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Assay and Test Kit for the Electrochemical Detection of Lead in Municipal Water Supplies

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This report summarizes the initial results of a drinking water testing project using a homebuilt potentiostat, the CStat Series II. The project has been divided into two phases, the first of which is mostly complete. The primary phase one goal was to develop and characterize a simple assay in the laboratory for two contaminants of interest in municipal water supplies: lead and copper. This goal included hardware optimization, development of an initial field kit and strategy, and logistical planning for future field trials. Current results and future directions are discussed.

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EXECUTIVE SUMMARY

Testing and evaluation of drinking water supplies in the field is of importance to the military. While benchtop-scale testing and large purification units are available for use at forward operating bases, this equipment is not suitable for individual or squad level missions due to size, weight, and power (SWaP) requirements. NRL has previously developed a miniaturized, hand held, and battery operated potentiostat along with assays and data analysis methods for the detection of several classes of contaminants in salt water for diver safety applications including heavy metals, herbicides, pesticides, and toxic industrial compounds [1]. Recent preliminary laboratory studies have shown that these assays can also effectively detect trace amounts of lead spiked into samples of rural well water. Our vision is a field kit that can be carried by an individual warfighter in a small Pelican case.

This report summarizes the initial results of a drinking water testing project using a homebuilt potentiostat, the CStat Series II. The project has been divided into two phases, the first of which is mostly complete. The primary phase one goal was to develop and characterize a simple assay in the laboratory for two contaminants of interest in municipal water supplies: lead and copper. This goal included hardware optimization, development of an initial field kit and strategy, and logistical planning for future field trials. Current results and future directions are discussed.

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1. INTRODUCTION

Individual warfighters on one week missions without re-supply require several liters of drinking water each day, much of which must be obtained from indigenous sources in order to minimize logistical burden. Effective purification methods for drinking water exist and are portable; these include iodine, chlor-floc tablets, and filtration membranes [2]. In contrast, there are currently no fielded technologies for use by an individual warfighter to evaluate the performance of field purification methods, or to assess the hazards posed by indigenous drinking water supplies. This topic is sufficiently important that it was the focus of a recent conference (February, 2022) sponsored by Army DEVCOM Soldier Center with participants representing nineteen different military and DoD groups including the Army Futures Command and the Marine Corps Systems Command. There is also significant academic interest in this topic aimed at crossover civil applications such as the evaluation of municipal and rural drinking water supplies [3], environmental monitoring [4], and the evaluation of wastewater treatment [5].

Current gold-standard analytical methods are ubiquitous but not easily brought into the field. These include various forms of mass spectrometry (GC-MS, LC-MS, ICP-MS), certain optical methods (ICP-OES, AAS), and cell culture. Portable sensors are also available; they can be broken into very broad classes that include (1) miniaturized spectrophotometers including Raman and UV-VIS spectroscopy; along with flow cytometry and turbidity measurements, (2) simple optical sensors including colorimetric and fluorescent methods, (3) electrochemical sensors, and (4) biosensors. All of these portable instruments have disadvantages. Miniaturized spectrophotometers are exquisitely sensitive but require lensing and other precisely aligned optical components that are easily disturbed with rough handling. They are also very expensive and can have difficulty with turbid samples. Colorimetric and fluorescent methods are simple and inexpensive and are often packaged as test strips, but tend to be single-target assays. Biosensors are a special case; they are defined as a sensor with a biological recognition element (eg, enzymes, DNA, whole cells, etc.) linked to a transducer such as those described in the first three categories listed above [6]. In addition to all of the previously stated disadvantages, biosensors sometimes require very long response times and often require a cold chain for reagents. Experts from the DEVCOM conference agree that the effectiveness of both COTS and research versions of portable instrumentation for water quality assessments in the field have not been fully explored. There is a need for a small, battery powered instrument.

This report focuses on electrochemical detection. Laboratory built potentiostats have been popular for about a decade [7]. They are inexpensive, small, and low power, and can be used for both single target and multiplexed assays. Additional cost savings are realized through the use of commercially available and disposable screen printed electrodes. However, it can be challenging to de-convolute the resulting voltammograms. If multiple targets are present in a field sample, and especially if they overlap, quantitative evaluation can be difficult. The ultimate goal of this project is to develop and demonstrate a portable electrochemical test kit containing a multiplexed assay for a variety of species of interest that may be found in contaminated drinking water. The focus of this report is two species: lead and copper.

1.1 Background

Lead contamination in drinking water is a problem that has existed for thousands of years. The ancient Romans discovered that lead made excellent water pipes. The metal is soft and malleable, easily drawn into cylindrical shapes, and the interior resists mineral deposits in a way that other materials do not. For example, cross sections of pipes made from materials such as copper or galvanized steel can develop significant flow restrictions after just a few decades of use with hard water. In contrast, lead pipes installed in the 1900s and used for over a century show a clean cross section. These useful properties made lead the material of choice in Roman, Medieval, English, and early American waterworks. Only recently have replacement materials become widespread.

