An On-Site Heavy Metal Analyzer With Polymer Lab-on-a-Chips for Continuous Sampling and Monitoring

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Abstract—An on-site analyzer system for monitoring of heavy metals has been presented. This analyzer can automatically perform long-term continuous water sampling and on-site heavy metals measurement using an array of disposable polymer lab-on-a-chips (lab chip) and a continuous flow sensing method. The system consists of a plastic fluidic motherboard with a microchannels network, microvalves and pump, control circuits, a wireless communication module, a potentiostat, LabVIEW control, and seven disposable heavy metal lab chips. Square wave anodic stripping voltammetry was performed using a microfabricated planar bismuth electrode on the chip for detecting heavy metal (e.g., cadmium, Cd) concentrations. Sensing performance sensitivity was improved with by the continuous flow sensing method propelled by the analyzer. On-site measurement of the Cd concentration change of the soil pore and ground water samples from a lab-scale reactor was automatically performed to evaluate the performance of the analyzer with lab chips.

Index Terms—Bismuth electrode, environmental monitoring, heavy metal sensor, lab-on-a-chip.

I. INTRODUCTION

Oil pore and ground water pollution by heavy metals is a major environmental concern, especially around heavy metal waste disposal sites, such as mining, pigment, and battery factories. Unlike organic pollutants, most of the important environmental heavy metals tend to accumulate in soils because of their chemical stability for long periods and their nonbiodegradable nature [1]–[4]. Typical environmental monitoring methods for these contaminants are realized by collecting samples in the field in person and transporting them to centralized laboratories for analysis. Unfortunately, long time delays associated with centralized analysis of samples retrieved from the field may result in alteration of the sample’s constitution as a result of chemical, biological, and physical reactions. Furthermore, centralized sampling and detection methods can be very costly and time consuming. Therefore, one of the best preventive measures is to rapidly and continuously monitor heavy metals in the field automatically [5].

Various sensing methods have been developed for on-site measurement of heavy metals, such as optical [6], piezoelectric [7], and ion selective electrodes [8], [9]. One of the most promising methods is anodic stripping voltammetry (ASV), which satisfies almost all requirements for on-site measurement of heavy metals. ASV is a voltammetric method for quantitative determination of specific metal ionic species. The analyte of interest is electroplated on a working electrode during a deposition step, and oxidized from the electrode during a subsequent stripping step. The oxidation current is measured during the stripping step. The oxidation of species is registered as a peak in the current signal associated with the potential at which the species is known to be oxidized [10]. Very sensitive sensors that employ mercury or mercury precursors as the working electrode have been developed for ASV to measure heavy metals [11]–[16], however, the toxicity of mercury and its precursors make them undesirable for disposable on-site environmental sensing applications.

An environmentally friendly heavy metal sensor using a microfabricated bismuth (Bi) working electrode has been developed in our group [17]. Bi was introduced as an alternative working electrode material to replace mercury in ASV for heavy metal sensing in different applications [18]. Compared with mercury, Bi is an environmentally friendly element, with a very low toxicity, and has widespread pharmaceutical use [19]–[25]. The Bi film electrodes are most commonly prepared by plating Bi ions onto a carbon substrate. Bi (III) ions (0.25–1 ppm) are directly added into the sample solution, and simultaneously deposited on the carbon substrate with target heavy metals [18]. However, in some cases, this method is not applicable for on-site heavy metal measurement in natural environment because it requires the presence of Bi in the sample solution. By using the MEMS and polymer micromachining techniques [26], [27], our newly developed sensors are ready-to-use, have a high yield, and are low cost, which is extremely suitable as a disposable sensor for on-site environmental monitoring applications.

To achieve a better sensing performance and comprehensive functions, variety of integrated sensing systems and analyzers built on single or array of sensor have been developed and reported in different application areas [5], [28]–[37]. Based
on our newly developed environmentally friendly disposable heavy metal sensor, a fully automatic analyzer was developed for on-site heavy metal monitoring, as illustrated in Fig. 1. This analyzer designed for on-site mounted at the heavy metal waste contamination site, and used for the continuous sampling and monitoring of heavy metals from the contaminated soil pore and ground water without user intervention. It has low-power consumption and charging capability for long-term use; and also has the flexibility to adjust the sampling and detection scheme on demand by choosing from different electrochemical detection methods and chips.

