Point-Of-Care Device for Quantification of Zinc in Serum

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Abstract— **With 42,000 cases reported annually in the United States and an approximate 10% mortality rate, [1] pediatric septic shock is a major health problem that is often difficult to treat effectively. Several studies have shown that children experiencing pediatric septic shock often have critically low levels of serum zinc (Zn), suggesting supplementation of Zn to be an effective therapeutic strategy. However, to protect the safety and well-being of the patient, it is extremely important to monitor blood serum concentration of Zn during supplementation in order to ensure that levels remain at or near the physiological norm and thus minimize the risk of heavy metal toxicity from over supplementation. Current methods for quantifying Zn in serum typically require sending serum samples to external laboratory facilities resulting in turnaround times ranging from hours to a few days. Therefore, timely monitoring of Zn levels in serum is often not possible in the clinical setting which ultimately limits the ability to use Zn supplementation as a therapeutic intervention. This paper reports on the development of a Point-of-Care device for rapid electrochemical measurement of Zn in serum. The device is centered on a three electrode sensor which uses Anodic stripping voltammetry (ASV) for sensing Zn levels. The Cu based sensor is read using a reader that has been developed using commercially available embedded system parts in combination with custom analog circuitry that is able to produce quantification results in approximately 6 minutes.**

I. INTRODUCTION

The human body relies on zinc as an essential element that plays a key role in the proper functioning of the immune system, insulin secretion/action, and production of various hormones and enzymes [2][3]. A reduction in the concentration of zinc (Zn) in blood serum has often been associated with infections and inflammations. Even more important to this research is the factor that children with pediatric septic shock and critically ill adults often demonstrate vitally low levels of zinc in blood serum [3]. Therefore, it has been proposed by several researchers that Zn supplementation may be an effective therapeutic strategy to treat these patients [4]. Therein lays the need for an effective way to monitor zinc levels in the patient to ensure that over supplementation does not result in heavy metal (Zn) toxicity. Currently there are two primary methods for quantification of Zn in serum – Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). The problem with these methods is that they involve specialized equipment and a highly trained operator, which requires the sample be sent to an external lab facility for processing. The measurement turnaround times often vary from hours up to a few days. Unfortunately, this is not acceptable in a clinical setting when dealing with critically ill patients. In order to combat this challenge a portable, rapid, and inexpensive Point-of-Care (POC) device to quantify serum zinc is needed.

A first generation proof of concept device developed by Sukhavasi et al. validated the use of Square Wave Anodic Stripping Voltammetry (SWASV) to detect and measure Zn [5]. In this initial phase, a Bismuth, Ag/AgCl, and Au based sensor was developed. [6] In the second generation device the sensor has been redesigned using Cu and $CuCl_2$, which improves the sensors performance and enables implementation into a disposable sensor cartridge that can be mass produced [7]. Details of the sensor design are presented in section II. Section III describes the second generation zinc chip reader that has been developed to drive the sensor, collect characteristic curves, and perform on-board data analysis to quantify zinc. Improved accuracy and the inclusion of on-board analysis of the characteristic curves are two key features that have been added to this generation of the zinc chip reader. Section IV describes the signal processing that has been added to analyze the characteristic curves and turn them into a measurement of concentration displayable on the readout. Section V describes the test methods and presents preliminary results for performance testing of the second generation device. Section VI summarizes advancements that have been made and discusses future work.

II. CU BASED SENSOR

A variety of electrodes including hanging drop mercury [8], mercury thin film [9], and glassy carbon [10] have been reported in the literature for the detection of Zn. Bismuth sensors such as those developed in previous work [6] had become popular due to their non-toxic nature, which makes it a good candidate for POC devices at or near the patient's bedside, while complicated fabrication step are required to

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achieve high accuracy. The new Cu based sensor takes this one step further by using non-toxic materials that are low-cost and simple to fabricate [7].

The sensor was fabricated by means of micro-fabrication techniques including photolithography, wet-etching, electro-deposition and soft-lithography. Metal layers of 20 nm titanium (Ti)/ 200 nm copper (Cu) were evaporated on glass slides cleaned by piranha etching. The new reference electrode was fabricated by chloridizing Cu to form a $Cu/CuCl_2$ electrode. A polymer well was fabricated in polymethylsiloxane (PDMS) using basic soft-lithography techniques. PDMS well and the glass substrate containing electrodes are bonded together using plasma discharge. The resulting sensor (shown in Fig. 1) is a three electrode electrochemical sensor that includes Cu auxiliary and working electrodes and a Cu/Cl_2 reference electrode. More details on the fabrication process can be found in the publication by Pei, et al [7].
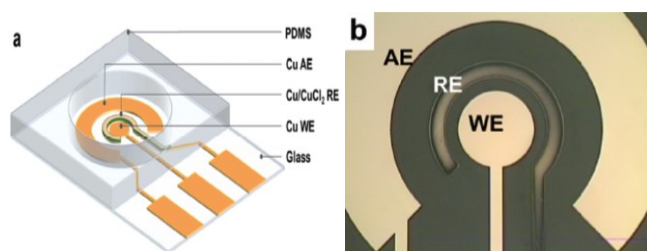

