Lab-on-a-Chip Sensor with Evaporated Bismuth Film Electrode for Anodic Stripping Voltammetry of Zinc

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Abstract
In this work, we report on the development of a lab-on-a-chip electrochemical sensor that uses an evaporated bismuth electrode to detect zinc using square wave anodic stripping voltammetry. The microscale electrochemical cell consists of a bismuth working electrode, an integrated silver/silver chloride reference electrode, and a gold auxiliary electrode. The sensor exhibits a linear response in 0.1 M acetate buffer at pH 6 with zinc concentrations in the 1–30 μM range and a calculated detection limit of 60 nM. The sensor successfully detected zinc in a bovine serum extract and the results were confirmed by independent AAS measurements. Our results demonstrate the advantageous qualities of this lab-on-a-chip electrochemical sensor for clinical applications, which include small sample volume (μL scale), reduced cost, short response time and high accuracy at low concentrations of analyte.

Keywords: Anodic stripping voltammetry, Zinc, Lab-on-a-chip sensor, Dopamine, Evaporated Bi film electrode
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1 Introduction
Zinc (Zn) is an essential trace element that plays a critical role in human metabolic and immune systems [1–6]. Abnormally-low levels of Zn are often associated with inflammation and infection [7–9]. While Zn homeostasis can be easily restored through Zn supplementation [10–13], overdosing is a serious threat that can lead to copper deficiency and neurologic disease such as myelopathy or Alzheimer’s [6,14,15]. Thus, constant monitoring of Zn levels in patient’s blood becomes of critical importance for the supplementation strategy to work. Traditionally, such measurements are performed in blood serum with total Zn concentrations in the 65 to 95 μg/dL (9.9 to 14.5 μM) range [16], using conventional methods such as atomic absorption spectroscopy (AAS) [17,18] and inductively coupled plasma mass spectrometry (ICP-MS) [19,20]. Both of these methods provide accurate measurements in diluted serum or blood, but require bulky and expensive instruments and specialized personnel to operate them. Furthermore, shipping of samples to a centralized lab can present significant time delays of potentially time-sensitive information. Due to these challenges, conventional methods are not suitable for bed-side monitoring of Zn levels in blood for some patients in clinical settings, and thus, a low-cost instrument with rapid response is in great demand.

Compared with spectroscopic methods, anodic stripping voltammetry (ASV) rises as a promising alternative for measurements of trace metals such as zinc, lead and cadmium [21,22]. This technique is more time- and cost-effective while providing limits of detection (LOD) in the sub-nanomolar range. Previous work has been mainly based on mercury electrodes because of the stable performance and low detection limits [23–26], but handling and disposal issues stemming from mercury toxicity now impede widespread application.

Bismuth (Bi) has been gaining popularity in recent years as an alternative electrode material for electrochemical measurements. It forms low-temperature alloys with heavy metals to enable preconcentration and offers a wide negative potential window. Most importantly, it is much less toxic than mercury and is environmentally-friendly [27]. Wang et al.[28] and Guo et al.[29] compared performance of in situ deposited Bi films on carbon-based electrodes to mercury film electrodes (MFE) for ppb level Zn measurements in acetate buffer and found responses to be comparable. Kefala et al.[30,31] used in situ and ex situ deposited Bi with or without the presence of Nafion coating on glassy carbon electrode, and achieved a sub-ppb (nM) detection limit for Zn.

Still, detection of Zn in complex sample matrices such as serum or whole blood by ASV remains challenging. Direct measurement of Zn in serum is not possible be-
cause a large fraction of Zn is bound to protein and will not appear upon analysis. Thus, an extraction procedure is needed to reduce or even remove the organic components and release Zn into the aqueous phase [32,33]. Wei et al. [34] developed an improved extraction procedure for stripping analysis that achieves 97% recovery of Zn. This approach enabled us to accurately detect concentrations of Zn in bovine serum and has the potential to be a viable technique for human serum or blood.

