Title

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Permalink

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Journal

IEEE Sensors Journal, 22(4)

ISSN

1530-437X

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Publication Date

2022-02-15

DOI

10.1109/jsen.2021.3126732

Peer reviewed

Engineered Electroactive Solutions for Electrochemical Detection of Tuberculosis-Associated Volatile Organic Biomarkers

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Abstract

Rapid screening of tuberculosis by evaluation of associated volatile organic biomarkers in breath is a promising technology that is significantly faster and more convenient than traditional sputum culture tests. Methyl nicotinate (MN) and methyl p-anisate (MPA) have been isolated as potential biomarkers for mycobacterium tuberculosis and have been found in the breath of patients with active pulmonary tuberculosis. A novel approach to detection of these biomarkers in liquid droplets (e.g. from breath condensate) using inexpensive screen-printed electrodes is presented. Previous modelling studies suggest that these biomarkers complex with certain transition metals of particular valence state. This interaction can be exploited by mixing the biomarker sample into an electroactive solution (EAS) containing the functional metal ion and observing the change electrochemically. The study focuses on low biomarker concentrations, determined to be clinically

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relevant based on preliminary GC-MS studies of the levels found in patient breath. It was found that both the cyclic voltammogram and square wave voltammogram of copper(II) change significantly when as little as 0.1 mM MN is added to the solution, with analysis times of less than 2 min. Copper(II) exhibits three separate peaks during square wave voltammetry. The location and area of each peak are affected differently as the concentration of MN increases, suggesting a reaction with specific oxidation states of the metal. In this way, a "fingerprint" method can be used to identify biomarkers once their known interaction is established.

Keywords

Breath Biomarkers; Electrochemical Sensors; Point-of-care Diagnostics; Translational Medicine

I. Introduction

The World Health Organization (WHO) has cited tuberculosis (TB) as a global health emergency. This deadly but treatable disease affects nearly one-third of the world's population [1]. Unfortunately, the vast majority of these cases occur in developing countries where medical care is not easily accessible geographically or economically. A great need exists for inexpensive, point-of-care (POC) diagnostic testing. Developing a rapid non-sputum biomarker-based test has been given high priority by the WHO, as sputum testing causes a variety of problems in very young and otherwise ill patients. In the report of a consensus meeting, they summarized the target product profile for this type of diagnostic test. They described the "optimal" test as one which could achieve similar sensitivity and specificity as the Gene Xpert assay, generates a result in less than 20 minutes, is disposable with minimal maintenance and costs less than 4 USD per unit test for reagents and consumables [2].

Screening of disease by means of volatile organic biomarkers (VOBs) in breath has been well documented for a variety of diseases both pulmonary and non-pulmonary [3]–[6]. Methyl nicotinate (MN) and methyl p-anisate (MPA) have been identified as potential biomarkers in patient breath for detecting TB using gas chromatography coupled with mass spectrometry (GC-MS) [7], [8]. Unlike other known biomarkers of TB, such as methyl phenylacetate and 2-methoxybiphenyl, MN and MPA are not present in cultures of similar mycobacteria, and are not expected to be readily found in the breath of healthy individuals. Identification by these biomarkers in the breath is a highly sought-after solution, as the sample collection is noninvasive, does not require a sterile environment (important for POC applications) and any patient regardless of age or health can produce a viable sample. Although useful in understanding the role of biomarkers in disease detection, GC-MS is often not viable in POC sensing of tuberculosis due to its size, cost, and need for highly trained technicians to operate.

Gas-phase electrochemical sensing of these biomarkers via amperometry has been proposed [9], however the use of ambient air requires the experiments to be run without a separate reference electrode, making these gas-based experiments difficult to reproduce and highly dependent on the working electrode, which can vary greatly between batches. One way to

mitigate this issue is to use a liquid electrolyte solution utilizing three distinct electrodes. Because these biomarkers are semi-volatiles, they are relatively soluble in water and straightforward aqueous electrochemistry becomes a viable option. This is much less expensive and requires simpler sample preparation than if organic electrochemistry was required. These types of water-soluble volatiles have been seen in up to millimolar concentrations in breath condensate [10], which is simple to collect and store. According to Davis et al, as little as 10 minutes of normal breathing can yield up to 2 mL of sample, more than is necessary for standard electrochemical techniques.