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Municipal water systems in large US cities mostly pre-date the availability of alternative materials. In a municipal network, water is purified, filtered, disinfected, and sometimes fluoridated at the treatment plant. When leaving the plant, the water is certified to be clean and delivered to the city through water mains. In most areas, these water mains have been replaced with safe materials. However, at the street level, many older houses continue to use lead service pipes, and they may contain copper interior plumbing. A service pipe is the segment that connects an individual house or building to the common water main. There is a much larger number of service pipes than water mains in a municipal network, and service pipes are not easily replaced by the local government because they mostly run under private property and typically go through a basement wall to the interior of the dwelling. Houses in the US built before 1950 are likely to have lead service pipes. Voluntary phase out began in the 1950s, but lead pipes continued to be installed in some cities (such as Chicago) until banned by Congress in 1986.

The best solution to the problem is replacement, but digging up pipes is expensive. This issue was directly addressed by an article recently published by the Brookings Institute [8]. The authors report that as of 2016, nearly 7% of all households in the US have lead service pipes, and that an estimated 400,000 schools and childcare facilities are affected. Clearly, this is a serious problem. It has been addressed in various ways by different localities. In Madison, WI, approximately 8000 lead service pipes were replaced. The project started in 2001 and took 11 years to complete at a cost of $15.5 million to the city and $1300 to each homeowner. In Newark, NJ, the city replaced 18,500 lead service pipes in just 2 years. It was free to homeowners but cost the city $120 million and required an ordinance to be passed that allowed the work to take place without the homeowner's permission. Most cities in the US are unwilling or unable to foot this bill, leaving part or all of the responsibility to the individual homeowner. Due to the expense of replacement, many homeowners have not addressed these problems, even with financial incentives offered by their city or state government.

Focusing on the local area, in 2016 the District of Columbia (Washington, DC) introduced a publicly available lead map [9] at the house level indicating the composition of all of the service lines in the entire city to the best of their knowledge. At the time of writing, this map is the only one of its kind available in the United States. Moreover, the District has a very aggressive lead monitoring and pipe replacement program. One example features a house in northeast Washington, DC that was built in 1911. In 2018 the house was found to have a lead service pipe approximately 60 to 80 feet long. Free analysis provided by DC WASA indicated that the tap water in the house had low levels of lead, roughly 2 ppb. Replacement was recommended, but there was so much demand for this service that it took months to get the work scheduled. It turns out the pipe was buried nine feet underground. It took a crew of ten workers with shovels and a backhoe nearly 14 hours to dig up the pipe and replace it; this included shutting down a relatively busy street and tying the new pipe into the water main on the far side. The primary author of this report estimates the total value of the job was well over $10 K. Sadly, this is in line with EPA estimates, which state that average replacement cost is $4700, with actual values typically observed between $1200 and $12,300 per line. With DC government incentives, the out of pocket cost to replace this pipe was significantly less, at $2 K. However, even with these steep discounts this price is not affordable to many residents in the District. Alternatives such as using filtered water are in widespread use.

There are no safe lead levels in water. Most adults can tolerate some lead content in drinking water without acute health effects. The biggest problems are fetal growth in pregnant women and in children under the age of 4, when brain development is taking place. In these cases, any finite lead exposure has been shown to lead to negative outcomes. Chronic low level exposure in children is especially serious. Brookings cites a study of Swedish students [10] which found that an increase in blood lead levels from 5 to 10 micrograms per deciliter is associated with a decrease in 9th grade GPA of 2.2 percentile points, and a 2.3% decrease in the likelihood of graduating high school—academic consequences that imply a 5.5% decrease in average earnings in young adulthood. In addition, they cite reports that claim a 1% increase

in blood lead level has been shown to raise the probability of teenage aggressive behavior by 4% and criminal behavior by 5%, and for violent crimes, the associated increase is as large as 8%.

Congress has taken some action to reduce chronic lead exposure, such as to phase out lead in gasoline (1973), to ban lead in residential and consumer paints (1978), and eliminate lead solder in food cans (1995). These actions have been very effective. The Brookings study suggests that the phasing out of leaded gasoline in the US in the 1980s resulted in a significant decrease in violent crime in the 1990s. However, a JAMA Pediatrics study [11] found that despite a government report showing that the median lead concentration in the blood of children in the US aged 1 to 5 years dropped from 15 micrograms per deciliter in 1976-1980 to 0.7 micrograms per deciliter in 2013-2014, more than half of the 1.1 million American children under 6 still tested positive for lead in their blood.

