Development of a Point-of-Care Device to Quantify Serum Zinc to Aid the Diagnosis and Follow-up of Pediatric Septic Shock

Sowmya Sukhavasi, Pretha Jothimuthu, Ian Papautsky, and Fred. R. Beyette, Jr., IEEE Member

Abstract— In the Unites States Pediatric septic shock is a major health problem with about 42,000 cases per y ear and a mortality rate of about 10% [1]. Studies have indicated th at children with pediatric septic shock have demonstrated critically low levels of serum z inc (Zn) and supplementation of Zn is being suggested as a thera peutic strategy. However, to protect patient safet y, it is v ital that Z n levels be monito red during supplementation to insure th e Zn concentration levels remain at or near physiologic normal levels. Currently Atomic Absorption Spectroscopy (AAS) is used to quantify Zn levels in serum samples. Unfor tunately, AAS fr equently involves sending serum samples to external laboratory faciliti es which yields measurement turnaround time that r ange from hours to days. Thus, timely monitoring of Zn levels is c ritical to preventing over supplementation th at could result in critical conditions such as heavy metal (Zn) toxicity. This paper reports on the development of a Point-of-Care device for rap id electrochemical measurement of Zn. The prototy pe device is able to accurat ely quantify Zn in serum w ith a turn-around time of about 30 minutes. The de vices is based on a th ree electrode sensor which uses Anodic stripping voltammetry (ASV) for sensing Zn levels. The ASV electrode sensor is read using a r eader that has be en developed using c ommercially available embedded system components and custom anal og circuitry.

I. INTRODUCTION

Zinc is an essential element that affects functioning of the immune system, insulin secretion/action, various hormones and enzymes [2-3]. Reduced serum zinc (Zn) concentration has been associated with infections and inflammations. Also, studies have indicated that children with pediatric septic shock and critically ill adults have demonstrated critically low levels of serum Zn [3]. It has been suggested that Zn supplementation could be a very effective therapeutic strategy [4] provided there exists the possibility to adequately monitor serum Zn levels to insure that over supplementation does not result in heavy metal (Zn) toxicity. Currently Atomic Absorption Spectroscopy (AAS) is used to quantify Zn in serum. However, this quantification method, which frequently requires sending samples out to an external lab facility, can have measurement turnaround times that vary from hours to days. Unfortunately, this delay cannot be tolerated particularly while dealing with critically ill patients. To overcome this challenge a portable, rapid and inexpensive Point-of-Care (POC) device to quantify serum Zn has been developed.

The proposed POC device consists of an electrochemical sensor and a zinc chip reader (integrate with the sensor and external memory to store the experimental data). Anodic stripping voltammetry (ASV) is the electroanalytical method used to detect and measure Zn. Section II details the ASV method and the sensor used in this work. Section III describes the device hardware of the zinc chip reader used to drive the sensor and collect characteristic curves that can be used to quantify the zinc present in the sample. Section IV describes the experimental set-up and the method used to conduct experiments. Finally, Section V summarizes the preliminary testing results and future work that is currently afoot.

II. ELECTROCHEMICAL SENSOR AND ANODIC STRIPPING VOLTAMMETRY SION

The sensor (Figure 1) is a three electrode device that includes a Bismuth sensing (working) electrode, a Ag/AgCl reference electrode and Gold counter electrode. Other electrodes such as hanging drop mercury [5], mercury thin film[6], glassy carbon [7] have been reported in literature for the detection of Zn. However, bismuth has become popular recently due to its non-toxic nature, making the sensor convenient to be used in a Point-of-Care setting at or near the patient's bed side.

In our work, the sensor was fabricated by means of microfabrication techniques including photolithography, wet-etching, electrodeposition and soft-lithography. Au coated glass slides were patterned using photolithography to form the Au microelectrodes. A polymer well was fabricated using soft lithography technique was bonded to the substrate to form the electrochemical chamber. In the future, the well will be replaced with a microfluidic channel network. Electrodeposition was used to selectively deposit Bi and Ag/AgCl on the patterned Au electrodes to form the
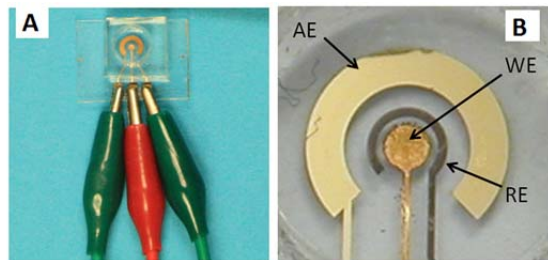

