

POINT-OF-CARE MEASUREMENT OF ZINC IN BLOOD SERUM

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ABSTRACT

This work reports on the development of a point-of-care (POC) system for electrochemical measurement of zinc in blood serum. Critically ill pediatric patients have consistently demonstrated abnormally low blood zinc levels. Supplementation of zinc as been proposed as therapeutic strategy. The conventional approach using atomic absorption spectroscopy is sensitive but has a long time around (24-48 hrs). Thus, sensing blood samples to a centralized laboratory for analysis is not a viable option when patient's zinc blood levels must be monitored on the time scale of hours. Our electrochemical sensor relies on an environmentally-friendly bismuth electrode for zinc determination by anodic stripping voltammetry in the physiological range of 65~95 $\mu\text{g/dL}$. Ultimately, the POC system allows rapid (10~20 min) zinc measurements in blood serum, and with further development and integrated sample preparation may be converted into a self-check platform for bedside monitoring.

KEYWORDS: Electrochemical sensor, zinc, anodic stripping voltammetry, bismuth, blood serum

INTRODUCTION

Zinc (Zn) is an essential trace element required for the normal functioning of hormones, enzymes and transcription-related factors [1,2]. Zinc deficiency has been associated with outcomes in critically ill patients, especially in pediatrics [1-3]. The existing "gold-standard" for monitoring are atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectroscopy (ICP-MS). However, 24 to 48 hrs time delay associated with such measurements presents a critical challenge for any Zn supplementation therapy [1] as inadequate monitoring can easily result in over-supplementation leading to adverse effects due to toxicity associated with zinc heavy metal.

Stripping voltammetry has been used to detect and measure Zn on the macroscale using working electrodes such as hanging drop mercury electrode (HMDE) [4], mercury film electrode [5], perfluorinated anion-exchange polymer mercury film electrode [6] and pencil-lead bismuth film [7] electrodes. Bismuth, a non-toxic electrode material has been replacing the toxic mercury electrodes and in this work, an electroplated bismuth film on gold was used as the working electrode including an integrated microfabricated Ag/AgCl reference electrode and a gold counter electrode. Anodic stripping voltammetry (ASV) is the electroanalytical technique that has been popularly used for measurement and detection of metal ions because it is a highly sensitive technique. This technique has been proven for lead measurements in blood but Zn is electronegative and presents problems like hydrolysis.

EXPERIMENTAL

The POC system consists of a lab-on-a-chip (LOC) sensor and a reader. The microfabrication procedure is shown in Fig. 1 and takes advantage of soft-lithography and electrodeposition. E-beam evaporation was performed to make gold-coated glass slides and these were patterned to form three electrodes by means of photolithography and electrodeposition was performed to form the three different electrodes of the sensor. The three-electrodes (Fig. 2) are a bismuth (Bi) working electrode, Ag/AgCl reference electrode, and Au auxiliary electrode. Electrodeposition of the Bi film electrode are performed using a Bi plating solution

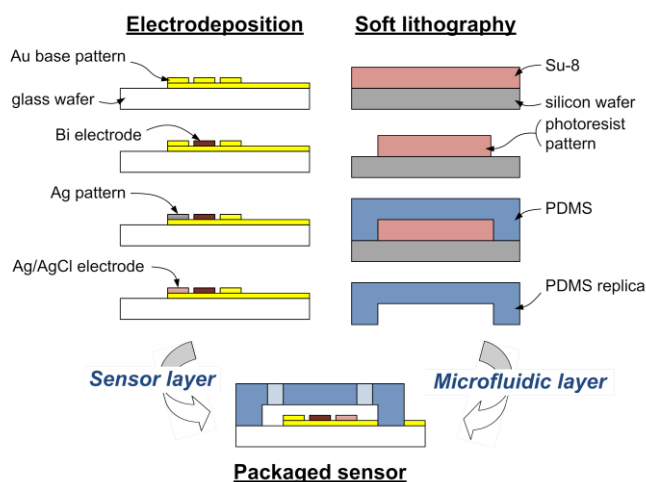


Figure 1. Schematic illustration of the microfabrication procedure for the sensor.

