MICROELECTRODE ARRAYS IN THIN-LAYER CELLS FOR RAPID, SENSITIVE, AND CALIBRATION-LESS DETERMINATION OF ARSENIC IN μL VOLUMES BY ANODIC STRIPPING COULOMETRY

M.M. Marei^{1*}, T.J. Roussel¹, M.M. Crain¹, R.P. Baldwin², and R.S. Keynton¹

¹Bioengineering Department, University of Louisville, Louisville KY, USA

²Chemistry Department, University of Louisville, Louisville KY, USA

ABSTRACT

Recent contamination events have emphasized the need for widespread heavy metal monitoring over extended periods of time. The customary and laborious random “grab sampling” for later analysis at central laboratories does not adequately satisfy this need. Our group is developing an electroanalytical approach that allows highly sensitive, calibration-less measurements to quantify heavy metals in microliter volumes. This approach offers promise for economical, miniaturized, remotely-deployable sensor networks. This study reports the application of microfabricated gold microelectrode arrays within a thin-layer cell and demonstrates sufficient sensitivity to detect sub-5-ppb arsenic concentrations in a microfluidic sample volume ($< 5 \mu\text{L}$).

INTRODUCTION

A number of recent events have highlighted the need for heavy metal monitoring at a large number of sites in a simultaneous fashion over extended periods of time. Such events include contamination of drinking water from buildings with Pb plumbing [1], accidental spills of wastewater from storage sites into the environment [2], and the recently enacted regulations for heavy metals in power plant effluents [3]. In addition to these recurring events, heavy metals such as As, Pb, Hg, and Cd, have been of longstanding concern and are ranked #1, #2, #3, and #7, respectively, on the Agency for Toxic Substances and Disease Registry 2015 Priority List of Hazardous Substances at the >1300 National Superfund sites [4].

Current heavy metal monitoring and analysis methods rely on sophisticated techniques such as inductively coupled plasma mass spectrometry and graphite furnace atomic absorption spectroscopy, which are performed in central laboratories [5, 6]. This approach necessitates sustained, labor-intensive and coordinated “grab-sampling” efforts to collect and transport samples to the central laboratory, placing severe limitations on the number of sites that can be monitored and the sampling frequency at these individual sites. This need for more frequent monitoring at a multitude of sites has, in fact, led to several types of portable ‘suitcase’ instruments; however, this approach also relies on regular site visits by a mobile technician.

The desire to reduce reliance on the above labor-intensive sampling practices has led to a number of automated ‘on-line’ metal analyzers [7-11]. In automated analyzers, electrochemical methodologies have been widely practiced since the associated instrumentation is amenable to direct control and automation by integrated electronics, and the electrochemical stripping analysis of heavy metals is a well-established and highly sensitive technique. A recent review of 172 on-line stripping methods spanning a 12 year period, however, reveals the ubiquity of automating continuous-flow, bench-scale (mL) analyses and the need for frequent calibration with standards or blank solutions [12].

At present, the automated electrochemical heavy metal analyzers based on the above approaches are necessarily complex and costly, in order to accommodate the calibration and sample pretreatment functions for large sample volumes. And even with this capacity for automation, such analyzers are effectively cabinet-sized

appliances intended for centralized, industrial process monitoring, which from an economic perspective, makes them unsuitable for environmental and/or decentralized monitoring applications.

Our group has therefore been developing an autonomous, economical, remotely deployed sensor platform for continuous monitoring of heavy metals in water intended for use at the superfund sites, which may also be suitable for other decentralized heavy metal monitoring applications. The approach combines elements of microfabrication, microfluidics, and electrochemistry which synergistically provide inexpensive, simple to automate, mass-producible, redundant sensor networks with minimal requirements for on-site power and reagents [13-16]. This approach has been designed from the outset to take advantage of the well-known but little-used electrochemical coulometry method, which is one of very few analytical chemistry techniques that is capable of calibration-free measurements [14, 16].

Further, coulometry can be combined with another electrochemical technique: stripping analysis, which is a well-established and remarkably sensitive technique for the analysis of heavy metals. The remarkable sensitivity of stripping analysis arises from the preceding pre-concentration step in which dissolved species such as metal ions are accumulated on the electrode surface. Naturally, the sensitivity is greatly enhanced when a large volume of sample is analyzed, since more of the dissolved species can be pre-concentrated on the electrode surface. Given that even trace (low ppb) heavy metal concentrations are of environmental concern, it stands to reason that the required sensitivity is particularly challenging to attain in microfluidic devices, as we propose.