We developed a miniaturized lab-on-a-chip sensor with electrodeposited Bi thin film electrode for measuring Zn to overcome shortcomings of the existing Bi electrodes. Our sensor size is approximately 15 mm × 19 mm, requires only microliters of sample, and performs analysis in less than 15 min. These features make it ideal for point-of-care applications. The sensor consists of a gold auxiliary electrode (AE), a silver/silver chloride (Ag/AgCl) reference electrode (RE) and a bismuth working electrode (WE). The layout of electrode patterns was generally akin to our earlier work [35] with adjustment of the connection pads. Meanwhile we developed a user-friendly interface that integrated an edge board connector and a mini-USB port to provide simplified connection and accessibility [36]. In this work, we focused on measurement of Zn and achieved a 60 nM (3.9 ppb) (calculated by 3σ/slope) LOD in 0.1 M, pH 6 sodium acetate buffer. We also demonstrated successful measurement of approximately 4 μM Zn in extracted bovine serum using the standard addition method and confirmed the result by separate AAS measurements.

2 Experimental

2.1 Reagents

Reagents were purchased from commercial vendors. Piranha solution was prepared from 7 H2SO4:3 H2O2 (volume ratio) to clean glass substrates. Two types of wet etchants were used to obtain electrode patterns: gold etchant was made from HNO3 :2 HF:7 DI water (volume ratio) to clean glass substrates. Two types of wet etchants were used to obtain electrode patterns: gold etchant was made from HNO3 :2 HF:7 DI water (volume ratio) to clean glass substrates. Two types of wet etchants were used to obtain electrode patterns: gold etchant was made from HNO3 :2 HF:7 DI water (volume ratio). The salts – I2, KI (w/w/v); titanium etchant was made from H2O2:2 HCl:1 DI water (volume ratio). The salts – I2, KI – and acids – HNO3 and HF – were purchased from Fisher Scientific. Bismuth target (Bi pieces, 1–12 mm, 99.999% trace metal basis) was purchased from Sigma Aldrich. We purchased silver plating solution (Silver Cyless II 10.00 GM per 1.00 GL) from Technic Inc., and zinc stock solution (Zinc Standard for AAS, 1000 mg/L in 2% nitric acid, Fluka) from Sigma Aldrich. A 1 M KCl solution was prepared from salt (Fisher Scientific) and used to chloridize the Ag reference electrode into Ag/AgCl. Acetate buffer (pH 4.65, 0.1 M) was purchased from Fluka and adjusted to pH 6 with NaOH and acetic acid (Fisher Scientific). Hyclone Characterized Fetal Bovine Serum from Thermo Scientific was used for the serum extraction procedures.

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2.2 Serum Extraction

First, 10 mL (5 mM) dithizone in chloroform was deprotonated by mixing with 10 mL (pH 9) 1 M ammonium/0.5 M ammonium buffer solution. Then the deprotonated form of dithizone was mixed with the solution containing serum and 0.5 mL of 0.05 M potassium thiocyanate in ethanol, followed by sonication for a period of 5 min. Afterward, the solution was transferred into a 50 mL plastic tube and centrifuged for 10 min at a rate of 4000 rpm to separate the two phases. The organic phase was collected and sonicated with 10 mL of 1 M sulfuric acid for another 5 min. After extraction procedures, we adjusted the pH of the sample to 6 using NaOH and diluted the sample with pH 6, 0.1 M acetate buffer by a factor of 2.

2.3 Device Design and Fabrication

Steps for fabrication of the electrochemical sensor varied, depending on the type of Bi film used for WE. The electrodeposited Bi WE process included a single evaporation step to deposit gold metal layer, followed by a single photolithography patterning step to define electrode shape, second photolithography step to define sacrificial mask, a second e-beam evaporation step to deposit a thin film of bismuth, and a lift-off process to pattern the Bi WE (Figure 1b).