Fig. 1. (A) Photograph of the electrochemical sensor with connectors and (B) close-up image of the sensor where AE is Gold auxiliary electrode; RE is Ag/AgCl reference electrode; WE is Bi working electrode

working and the reference electrodes respectively. The Bi plating solution was made up of 500 mg/L of Bi AAS solution containing Bi (III) ions along with acetate buffer of pH 4.65 (0.1 M). The Bi film was plated on the circular electrode by means of controlled-potential electrodeposition by the application of -800 mV for 4 min. The Ag/AgCl reference electrode was made by controlled-current electrodeposition by the application of 5 mA/cm^2 current density for 60 s and then this was chloridized using 1M KCl solution for 30s.

Anodic Stripping Voltammetry (ASV) is the electroanalytical technique widely used for detection of heavy metals in solution as it has the lowest detection limit. In particular, Anodic Square Wave Stripping Voltammetry (ASWSV) has been used for detecting and measuring Zn. The ASWSV technique is carried out in three phases. In Phase one the dissolved metal in the sample is accumulated on the working electrode by controlled potential electrolysis, this is called the *pre-concentration* phase. In this phase the Zn^{+2} metal ions are reduced to elemental metal Zn^0 as a result a metallic film is formed over the Bismuth working electrode. And during this step the sample solution is constantly stirred to ensure that all the metal ions are deposited on the electrode surface. The second phase is the *Quite time*, this is similar to the preconcentration step except that the stirring is turned off. In the last phase the deposited metal is stripped off of the electrode surface by applying a stair cased square wave in positive direction. This is called the *stripping phase*. During which the metal is oxidized back to its ionic form. This oxidation process generates an anodic current that can be measured by a sensitive ammeter to reveal peaks (i.e. increased current flow) at stripping voltages that are characteristic to the metal specie being oxidized. Thus by measuring the magnitude of the peak at the stripping potential associated with Zn oxidation, it is possible to quantify the amount of Zn that was accumulated out of the solution in the first phase of the measurement.

III. DEVICE HARDWARE DESCRIPTION

To use the zinc sensor described in section II, the sensor must be connected to a potentiostat that is capable of providing the necessary driving potentials and measuring the current associated with striping metal ions off of the electrode. To facilitate the development of a Point-of-Care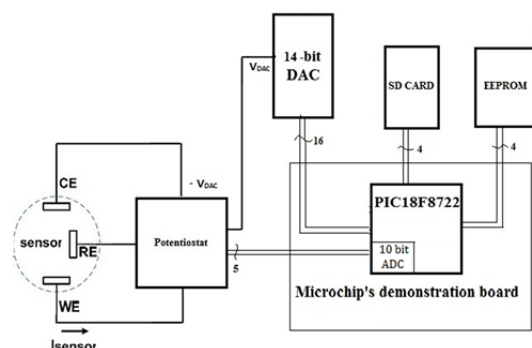 device, a custom chip reader has been developed to perform the two functions associated with the potentiostat needed to drive zinc sensor described in section II. First, the chip reader generates the required potential profile to drive the zinc sensor during the three phases of ASWSV cycle. Second, the chip reader provides real time data acquisition necessary to detect the sensor current. In this function the data acquisition system captures a digitized representation of the current values and stores that data for subsequent signal analysis required to quantify the zinc concentration in the sample under test.

Figure.2 shows the block diagram of the device instrumentation developed for the zinc chip reader. As described above, this circuitry centers around the potentiostat interface and a embedded system which dictates the actions of the entire hardware device.

A. The Embedded System

The embedded system consists of a PIC 18 explorer board by Microchip® and other commercially available peripheral devices such as a DAC, 256K byte EEPROM and a SD card module. At the core of the explorer board is a PIC18F8722 microcontroller that is programmed by software to control and schedule when each section of the hardware is activated. The PIC ports interfaces with the 14 bit DAC which generates the required potential profile for ASWSV. The PIC also has an integrated 10 bit Analog to Digital Converter (ADC) module which has been used to digitize the measured current value. Quantification of zinc is done by measuring the peak current value from an anodic stripping voltammogram, which is plotted as measured current against the input potential. The peak current is compared against a calibration curve to obtain the concentration of Zn. Thus it is required to store the real time data acquired after each half cycle of the stair cased square wave during stripping. For this purpose an EEPROM has been used to temporarily store data and once the experiment has been completed all the data will be transferred to a SD card in a text file format, which can then be plotted and further analyzed on any computer. The PIC accumulates 64 Kbytes (one page of the 25LC256 EEPROM) of this real time data (Voltage and Current) values acquired during the stripping phase and then schedules the write to EEPROM. After the final stripping phase is completed the PIC and the software are responsible to transfer data onto the SD card. The EEPROM and the SD card are interfaced to the PIC microcontroller using the two Serial Peripheral Interface (SPI) ports available in PIC18F8722.