In this work, it is shown that sufficient sensitivity is attainable within a microfluidic device (analysis volume $< 5 \mu\text{L}$) to detect sub-5ppb arsenic concentrations, which even meets the EPA mandated regulations for arsenic. Further, the overall analytical sensitivity and practical performance of the sensor is shown to be responsive to parameters which, in principle, can be controlled during design and manufacture of the sensor.

THEORY

The basic premise of the sensor relies on Faraday’s 1st law of electrolysis, first proposed by Michael Faraday in 1834 [17]:

$$Q = n \times F \times N \quad \text{Eqn. 1}$$

where Q represents the charge, n represents the number of exchanged electrons per mole of analyte, F is the Faraday constant, and N is the number of moles of analyte. The number of moles of analyte can be represented as:

$$N = C \times V \quad \text{Eqn. 2}$$

where C represents the analyte concentration and V represents the sample volume. For a stripping process, according to **Eqn. 1** and **Eqn. 2**, the stripping charge (Q_{strip}) ought to correspond exactly to the prevailing analyte concentration according to:

$$Q_{\text{strip}} = n \times F \times C \times V \quad \text{Eqn. 3}$$

when the redox reaction (n) and the cell volume (V) are known, so

long as sufficient time is allowed for electrolysis to proceed to 100% completion during the pre-concentration step. In order to achieve complete pre-concentration of dissolved metals rapidly (in about one minute), our group has developed a thin-layer cell, the schematic of which is shown in **Figure 1**. The cell features a working electrode as one wall of a thin-layer fluidic cell, which is isolated from the counter/reference electrode compartment by a semi-permeable membrane.

Figure 1: Partial schematic of thin-layer cell for coulometry with structural layers omitted for clarity

One practical limitation of **Eqn. 3** is that, under experimental conditions, the measured charge (Q_{total}) represents not only the faradaic processes of interest (e.g., the stripping of plated metals, Q_{strip}), but also any background and non-faradaic processes (e.g., double layer charging current at the electrode/electrolyte interface, Q_{dl}). Accordingly the subtraction of Q_{dl} is necessary for accurate estimation of Q_{strip} . According to:

$$Q_{strip} = Q_{total} - Q_{dl} \quad \text{Eqn. 4}$$

Although direct measurement of Q_{dl} can be obtained by performing the experiment under identical conditions in a blank solution, a simpler approach for in-the-field analysis is to perform this measurement of Q_{dl} directly in the sample solution. Accordingly, our group has developed a method we have termed *double potential step anodic stripping coulometry* (DPS-ASC) where the deposition potential is applied briefly (100 msec), followed by the stripping potential (pulses 1 and 2 of **Figure 2**). Since no appreciable metal pre-concentration occurs during this rapid step, the total charge (area under the curve) for pulse 2 is an approximation of the Q_{dl} . This pulse sequence is repeated, only with a much longer deposition (pre-concentration) pulse (60 seconds, pulse 3 of **Figure 2**) to exhaustively plate all dissolved metals in the thin-layer cell volume. Subsequent stripping in Pulse 4 of **Figure 2** represents Q_{total} . The charge corresponding solely to Q_{strip} can be visualized by overlaying pulses 2 and 4 (Q_{dl} and Q_{total} , respectively) on the same axis, which in principle, is equivalent to the operation shown in **Eqn. 4**.

Figure 2: Illustration (not to scale) of time vs. recorded current for DPS-ASC. Positive pulses are -500mV deposition potential; Negative pulses are +500mV stripping potential. Signal obtained by overlaying pulses 2 and 4 on the same axis and integration to obtain charge within crescent (Figure 3)

EXPERIMENTAL

The flow cell consists of two polycarbonate fixtures, rubber gasket layers, a 100 μm thick pyrolytic graphite sheet (Panasonic Electronic Components, Secaucus, NJ) as a counter electrode (CE), and a 200 MWCO membrane (SelRO MPF-34, Koch Membrane Systems, Inc., Wilmington, MA). An access hole on the top polycarbonate fixture allows insertion of a custom-made Ag/AgCl 0.8 mm OD miniature reference electrode (RE), as previously described in [14]. Assembly yields a three-electrode, membrane-separated, dual-compartment cell with independent stoppable flow paths (**Figure 1**).

As_2O_3 AAS standard solution (1000 ppm) is diluted into a supporting electrolyte solution containing 10 mM HNO_3 and 10 mM NaCl to make the concentrations indicated in the text below.

Electrochemical measurements were carried out using a BASi Epsilon potentiostat (Bioanalytical Systems, West Lafayette, IN). In all cases, a 10 second pre-conditioning step (at the stripping potential) is followed by the sequence of potentials in **Figure 2**. Briefly, this consists of an in situ blank deposition for 100 msec followed by stripping for 320 msec, then deposition for 60 seconds to exhaust the cell volume of all dissolved metals followed by stripping for 320 msec. The deposition and stripping potentials for arsenite are -500 mV and + 500mV, respectively.