Consensus opinion among experts is that a serious effort should be made to eliminate lead from drinking water supplies. But as noted above, due to the sheer size and expense of the project it is unlikely to be completed in the near future. When service pipe replacement is not possible, drinking water monitoring is key. The question becomes: at what level should a threshold be set? The Lead and Copper Rule (LCR) was introduced by congress in 1991; it sets strict limits on the concentration of these two metals allowed in tap water [12]. While the maximum contaminant level goal is zero, a somewhat arbitrary action limit of 15 parts per billion (ppb) has been set for lead. In the District, the unwritten rule of thumb is that monitoring is necessary for any positive lead value over 5 ppb. Copper ions typically come from copper piping that is popular and still used in interior household plumbing. The limits for this metal are much higher; the action level is set at 1300 ppb. Additional EPA limits for other substances may be found on their website [13]; these are typically relevant for well water and other rural sources.

As a final note, these numbers and figures are for the United States only. However, lead water pipes are still in widespread use in other parts of the world and contamination can be severe. This is a real issue for the warfighter: in some locations, even tap water is unsafe to drink without additional purification. Although acute responses typically come from the presence of bacteria, long term chronic health effects can result from consistently drinking contaminated municipal water, or from drinking untreated well water, especially in areas where certain herbicides and pesticides are in use. For both civil and military applications, a reliable, fast, portable, and affordable field test kit is needed.

2. EXPERIMENTS AND RESULTS

The goal for phase one of this project is the validation of a simple electrochemical assay for lead and copper in tap water that can eventually be taken into the field. There are two sources of water that must be considered: municipal and rural (well) water. The origin of copper and lead in municipal supplies is exclusively from service and interior household pipes. But these heavy metals can enter environmental waters from other routes. For example, runoff from industrial processes and mining activity can contaminate both surface and groundwater. Over the duration of this project, we intend to consider water from a variety of locations to demonstrate effectiveness against these different contaminant sources.

Development of a field-portable assay for trace lead in tap water at EPA relevant levels is a challenging problem because 15 ppb is a very small number. Historically, lead has been detected electrochemically using mercury. The assay works because lead forms an amalgam with mercury that is electrochemically active; since mercury is also the working electrode in the assay it is exquisitely sensitive. Unfortunately, mercury is as toxic as lead, if not more so. Transportation and handling of mercury by the warfighter is undesirable; in a civilian kit for household use it is out of the question. In 2000 it was observed that trace amounts of lead could also be electrochemically detected on bismuth, an element that is significantly less toxic than mercury [14, 15]. Since these initial groundbreaking studies, there has been a flurry of activity in the scientific literature on the development of various types of bismuth electrodes, mostly for trace heavy metals detection [16]. These experiments fall into general classes: solutions containing bismuth salts that are plated onto the working electrode at the time of the

experiment; pre-formed bismuth electrodes such as a plated metal or an oxide film; and other hybrid structures such as coated nanorods. Screen printed bismuth film electrodes are commercially available in a disposable format for a very reasonable cost.

2.1 The Design Cycle

The ultimate goal of this effort is six sets of field trials, which serve two purposes. First, they allow us to collect data outside of the lab using our custom built potentiostat prototype, the CStat Series II. This experience is very important for design evaluation and to implement improvements in the hardware, software, and firmware. Second, the field trials allow us to slowly increase the complexity of our assay and test it under realistic conditions and on realistic samples, which may or may not be contaminated with trace compounds of interest. In addition to field testing, samples will be retained and brought back to the laboratory for gold standard testing.

Due to the iterative nature of this process, a design cycle (Figure 1) has been introduced to model the expected journey through this project. It consists of four elements: hardware development, assay development, assembly of a field kit, and field trials. We expect to go through the full cycle at least six times, once for each set of field trials. However, the journey will not always be linear. This is illustrated in the figure.

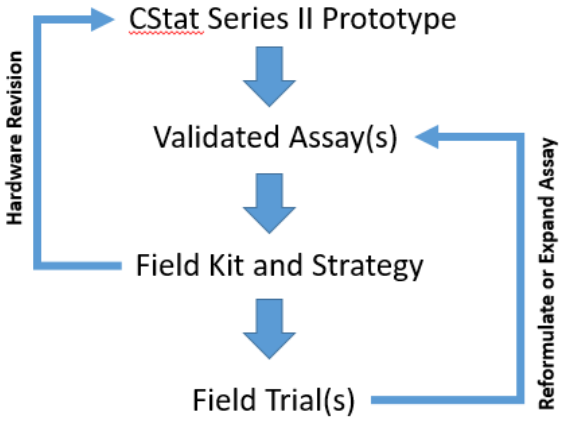

Figure 1. The design cycle for this project. Step 1 is hardware development, including firmware and software. Step 2 is a laboratory validated assay. Step 3 is implementation into a field kit. Step 4 is field trials. The path through the design cycle can be non-linear.

The entry point for this project was a freshly built and relatively untested instrument: the CStat Series II v3.84, which was the first working prototype from the second generation of NRL's custom built potentiostat. We also had an initial assay for lead on graphene electrodes, albeit one that did not yet meet the EPA limits for lead in tap water.