Traditional electrochemical detection requires direct oxidation at potentials within a relatively small range (±2V). The number of VOBs able to be detected in this way is therefore very limited due to their tendency to be chemically stable at these potentials. A common solution to this problem is the use of high-temperature metal oxide sensors (MOS) operating at high potentials, which have been used to study VOB patterns in a variety of fields. Although the exact mechanisms differ between device configurations, they are believed to work by measuring changes in conductivity induced by either a reaction with oxygen ions or by competitive adsorption/ replacement of those ions at the MOS surface [11]. Unmodified MOS's therefore cannot directly detect individual biomarkers, and in clinical testing have not shown sufficient accuracy (sensitivity and specificity) to be relevant in differentiating between TB-negative and positive patients [12]. Similarly, chemiresistive sensors based on conductive nanoparticles have also been used to distinguish between the VOB breath profiles of healthy individuals and patients for diseases such as lung cancer [13] and TB [14]. Arrays comprised of MOS's and other chemiresistive sensors (often known as an electronic nose or e-nose) have been trained and tested on the breath of patients afflicted with many pulmonary diseases, including TB. Reviews of these studies [15], [16] have concluded that although this promising technology is convenient and inexpensive, much work is left to be done to improve both sensitivity and specificity of the methods. Because these types of sensors do not directly measure disease-specific biomarker, but rather use pattern recognition to match similar chemiresistive profiles, they require robust, highly specific training sets and near perfect gold standard testing, both of which are difficult to obtain in regions where TB is most prevalent. This pattern recognition technique is also highly susceptible to confounders, and metal-oxide based sensors have been found to be especially sensitive to humidity changes [15].

Modelling studies performed by Ray et al. [17] suggest that TB biomarkers will interact selectively with transition metals in particular oxidation states. These interactions can be used by studying the change in electrochemical activity of a given metal ion when the biomarker is present. This gives an advantage over methods in which the direct oxidation of the biomarkers is measured [18] for two important reasons: first, it does not require biomarkers to be electrochemically active, making it more universally applicable; second, in a complex sample, other components with similar oxidation voltages will be indistinguishable. If these components are present in larger amounts, they can obstruct or even completely mask the signal of the biomarkers. Because early detection is so important, these biomarkers are expected to be present in very low amounts in suspected TB patients. Preliminary studies using GC-MS to determine MN levels in patient breath have estimated concentrations in the range of 0.1 to 10 mM in the corresponding condensate [19], much

lower than the range tested by Metters et. al. The use of a separate electroactive species such as copper, which has very well-defined electrochemical activity, allows for a much larger signal which will not be obscured in a complex breath condensate sample. This is because most aromatic organic compounds, especially those with benzyl and pyridine groups (such as MN and MPA) have oxidation potentials more than 1 V greater than those of low-valence transition metals [20]. It is hypothesized that operating in this lower potential range will minimize competing signals from electrochemically active VOBs in the breath. For these reasons an engineered electroactive solution (EAS) was designed to be added to aqueous biomarker solutions in order to study the change in electrochemical activity of the metal ion. The solution is simple, composed only of an inexpensive transition metal salt (copper chloride) and supporting electrolyte (sodium chloride) at neutral pH. This solution along with an inexpensive, commercially available carbon screen printed electrode (SPE) together make up the sensor kit, which at scale-up would cost less than 2 USD. Other copper-based sensors (specifically copper oxides) have become popular in a variety of gas-phase sensing applications in recent years [21]. In this work we look to broaden their application into liquid-phase sensing in order to provide better performance (specificity, operating conditions, etc) than typical metal oxide sensors.

In this work, cyclic voltammetry (CV) and square wave voltammetry (SWV) were used to study the basic behavior of the copper EAS as well as the change in activity when TB biomarkers were present. Cyclic voltammetry is a powerful technique which can be useful in studying a specific oxidation-reduction reaction of a reversibly oxidative species. However, it is limited in its ability to study multiple species because of diffusive activity during testing. Therefore, a pulsed technique (SWV) was also studied in order to reduce diffusion effects, increase signal and better separate peaks of separate oxidative species. The results of both studies are presented and discussed.

II. Materials and Methods

A. Potentiostat Hardware/Software

Liquid solutions were electrochemically analyzed using a hand-held potentiostat (PalmSens, EmStat) and the associated software provided (PalmSens, PS Trace 5.8.1704). Integration of peaks to determine the area under the curve (AUC) values was performed using the PS Trace software Integration tool.