II. SYSTEM OVERVIEW

The design of the analyzer is comprised of a plastic fluidic motherboard with a microchannel networks, valves, and a pump, printed circuit boards (PCBs) for control circuits, a wireless communication module, a potentiostat, and LabVIEW programs with data acquisition (DAQ) card. The analyzer is loaded with an array of seven disposable heavy metal lab-on-a-chips (lab chip). The size of the analyzer is 30 cm × 23 cm × 7 cm, and the weight is around 3 kg. The power consumptions of each major analyzer component are listed in Table I. The total power consumption of the analyzer at idle status is 0.24 and 2.64 W depending on whether the wireless module is turned off or on, and the power consumption during pumping is 1.44 or 3.84 W (wireless ON). Latching type magnetic solenoids valves, which only consume power when they change the valve status, are specially chosen to reduce the power consumption. The block diagram of the system is shown on Fig. 2.

1) **Fluidic motherboard**: A micromachined polycarbonate (PC) plate was used as a motherboard for all the fluidic components. The surface mounted micro valves (LHLX0500200B, The Lee Company, IL, USA) and peristaltic pump (SP100VO, ATP Instruments, IL, USA) with the connecting microchannel network, and the in/outlets form the fluidic system for sampling and sample transfer are all machined or assembled on the motherboard.

2) **Control circuits**: Analog control circuits were designed and implemented on two PCB boards to achieve the major functions of voltage regulation, valve driving, pump driving, and multiplexing between the sensor outputs and the potentiostat.

3) **LabVIEW programs**: The user interface was written using LabVIEW, and provides functions to setup the electrochemical detection, setup the sampling, monitor the system status, and provide a readout, display and analysis, and storage of the obtained data through a DAQ card (NI USB-6008, National Instruments, TX, USA) to PDA/UMPC device.

4) **Wireless module**: A Wireless USB module (GUWH104Kit, IOGEAR Inc., CA, USA) was used for communication between the analyzer and the LabVIEW Program on the remote PDA/UMPC.

5) **Potentiostat**: A handheld electrochemical interface (PalmSens, Palm Instruments BV, The Netherlands) was connected to the control circuit boards and used as the potentiostat for the electrochemical measurement.

6) **Polymer lab chip array**: The lab chips were fabricated using MEMS and polymer micromachining techniques. Seven chips can be easily loaded onto the fluidic motherboard with good fluidic and electrical connection, and fixed by a cover plate using embedded magnets. Each lab chip has three redundant sensors, which can individually measure the same sample and obtain the average value and standard deviation.

For a typical measurement, the basic working procedure is: 1) choose the electrochemical method (e.g., square wave ASV) and parameters (e.g., scan range, frequency); 2) choose the fluidics control scheme and parameters for sampling (e.g., flow rate, sensing time interval between each chip, washing step time); 3) sample from the first selected inlet and pump the sample to the first chip; 4) measure the first sample at the first chip with the three sensors, and store the measurement data;
III. HEAVY METAL LAB CHIP ARRAY

Disposable polymer lab chip with microfabricated planar Bi electrode (Fig. 3) for on-site detecting heavy metals is one of the major components of the analyzer in this work.

All chips are single use and fully disposable; therefore, eliminate the need for surface renewal steps between measurements, and avoiding “memory effects,” which commonly occur during ASV [38]. In order to minimize potential environmental contamination by disposable chips, microfabricated planar Bi electrode replaces toxic mercury as the working electrode for heavy metals detection.

The fabrication of the chip involves two processes, as shown in Fig. 4: a photolithography and metal patterning step for the sensor chip, and a UV LIGA [26], [27] and polymer injection molding for the microfluidics chip.