The next four sections of this report focus on the different steps in the design cycle, and detail our current results for each. For logical clarity, the steps are not addressed in order, based either on the chart or chronologically. We start by describing the field kit and strategy; then move to the assay; continue by describing hardware, firmware, and software development; and finally end with a discussion on the logistics for the upcoming field trials.

2.2 Field Kit

Laboratory samples typically consist of clean water spiked with known amounts of specific contaminant(s). This clean background makes it possible to isolate and understand signals from each analyte of interest and to construct calibration curves. In contrast, samples obtained in the field will have

an unknown background from dissolved minerals and other trace components unique to the location and time at which the sample was collected. The electrochemical detection method employed in this work is anodic stripping voltammetry; voltage is swept over a range and the resulting current is measured as a function of the voltage. At some voltages in this sweep, the background may be close to zero and signal is easily interpreted. At other voltages in the sweep, there might be a non-zero background. If the signal from the contaminant is additive with the background, it can still be extracted from the sample from carefully constructed experimental techniques, even if the background current is not known a-priori. However, sometimes interferents will form complexes with the contaminant, chemically react with the contaminant, or otherwise mask the signal, at which point detection becomes difficult.

The strategy we have chosen for this work is the method of successive additions. It works as follows. Rather than performing a single test, the sample is divided among multiple vials, each of which is spiked with a known amount of the analyte(s) of interest in different amounts. A least squares fit is applied to the resulting data. The concentration of analyte in the original sample is found as the negative of the value where this line crosses the x-axis as illustrated in Figure 2.

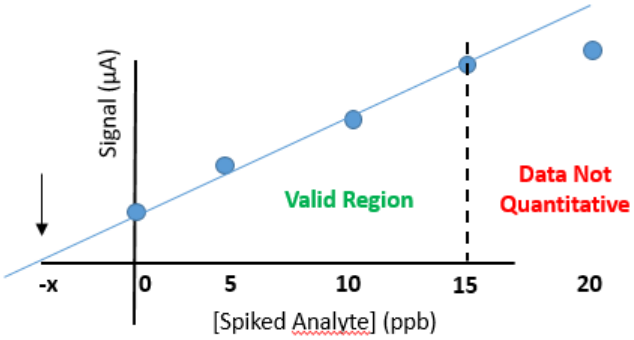

Figure 2. The method of standard addition. Here, the sample is divided into four parts, each of which is spiked with a known amount of analyte: zero, low, medium, and high concentration. A least squares regression line is fit to the data. The point at which the line crosses the x-axis is the negative of the concentration of analyte in the original sample. The experimentally determined analyte concentration is labeled in the Figure as (-x). Note that these are imaginary data points for illustrative purposes only and the x-axis concentrations shown here may differ from actual assay results. That is: the actual assays can be linear and quantitative beyond 20 ppb concentration.

In order for this method to work, laboratory calibrations must verify that the total concentration of analyte in the sample plus the spiked amount produces a linear response. If the total concentration is outside of the linear region the regression line will fit poorly, but we can still make a qualitative conclusion: don't drink the water.

Interferents can be problematic for this method. In most cases, metal ions tend to have well defined electrochemical peaks that are separated from each other. Copper is an exception for bismuth electrodes and will be discussed further below. In contrast, organics such as herbicides, pesticides, and other compounds can have very broad peaks. We plan to split the sample and detect these families of compounds separately. For metals, the addition of hydrogen peroxide plus UV light can digest organic compounds, remove them from the background, and prevent them from interfering with the assay [17]. This is performed at low pH. If the goal is to detect organics, a chelating agent (such as EDTA) is added to bind to the metals and render them electrochemically inactive. This must be done at high pH, where the complexing agents are most effective.

Sample preparation is relatively simple for the method of standard additions. Buffer solutions containing any required reagents such as hydrogen peroxide or EDTA can be prepared in the laboratory ahead of time. Water samples collected from municipal supplies do not need to be filtered. Any water collected from a rural source such as a well or environmental surface water should be immediately pushed through a 0.45 µm syringe filter to eliminate bacteria and solid particulates. The sample can then be

added to the prepared buffer solutions. When working in the field, at least half of the collected sample should be retained in pre-cleaned bottles and brought back to the laboratory for confirmatory testing.

For municipal tap water testing, the assay only needs to be sensitive to lead and copper. Our strategy is as follows: four 50 mL falcon tubes are pre-filled with 2.5 mL of 10x concentrated buffer solution, each clearly labeled as spiked with a different amount of lead and copper: zero, low, medium, and high concentration. That is, the concentrations of all species in the buffer are designed to reach a certain level when diluted by a factor of 10. Typical procedures for collecting municipal samples requires the water to be sitting in the pipes unused for between 8-24 hours. The tap is turned on and a 500 mL water sample is immediately collected. This step should be complete within 30s of turning on the tap. The sample is manually shaken, and then aliquots are distributed to fill each Falcon tube to the 25 mL line. The tubes are capped and manually shaken. In the present version of the assay, approximately 10 mL of each of these vials is poured into a glass sample tube. Anodic stripping voltammetry is performed with a single bismuth metal electrode sequentially on each tube, starting with the lowest spiked concentration and ending with the highest.