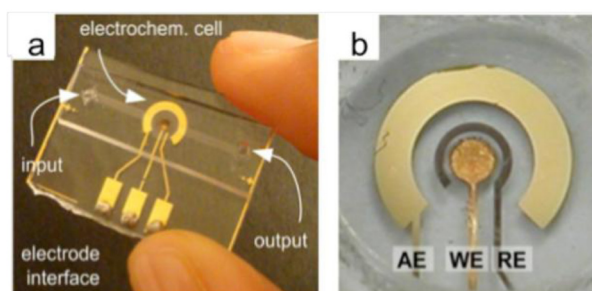


Figure 2. (a) Photograph of the sensor chip and (b) close-up image of the electrochemical cell where AE = gold auxiliary electrode; RE = Ag/AgCl reference electrode; WE = Bi working electrode.

of 500 mg/L concentration in acetate buffer (pH 4.65) using controlled-potential deposition at -0.8 V for 3 min (using BAS potentiostat). Soft lithography technique was used to make an electrochemical chamber using polymer polydimethylsiloxane (PDMS) which was bonded over the microfabricated sensor region by means of plasma discharge.

The LOC sensor is connected to the POC chip reader (Fig. 3) which includes the potentiostat circuitry and signal processing circuitry. It is implemented using a PIC18f8722 microcontroller, 10-bit DAC (AD7840) and op-amps. The electroanalytical technique used for the measurement of Zn is anodic stripping voltammetry (ASV) which consists of the preconcentration step and the stripping step. The preconcentration potential and the preconcentration time for the deposition of the Zn ions was varied and the optimum parameters for these two variables were optimized. The Zn measurement process starts with a preconcentration phase where a -1.6 V bias is applied between the WE and AE, followed by the stripping phase where current flowing between the WE and RE is measured as potential is swept from -1.6 V to -0.6 V.

RESULTS AND DISCUSSION

The electroplated bismuth working electrode of the sensor has a working potential window range of -1.6V to -0.3V due to the hydrolysis that occurs at voltages below -1.6V and the stripping of the bismuth film at -0.3 V. A 10 μM concentration of Zn was used to determine the optimum preconcentrated time by varying the preconcentration time for 30s, 60s, 300s, 600s and 900s with stirring. The results showed that 600s was sufficient to preconcentrate all the

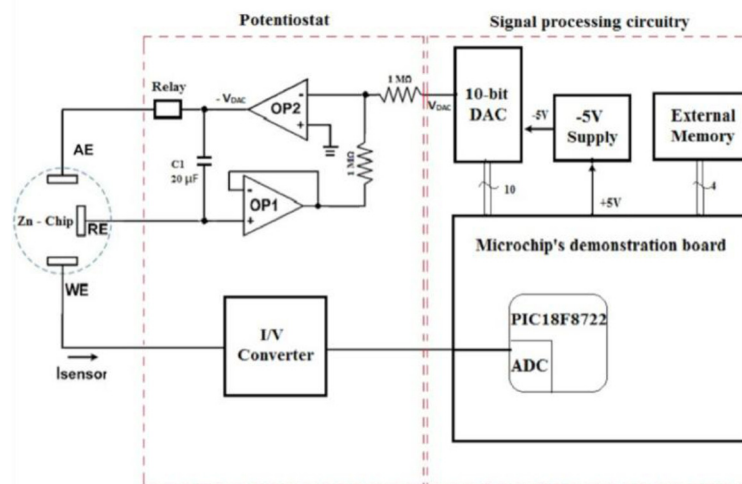


Figure 3. Block diagram of the point-of-care chip reader illustrating Zn chip connected to the potentiostat box (used for controlling bias potential and to measure the current), and the signal processing unit (used to supply power, digitize output, and interface with external memory and/or computer).

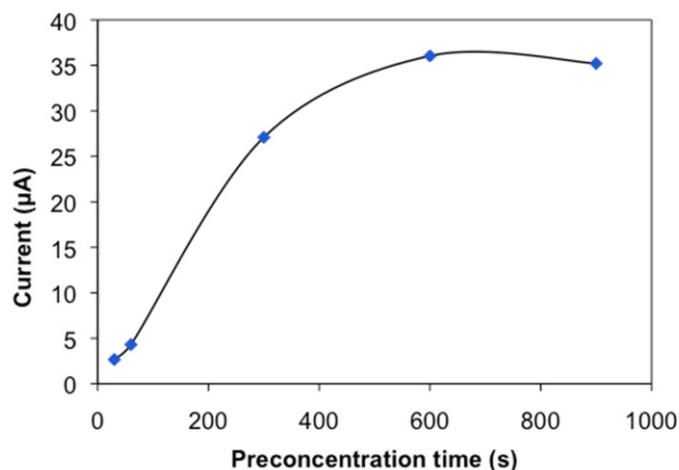


Figure 4. Relationship between preconcentration time and measured current values for 10 μM Zn concentration.