B. Electrode Information

Screen-printed electrodes (SPEs) utilizing unmodified carbon-paste working and counter electrodes and a silver metal reference electrode were used in all measurements (DRP-110, 4mm diameter WE, Metrohm-DropSens). Electrodes from three distinct lots were used for various portions of the study, as noted in the Results section.

C. Sample Preparation

Aqueous solutions were prepared using deionized water (8MΩ type 1 ultrapure, PURELAB®Classic). Base electroactive solutions consisted of aqueous copper (II) chloride (Alfa Aesar, anhydrous, 98% min) as the active metal and sodium chloride (Fisher

Chemical, 99.0%) as a supporting electrolyte. The concentration ratio of active metal to supporting electrolyte was determined based on both theoretical and experimental work performed by Dickinson et al. [22], who observed the ideal ratio to be between 100 and 1000 to ensure proper diffusion for monovalent salts. A ratio of 1000 denotes "full" support, and a ratio as low as 100 shows no significant difference in the results of electrochemical experiments. Therefore, base electroactive solutions were prepared in the following concentrations:

- EAS 1 – 0.1 mM $Cu(II)Cl_2$, 100 mM NaCl, aqueous

- EAS 2 – 1.0 mM $Cu(II)Cl_2$, 100 mM NaCl, aqueous

To these electroactive solutions, varying amounts of analyte (methyl nicotinate, Acros organics 99%; methyl anisate panissaeuremethylester 99%) were added to prepare test solutions. All test solutions were freshly prepared/ diluted from more highly concentrated stock solutions within a day of testing. Expected clinically relevant biomarker concentration ranges were chosen based on preliminary GC-MS studies of active TB patient breath.

D. Electrochemical Analysis

A 150 μL droplet of sample was used for each test, and each SPE was used only once. Final electrochemical method parameters were chosen in order to obtain the best separation of redox peaks without a significant loss of signal as well as to minimize solvent background. Analysis time was also taken into consideration, as turnaround time is an important factor in point-of-care diagnostics.

1) Cyclic Voltammetry: Cyclic voltammograms of EAS 1 were obtained using the SPE described with the following parameters:

- Potential range: +0.5 V to −0.4 V.

- Scan rate: 50 mV/s

- Analysis Time: 1m 48s

2) Square Wave Voltammetry: Square wave voltammograms of EAS 2 were obtained using the SPE described with the following parameters:

- Potential range: −0.5 V to +0.4 V

- Amplitude: 25 mV

- Frequency: 2.0 Hz

- Step Size: 5 mV

- Analysis Time: 1m 35s

E. Sensor Pretreatment

Due to carbon ink binding factors present in most screen-printed electrodes which can cause sluggish electrode kinetics, it is recommended to pretreat the sensor for surface activation by SPE manufacturers [23]. This was hypothesized to be especially important when using

SWV (a single scan, non-equilibrium method). Cui et al. found that the simple, inexpensive procedure of applying anodic potentials in electrolytic solution was sufficient for surface activation [24]. According to their recommendations, the following pretreatment conditions were studied:

- Pretreatment 1: 1 M H_2SO_4, +1.6 V for 300 s

- Pretreatment 2: 2 M Na_2CO_3, +1.2 V for 300 s

During pretreatment, a 150 μL droplet was placed on a fresh SPE, then a constant anodic potential was applied for the specified time. After pretreatment, the SPE was rinsed thoroughly with DI water using a wash bottle for at least 20 s, then dried carefully by dabbing with a Kimwipe before testing. It was important to ensure the SPE was completely dry in order to maintain proper analyte concentrations. The performance of SWV using pretreated SPEs was then compared to those that were untreated.