Glass slides (3 in squares, 0.028 in thick, S. I. Howard Glass Co., Inc.) were cleaned using piranha solution, and coated by layer of 20 nm titanium and 200 nm gold using e-beam evaporation. Gold electrode patterns were created using photolithography (Shipley 1818 resist), followed by wet etching. For the evaporated Bi film electrodes only, a second photolithography step was used to create a thick sacrificial mask of photoresist, followed by a second evaporation step to deposit 200 nm layer of bismuth. This bismuth film was patterned using a lift-off process to achieve clean bismuth working electrodes, as shown in Figure 1b. To fabricate the polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) well, we used standard soft lithography and plasma bonding techniques (plasma surface treater, BD-20AC, Electro-Technic Products Inc.). All electrodeposition steps were performed after PDMS wells were bonded to glass substrates and cured. To form Ag/AgCl reference electrode [32], chronopotentiometry was first used to electrodeposited a thin layer of silver by the reduction of silver ion using 3 mA/cm2 current density for 60 s. This was followed by a second step where silver was oxidized in the presence of chloride ion for 30 s, thus depositing silver chloride on half of the electrode. Electrodeposition of the Bi film was carried out using chronoamperometry at ~0.8 V for 240 s following by rinsing with DI water for 60 s. Figure 1c shows a close-up
2.4 Procedure

A miniature USB Potentiostat (WaveNow, Pine Instruments, Inc.) was connected to a computer and also to an interface connector with an electrochemical cell inserted. Figure 1d demonstrates the PCB-based edge board connector interface with a sensor inserted, where the interface was connected to the potentiostat via a mini USB cable. We used 100 μL as the sample volume for all the experiments in acetate buffer. With cyclic voltammetry (CV) in pH 4.65, 0.1 M acetate buffer, the potential window of an evaporated bismuth WE was confirmed to be similar to that of an electroplated bismuth electrode. Based on previous optimization of ASV conditions in pH 6.0 M acetate buffer [36], we used the following parameters: −1.6 V as preconcentration potential with 600 s duration, stripping range from −1.6 V to −0.6 V, and waveform parameters of 70 ms for period, 4 mV for increment and 25 mV for amplitude. Zn concentrations from 1 μM to 30 μM in acetate buffer were used to construct the calibration curve.

For the detection of unknown Zn level in extracted bovine serum, we increased the sample volume to 200 μL and adjusted stripping parameters based on further optimization in serum extract to be −1.4 V for preconcentration potential, 900 s for duration, and 40 ms for period. Also, we introduced a surface preconditioning process before deposition to eliminate possible variations between each measurement, including four segments of CV scanning from −1.2 V to −0.4 V in the serum sample. We used the method of standard additions to determine the actual concentration of Zn in extracted serum.

3 Results and Discussion

3.1 Bismuth Film Electrodes

We carefully examined electrodeposition of Bi films to improve sensor performance in biological samples. In our earlier work, we electrodeposited the Bi WE at −0.8 V with agitation of the deposition solution for 240 s. The Bi film was approximately 160 nm thick with surface roughness of 230 ± 49 nm [35]. As demonstrated in Figures 2a and d, the rough Bi film suffered from severe crystallization which leads to poor coverage of most areas of the gold substrate; the film is thicker at the edge than the center of the circular electrode and has branches extending towards the RE, which causes huge variations in Zn accumulation on the WE during the deposition step because of surface area variability. When this Bi film was tested for ASV of Zn in sodium acetate buffer, it exhibited high LOD, which was calculated to be 6 μM, and it could only provide measurements in serum samples spiked with 20–60 μM of Zn.