For the sensor chip, Bi rods (99.999%) were purchased from ESPI Metals (OR, USA) and used as metal source for the e-beam evaporator (Temescal FC1800, BOC Edwards Temescal, CA, USA). Gold layer of 100 nm and Bi layer of 100 nm were deposited on the 3-inch blank cyclic olefin copolymer (COC) substrate using an e-beam metal evaporator. The cleaning of the COC surface before the metal deposition was normally achieved by immersing the COC wafer in acetone, methanol, and DI water each for 5 min. After blow-drying the COC wafers by using nitrogen gas, the COC wafers were placed in 60 °C oven overnight before metal deposition. After deposition, the wafer is cleaned again by using acetone, methanol and DI water, respectively. Shipley 1818 photoresist was spin-coated at the spreading speed of 500 rpm for 10 s and spinning speed of 3000 rpm for 30 s. The wafer was then placed in the 60 °C oven for 30 min for prebaking process. After the prebaking, the thickness of the Shipley 1818 is estimated to be 2 μm. UV exposure of the photoresist is performed 10 s by directly contacting to a Cr mask, which contains the designed electrode pattern. The exposed wafer is then immersed into a developing solution, which consists of one part of Shipley 351 solution and 5 parts of water. By slowly stirring the developing solution, exposed photoresist starts to dissolve into the solution. In order to obtain the designed electrode pattern, unprotected gold and Bi region should be removed from wafer surface. The electrodes were etched by Bi (2% H2SO4 and 2% H2O2) and gold (TFA) etchant to form the designed electrode structures. The Bi working electrode was covered with Shipley 1818 photoresist as protective layer.

After that, an electroplating process was used for making the Ag/AgCl reference electrode. A cyanideless silver electroplating solution is used to achieve a thin coating of silver over gold seed layer. During the silver electroplating, a platinum foil is used as the anode and the wafer is connected to the cathode. Current density is controlled at 5 mA/cm² for a uniform and dense silver layer. The electroplating process lasts for 20 s and a shining silver layer of 1 μm is achieved. The electroplating of silver chloride (AgCl) is achieved by using 1 M potassium chloride (KCl) solution. The platinum foil is connected to the cathode and the wafer, which has the silver layer, is connected to the anode. Same current density is applied for 15 s until the white/shining silver electrode turns into dark-red color, which is the natural color of newly formed AgCl. Until this step, the fabrication of electrode pair is constructed.

For the microfluidics chip, Omnicoat and SU-8 2075 photoresist (MicroChem Corporation, MA, USA) was first spin-coated on the 3-inch nickel disk to achieve 220 nm thickness, followed by a prebake process. After the photoresist layer was exposed to a UV source, it was baked again for SU-8 crosslinking. After developing in SU-8 developer (MicroChem Corporation, MA, USA), electroplating was performed in a nickel electroplating bath, using a two-electrode system with a nickel anode and the patterned nickel disk cathode. Finally, a nickel mold with a 200-μm-thick plating nickel microstructure was obtained after removal of the residual SU-8. The nickel disk with patterned channel structures was used as a master mold to replicate the COC polymer microfluidic chips by injection molding (BOY 22A, BOY Machines Inc., PA, USA). After drilling holes for fluidic interconnection at the inlet and outlet using a micro drill bit, the microfluidics chip was bonded with the sensor chip using UV adhesive bonding technique at room temperature to make the final lab chip.

Photographs of the fabricated chip are shown in Fig. 3(b), and illustrate three individual sensors with planar electrode arrays,
electrical connections, and microchannels. The entire chip size is 4 cm × 2.5 cm, and the sensing chamber has width of 3 mm, length of 15 mm, depth of 200 μm, and total volume of around 10 μL. All electrodes have length of 3 mm, width of 500 μm, and a spacing of 500 μm. Array of three sensors were easily microfabricated on one chip, and were used for simultaneous measurements to obtain the average value on one chip. The size of the chip fit the chip slots on the fluidic motherboard. When the chips are loaded into the analyzer, the fluidics inlet and outlet on the chip connect to the microchannels on the fluidics motherboard of the analyzer through O-rings, and the electrical contact pads on the chips are contacted to spring loaded connectors (Mill-Max Manufacturing Corporation, NY, USA) on the PCB board in the analyzer to form the electrical connection.