III.   Results and Discussion

A.   Cyclic Voltammetry (CV)

A cyclic voltammogram of 0.1 mM Cu(II)Cl2 without any biomarker present (EAS 1) was obtained such that only a single oxidation-reduction reaction was seen in the potential range. By the third scan the system reached a reproducible CV, which was consistent over multiple SPEs (see Fig. 1a for examples). Both the oxidation and reduction peaks were sharp with a center potential of about +0.045 V vs silver reference (hereafter referred to as SR). Sample solutions containing methyl nicotinate (TB biomarker A) at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mM were then tested and compared to the base EAS 1 solution with no biomarker. Fig. 1b shows the CVs of EAS 1 (0.1 mM Cu(II)Cl2) with and without biomarker present. The addition of only 1 mM methyl nicotinate results in a visually marked difference in the shape of the CV, causing a change in the peak potentials and currents of both the oxidative and reductive peaks. It is observed that the oxidation and reduction peak potentials of the CV undergo shifts proportional to the analyte concentration (see Fig. 2). The presumed cause of these changes is an interaction of the analyte with a specific oxidation state of copper. Preliminary results indicate that when methyl p-anisate (TB biomarker B) was added in similar concentrations, little to no shift in peak potentials was observed.

B.   Square Wave Voltammetry (SWV)

Based on results found using cyclic voltammetry, it was hypothesized that the biomarkers of interest interacted differently with the various redox couples of copper. To test this hypothesis, a pulsed voltammetry method was used to increase signal and sharpen the various peaks, something that is difficult to do in a normal sweep method. This method is used to remove diffusion effects and to increase signal by combining signal from both the forward and reverse reactions of a redox couple. A suitable potential range was determined experimentally which resulted in a clear separation of all three redox couples expected from copper using EAS 2 (1 mM Cu(II)Cl$_2$). The SWV of EAS 2 using an untreated SPE can be seen in Fig. 3.

The use of a quasi-reference electrode (silver) precludes the direct assignment of redox peaks by known reduction potentials. However, the peaks can be expected to appear in a similar location and pattern to those found using a traditional silver/ silver chloride (Ag/AgCl) reference electrode. Based on known solutions (containing only Cu2+, Cl- and Na+ initially) and the applied potential range, likely oxidation states of copper in solution during the course of the experiment are Cu2+, Cu+ and Cu0. Reduction potentials of the reactions likely to occur in this potential range for these species (vs Ag/AgCl) as obtained from Bard et al. [25] are listed in Table I. The peaks in Fig. 3a are named in order from lowest peak potential to highest as follows: peak 1 –0.35 V, peak 2 –0.19 V, peak 3 –0.01 V (vs SR). It can be assumed that if only the three stable oxidation states of copper are present in the solution, the order of reaction peaks present can be assigned in the same order as the reactions listed in Table I, from lowest peak potential to highest. Therefore, likely redox reaction couples have been assigned to the peaks as follows:

- Peak 1: $Cu^{2+} \leftrightarrow Cu^+$

- Peak 2: $Cu^{2+} \leftrightarrow Cu^0$

- Peak 3: $Cu^+ \leftrightarrow Cu^0$

This is further supported by comparing the peak potentials observed with those expected using a standard reference electrode. When a stable reference electrode is used, the change in reduction potential should be fairly consistent among all electroactive species. The difference in reduction potential for peaks 1, 2 and 3 (vs SR) compared to the assigned redox couples (vs Ag/AgCl) are 0.29 V, 0.27 V and 0.27 V, respectively. This represents a consistent shift in reduction potentials, as expected.

Sample solutions containing methyl nicotinate (TB bio marker A) at various concentrations between 0.1 and 5 mM were then tested and compared to the base EAS 2 solution with no biomarker. A noticeable change in the voltammogram was observed at concentrations as low as 0.1 mM for methyl nicotinate, the estimated limit of detection for this configuration and Cu(II) concentration. The most significant difference lies in the size of peaks 2 and 3. As the concentration of biomarker A increases, the area of peak 2 decreases while the area of peak 3 increases proportionally in this range (Fig. 4). This suggests a difference in reactivity with different oxidation states of copper for biomarker A, and from this a better comprehension of the reaction mechanism can be obtained. The changes in peaks 2 and 3 suggest that biomarker A acts a reducing agent, reducing the Cu^{2+} present to Cu^+. The resulting Cu^+ is then further reduced to Cu^0 as higher potentials are applied. This accounts for both the decrease in peak 2 as well as the increase in peak 3. A simple proposed reaction mechanism based on this observation is as follows:

$$Cu^{2+} + MN \rightarrow Cu^+ + MN^+$$

$$Cu^+ + e^- = Cu^0$$
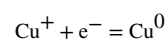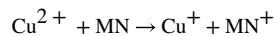
Further mechanism studies are needed to determine the exact interaction between copper and MN (TB biomarker A) and the final reaction products.

