

# COPPER-BASED SENSOR FOR POINT-OF-CARE MEASUREMENT OF ZINC IN SERUM

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## ABSTRACT

We report the first copper-based microsensor for electrochemical measurement of zinc in serum. Zinc is a trace metal which requires carefully monitoring, yet current methods are too complex for a point-of-care system. Electrochemistry offers a simple technique for metal detection, but the conventional electrode materials are difficult or expensive to microfabricate. Our sensor uses copper as a low-cost electrode material that is simple to fabricate. The sensor shows competitive performance in electrochemical detection. Anodic stripping voltammetry of zinc exhibited 91nM limit of detection and successfully determined its concentration in serum. These features make our sensor suitable for disposable, point-of-care applications.

**KEYWORD:** Copper, electrochemical sensor, zinc, serum, anodic stripping voltammetry

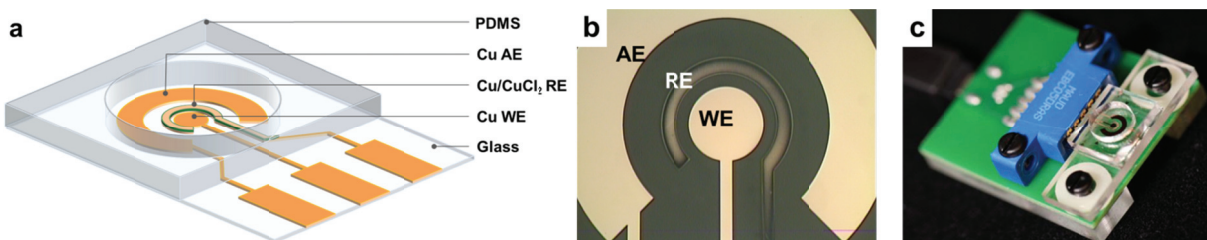
## INTRODUCTION

Zinc (Zn) is an essential trace metal playing key role in metabolism and is a critical component of many enzymes, hormones, and nucleic acid transcription-related factors. Zn imbalance can lead to a number of illnesses. Inflammation and infection, as well as depressed growth and chronic liver disease, are associated with reduced Zn level in serum. Pediatric and adult studies have consistently demonstrated abnormally low Zn levels in critically ill patients [1]. On the other hand, excess Zn intake can lead to copper deficiency and neurologic disease. Zn is traditionally measured in blood serum, with the physiological range of 65-95  $\mu\text{g/dL}$ . The importance and narrow safe range of Zn requires careful monitoring.

The current method for determination of Zn is either by atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectroscopy (ICP-MS). These methods are accurate, but not very efficient from the standpoint of timeliness, cost, the need for trained operators, and accessibility issues to centralized labs. Compared with AAS and ICP-MS, electrochemical detection techniques are easier to miniaturize. In particular, anodic stripping voltammetry (ASV) is a commonly approach for the detection of heavy metals such as lead and cadmium. The limit of detection (LOD) of this technique can be as low as  $10^{-12}$  M, which is sufficient for most trace metal detection needs.

Conventionally electrochemistry is performed using carbon-based or gold/platinum electrodes. Carbon-based electrode coated with bismuth is widely used as an environment-friendly alternative to conventional mercury electrodes, with comparable potential window and performance in the highly negative range[2]. Previously, we reported a gold-based microsensor with integrated Ag/AgCl reference and bismuth working electrode for detecting Zn using ASV[3]. The sensor worked reliably in acetate buffer with LOD of 1  $\mu\text{M}$ . However, in the extracted serum for detection of Zn, large variability was observed in ASV[4], making determination of Zn concentration in extraction samples challenging. Reliable sensors using low cost material with simple fabrication are required for disposable, point-of-care applications.

In this paper, we present a new copper (Cu) -based sensor with Cu working and auxiliary and Cu/CuCl<sub>2</sub> reference electrodes. Our experiments demonstrated that the Cu-based sensor is sufficiently stable for at least one electrochemical experiment. By optimizing experimental parameters, the Cu-based sensor exhibited low LOD for Zn, measured at 91 nM (5.95 ppb) for ASV in acetate buffer, good sensitivity of more than  $1\mu\text{A}/\mu\text{M}$ , and good linearity in the range below 5  $\mu\text{M}$ . In the experiments of extraction samples, we were able to determine the concentration of Zn in bovine serum using standard addition approach.



