientist (J. Amberg) to design two collaborative cross-validation studies to further improve eDNA monitoring for early detection of zebra/quagga mussels (including new qPCR assays, different PCR inhibitor removal approaches, and the new LAMP PCR technology and assay for zebra mussels.
    c. Finished validating a new qPCR assay for the invasive aquatic pondweed species. We processed, analyzed, and wrote a report on pondweed testing of samples from the Swan Valley for the U.S. Forest Service.
    d. Collected preliminary data on digital droplet PCR (ddPCR), a new method testing eDNA samples for presence of a target species, using an assay designed to detect zebra and quagga mussels.
    e. Generated and analyzed data using two qPCR assays (rainbow trout, Euarasian milfoil) for samples comparing high and low volume eDNA techniques for lakes. Added this data to a manuscript in preparation for submission.
    f. Helped write and submit a grant proposal to the Lake County Conservation District to sample for early detection of zebra mussels around Flathead Lake.
    g. Prepared advertisement (through UM HR department) to hire a new eDNA coordinator/technician to hire in our lab.

Outreach:
Continued 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. Corresponded with Montana Fish Wildlife and Parks (FWP), USGS, USFS, county weed districts, and other agencies about continuing and improving AIS eDNA monitoring.

Total Amount of Expenditures as of July 17, 2016:

Total Budget	$314,979.46
All Expenditures	$280,772.72
All Encumbrances	$23,240.00

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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:

An automated system for the measurement of dissolved oxygen in freshwater was purchased.

Progress towards Milestones:

A. **Sub-objective 1 (Quarter 7):** The objective during this period has been to continue laboratory and field-testing of the autonomous alkalinity system (SAMI-alk). Post-doctoral researcher Chun-Ze Lai has conducted these tests with assistance from Research Associate Cory Beatty. Graduate Student Reba Van Beusekom is also working on a benchtop version of the instrument. This period included these specific activities:

- The autonomous alkalinity system has been tested with different ionic strength backgrounds. The measured values match very well with those determined on a benchtop system. The long-term stability has also been estimated by continuous monitoring a freshwater sample for 8 days. We obtained excellent reproducibility (± 2.1 $\mu mol \cdot kg \cdot soln^{-1}$ which is equal to ±0.1%) over the entire period.
- The autonomous alkalinity system low temperature tests (<15°C) have not been as accurate as those at higher temperatures as described in the previous quarterly report. We have not been able to definitively determine the cause of the error but evidence suggests that the indicator solubility is exceeded at low temperatures. If the total indicator concentration decreases, it will lead to systematically low alkalinity values as observed.
- We were not able to repeat the Clark Fork River deployment described in previous reports because the grant could not be extended beyond June 30 when river flows were safer.
- Reba van Beusekom has continued to test a simplified version of the SAMI-alk that does not require a stirred optical cell, significantly simplifying the current prototype. During this quarter we have discovered that indicator adsorption on

the walls of the tubing can significantly influence the results. These findings are the focus of future research that will hopefully be funded by other sources.

- We have continued to assist the Amish/Luikart team with the design of an autonomous eDNA sampling system, in collaboration with Sunburst Sensors.

B. **Sub-objective 2 (Inception – June 1, 2017):** Analyze data, prepare intellectual property documents, prepare research publications, and commercialize any products.

- We have sold an autonomous alkalinity system to a NOAA group focused on testing new autonomous equipment (a $50,000 contract to Sunburst Sensors). The instrument will be deployed in the Arctic Ocean for 40 days in late July 2017. The instrument has generated a lot of interest from the marine research community but we are not prepared to broadly sell the instrument because further optimization is required.

- A related manuscript has been published:

  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, *Global Biogeochem. Cycles*, *31, 663–677, doi:10.1002/2016GB005578*.

  The paper was highlighted on the cover issue of the journal *Global Biogeochemical Cycles*, http://onlinelibrary.wiley.com/doi/10.1002/gbc.20452/epdf and the Americal Geophysical Union newsletter *EOS* - https://eos.org/research-spotlights/why-is-there-so-much-carbon-dioxide-in-rivers?utm_source=rss&utm_medium=rss&utm_content=why-is-there-so-much-carbon-dioxide-in-rivers.

Total amount of expenditures as of 6/01/17:

Total Budget:	$290,971	
All Expenditures:	$246,479	Salary, benefits, tuition and supplies
All Encumbrances:	$35,000	Salary, benefits and tuition