1) SPE Lot Variability: Over the course of the study three distinct test sets on different dates, each using different SPE lots, were performed. In the first set (Lot#1) single tests at each concentration were performed to establish proof-of-concept and test possible sensor pretreatments. In the next two sets (Lot#2 and Lot#3) the tests were run using a variety of biomarker concentrations, and all tests were performed on triplicate SPEs to verify reproducibility. A comparison of the SWV results between the different SPE lots (Fig. 5) show that although some variability can be seen, the results appear consistent and trends remain the same over different lots and over time.

2) Selectivity: The changes in peak area observed when biomarker A was added, as well as the observed shift in peak potential, can be used to determine a "fingerprint" specific to a given biomarker. The addition of these extra peaks obtained in SWV gives greater selectivity than the single peak obtained in the CV experiments. This increase in selectivity is crucially important in the complex breath matrices for which this sensing platform is designed in order to distinguish signal from low-level analytes. Once again biomarker B was used to test the effect of adding a particular biomarker concentration to the EAS. Fig. 6 compares both the peak area and potential for peaks 2 and 3 of the EAS 2 SWV when 1 mM of each biomarker is added. Very little change in peak 2 is observed with biomarker B, and the shift in potentials for both peaks was significantly larger for biomarker A than biomarker B.

3) Pretreatment/ Reproducibility of SPEs With SWV: The effect of SPE pretreatment on the resulting SWV using EAS 2 (1 mM Cu(II)Cl$_2$) is shown in Fig. 6a. Pretreatment using 1 M H$_2$SO$_4$ (pretreatment 1) yielded larger, more defined copper redox peaks than pretreatment using 2 M Na$_2$CO$_3$(pretreatment 2). However, the untreated SPE exhibited two out of three redox peaks that were larger and better defined than pretreatment 1. Pretreatment 1 for testing using EAS 1 (0.1 mM Cu(II)Cl$_2$) increased the overall signal magnitude, however peaks 1 and 2 became obscured in the background signal (see Fig. 7b).

It was then hypothesized that pretreatment may improve reproducibility of the SWV signal due to inconsistencies in the SPE surface. The effect of pretreatment 1 on the reproducibility of the SWV curves compared to untreated SPEs can be seen in Fig. 8. Fig. 8a shows the results of three new, untreated SPEs tested using EAS 2, and in Fig. 8b four different SPEs were first pretreated, then immediately run using the same solution and test parameters. It was concluded that pretreatment 1 did not make a significant difference in the reproducibility of the sensors under these conditions.

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IV. Conclusion

Both square wave voltammetry and cyclic voltammetry of copper-based electroactive solutions on inexpensive, single-use screen-printed electrodes showed a significant change when small amounts of methyl nicotinate (a biomarker of TB) were added. Analysis times for both CV and SWV experiments were less than 2 min each, exceeding target goals for rapid biomarker testing of TB. The resulting voltammograms were semi-quantitative for biomarker concentration, exhibiting a predictable change in peak area and/or shifted potential at concentrations as low as 0.1 mM.

The technique was shown to be selective for methyl nicotinate over methyl p-anisate, another unique TB biomarker. The CV experiments were highly reproducible between SPEs at reaction equilibrium, however the selectivity of this type of experiment is limited due to the use of only one redox reaction and the added issue of diffusion. The use of multiple redox peaks via SWV shows promise as a "fingerprint" method of identifying biomarkers, as it shows how a given biomarker interacts with each redox couple of copper in the range tested. In future work, the specific pattern observed in the area of each peak when MN was present can be transformed into a simple sensor read-out using a specifically coded application that calculates the change in peak areas and potentials from a blank EAS run on the same day. The output would then read "positive" if the changes fell within the expected range for MN, as well as give an expected concentration based on the signal magnitude and a base calibration.

The observation that MPA added at the same concentration as MN invokes a much smaller response in the EAS peaks suggests the specificity of the MN response compared to other similar aromatic compounds commonly found in breath. The response of the peaks to potential interferents common in human breath, and especially those that may be chemically similar to MN such as nicotine derivatives found in the breath of patients who smoke cigarettes, requires further investigation.