2.3 Assay

Electrochemistry takes place on a working electrode, which typically has a composition and microstructure specifically chosen to be advantageous to the detection of the compound(s) of interest. However, the main goal for this project is to evaluate the CStat Series II potentiostat. For this reason, we are trying to keep as many of the other components as possible commercial-off-the-shelf (COTS). While there are a number of reports in the literature describing novel bismuth electrodes with large surface area, embedded catalysts, or with other novel properties [16], we have chosen to use simple bismuth metal screen printed electrodes, commercially available for about $6 each from Dropsens.

When the project started, we had a limited stock of bismuth metal electrodes upon which initial data was generated. These particular electrodes are no longer available for purchase. We have ordered replacement material, which comes in the form bismuth oxide and require an extra activation step in 0.1 M KOH for 600s at -1.2 V to transform the film to bismuth metal. The material has not yet arrived at NRL. Data in the following sections are reported for the older bismuth metal film electrodes. The newer bismuth oxide electrodes will need to be characterized before being used at the first upcoming field trial in Washington, DC.

2.3.1 Lead (Pb)

The EPA action level for lead is just 15 ppb, which is a challenging concentration to detect using any portable method. Given the expected amount of optimization to reliably obtain these limits, we chose to characterize this contaminant first. Laboratory validation requires us to construct a calibration curve under a set of selected operating conditions. Traditionally the detection of heavy metals occurs in 0.1 M acetate buffer at pH 4.5. Initial studies with screen printed bismuth metal electrodes indicated that this was not very sensitive. We were able to obtain significantly better data using phosphate buffer. A few different buffers were tested around pH 7. The best results are shown in Figure 3. We suspect that the age of the electrodes and their composition may be a contributing factor here; freshly activated bismuth oxide electrodes may perform differently.

Results for potassium phosphate buffer at pH 7.0 were promising, especially at very low concentrations. While the goal is to hit the EPA action limit of 15 ppb, it is unlikely that we will randomly find a source in Washington DC with concentrations this high. It is more likely that there will be low residual lead levels around 5 ppb or less. Although the calibration curve becomes non-linear above roughly 10 ppb, this assay should be sufficient for our initial field trials in October.