To reduce surface roughness of the Bi films and improve repeatability of the sensor, we optimized the fabrication procedures as reported by Pei et al. [36]. While it is a common practice to stir solution during electrodeposition to minimize mass transport limitations, we found that using quiescent solution [37] yielded bismuth films with smoother surfaces and cleaner edges, reducing the formation of long branches which might short circuit the closely placed WE and RE. We de-oxygenated the bis-
muth plating solution [38,39] to increase the film thickness to about 200 nm and achieve better film quality. As shown in Figures 2b and e, the improved electroplated film completely covers the Au surface, and the surface is smoother than those in a and d with less Bi crystals on the center. However, Bi still tended to aggregate along the rim, forming extra mesh-like membranes that extend into the gap between WE and RE. Thus the surface roughness was still not favorable, calculated as 132 ± 98 nm; the large standard deviation revealed huge differences in surface morphologies between the center and the thick and rough rim area. The variations of Bi surface still introduced large uncertainty into the voltammograms in acetate buffer, and the inability to directly measure Zn levels in serum was not solved by this strategy.

To further improve the film quality, we evaporated bismuth on gold, which was expected to yield films of the highest quality possible. Electrode patterns were formed by lift-off to guarantee a clean edge for the WE. As demonstrated in Figures 2c and f, evaporated Bi electrodes offer a significant improvement over the earlier electrodeposited Bi electrodes. The Au surface was evenly covered by about 300 nm of Bi film with hardly any defects and the surface roughness was greatly reduced to 40 ± 2 nm. Also the shape and location of the Bi film was precisely matched to the Au pattern. Consequently, the electrode performance improved significantly. The variation of performance in buffer was reduced and acceptable results could now be achieved in serum matrix.

To demonstrate the overall improvement of the evaporated Bi electrode, we compared the performances of the three types of Bi WE with ASV determinations of Zn. Figure 2g shows the Zn stripping peak current and reproducibility for electrodes fabricated using these different approaches. Compared to the results from the first generation of electroplated bismuth film in the left column, the improved bismuth electroplating procedures created finer and neater films, which caused a minor increase in the Zn peak current from 13 µA to 14.5 µA and a dramatic decrease in coefficient of variation from 42% to 8% as shown in the middle column. After we used e-beam evaporation to deposit the Bi film, the peak amplitude of the signal current dropped from 14.5 µA to ~12 µA due to the large decrease in film roughness which is related to the electrode surface area, while the variation was minimized to less than 2% as shown in the right column.

3.2 Calibration of Evaporated Bismuth Electrodes in pH 6 Acetate Buffer

We first used cyclic voltammetry (CV) to confirm the potential window of our Bi electrode. Voltammograms in pH 4.65 acetate buffer showed a flat region from −0.3 V to −1.4 V, which agrees with our previous results [36] as well as others [28]. Next, to construct a calibration plot, square wave ASV (SWASV) of zinc was performed in the concentration range of 1–30 µM in acetate buffer. We used SWASV parameters listed in the experimental section which are the same for electroplated Bi electrode. For most concentrations, we repeated experiments three times (n = 3) using a new disposable device each time to obtain standard deviation. For 1 µM, which was the lowest Zn concentration sample, an n = 5 was used to calculate the detection limit based on 3σ/slope.
The Bi WE was successfully calibrated in 0.1 M pH 6 acetate buffer, and the voltammograms were compared with electroplated Bi WE. The voltammograms in Figure 3a show the Zn stripping peaks at approximately −1.25 V for higher concentrations, but shifting positively with decreasing Zn levels. One possible reason for this is that oxidation of the first monolayer of Zn that is deposited directly on Bi requires larger energy than stripping of subsequent layers of Zn that have deposited on Zn. However, the differences are not sufficient to cause double stripping peaks. Consequently, Zn stripping peaks in the 10−30 μM range exhibit shoulders on the positive side which broaden the peaks. As Zn levels decrease, Zn atoms stripping from Bi surface become the primary source, thus the peaks gradually migrated to nearly −1.1 V [40–42]. Also, the Zn stripping peaks are ~100 mV more positive than those on electroplated Bi electrodes [32]. This shift might be attributed to the differences in fabrication of the reference electrodes. In one case, the Ag/AgCl RE electroplating was the final fabrication step. In another case, the Ag/AgCl RE was plated one step prior to the electroplating of Bi. The inevitable variations involved in the soaking and rinsing steps could potentially shift the half-cell potential of the reference electrode by altering the composition of the Ag/AgCl layer to a certain extent, resulting in shifted Zn peaks.