Based on the peak patterns observed when MN is present, a preliminary mechanism can be deduced for the interaction of the biomarker with copper(II). This allows for a more specific identification, making it a better candidate for use in a chemically complex environment such as breath condensate. SWV also showed a significant change to the EAS with methyl nicotinate concentrations as low as 0.1 mM, the current limit of detection. Recommended pretreatment/ activation of the SPE surface did not show obvious improvement of sensor signal or reproducibility.

The novel sensing platform is currently undergoing studies for improvement of detection limits and selectivity. These include studies using different transition metals, different electrolytes, the effect of pH, signal from possible confounding compounds, etc. Parallel studies examining the transfer of gaseous VOBs in the breath to aqueous solution are also ongoing and will further instruct necessary detection limits and possible confounders.

Acknowledgment

Biography

Christina N. Willis Dr. Christina Willis received a B.S. in Chemistry from Utah State University (2014) before pursuing both an M.S. (2016) and Ph.D. (2021) in Chemical Engineering at the University of Utah. During her time at the University of Utah, she worked under Dr. Mohanty in the Advanced Materials and Microdevices lab with a research focus on the development of point-of-care/use electrochemical sensors. After defending her dissertation in Oct. 2020, she accepted a new position at Northrop Grumman and currently works as a Principal Research Scientist in the Research and Development group.

Shaylee R. Larson Shaylee Larson is a senior chemical engineering undergraduate at the University of Utah. Shaylee has cultivated a great passion for scientific research with the Mohanty group where she develops electrochemical sensing techniques. She hopes to continue her research career and aspires to further her studies as a graduate student in the coming academic year.

Alfred Andama Dr. Alfred Andama is a Medical Laboratory Technologist and the Chief of Teaching Laboratories in the Department of Internal Medicine at Makerere University College of Health Sciences. He received a Diploma in Medical Laboratory Technology (2000), then a Bachelor of Science in Biomedical Laboratory Technology (2007) and thereafter a Master of Laboratory Science and Management (2012). His PhD was in Medical Microbiology focused on Tuberculosis (TB) Diagnostics (2021). All the above studies were completed at Makerere University.

Alfred joined Makerere University service in 2000 as a Technical Assistant, and has since grown through the ranks to become the Chief of Teaching Laboratories in the department of Internal Medicine. His research interests focus on evaluating TB diagnostics, either in their early or late stage of development, as well as testing completed diagnostic tools in clinical settings in order to gain real world experience.

Devan Jaganath Dr. Jaganath is an Assistant Professor of Pediatric Infectious Diseases at the University of California, San Francisco (UCSF). He completed his medical training at the University of California, Los Angeles (UCLA), Master's degree in public health and Pediatrics residency at Johns Hopkins University, and Infectious Diseases fellowship at UCSF. His research interests are in the development and evaluation non-sputum, point-of-care diagnostics for tuberculosis disease.

Manoranjan Misra Dr. Mano Misra is a professor in the Department of Chemical and Materials Engineering at the University of Nevada-Reno. He received a B.S degree in Chemistry from Utkal University, India (1970), an M.S in Metallurgical Engineering from

the South Dakota School of Mines in (1977) and a PhD in Metallurgical Engineering from the University of Utah (1981). Professor Misra has spent the over 30 years in materials related research with over 250 publications. His current research interests include applying advanced materials for medical diagnostics including tuberculosis, colorectal cancer and COVID-19.

Adithya Cattamanchi Dr. Cattamanchi is a Professor of Medicine and Epidemiology at the University of California San Francisco (UCSF). He received a BA in public policy studies from Duke University (1997), before pursuing a MD (2003) and a Masters in Clinical Research (2010) from UCSF. Dr. Cattamanchi also completed residency training in internal medicine and a fellowship in pulmonary and critical care medicine at UCSF. He joined the UCSF faculty as an Assistant Professor in 2010. His research interests focus on the development and evaluation of novel diagnostics for tuberculosis, and implementation of evidence-based care for tuberculosis in high burden settings.

Swomitra K. Mohanty Dr. Mohanty is an Associate Professor in Chemical Engineering and Materials Science Engineering at the University of Utah. Received a BA in biology from the University of Chicago (1997), before pursuing a MS in electrical and computer Engineering from Georgia Tech (2003), and a PhD in biomedical engineering from the University of Wisconsin-Madison (2008). Professor Mohanty also did his postdoctoral studies at UC-Berkeley in the Department of Mechanical Engineering (2008–2010).