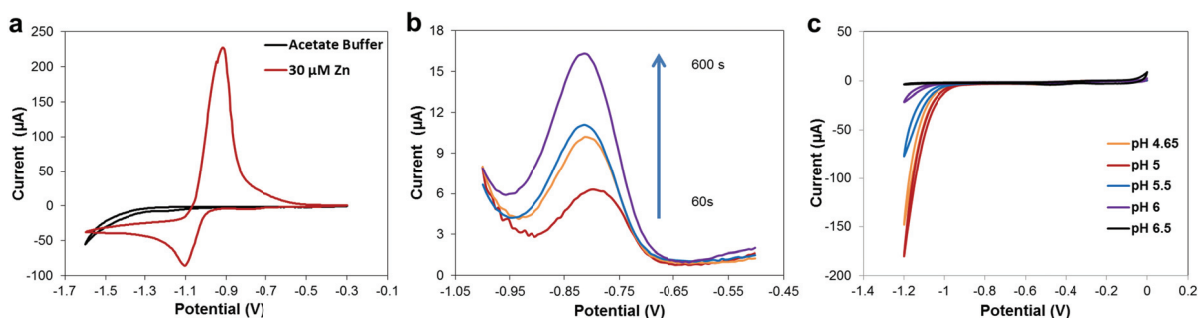
**Fig. 1.** (a) Illustration of the sensor. WE: working electrode, AE: auxiliary electrode, RE: reference electrode. (b) Image of the three electrodes. (c) Photograph of the sensor with a platform connecting to potentiostat.

## FABRICATION

The sensor, schematically shown in Fig. 1a, was fabricated using a combination of lithographic and deposition techniques. Metal layers of 20 nm titanium (Ti) /200 nm Cu were evaporated on glass slides cleaned by piranha etching. Three electrode patterns with contact pads were formed using photolithography and wet etching. A new reference electrode, Cu/CuCl<sub>2</sub>, was simply fabricated by chloridizing Cu with 69  $\mu$ A for 30s. The electrodes layout is shown in the photograph of a completed sensor in Fig. 1b. A polymer well was fabricated in polydimethylsiloxane (PDMS) using standard soft lithography methods. It was bonded to glass substrates contacting electrode patterns using plasma discharge. A platform was fabricated to simplify the connection between sensors and potentiostat. An edge board connector and a mini USB port were soldered and connected on a PCB board. To perform electrochemical experiments, as shown in Fig. 1(c), the sensor was inserted into the edge board connector, and connected to potentiostat (WaveNow, Pine Instrument) using a mini USB cable.

## RESULTS AND DISCUSSION

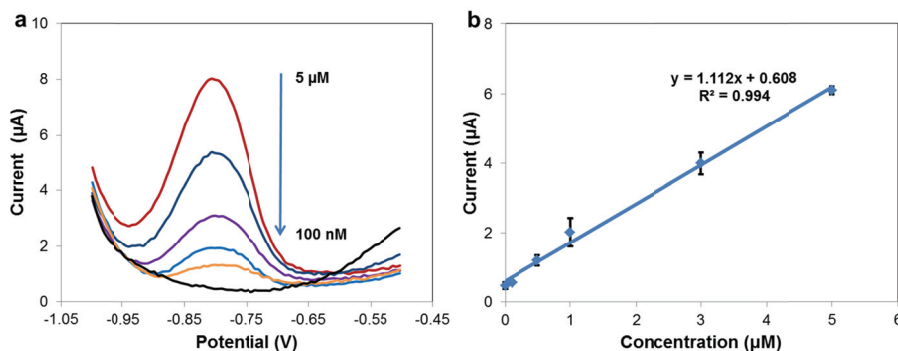
This is the first sensor using Cu as the material for both working and auxiliary electrodes, with an integrated Cu/CuCl<sub>2</sub> reference electrode. Cu is not common material for electrochemical sensors since it is easily oxidized. Therefore, it is important to first demonstrate that the new sensor is able to perform electrochemical experiments properly.



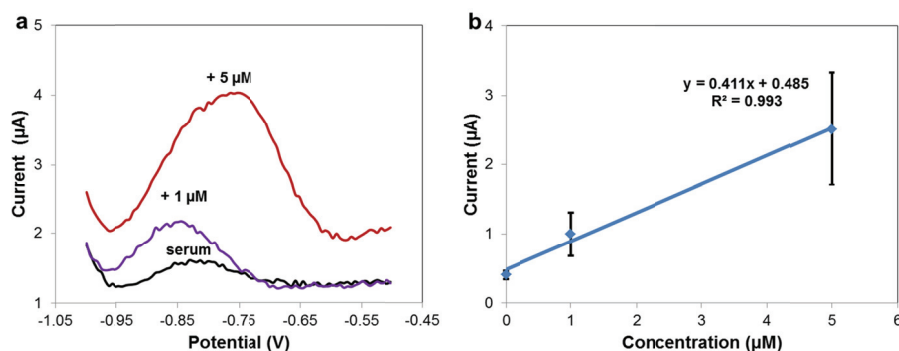
**Fig. 2.** (a) Cyclic voltammetry (CV) performed in acetate buffer (0.1 M, pH 6) and 10mM Zn. Scan rate 100 mV/s. (b) ASV of 10 $\mu$ M Zn in acetate buffer (0.1 M, pH 6) with increasing duration for pre-concentration. The potential of Zn peaks are  $808.9 \pm 8.5$  mV. Pre-concentration potential -1 V, amplitude 60 mV, period 50 ms, increment 6 mV. (c) CV in 0.1 M acetate buffer of different pH. Scan rate 100 mV/s.