A calibration plot was constructed based on Zn peak heights versus concentration, and then detection limit was calculated. The curve in Figure 3b shows a favorable linear relationship between peak height of stripping current and Zn concentration; the correlation equation was $I(\mu A) = 0.5782 \times [\text{Zn} (\mu M)] - 0.4192$ ($R^2 = 0.99$ for $n = 7$). The LOD was calculated to be 60 nM, exhibiting a significant improvement over the electroplated Bi WE [32] despite the decrease in sensitivity from 1.48 μA/μM to 0.58 μA/μM. This substantial improvement is due to the more precise process control of the evaporation technique, which allowed us to create smoother films with identical shapes on the structural layer, thus minimizing variation of surface roughness, actual surface areas and shapes of the Bi film. As a result, we achieved enhanced reproducibility and lower LOD.

### 3.3 Optimization of ASV Parameters in Extracted Serum

Further adjustments were necessary for application of the Bi WE in serum matrix since the behavior was quite different from acetate buffer. Initially we did several measurements in 100 μL of extracted serum diluted one time by acetate buffer, using the same parameters as optimized in acetate buffer. Bubbles formed on the working electrode surface during deposition at −1.6 V, which disrupted Zn deposition by blocking the solution from the electrode surface. The resulting voltammograms were not analytically useful, as Zn peaks were distorted and obscured by a large H⁺ reduction wave leading to poor reproducibility.

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To remedy this, we optimized the voltammetry parameters starting by examining a series of deposition potentials: −1.6 V, −1.5 V, −1.4 V and −1.3 V. For −1.6 V, the H⁺ reduction current was the highest and variability was the largest. While signal was almost the same for the rest of the tested potentials, depositions at −1.3 V and −1.5 V exhibited larger variability in Zn peaks. Thus, we chose −1.4 V deposition potential, which was sufficiently negative to efficiently deposit Zn, but not too negative to introduce large H⁺ reduction current. Figure 4a shows Zn stripping current amplitudes and variations for each potential, indicating that −1.3 V, −1.4 V and −1.5 V could all be considered, but −1.4 V was preferred for its stability.

Different preconcentration times were also examined, as illustrated in Figure 4b. The peak current for Zn stripping increased rapidly with deposition time for the first 600 s before plateauing at 900 s. Although 600 s deposition provided adequate signal current, we selected 900 s due to the improved stability and consistency in the voltammograms despite a negligible drop in Zn peak amplitude. The 900 s accumulation may seem long, especially compared with 60–120 s typically reported in literature. This is due to the limited agitation in the electrochemical cell, and thus poor mass transport of metal ions to the
electrode surface. While we are using a vibrating platform to agitate sample inside our small electrochemical cell, the resulting mass transport is not nearly as efficient as in bulk, stirred samples. In essence, our analysis is performed in a nearly-quiescent state, which limits mass transport and necessitates a longer preconcentration time.