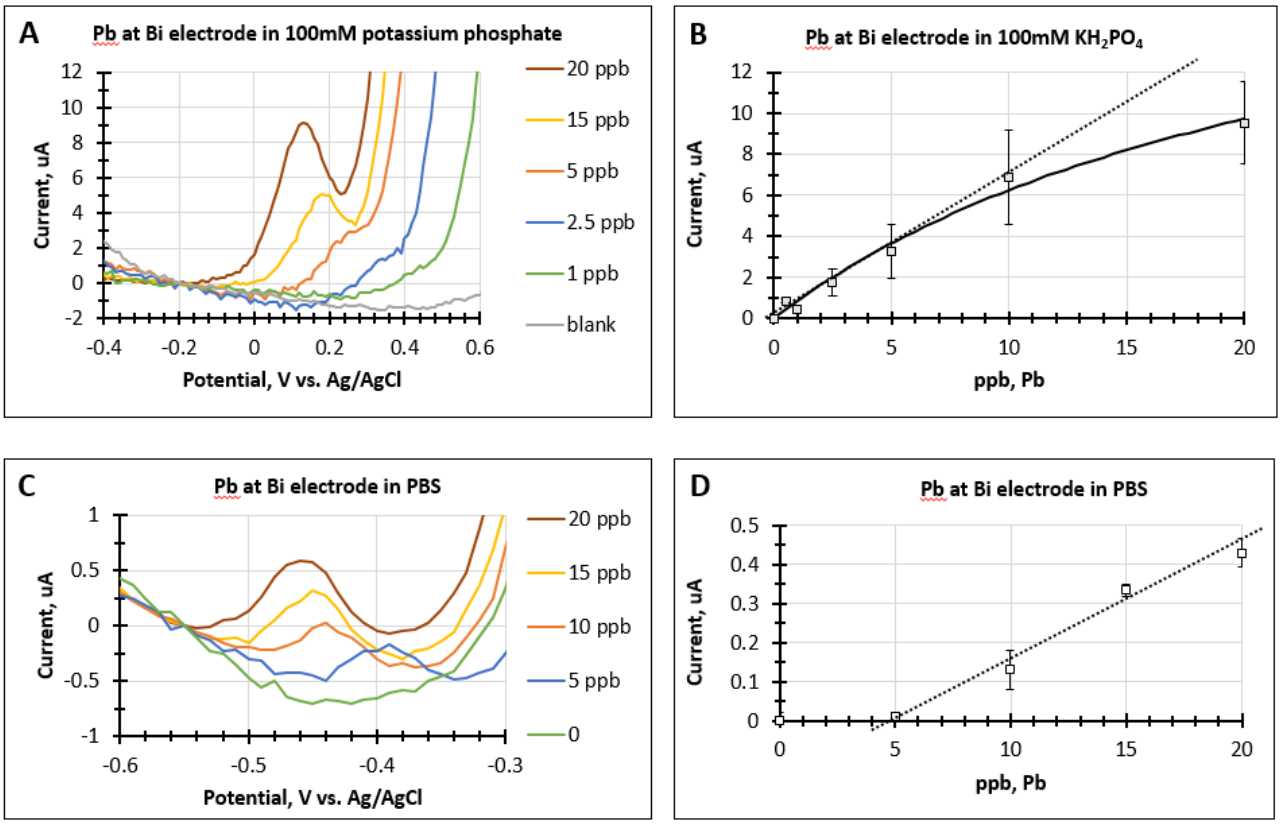

Figure 3. Results for lead (Pb) on screen printed bismuth metal electrodes. Using a 100 mM potassium phosphate buffer, square wave voltammograms (A) and the calibration curve (B) indicate suitability for measurements at low ppb lead levels expected to be found in municipal water supplies. Using a phosphate buffered saline buffer, square wave votammograms (C) and the calibration curve (D) are linear at slightly higher concentrations and might be more appropriate for well water analysis. All calibration curves were determined using peak height. For reference, the EPA action level for lead in municipal water supplies is 15 ppb.

Results using PBS as the buffer have more long term potential, given that the data remains linear at higher concentrations. We can take advantage of this by spiking the buffers in the standard addition with higher doses of lead. However, the fact that the least squares fit does not go through zero is concerning. There are two issues here. First, the current in the blank is not zero; it is actually negative. It may be necessary to shift the baseline before generating the calibration curve. The second issue is that the location of the peak shifts as the concentration changes. Rather than measuring at a fixed voltage, it may be necessary to locate the voltage of the peak maximum in each measurement.

2.3.2 Copper (Cu)

Copper has proven to be much more difficult to measure than lead. Given the performance of graphene, our initial impression was that performance on bismuth would be similar. With a 1300 ppb EPA action level, reaching the proper limits of detection should be very easy. However, experiments using bismuth metal electrodes showed no peaks at all. A literature search suggested that copper is very difficult to detect because under some solution conditions its peak overlaps with the wide bismuth electrochemical peak and is completely masked [18].

The most common concern in literature is not the detection of copper on bismuth. Rather, the problem is that copper tends to be an interfering species in many assays [18]. A study exists that demonstrates a method to shift the copper peak away from the signal of other species [19]. The addition of hydrogen peroxide in small amounts (0.040 %) shifts the peak of copper to a much higher voltage than

the wide bismuth peak itself in acetate buffer at pH 4.5. This also effectively makes it quantifiable. We intend to reproduce those experiments soon to see if an assay can be developed.

2.4 Hardware Development

Our research group has a long history of performing electrochemistry in challenging environments. A release version of NRL's first generation hand-held potentiostat, the CStat, was produced in 2017. Originally developed for the detection of explosives in sands and soils [20], it was subsequently extended to the detection of contaminants in salt water for diver safety over the next few years [21]. At that point, we realized that a new potentiostat design would be advantageous for trace detection in water. This was the origin of the second generation potentiostat, the CStat Series II. The first version was the v3.80, although it had far too many bugs to be used in the laboratory. The first working version of this instrument was prototype v3.84, which was available around the time that the project started. Both laboratory validation of the instrument was necessary as well as ensuring that assays could be run on it and that results would be comparable to a COTS benchtop potentiostat, in this case a model 760E from CH Instruments.

Since the project started, several revisions have been designed and built. The v3.85 and v3.86 have been assembled and tested in the laboratory, and the v3.875 is at the foundry. All versions are 4-wire potentiostats designed to be used in the field for trace metals analysis. In addition, they are capable of supporting a daughterboard, although currently the only designed daughterboard is for environmental water testing for aquaculture purposes. Following the idea of a design cycle, experiment and assay development guides hardware improvements.

2.5 Logistics for Field Trials

The final aspect of phase one of the project is planning for field trials, which start in October 2022. We currently have leads for five of the upcoming six trials. The originally proposed schedule is shown in Table 1.

Test	Target List (cumulative)	Limit [ppb]	Proposed Site
1	Lead (Pb^{2+})	15	Municipal (DC)
	Copper (Cu^{2+})	1300	
2	Total Chromium (Cr^{3+}, Cr^{6+})	100	Military Base
3	Total Arsenic (As^{3+}, As^{5+})	10	Small Town Well Water
4	Cadmium (Cd^{2+})	5	Environmental Water (Southwest)
	Mercury (Hg^{2+})	2	
5	One or more selected herbicide		Rural Well Water (Midwest)
6	One or more selected pesticide		TBD (Well or Environmental)

Table 1. Current list of upcoming field trials.