To evaluate the new sensor, we performed two electrochemical experiments. Cyclic voltammetry (CV) (Fig. 2a) showed the negative range of potential window for Cu-based sensor was 0.5 V positive than that of our previous bismuth sensor [3], while the position of Zn stripping peak also shifted  $\sim 0.35$  V positively. In ASV (Fig. 2b), Zn stripping peaks were -0.8 V and the potentials of peaks did not shift with duration varying from 60 s to 600 s, which demonstrates the stability of reference electrode and Cu auxiliary electrode. Since the sensor is aimed to be disposable for serum detection and the 600 s is long enough for most stripping analysis, these experiments demonstrated that the sensor is sufficiently robust.

After demonstrating the stable function of Cu-based sensor, more CV were performed in acetate buffer of different pH to explore the electrochemical characteristics of the sensor, as shown in Fig. 2c. The negative range of potential window of the sensor expands as pH approaching neutral. Taking CV in acetate buffer of pH 6 for example, the potential window is from 0 V to -1 V, providing the capability to detect metals like Zn.



**Fig. 3.** (a) ASV of Zn in acetate buffer, pH 6, 0.1 M, 100  $\mu$ L. Pre-concentration potential -1 V, duration 300 s, amplitude 60 mV, period 50 ms, increment 6 mV. (b) Calibration curve for Zn in buffer.



**Fig. 4.** (a) ASV of Zn extraction from bovine serum, 200  $\mu\text{l}$ . Pre-concentration duration 600 s. Others was the same as in buffer. (b) Standard addition curve for Zn in serum.

Calibration curves were constructed by performing ASV in 0.1-5  $\mu\text{M}$  Zn of 100  $\mu\text{l}$  in 0.1 M acetate buffer, as shown in Fig. 3a, using optimized parameters. Pre-concentration was done at -1.0 V for 300 s. The waveform parameters of square wave were optimized to: 60 mV amplitude, 50 ms period, and 6 mV increment. The calibration curve, shown in Fig. 3b, indicates the sensitivity of Cu-based sensor for Zn in acetate buffer is 1.101  $\mu\text{A}/\mu\text{M}$ , with good linearity ( $R^2=0.993$ ). Seven measurements of 100 nM Zn were performed for calculating the LOD, which was 91 nM or 5.95 ppb. These results indicate that the linear range is sufficient to bracket the physiological range of 10-15 $\mu\text{M}$  Zn in serum with dilution factor of 3-100.

The target of this sensor is to detect Zn in serum. A critical challenge is that performance of the sensor in diluted serum is different from that in buffer. For example, 20  $\mu\text{M}$  additional Zn in bovine serum diluted by 20 $\times$  is not detectable in ASV with pre-concentration duration of 120 s, let alone to detect Zn in serum. Meanwhile, the majority of blood Zn is sequestered in proteins, therefore Zn have to be first extracted and then be detected by ASV which can only determines free metal ions. So Zn was extracted by dissolving Zn-dithizone complex into organic phase and releasing Zn back to aqueous phase by sulfuric acid. Then the pH was adjusted to 6 for ASV measurements. The total dilution factor was 12.5 $\times$ . Standard addition approach was used to determine Zn concentration in the original sample. ASV was performed in extracted sample and spiked with additional Zn of 1 and 5  $\mu\text{M}$ , shown in Fig. 4a, using the same parameters as in buffer except for doubled volume and pre-concentration duration. The standard addition curve, shown in Fig. 4b, indicates the sensitivity of Cu-based sensor for Zn in extracted serum was 0.411  $\mu\text{A}/\mu\text{M}$ . Based on the standard addition curve, the concentration of Zn in blood serum can be calculated as  $0.485/0.411 \times 12.5 \approx 14.8 \mu\text{M}$ , which is within the physiological range.

## CONCLUSIONS

The new Cu-based sensor shows improved LOD by one magnitude for Zn detection compared to our previous sensor, and successfully determined the concentration of zinc in blood serum. The copper-based sensors feature competitive performance in electrochemical detection, low-cost electrode material compatible with microfabrication and PCB processing, and simple fabrication with potential for mass production. These features make the sensor perfectly suitable for disposable, point-of-care applications.

## ACKNOWLEDGEMENTS

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