Fig. 1. (a) Model of the electrochemical sensor and glass substrate and (b) close-up image of the sensor where AE is Copper Auxiliary Electrode; RE is $Cu/CuCl_2$ reference electrode; WE is Cu working electrode.

Anodic Stripping Voltammetry (ASV) is the electrochemical technique used widely for detection of heavy metals in solutions due to its low detection limits. In particular, Square Wave Anodic Stripping Voltammetry (SWASV) is commonly used to detect and measure Zn in solutions. SWASV is a three phase process. The first phase referred to as the *Pre-Concentration Phase,* involves accumulating the dissolved metal in the sample onto the working electrode by controlled potential electrolysis. In this phase the Zn metal ions are reduced to elemental Zn as a result of a metallic film that is formed over the working electrode. During this step, which lasts 5 minutes, the sample is constantly stirred using a laboratory vibration plate to ensure that most of the metal ions are deposited on the electrode surface. The second phase is the *Quiet Time*, which is similar to the pre-concentration phase except that the stirring is turned off. This step lasts approximately 15 seconds. In the last phase the deposited metal is stripped off of the electrode's surface by applying a stair-cased square wave in the positive direction. This is aptly called the *Stripping Phase*. During this third phase, which lasts approximately 25 seconds, the metal is oxidized back to its ionic form in the solution. The process of oxidizing the metal generates an anodic current that can be measured by a sensitive ammeter to reveal the peaks (i.e. increased current flow) at stripping voltages that are characteristic to the metal that is being oxidized. Using the magnitude of the peak at the stripping potential associated with Zn oxidation allows the quantification of the Zn concentration. Details on the test parameters can be found in the work of Sukhavasi, et al [5].

III. DEVICE HARDWARE DESCRIPTION

The Zinc Chip Reader provides the sensor with the required potential waveform to perform SWASV as described in section II. The device records the current output of the sensor with the corresponding input potential values. SWASV generates two current profiles, one for each half cycle. Current values in the positive half cycle are presented in the Forward current curve and the current values of the negative half cycle are presented in the Reverse current curve. The device records both Forward and Reverse currents and the Reverse current is subtracted from the Forward current to produce the SWASV curve. After capturing the SWASV curve, the reader hardware analyzes the curve to determine the peak current. Peak values for different concentrations are calculated and used to obtain a calibration curve. Based on the calibration curve, the device determines the concentration of an unknown Zn solution and displays the results. Push buttons are used to command the device to commence testing after inserting the solution into the sensor. A liquid crystal display (LCD) is used to inform the user regarding progress of a test and display the results. Finally, data points for the SWASV curves are stored in a SD card for future reference. A block diagram of the device hardware is shown in Fig. 2.

A. The Embedded System

Device hardware is built using a Microchip's Explorer 16 Development Board, a dsPIC33FJ256GP710 16 bit digital signal controller (DSC) and a custom designed printed circuit board (PCB) with off the shelf components. The explorer 16 development board is a low cost modular development system for the DSC with onboard 2-line-by-16-character LCD, push buttons, easy access to I/O pins of the DSC, and 5 V and 3.3 V voltage supplies. The main control unit of the system is the DSC. The DSC is responsible for providing the digital inputs for the digital to analog converter (DAC) to generate the square wave to perform SWASV, collecting and storing the voltage representation of the current output of the sensor using DSC's on chip 12 bit mono polar analog to digital converter (ADC) at 500ksps. An external level shifter circuit is used to convert the -5 V - +5 V analog output of the I-to-V converter to 0 - 3.3 V range that satisfies the input requirement of the DSC's ADC. The DSC's 30 Kbytes data memory, optimized hardware for faster data processing and digital signal processing library functions built especially for its optimized hardware are utilized to perform signal processing and analysis on the SWASV curve. A SD card reader is attached to the DSC using the SPI interface.