IV. FLUIDICS SYSTEM

To automatically perform sampling and fluid (i.e., samples and buffer solutions) transfer, a LabVIEW program controlled fluidic system has been developed. The fluidic system consists of a plastic fluidic motherboard with microchannel network, microvalves and pump, sampling ports with tubings and filters, buffer reservoirs and inlets, and waste outlet and reservoir. The schematic illustration of the fluidic system design is presented in Fig. 5(a).

The plastic fluidic motherboard (30 cm × 23 cm × 1 cm) was fabricated by computer numerical control (CNC) micro-machining and thermal bonding techniques. Fig. 5(b) shows the photograph of the plastic fluidic motherboard after thermal bonding. All components were then assembled on the fluidic motherboard. Fifteen magnetic solenoid valves are mounted on the fluidic motherboard surface and connected to microchannels of the motherboard; a peristaltic pump is connected to the microchannels of the motherboard through silicone tubings. Seven chip slots (4 cm × 2.5 cm × 0.2 cm) are machined on the top surface of the fluidic motherboard with fluidic and electrical interfaces for loading disposable lab chips. All components on the motherboard are connected via 500 μm square microchannels.

Fourteen valves are used for regulating the fluidic path among all seven sampling inlet and lab chip, and one three-way valve (V₀) is used for regulating different buffer solutions. For each measurement, only one fluidic path is opened, thereby eliminating cross-contamination between different measurements. When a certain fluidic path is chosen, the 2-way valve for this path is opened, while other 6 two-way valves are kept closed; the three-way valve switches to the sampling inlet and the sample is sucked from the inlet. After this measurement, the three-way valve changes to the buffer inlet, and the washing buffer is sucked from the buffer reservoir for the washing step to eliminate residue from the previous measurement.

The maximum flow rate of the fluidic system is 3.5 mL/min when the duty cycle of the peristaltic pump is 100%, and can be adjusted by reducing the duty cycle. The maximum flow rate is the average rate obtained by calculating the value of the total pumped sample volume in a fixed period of pumping time (e.g., 5 min). The flow scheme can also be chosen from either static measurement with no flow during the deposition step of the ASV, or continuous flow measurement, which keeps the sample flows during the increase the target metal deposition efficiency.

V. CONTROL CIRCUITS

Fig. 6 shows the major components of the analog control circuit used in the analyzer. The main functions of the circuit include voltage regulation, valve driving, pump driving, and multiplexing between the sensor outputs and the potentiostat. Note that the left pointing symbols, for example, correspond to the input signals generated from the LabVIEW program.

The voltage regulator consists of a switch-mode DC-to-DC converter (CC101212DFE, TDK-Lambda, Japan) that can generate multiple voltages different from the battery voltage. Capacitors C₁ and C₂ are used to reduce the ripples of the output voltages.

The basic form of the valve driving circuit consists of a bipolar transistor (MP54A2, Fairchild Semiconductor, ME, USA) with the valve connected between its collector and positive supply voltage. An analog switch (ADG436, Analog
Devices, Inc., MA, USA) however, has been inserted in between. This is to ensure that the driving current through the valve can flow in both directions, as required for the latching valve to work correctly. The usage of the latching valve greatly reduces the power consumption of the system as it only consumes power when it is switching. The diode is used to protect the transistor from back electromotive force. The pump driving circuit is similar to the valve driving circuit but in simpler format. No analog switch is needed as the pump is designed to move liquid samples in a single direction. A medium-power transistor is (TIP121, Fairchild Semiconductor, ME, USA) employed to drive the pump with adequate current (~100 mA).

The multiplexing circuit establishes the electrical paths between the electrodes of a given sensor and potentiostat inputs. Three sets of 32-to-1 multiplexers are used because there are 21 sensors in total and each sensor consists of three electrodes. Fig. 6 illustrates one such set, which is formed by two 16-to-1 multiplexers (DG406, Maxim Integrated Products, CA, USA) and one 2-to-1 multiplexer (MAX309, Maxim Integrated Products, CA, USA) for rapid prototyping purpose. Generally speaking, low leakage current, low crosstalk, and low on-resistance are the most desirable properties for the multiplexer.