RESULTS

The measured charge values (crescent shaped areas resulting from overlaying pulses 2 and 4) are shown for the indicated arsenite concentrations in **Figure 3**, where a macro-electrode comprised one wall of a 1.85 μL thin-layer cell. Under these conditions, the crescent obtained for the 100 ppb arsenite signal is discernably larger than the crescent obtained in the absence of any arsenite. However, it is also apparent that detection of the lower arsenite concentrations would benefit from a smaller crescent at 0 ppb arsenite. This is also reflected in numerical plots of the charge vs. arsenite concentrations shown in **Figure 5**. Despite a clearly linear dependence of charge on the arsenite concentration, it is evident that the non-zero intercept of the line contributes significantly to the signal at the lowest arsenic concentrations. This is due to the fact that, in practice, the DPS-ASC background correction only provides an approximation of Q_{dl} .

Figure 3: Obtained charge for elliptical macro electrode as one wall of 1.85 μ L thin layer cell. Pulse 2 (grey) is background. Pulse 4 (black) is arsenic stripping and background. All traces overlay three sets of data.

Numerous experiments have shown that the value of the intercept (in the absence of any metal solutions) is directly related to the total electrode area. In order to reduce the electrode array we have therefore microfabricated microelectrode arrays (MEAs) by depositing silicon nitride on the gold surface, followed by traditional photolithographic patterning and a liquid-based HF vapor etching in the fume hood, yielding >99% electrochemically active MEA elements (see copper plated MEA in Figure 4). Photographs of the arrays are shown in Figure 4 and the array geometries are summarized in Table 1.

Figure 4: Side view schematic of the MEA sensor chip (top left); and optical microscopy photos of MEAs

Table 1: Summary of microelectrode array design geometries

chip design	number of electrodes	edge to edge distance	electrode area	geometric area*
Ellipse	1	NA	0.232 cm ²	0.23 cm ²
50 μ m	3008	50 μ m	0.059 cm ²	0.23 cm ²
20 μ m	6394	50 μ m	0.020 cm ²	0.23 cm ²
5 μ m	12711	50 μ m	0.002 cm ²	0.23 cm ²

The data obtained for the microelectrode arrays are shown in Figure 5, where the crescents for much lower arsenite concentrations were discernible from the crescent at 0 ppb arsenite. For instance, the 5 μ m array of Figure 5A allows clear visualization of sub-5-ppb arsenite concentrations. In all cases, the lines on either side of the crescent are overlaid triplicates from three trials (i.e., three grey traces and three black traces). This inter-trial reproducibility is a notable feature of the DPS-ASC method {RSD typically <5%}. In addition, the data of Figure 5 show that the stripping signal is much faster on the microelectrode arrays than the single macro-electrode. This observation is in agreement with the accepted behavior for microelectrodes [18].

Figure 5: Signals obtained with 5 μ m array (A); 50 μ m array (B). Note scale bars compared to figure 3 and discernibility of <5 ppb arsenic concentrations. All traces overlay three sets of data.

To enable a more direct comparison to the macro-electrode, the charge values shown in Figures 3 and 5 are plotted vs. the arsenite concentration in Figure 6. As noted above, the error bars reflect the inter-trial reproducibility. The variations in the slope of the arsenite calibration lines of reflect the fact that the volume of

Figure 6: Calibration plot of charge (crescent area) vs. concentration at macro electrode (black), 50 μ m array (blue), 20 μ m array (green), and 5 μ m array (red). Note improved accuracy/lower intercept of MEA in inset.

the thin-layer cell varied slightly from assembly to assembly. Since the slope is defined as the analytical sensitivity, thin-layer cells of different volumes may, in the future, be designed to meet performance requirements for different samples. Nonetheless, the stripping charge obtained under each set of conditions were linear over a wide range of the concentrations. At higher concentrations, the slope of the lines decreased (not shown) since metallic As acts as an insulator, in agreement with previous observations [19].

As shown on the inset of **Figure 6**, the intercepts at 0 ppb arsenite decreased with the electrode area. Correspondingly, the charge values within the crescent at 0 ppb decreased in proportion to the decreasing electrode area, as shown in **Table 2**. Further, the charge obtained at this intercept value represented a smaller fraction of the measured charge, which is particularly important at the lowest arsenite concentrations.

Table 2: Charge within crescent at 0 ppb arsenite as a smaller portion of the measured charge at 100 ppb (100 ppb not measured on the 20 μm array, 50 ppb shown).