B. The Potentiostat

The purpose of the potentiostat circuit is to maintain the electrochemical cell potential as same as the inverted output of the DAC in spite of cell resistance fluctuations during the experiment. The other important function of the potentiostat is to measure the current through the cell. Potentiostat does this by means of three operational amplifiers configured as a potential controller, voltage follower and a current follower/I to V converter. Potential waveform from the DAC is applied at the inverting input of the potential controller where the potential is inverted and fed in to auxiliary electrode. Voltage follower, connected to the reference electrode
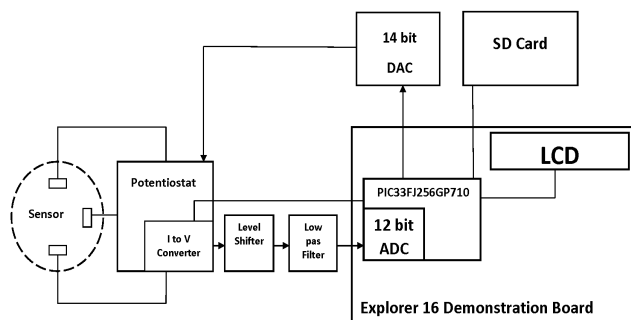
Fig. 2. Block diagram of the point of care zinc chip reader

applies the cell potential back to the inverting input of the potential controller. Since the non-inverting input of the potential controller is kept at ground, the sum of the voltage follower output and the output potential of the DAC at the inverting input should be zero. To satisfy this condition, the voltage follower adjusts the output to maintain the cell potential equal to the inverted DAC potential. The high input impedance of the voltage follower directs all of the cell current to the working electrode. The current follower collects the current flowing through the working electrode and directs it through its feedback resistor generating a voltage proportional to the current [11]. The DSC selects the resistor for the feedback resistor of the current follower out of an array of eight resistors based on the current to get a readable voltage at all values of the expected current range.

IV. SIGNAL PROCESSING

The reverse current captured during SWASV is subtracted from the forward current to obtain the SWASV curve. The magnitude of the peak of the SWASV curve is proportional to the zinc concentration [6]. Peak currents for five known concentrations are calculated and a calibration curve is obtained. The calibration curve is used to quantify zinc concentration in an unknown solution based on its peak current.

A. Generating the SWASV curve

The ADC of the DSC collects the voltage equivalent of the current in real time and the DSC converts them back into current based on the ADC values and the resistor value used in the I-to-V converter. The DSC then stores the forward and reverse current values in a data buffer. Once the SWASV is completed, the DSC does a boxcar averaging on each current profile to eliminate any outliers which can appear as false peaks in the SWASV curve. Once the positive and the negative currents are smoothed out the difference of the two current profiles are calculated and stored in a separate data buffer.

B. Determining the magnitude of the peak

Peak current is determined as shown in Fig. 3. For all concentrations, SWASV curves have two minimum current values and one peak current. Two minimum current values are used as the reference points to draw a baseline. The distance from the peak to the base line is considered the peak current. The magnitude of the peak current is proportional to the Zn concentration. The base line current increases as the

concentration increases [6]. Therefore peak current acquired by this method gives a better representation of the Zn concentration than the peak current itself. Equation (1) is used by the DSC to determine the peak of the SWASV curve.

$$peak\ current = BI - FC - EF/DF(AG - FC)\quad(1)$$

BI is the maximum current value, AG is the minimum current value between the first and maximum current values and FC is the minimum current value between maximum current and the last current value.

C. Calibration curve

Peak current is expressed as a polynomial function of Zinc concentration. Coefficients of the polynomial are determined using the polynomial least square method. Least square method converts the polynomial to a matrix where the coefficients are set so that the deviation of the data obtained by the polynomial is a minimum compared to the actual data. The matrix is solved using the Gauss elimination method to determine the coefficients. Once the coefficients for the best fitted polynomial are determined, the bisection method is used to find the root/concentration of the test sample based on its peak current. The bisection method assumes f(x) values on either sides of a root are of opposite signs. If the root is within the interval [a, c] and the midpoint being b, the signs of f(a)f(b) and f(b)f(c) is checked to find the subinterval that contains the root. This step is iterated until the size of the interval is within a predetermined tolerance. The midpoint of the last iterations gives the best approximation of the root. The device is programmed to find a second order calibration curve using five samples and the tolerance of the bisection method is set to 1e-12.
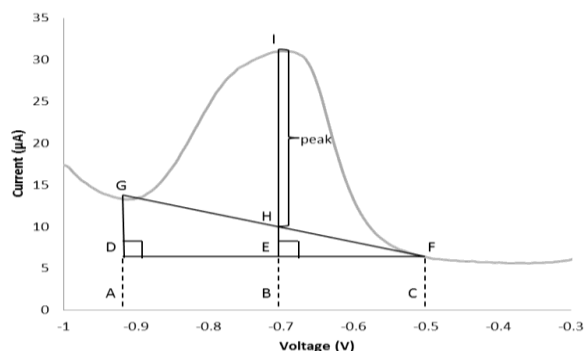