VI. RESULTS AND DISCUSSION

The first prototype of the analyzer was developed in our group, as shown in Fig. 7. The customized LabVIEW user interface including system status, electrochemical detection setup, and fluidics control setup is shown in Fig. 8.
The detection and quantification of Pb (II) and Cd (II) using the Bi electrode based heavy metal sensor have been reported previously [17]. Pb (II) and Cd (II) were statically performed using SWASV inside the microchannels, with limit of detection of 8 ppb for 60 s deposition for Pb (II), and 9.3 ppb for 90 s deposition for Cd (II) [17].

By using the analyzer and polymer lab chips, the performance of the heavy metal sensor using microfabricated planar Bi working electrode was characterized by cyclic voltammetry (CV), as shown in Fig. 9. The scan range of CV is from 0.5 V to −1.5 V with a scan rate at 100 mV/s. The Bi working electrode showed a flat potential window from −1.3 V to −0.3 V versus the on-chip Ag/AgCl reference electrode in CV. The hydrogen evolution and Bi oxidation potential are found at about −1.3 V and −0.3 V, respectively. The reduction peak of the Bi oxides and hydroxides, which is formed at the positive scan, is at around −1.0 V. This potential window is ideal for measuring lead (Pb), cadmium (Cd), and zinc (Zn) using ASV.

In this work, array of lab chips were incorporated into the analyzer with customized user interface to perform the continuous measurement for on-site applications. As an example, in Fig. 10(a), SWASV was automatically performed to measure 125 ppb Cd (II) standard solution in PBS buffer (pH = 7.43) by one chip. The measurements were performed serially by all three individual sensors on one chip for the same sample. The SWASV conditions are: $E_{\text{dep}}$: −1.2 V for 30 s, $E_{\text{swv}}$: 25 mV, $E_{\text{step}}$: 5 mV, $f$: 100 Hz, flow rate during deposition: 350 µL/min.

In Fig. 10(a), clear Cd (II) reduction peaks were displayed on the UMPC screen at −0.877 V versus on-chip Ag/AgCl reference electrode with a low standard deviation on both the peak current height and peak current potential obtained from three sensors. The average value (14.2 µA, $n = 3$) and standard deviation (0.6 µA, $n = 3$) of peak currents were calculated for calibration. The calibration curve using the same condition from 125 to 1000 ppb ($R^2 = 0.973$) is shown in Fig. 10(b) with the sensitivity of 24 nA/ppb. All data were stored on the PDA/UMPC for further analysis.

One of the important features of the analyzer for measuring heavy metals is that it can increase the output sensor signal by using a continuous flow sensing method. During the deposition step of the ASV, the analyzer can keep the sample continuously flow through the chip at controlled flow rates, and stop the flow during the stripping step for measurement. Using this method, the deposition efficiently can be increased due to the accelerated mass transfer of the heavy metal ions to the Bi working electrode surface compared with static measurement method inside the microchannel. In the static measurement case, the mass transfer of the heavy metal ions to the working electrode is limited by the diffusion only. Fig. 11 shows the increase of the output peak current by increasing flow rates during the ASV deposition step when measuring 500 ppb Cd (II) samples in PBS buffer (pH = 7.43). The SWASV conditions are: $E_{\text{dep}}$: −1.2 V for 20 s, $E_{\text{swv}}$: 25 mV, $E_{\text{step}}$: 5 mV, $f$: 100 Hz. A clear trend of peak current increase is observed with the flow rate increase during the deposition step. Although the increase of the flow rate will cause a larger volume of sample consumption, it is not a problem for on-site environmental monitoring by considering the usually unlimited environmental sample (e.g., ground water) in the nature.