[Arsenite] (ppb)	Charge within crescent (μC) (relative standard deviation)			
	macro-electrode	50 μm array	20 μm array	5 μm array
0	1.54 (2.4 %)	0.49 (11.1 %)	0.22 (2.6 %)	0.039 (3.4 %)
50		1.06 (2.2 %)		0.26 (3.2 %)
100	2.19 (1.6 %)		0.86 (1.5 %)	0.42 (9.2 %)

CONCLUSIONS

This paper describes progress towards sensing trace arsenite concentrations in microfluidic volumes utilizing *double potential step anodic stripping coulometry* (DPS-ASC). The use of microelectrode arrays is shown to significantly enhance the capability to perform As measurements below 5 ppb. In addition to the performance enhancement attained by utilizing microelectrode arrays to reduce the area of the working electrode and the contribution of Q_{dl} to the measurement, it was confirmed that the slope of measured standards reflects the volume of the assembled electrochemical cell. Thus, for a given microelectrode array geometry, improvements in the overall sensor performance at the lowest concentrations can be further enhanced by designing thin-layer cells with larger volumes. Finally, we are incorporating the performance of this cell into an autonomous, low-power, calibration-less system intended for autonomous use in remote locations.

ACKNOWLEDGEMENTS

This work was supported by the Department of Bioengineering Lutz Endowment and the Earl and Mary Lou Kohnhorst Endowment

REFERENCES

- Nichols, A.Y.M., *Beyond Flint: Excessive lead levels found in almost 2,000 water systems across all 50 states*, in *USA Today*. 2016, Gannett Satellite Information Network, LLC.
- Turkewitz, J., *Environmental Agency Uncorks Its Own Toxic Water Spill at Colorado Mine*, in *New York Times*. 2015, The New York Times Company.
- EPA, *Effluent Limitations Guidelines and Standards for the Steam Electric Power Generating Point Source Category: Final Rule* E.P. Agency, Editor. 2015.
- ATSDR, *Priority List of Hazardous Substances*, ATSDR, Editor. 2016, Division of Toxicology and Human Health Sciences: Online.
- EPA, *Analytical Methods for Drinking Water*. 2016.
- Skoog, D.A., F.J. Holler, and T.A. Nieman, *Principles of instrumental analysis*. 5th ed. Saunders golden sunburst series. 1998, Philadelphia; Orlando, Fla.: Saunders College Pub. ; Harcourt Brace College Publishers.
- ADI. *ADI 2025VA Process Analyzer*. 2016; Available from: <http://www.metrohm.com>.
- Applitek. *VPA Voltammetric Process Analyzer*. 2016 [cited 2016 4/1/2016]; Available from: <http://www.applitek.com>.
- Cases-Utrera, J., et al., *Development of an Automated Heavy Metal Analyser*. *Electroanalysis*, 2015. **27**(4): p. 929-937.
- Istran. *EcaMon Series Analyzers*. 2016 [cited 2016 4/1/2016]; Available from: <http://www.istran.sk>.
- Mpderm Water. *OVA Series Analyzers*. 2016 [cited 2016 4/1/2016]; Available from: <http://www.modernwater.com>.
- Economou, A., *Recent developments in on-line electrochemical stripping analysis-An overview of the last 12 years*. *Analytica Chimica Acta*, 2010. **683**(1): p. 38-51.
- Carroll, S., et al., *Microfabricated electrochemical sensors for exhaustive coulometry applications*. *Sensors and Actuators B-Chemical*, 2011. **160**(1): p. 318-326.
- Marei, M., et al., *Electrochemical and microfabrication strategies for remotelyoperated smart chemical sensors: Application of anodic strippingcoulometry to calibration-free measurements of copper and mercury*. *Analytica Chimica Acta*, 2013. **803**(1): p. 47-55.
- Marei, M.M., et al., *Electrochemical Dissolved Oxygen Removal from Microfluidic Streams for LOC Sample Pretreatment*. *Analytical Chemistry*, 2014. **86**(17): p. 8541-8546.
- Marei, M.M., et al., *Calibration-less measurement of As(III) with in situ background subtraction by double potential step anodic stripping coulometry for continuous remote monitoring applications*. *Electroanalysis* (Submitted), 2016.
- Faraday, M., *Experimental Researches in Electricity VII*. *Philosophical Transactions of the Royal Society*, 1834(124): p. 77-122.
- Wang, J., *Stripping Analysis: Principles, Instrumentation, and Applications*. VCH Publishers, Deerfield Beach, FL, 1985.
- Feeney, R., et al, *On-Site Analysis of Arsenic in Groundwater using a Microfabricated Gold Ultramicroelectrode Array*. *Analytical Chemistry*, 2000. **(72)**: p. 2222-2228.

CONTACT

*M.M. Marei; mmmare01@louisville.edu