B. The Potentiostat

The potentiostat is a very important electronic circuit responsible for controlling the potential difference between Reference Electrode (RE) and Working Electrode (WE) by sampling the cell potential and varying the Auxiliary Electrode (AE) potential accordingly and it also measures the anodic current from the WE [8]. Figure 3 shows the circuit schematic of the potentiostat.

The operational amplifier OP1 is the control amplifiers that control the potential difference between RE and WE. OP2 is essentially a buffer amplifier that samples the cell
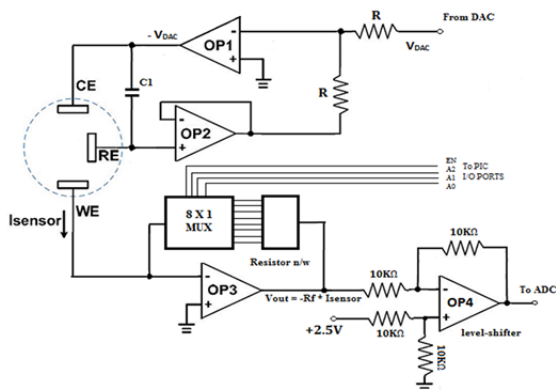

Fig. 2 Block diagram of the Point-of-Care Zinc chip reader

Fig. 4 Circuit schematic of the potentiostat
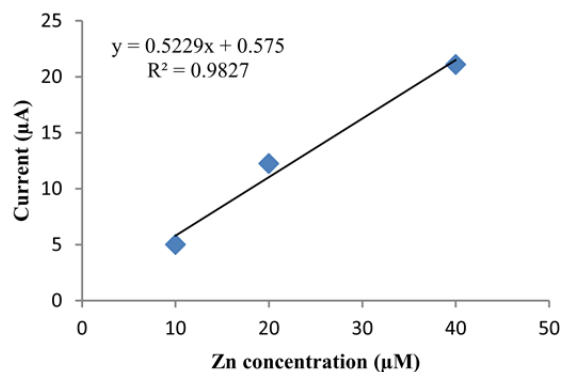

Fig. 3 Calibration curve of the measured current values of Zn in acetate buffer of pH 6 for three different concentrations - 10μM, 20 μM, 40 μM.

potential by sampling the voltage at RE. OP2 ensured that no current will be flowing through RE as it is connected to the input terminal of an operational amplifier which has very large input impedance. Another property of operational amplifiers is that it changes its output such that both the inputs are equal (i.e to keep the difference between the two input equal to zero). Hence if the cell potential is different from the applied potential, V_{DAC} which is generated by the DAC, the output of OP1 will change accordingly to restore the cell potential back to V_{DAC}. As shown in Figure. 3 OP3 keeps the WE at virtual ground and OP2 ensure that all the current flows through WE. OP3 is configured as a I-to-V converter. The current I_{sensor} from WE is converted to a corresponding voltage according to the following expression

$$V_{out} = - I_{sensor} * R_f$$

where R_f is the value of the feedback resistance.

Since the range of current depends on the concentration of Zn in the sample solution, instead of using a single feedback resistor a network of resistors ranging from ($1K\Omega$ to $4M\Omega$) is used along with an 8X1 multiplexer. Table 1 shows the feedback resistor values, corresponding current range and the select lines. The multiplexer's select lines are controlled by the PIC and the software is programmed to automatically scale the I-to-V conversion by changing the select lines depending on the measurements made. The 10bit ADC on PIC18f8722 can measure only positive voltages between 0V to 5V. A level shifter has been incorporated using OP4, it

up-shifts the V_{I-V} by 2.5V. The output of the level shifter is connected to the ADC for digitization. The software then converts the measured voltage back to its corresponding current value and stores it in the EEPROM.

A Printed Circuit Board (PCB) has been designed to host the potentiostat circuit and hardware peripherals such as DAC, EEPROM, SD card module. The PCB is connected to the PIC explorer board through its I/O expansion slot.