From 2011–2014, he joined the University of Utah Department of Chemical Engineering as a Research Assistant Professor and in 2015 he accepted an offer as a tenure track faculty member in both the Department of Chemical Engineering and Materials Science Engineering. His research interests focus on synthesizing engineered nanomaterials for applications in point-of-use/care sensing methods, specifically targeting environmental contaminants, and diseases that disproportionately affect low resource settings such as tuberculosis and pneumonia.

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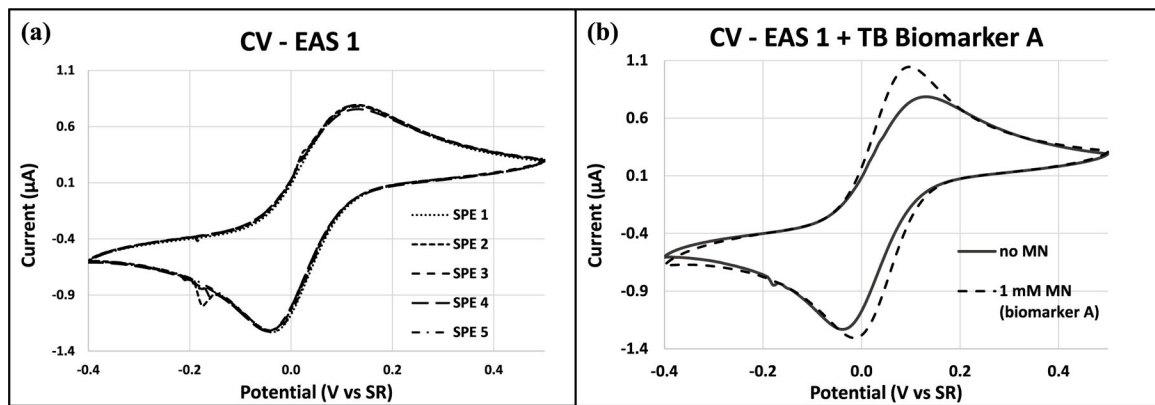
Fig. 1.

The third (equilibrium) cyclic voltammetry scans of (a) EAS 1 alone (containing copper(II)) using five different screen printed electrodes (SPEs) on different test days, and (b) EAS 1 with and without MN present (biomarker A). Little to no difference is observed in the CVs of the EAS alone over multiple SPEs, indicating a high level of reproducibility in the sensor kit/ test method. Both the peak current and potential visibly shift when MN is added at 1 mM. (SPE Lot#1).
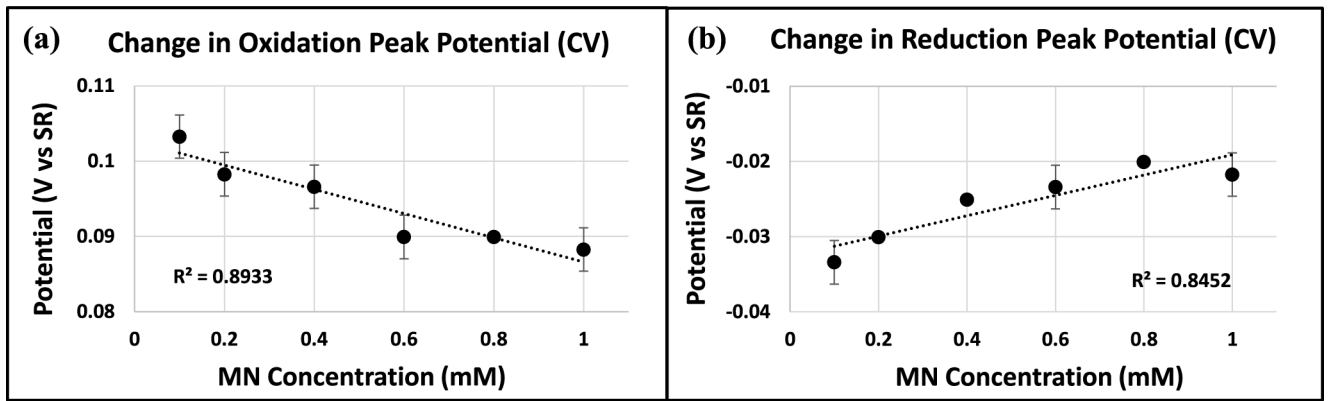
Fig. 2.