Fig. 3. Shows typical characteristics of SWASV. Properties of similar angle triangles are used to derive IH (peak current)

V. RESULTS

SWASV is performed on 100 μL 0.1 M acetate buffer solution spiking the buffer solution with different concentrations of zinc ranging from 15-40 μM. Optimized parameters for SWASV are 300 s pre-concentration at -1.0 V, 60 mV amplitude, 50 ms period and 6 mV increments. Samples with concentrations of 15, 20, 25, 30, and 40 μM are used for calibration. The user presses a push button to commence the SWASV after introducing each sample to the device. Once the five calibration samples are done, the

device prompts the user to insert the test sample. Based on the calibration curve and peak current of the test sample, the device determines concentration and displays it on the LCD.

Fig. 4 represents the SWASV curve for a 20 µM sample. Equation (1) was used by the device to determine the peak current for the sample to be 5.896 µA. Using MS Excel to process the data points stored on the SD card, the peak current is confirmed to be 5.878 µA.

Equation (2) represents peak current (µA) as a second order polynomial function of Zn concentration (C) (µM) determined by the device.

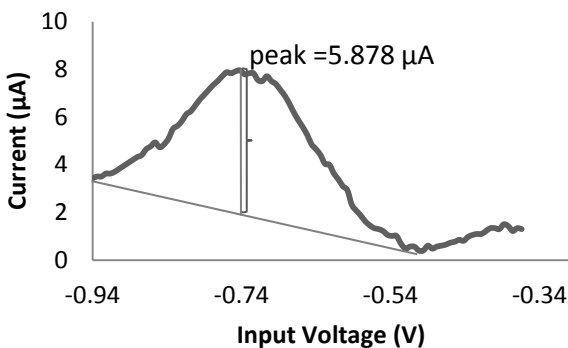$$Peak\ Current = 0.007C^2 + 0.025C + 2.885 \qquad (2)$$

Fig. 4. SWASV curve for 20 µM. 5.878 µA is the peak magnitude determined externally after data is exported to Excel.
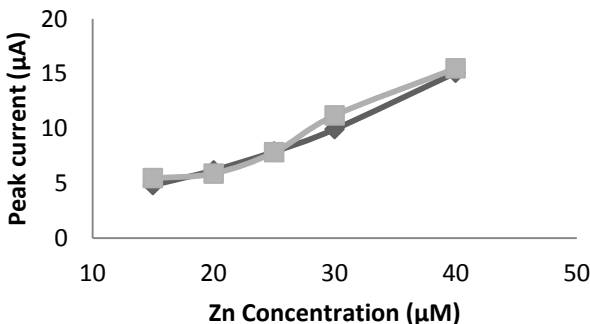

Fig. 5. Dark grey is the calibration curve in equation determined by the device and the light grey curve is the calibration curve obtained using Excel by using the peak concentration values. Both curves are from the same set of tests.

According to Fig. 5, the calibration curve obtained externally in MS Excel for the same set of tests correlates well with the calibration curve obtained by the device internally. Table 1 shows the concentrations determined by the device. The device needs be optimized to obtain peaks with higher repeatability. A single faulty peak of a calibration sample can shift the entire calibration curve deviating the concentrations obtained using the curve from the actual values. More work is being done to increase the repeatability. Additionally, more samples will need to be taken to obtain a more accurate calibration curve for the averaged peak values.

TABLE I.   CONCETRATIONS DETERMINED BY THE DEVICE

Actual Concentration (µM)	Concentrations (µM) determine by the device			Average	Standard Deviation
	Experiment 1	Experiment 2	Experiment 3		
15	19.07	18.38	19.30	18.91	0.478
30	34.96	36.89	36.41	36.08	1.008
40	38.8	40.72	39.76	39.76	0.960

VI. Conclusion

The Point-of-Care device presented here is capable of quantifying Zn concentration in a buffer solution for concentration values from 15 µM to 40 µM. The duration to perform a single test after calibration is less than 6 minutes. Improved Cu based sensor can be used as a low cost inexpensive disposable cartridge that is mass producible using PCB fabrication methods. Future work includes developing the sensor to quantify Zn in serum, assembling all the electronic components on a single PCB, integrating the vibrator plate into the device and device packaging.

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