IV. CURRENT RESULTS AND ONGOING WORK

All the measurements are made with a preconcentration time of 600s and a quite time of 20s. Followed by a potential scan from -1.6V to -0.6V at a frequency of 15Hz, step size of 4mv and pulse amplitude of 25mV. During measurements, samples were stirred using a touch mixer. With these parameters preliminary testing was done by spiking a pH6 acetate buffer with three different concentrations of Zn. The sample volume required is 200μL, which is very low. It was noted that stripping peak for Zn occurred approximately around -1.36V. The measured current at WE increased with increase in concentration as expected. Figure 4 shows the calibration curve with three different concentrations 10μM, 20μM and 40 μM and it has a sensitivity of 0.5229 μA/μM. In order to check the repeatability of the system three measurements have been made for each concentration. Table. 2 reports the results and the standard deviation for each concentration.The physiological range of Zn is 65 – 95 μg/dL, or 10 – 15μM, is enclosed in the above measurements. Hence by using this system we can detect Zn in biological samples.

Table 2 Listing of feedback resistors, their associated current ranges and the MUX address settings required to activate each current range.

Feedback resistor value	Current range measured		8X1 MUX select bits		
	Lower limit	Higher limit			
$1K\Omega$	400μA	1.5mA	0	0	0
$3.3K\Omega$	121μA	454μA	0	0	1
$10K\Omega$	40μA	150μA	0	1	0
$30K\Omega$	13μA	50μA	0	1	1
$100K\Omega$	4μA	15μA	1	0	0
$330K\Omega$	1.21μA	4.24μA	1	0	1
$1.25M\Omega$	320nA	1.2μA	1	1	0
$4M\Omega$	100nA	375nA	1	1	1

Table 1 Current values for different concentrations of Zn

Concentra-tion	Net current (μA)				Standard devia-tion
	Experiment 1	Experiment 2	Experiment 3	Average	
40μM	21.025	22.053	20.197	21.0917	0.7592
20μM	12.975	11.782	11.953	12.2367	0.5267
10μM	5.0975	4.697	5.213	5.0025	0.2211

However, in biological samples most (approximately 60-70%) of the Zn is bound to protein. To measure the total concentration of Zn in such a sample would require a sample preparation procedure that beaks-up proteins and releases metal. Currently, work is in progress to digest the sample by acidification to release the bound Zn followed by buffering back to normal measurement pH. Also the integrated 10-bit ADC in the PIC18F8722 will be replaced by a higher resolution ADC to improve the accuracy of measurements.

V. Conclusions

The Point-Of-Care system is able to detect and quantify Zn with a significantly shorter turn-around time compared to the use of AAS which takes up to 2 days. The device reported hear can report a result in approximately 30 minutes. This device requires very low sample volume of 200μL(critical for pediatric patients) and it is portable making it usable at patient's bedside.

References

[1] R.S. Watson, J.A. Carcillo, W.T. Linde-Zwirble, G. Clermont, J. Lidicker, D.C. Angus, " The epidemiology os severe sepsis in children in the United States", *American journal of respi ratory and critical care medicine,* vol. 167, p. 695-701,2003.

[2] K.H. Ibs, L. Rink, J. Nutr., "Zinc-Altered Immune Function", vol.133, p. 93-100, 2003.

[3] N.Z. Cvijanovich, J.C. King,H.R.Flori, G.Gildengorin, H.R. Wong, "Zinc homeostasis in pediatric critical illness",Pediatric Critical Care Medicine , vol.10, p. 29-35, 2009.

[4] D.K. Heyland, N. Jones, N.Z. Cvijanovich, H. Wong, J. Parenter. "Zinc Supplementation in Critically Ill Patients: A Key Pharmaconutrient?",Enteral Nutr. , vol. 32, p. 509-516, 2008.

[5] Y. Israel, T. Ofir and J. Rezek, "Determination of trace impurities in high-purity reagents by mercury thin-film anodic-stripping voltammetry," *Mikrochim. Acta,* vol. 69, p. 151-163, 1978.

[6] W. Martinotti, G. Queirazza, A. Guarinoni and G. Mori, "In-flow speciation of Copper, Zinc, Lead and Cadmium in fresh waters by square wave anodic stripping voltammetry Part II. Optimization of measurement step," Anal. Chim. Acta, vol. 305, p. 183-191, 1995.

[7] A. T. Maghasi, H. B. Halsall, W. R. Heineman and H. L. Rodriguez Rilo, "Detection of secretion from pancreatic islets using chemically modified electrodes," *Anal. Biochem.,* vol. 326, pp. 183-189, 2004.

[8] J. Brad and L.R. Faulkner, *Electrochemical Methods: Fundamentals and Aplications,* 2nd ed. New York: Wiley, 2001.