In conjunction with deposition time, we examined sample volume used in our electrochemical cell. The results show that the stripping peak current is not proportional to sample volume (Figure 4c). While at first glance this may be surprising, the results in Figure 4c are consistent with the mass-transport limitations of our system. For the 50, 100, and 200 µL samples, the height of liquid in a 4.5 mm radius PDMS well is 0.79, 1.57, and 3.14 mm, respectively. Neglecting any convection and considering the sample as quiescent, we can calculate the diffusion length for Zn$^{2+}$ ions in each sample. The diffusion length $\delta_d$ is given as $\delta_d = (Dt)^{1/2}$, where $D$ is the diffusion coefficient of Zn$^{2+}$ ions in electrolyte, and $t$ is deposition time. From literature, diffusion coefficient can be estimated as $D = 10^{-9}$ m$^2$/s [43]. Using $t = 600$ s, the diffusion length can be approximated as $\delta_d = 0.77$ mm. Considering that the height of liquid layer is nearly the same as the diffusion length for the 50 µL sample, nearly all of the Zn$^{2+}$ ions must accumulate on the electrode surface. Increasing the height of the liquid layer (e.g., increasing sample volume to 100 µL and 200 µL) does not increase peak current since diffusion length cannot extend that far, so the additional sample volume is not accessible to the electrode. As a result, data in Figure 4c do not show a substantial change in signal from 50 µL to 200 µL sample volume. While in this work we used a 200 µL sample volume for all experiments, due to a minute increase in peak current, it remains possible to reduce the sample volume down to 50 µL in future applications without sacrificing signal strength.

We used a CV-based preconditioning process within the stripping range of Zn for Bi WE to achieve better reproducibility. Although we found evaporated Bi films to be thicker and to have less surface roughness than electroplated films, they occasionally produced abnormally low currents at relatively high concentrations of Zn, indicating that the Bi films exhibited some device to device variation. This variability may have been due to the intermetallic interaction between Bi and Zn, where small differences in the surface produce large variations in Zn deposition. We found that the CV pretreatment of the electrode alleviated this problem to some extent. Others [44–46] have also used this approach to achieve enhanced electron transfer, higher sensitivity, and more stable performance. Our adopted procedure consisted of scanning cyclic voltammetry from $-1.2$ V to $-0.4$ V at 100 mV/s sweep rate for four segments in serum sample before the preconcentration step. This step allowed the surface to equilibrate to the conditions that we used for our analysis, and the voltammograms then remained flat around the Zn stripping potential. After preconditioning, we observed more stabilized stripping curves and smaller variations among different runs of our experiments.

3.4 Zinc Detection in Extracted Serum

To demonstrate sensor operation with biological samples, unknown Zn concentrations were detected in bovine serum extracts using the method of standard additions on pretreated Bi WE (Figure 5). These Zn peaks broadened from 300 mV to approximately 500 mV, due to changes in sample composition such as the residual organic components, increased sample viscosity, and decreased ionic strength. An approximately 50 mV positive migration of Zn peaks was observed as concentrations decreased from 5 µM to 1 µM.
which is also the trend observed in acetate buffer. A similar trend was observed by us using electroplated Bi electrodes [32].

The Zn peaks in serum appear to shift from $-1250 \text{ mV}$ to a more positive potential of approximately $-750 \text{ mV}$, when compared with stripping in acetate buffer. This is shown in Figure 5c. The major reason for this shift is the several orders of magnitude difference in the chloride concentration between the two sample matrices. The chloride concentration in serum may vary, but generally remains in the 96–106 mM range [47]. The acetate buffer, on the other hand, has little chloride, primarily arising from the impurity of the sodium hydroxide salt used to adjust the pH (0.001% from Certificate of Actual Lot Analysis, or $4.7 \times 10^{-7} \text{ M}$ calculated based on the amount of sodium hydroxide added for the pH adjustment). Another possible source could be Cl$^-$ dissolved from the naked Ag/AgCl RE, but since the deposition time in acetate buffer is only 10 min, it is not possible for the dissolution of AgCl to reach equilibrium. Thus, the impurity in NaOH is the main source of chloride in the acetate buffer. Using the Nernst equation, the difference in RE potential between serum and buffer due to chloride ion concentration is calculated to be $\sim 300 \text{ mV}$, which is the majority of the observed positive shift in Figure 5c. This also suggests that it may be feasible to shift the Zn peaks in acetate buffer to the same potential as in serum extract by adding Cl$^-$ to the buffer. Manually shifting the voltammograms obtained in acetate buffer by 300 mV positively, as indicated in Figure 5c, we can observe the overlapping background currents of the two sample matrices.