Known logistical details for each of these trials are discussed below. Note that the proposed sites have been updated from their original entries in Table 1.

2.5.1 Upcoming Trial 1: Washington, DC

Details for the first set of field trials in Washington, DC have been finalized. The location is a private residence owned by a friend of the primary author, near the Logan Circle area in northwest Washington, DC. The actual address is not listed here for privacy reasons. At the time of writing, field trials are scheduled to be performed on Sunday, October 30. Based on the current validated assays, only lead will be tested. We will retain a sample and bring it back to the laboratory for further verification by gold standard methods.

2.5.2 Upcoming Trial 2: Local Army Base

Working with our contacts at Army DEVCOM and Army ERDC, we intend to run a set of trials on water obtained at an Army base. This second set of trials would take place in mid-December 2022 and would add total chromium to the list of contaminants. There is no particular reason to expect chromium in the tap water, but it should be noted that parts of the site at Edgewood are heavily contaminated by a number of substances and it is entirely possible that we may find it, if environmental sampling is included. In addition, we may find challenging background material in environmental samples. It is certainly worth retaining an environmental sample, if they will allow it and it is safe to enter the collection area. The most likely test case for tap water from this location is that we spike it with the contaminants of interest.

2.5.3 Upcoming Trial 3: Military Site in Texas

An opportunity has come up from DTRA for a trip to the YFZ ranch, a field site in rural Texas that may allow for water testing. While an exact date has not been set, it will probably take place in April 2023. The ranch was originally owned by a cult. It is now used for a variety of chemical and other testing. It is important to note that DTRA will not allow us to bring any collected water off-site, so these field trials will not have any gold standard comparison.

We had originally suggested that water for the third field trial would come from a small town. While additional on-site trials at a private residence in this area could be difficult without a contact, we can retain samples from different locations such as restaurants or gas stations and bring these back to NRL for testing.

2.5.4 Upcoming Trial 4: American Southwest

Actual location is still to be determined. The proposed date is May 2023. Environmental waters in the American southwest can have heavy mineral content and in a number of areas there is contamination from mine runoff. All previous targets including chromium and arsenic, as well as the new targets of cadmium and mercury might be found here.

2.5.5 Upcoming Trial 5: Rural Ohio

We have recently connected with a group known as the Cleveland Water Alliance, a tech incubator made up of small companies local to the Ohio area. They are very interested in the testing and monitoring of environmental water in the great lakes watershed and have invited us to perform testing on site. There is also a potential (civil) transition opportunity here. Due to their interest in PFAS, we will also leverage work on a separate NISE project that ended in September 2022 for additional testing on this trip. The Cleveland Water Alliance has indicated that the best time for sampling at this location is July, 2023. The primary target will be herbicides.

2.5.6 Upcoming Trial 6: Rural Kansas

We will leverage our CRADA partner Foothold Labs, which is located in Kansas, for the last set of field trials. They have provided us with rural well water samples in the past and have identified a number of field sites for potential collection. The one we would like to use is a campground located at Hillsdale, KS. Proposed date is September, 2023. The primary target will be pesticides.

3. FUTURE WORK

Phase two of the project has additional goals, especially in regards to assay development and data analysis. Once we begin looking at herbicides and pesticides in the spring of 2023, it is expected that

samples will need to be split into two different streams: trace metals and organics. This will probably require the use of two different electrode materials as well as very different solution conditions.

3.1 Assay Complexity

When measuring metals on bismuth electrodes, there are a number of relevant targets that are simple to measure such as lead and cadmium. However, not all targets are easily detected on bismuth. Some require formation of a complex or a chemical transformation in order to make the signal visible. A good example is chromium. A two step process has been reported that may be able to detect it on a bismuth film electrode [22]. First, all chromium(VI) in the sample is reduced to chromium(III) with 0.25 M potassium nitrate. Next, all of the chromium(III) is complexed with 5 mM diethylenetriamine pentacetic acid (DTPA). While this has been shown to create a peak on bismuth in pH 6 acetate buffer that can be used to quantify "total chromium", it is unclear how the signal will shift or otherwise affect all of the other peaks (lead, copper, arsenic, etc.), whether it can be used at pH 4.5, or whether it is affected by the addition of 0.040 % hydrogen peroxide that may be required for copper detection or digestion of organics. All of this will require experimental verification. If the assays are not compatible with each other, then the chromium test will need to be run in a separate flask.

We expect that a similar procedure may be required for arsenic. At a minimum, all arsenic species must be reduced to arsenic(III), resulting in a "total arsenic" measurement. Whether or not it needs to be transformed or complexed to another species for detection is still an open question.