An important performance metric for the “one-shot” disposable chip is reproducibility of the different sensors. Good relative standard deviation (RSD) was obtained in measuring 250 ppb Cd (II) samples using SWASV. The RSD of the peak
Fig. 10. (a) Screen shot of SW ASV measurement of 125 ppb Cd (II) in PBS buffer, which is automatically performed by three sensors on one chip using the analyzer with the customized LabVIEW program. SWASV conditions: $E_{\text{dep}}$: -1.2 V for 30 s, $E_{\text{sw}}$: 25 mV, $E_{\text{step}}$: 5 mV, $f$: 100 Hz, flow rate during deposition: 350 $\mu$L/min. (b) Calibration curve by the same conditions. Current height is 7.4% ($n = 6$) and RSD of the peak potential is 1.2% ($n = 6$).

Before deployment to waste disposal sites, the analyzer was evaluated by monitoring the Cd (II) concentration change in a lab-scale reactor. This reactor simulates pollution of soil pore and ground water around heavy metal waste disposal sites. In this reactor, 5 ppm concentration of the Cd (II) contamination source were continuously supplied to the soil pore and ground water at a flow rate of 0.035 mL/min for up to nine days, and the average flow rate of the groundwater is 2.8 mL/min.

Cd (II) concentration from 28–280 ppb ($R^2 = 0.986$) was first calibrated using SWASV with the analyzer and lab chips, as shown in Fig. 12(a). The SWASV conditions are: $E_{\text{dep}}$: -1.2 V for 90 s, $E_{\text{sw}}$: 25 mV, $E_{\text{step}}$: 5 mV, $f$: 100 Hz, flow rate during deposition: 0 $\mu$L/min. The calibration range is the estimated Cd (II) concentration range from the reactor. The sensitivity is 65 nA/ppb and the limit of detection is 9.3 ppb ($S/N = 3$) at the deposition time of 90 s. No major inferences were found in this experiment. The analyzer and the chips were then used for automatic on-site Cd (II) monitoring of the soil pore and ground water samples from the reactor, which was contaminated with a 5 ppm Cd (II) source for seven days. The obtained peak currents are not provided in the text.

Fig. 11. Peak current change at different flow rates during the deposition step of SWASV when measuring 500 ppb Cd (II) sample in PBS buffer ($pH = 7.43$) using the analyzer and disposable lab chips. SWASV conditions: $E_{\text{dep}}$: -1.2 V for 20 s, $E_{\text{sw}}$: 25 mV, $E_{\text{step}}$: 5 mV, $f$: 100 Hz.

Fig. 12. On-site measurement of Cd (II) in soil pore and ground water with Cd (II) contamination from a lab-scale reactor. (a) Calibration curve of Cd (II) at the estimated concentration range and (b) calculated results of the Cd (II) concentration change in the soil pore and ground water samples over seven days. All the samples were 1:1 mixed with 0.1 M acetate buffer. SWASV conditions: $E_{\text{dep}}$: -1.2 V for 90 s, $E_{\text{sw}}$: 25 mV, $E_{\text{step}}$: 5 mV, $f$: 100 Hz, flow rate during deposition: 0 $\mu$L/min.
were converted to the Cd (II) concentrations by the calibration curve in Fig. 12(a), and are presented in Fig. 12(b) at the range of 15–50 ppb. These data demonstrate that this lab chips along with analyzer could be used for fast and on-site monitoring of heavy metal such as Cd (II) and Pb (II) in real environmental samples such as waste disposal.

VII. CONCLUSION

In conclusion, an analyzer with polymer lab chip array has been successfully developed for automatic continuous sampling and on-site heavy metal measurement, and tested by monitoring of Cd (II) in the soil pore and ground water samples from a lab-scale reactor. Sensing performance was improved by continuous flow during ASV measurement using the analyzer and lab chips. This analyzer has the advantages of compact size, low weight, low power consumption, rapid detection, easy use, flexible sampling and detection scheme, and automatic controls, and can therefore be deployed on-site for long-term heavy metal detection. Furthermore, various different electrochemical detection methods and sampling schemes can be adapted for this analyser, providing flexibility for on-site monitoring of other environmental pollution species.

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