The reason for the additional $\sim 150 \text{ mV}$ shift of Zn peaks remains unclear, but we can tell from the voltammograms that it is related to the changes of the RE surface in the serum extract. Further examination of the RE changes in different matrices and following sample preparation procedures is needed, since the electrodes are unprotected in the sample. Nevertheless, this uncertainty is rather stable because the voltammograms based on multiple repetitions of measurements at each data point are consistent. Furthermore, this shift does not actually affect our ability to detect Zn since we are measuring amplitude of the Zn peak current, and the Zn peak potentials do not exhibit direct correlations to the peak amplitudes.

Due to complexity of serum as biological sample, it is possible for other trace metals to interfere with anodic stripping of Zn. The metals that exhibit stripping peaks in the potential range similar to Zn include Mn, Cr, and Cd. Other metals present in serum, such as Cu or Pb, strip at more positive potentials and are not expected to interfere. The Cd and Mn peaks are expected to occur at $\sim 400 \text{ mV}$ more positive or $200 \text{ mV}$ more negative than Zn, respectively. These stripping peaks, however, are absent from the voltammograms. Cr is present in trace levels in serum, approximately 77–500 nM (4–27 ppb) [48]. These trace levels are at the LOD of our sensor, and thus any presence in the sample would lead to minute peaks that would not severely affect Zn stripping.

Thus, any interference by other trace metals can be neglected.

The method of standard addition was used to determine unknown Zn concentrations in serum samples. The resulting calibration curve in Figure 5b showed high linearity ($\sim 97 \%$) and sensitivity (0.13 $\mu$A/$\mu$M), indicating good sensing performance in serum. Zn concentration was determined to be 3.9 $\mu$M (260 ppb or 26 $\mu$g/dL) from the intercept of the plot in Figure 5b, which was in close agreement with an independent AAS measurement of 4.5 $\mu$M. Thus, the Bi WE exhibits the capability to mea-
sure Zn at much lower concentrations than the ~20 μM result reported previously by Kruusma et al. [49] using boron-doped diamond or by Kumar et al. [50] who used a hanging mercury drop electrode (HMDE) for measurements in the range of 49 μM–63 μM. ICP-MS techniques have been reported to detect Zn with a LOD of ~61 nM (4 ppb) in whole blood or serum by Barany et al. [20]. Although our miniaturized voltammetric sensors are currently unable to match the precision and limits of detection of modern spectroscopic and mass spectrometry techniques, the measurements that they are able to do are still in the physiologically relevant range, and they can potentially be used for rapid, inexpensive, point-of-care analyses.

4 Conclusions

In this work, we presented a microfabricated electrochemical cell for point-of-care measurements with an evaporated bismuth working electrode, a silver/silver chloride reference electrode and a gold auxiliary electrode. The analysis time required takes 10–15 min for each measurement, which is greatly reduced compared with AAS or ICP-MS. With improved film consistency and quality, the evaporated Bi WE provides favorable reproducibility and sensitivity for detection of zinc in 100 μL acetate buffer with calculated LOD of 60 nM (3.9 ppb). The sensor performance was also confirmed by AAS.

With the capability to detect Zn concentrations in microliter sample volumes, the sensor offers an alternative to the conventional methods and can be applied in situations where sample volume is limited. The sensor is 15 mm × 19 mm in size and offers a convenient connection to a portable potentiostat, which is suitable for various on-site monitoring applications. The expense for sensor fabrication increased because of the extra photolithography and e-beam evaporation, but the cost per single sensor was still low as a result of mass production (20 sensors per batch). Also, the sensor should be applicable to other matrices such as human serum or whole blood for point-of-care measurements.

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