As the number of targets continues to increase, we will eventually reach the limit of what can be detected in a single flask. This is of interest from a basic scientific point of view: most studies either target multiple ions in a "low hanging fruit" approach, or else try to transform a single target. These types of assays are rarely mixed.

It was mentioned previously that we fully expect to analyze trace metals and organics separately. Metals are considered first because their peaks tend to be simple. They are typically measured at low pH and the addition of hydrogen peroxide, possibly with UV light, can digest organics that might otherwise interfere with the assay. Organics considered in this project include selected herbicides and pesticides. These will be measured at high pH with EDTA, a chelating agent that will remove most metal ions from solution. We expect that these compounds will be probed with a gold screen printed electrode, although COTS carbon or boron-doped diamond electrodes may also be considered.

3.2 Hardware Development

When the project was proposed, our vision was a single electrode assay that could unambiguously determine the concentration of both copper and lead in tap water with just one measurement. Unfortunately, laboratory testing has indicated that this is not possible. The actual kit will need at least four separate measurements for trace metals. It is possible there could be more. This introduces two new problems: analysis time and complexity to the operator. A typical stripping analysis for trace metals requires a 5 minute accumulation time, followed by a cyclic voltammetry step that takes roughly 1 minute each. Better results could be obtained with 10 minute accumulation. For four separate vials, this requires roughly 25 minutes for the shorter assay, or 45 minutes for the longer one. This might be unacceptably long for a warfighter in the field.

One of the advantages of using a home-built potentiostat is that it can be customized to the experiment. We are considering designing a specific daughterboard for drinking water testing that controls a multiplexer. In this case, all four chambers will be held at the accumulation voltage simultaneously. Then, each of the electrodes can be swept in sequence (1 minute each), for a total sample time of 9 minutes or 14 minutes for the longer assay. Since all technical details of the scans as well as data collection are handled by the software, the user can perform the entire assay with the click of a button, eliminating most of the complexity for the operator and making it useable with little training. In

order for this strategy to work, we will also need to demonstrate sufficiently low electrode to electrode variability in the contaminant peaks.

In the interest of making our instrument as modular as possible, we are considering breaking the analog and digital components apart in the next design. In this case, the sample holder will include a circuit board with most of the analog potentiostat components as well as the multiplexer. A standard set of cables will connect it to the CStat Series II front end, which controls all of the digital aspects of the device. A daughterboard on the CStat Series II will directly control the multiplexer, and will provide power for a set of four magnetic stirrers that will be built into the testing station. Global supply chain issues has slowed down hardware development time, but we are carefully selecting parts for these new devices based on their availability for purchase.

Recently a memorandum of understanding (MOU) has been established between NRL, Army DEVCOM, and Army ERDC, with a specific interest in the results of this line of research. Using this modular approach it might be possible to connect our proposed analog front end to the ACEstat [23], the homebuilt potentiostat developed by our Army collaborators. Regardless of the final instrument choice, a modular approach opens the door for additional use cases of our test kit with other military groups.

A number of hardware decisions will need to be made over the next year. In the original proposal, we had indicated that a Pelican case containing all kit components would be designed by the end of phase one of the project. We have made a decision to delay this last deliverable until the hardware and sample chamber are in their final form and have been tested.

3.3 Alternate Assays and Machine Learning

The final component of this work is data analysis. In the case of metals, simple peaks are expected in known locations and the sample concentration can be determined by a regression line fit using the method of standard additions. All of this can be automated in software. But Figures 3A and 3C show that there is some question about the actual location of the peaks. This topic is informally known as “peak picking” and is an area we intend to address. For example, a Gaussian fit followed by partial or full area of the peak may provide a more sensitive (and/or more linear) fit than simply reading the signal amplitude at a specified voltage. A number of different options need to be explored. The use of machine learning tools can be used to optimize the fits, which may reduce the overall noise and increase the accuracy of the assay.

The second proposed analysis method is much more elegant. In this approach, we look for an envelope that is common to all “clean” water samples. Any electrochemically active contaminant is expected to create peaks outside of this envelope and samples can be immediately flagged without the need to actually identify the species. The result is a yes/no assay. While not as useful for civilians evaluating well water on their property, this could be of great use to the warfighter. To begin the process of developing this assay, we have initiated water collection from a variety of field sites in Washington, DC. We hope to obtain an envelope representing “clean” water by adding a large number of samples taken from a variety of locations throughout the duration of the project. To actually evaluate this method will require the remaining time in the project.

4. CONCLUSIONS

Starting from the CStat Series II prototype v3.84 and a demonstration assay for lead on graphene, we have performed laboratory testing and validation as well as several build iterations. A field kit and test strategy was selected. Field testing is set for municipal water in late October, 2022 in Washington DC. Logistical planning for many of the upcoming six field trials is complete. Finally, a discussion of future directions during the upcoming year was provided.

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