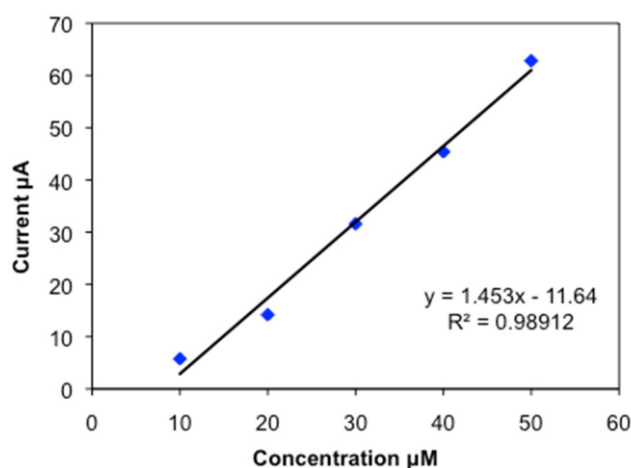
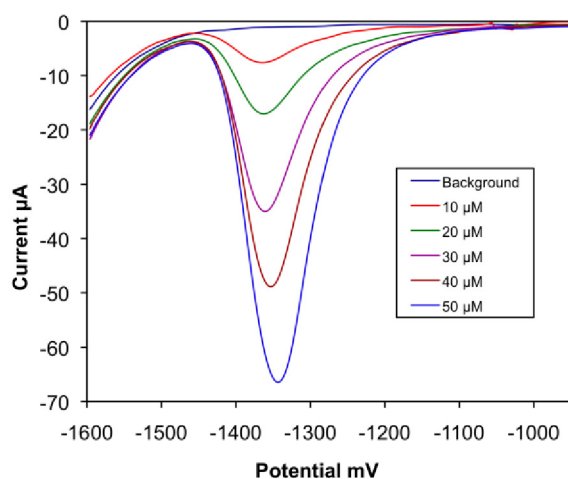


Figure 5. (a) Anodic stripping voltammetry and (b) calibration plot for measuring zinc in the physiological range (10-15 μM or 65-95 $\mu\text{g/dL}$) in acetate buffer at pH 6.

metal ions in the sample volume for this low concentration (Fig. 4).

Fig. 5a shows the background of acetate buffer of pH 6 and anodic stripping voltammetric peaks for increasing concentrations of Zn spiked in the same buffer. It was found that the Zn stripping peak occurs at -1.36 V and current value increased for increasing concentrations of Zn. A calibration curve (Fig. 5b) was constructed for Zn concentrations from 10 μ M (65 μ g/dL) to 50 μ M (327 μ g/dL), which brackets the physiological range, and shows sensitivity of 1.453 μ A/ μ M.

Metexchange agent was added to blood serum to release Zn ions sequestered in proteins (up to 40% can be bound). Metexchange is a commercially available product (ESA Inc.) which is mixture made up of chromium chloride 1.07 wt%, calcium acetate 1.43 wt% and mercuric ion 0.0028 wt% contained in acids and buffers. This is used for digestion of the serum matrix before measurement in order to detect all the zinc present in the serum. Fig. 6 illustrates a successful measurement of 100 μ M in a 200 μ L serum sample mixed with metexchange and buffered with sodium acetate shown in comparison to a background of serum and acetate buffer with no zinc.

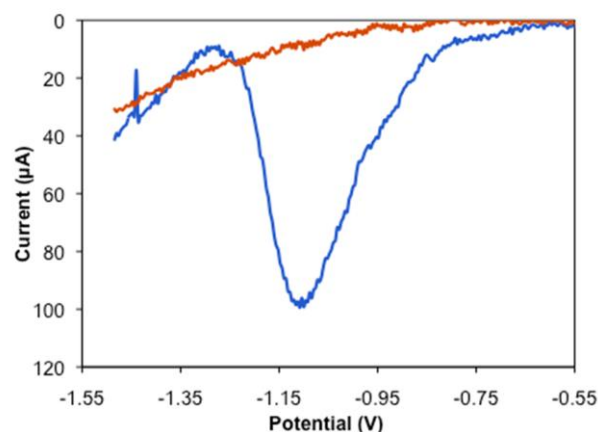


Figure 6. Anodic stripping voltammogram of serum buffered with sodium acetate buffer and metexchange illustrating stripping peak of 100 μ M zinc and the background.

CONCLUSION

The developed POC system permits measurement of zinc with several critical advantages, including reduced sample volumes (200 μ L from a drop of blood from a finger prick vs. 5 mL for AAS, which is especially important for pediatric patients) and reduced turnaround time (10~20 min vs. 1~2 days for AAS). Ultimately, this system will permit the quantification of Zn at the bedside of critically ill patients leading to improved recovery of critically ill patients.

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