The change in (a) the potential and (b) the current magnitude of the oxidation peak of copper observed in the CV of EAS 1 with varying MN concentration (biomarker A). The change in both was relatively linear ($R^2 > 0.84$) between 0.1 and 1 mM MN for EAS 1. Error bars shown represent the standard deviation observed in triplicate measurements. (SPE Lot#2).
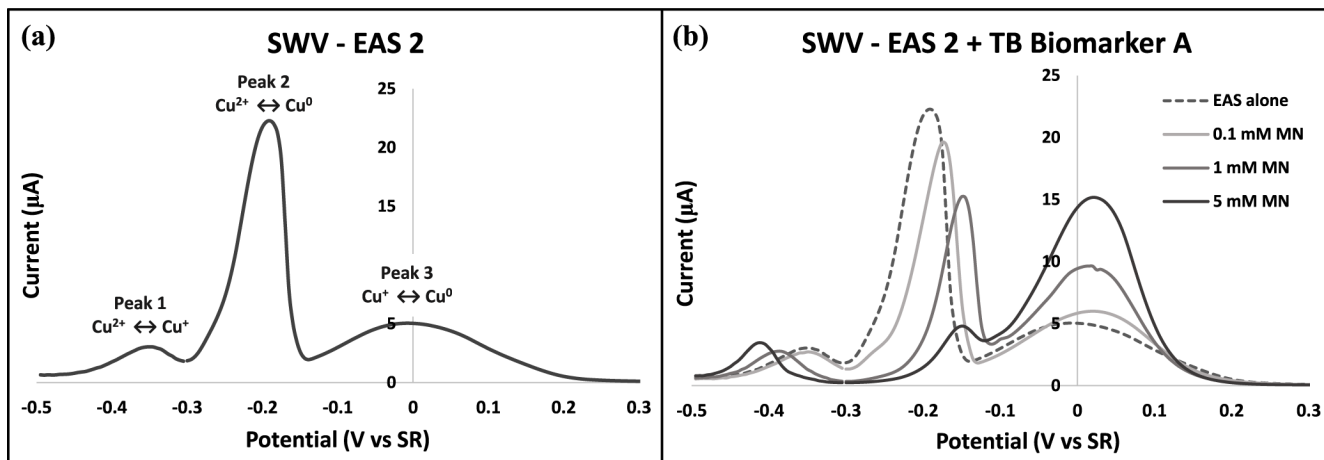
Fig. 3.

Examples of the square wave voltammograms of EAS 2 using (a) the EAS alone and (b) the EAS with MN (TB Biomarker A) added in various concentrations. Redox couple reactions were assigned to peaks based on their relative potentials; the peak areas of peaks 2 and 3 decrease and increase, respectively, as the biomarker concentration is increased. (SPE Lot#1).
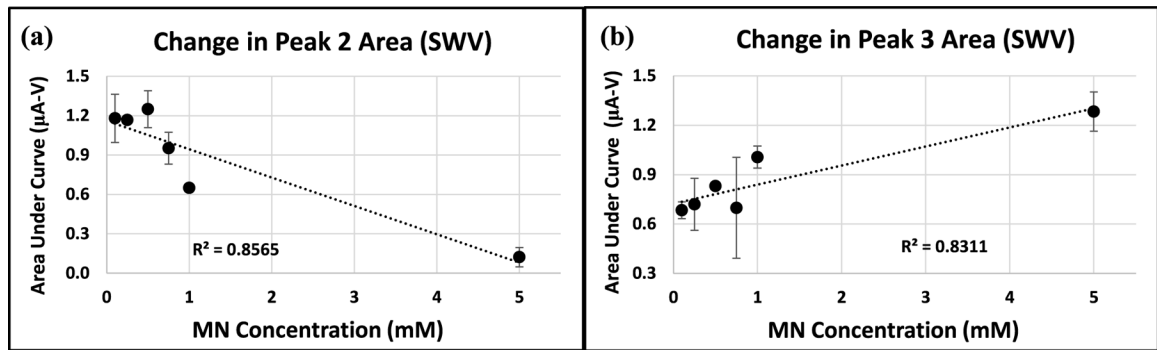
Fig. 4.

The change in the peak area for (a) Peak 2 and (b) Peak 3 observed in the SWV of EAS 2 with varying MN concentration (biomarker A). Error bars shown represent the standard deviation observed in triplicate measurements. (SPE Lot#3).
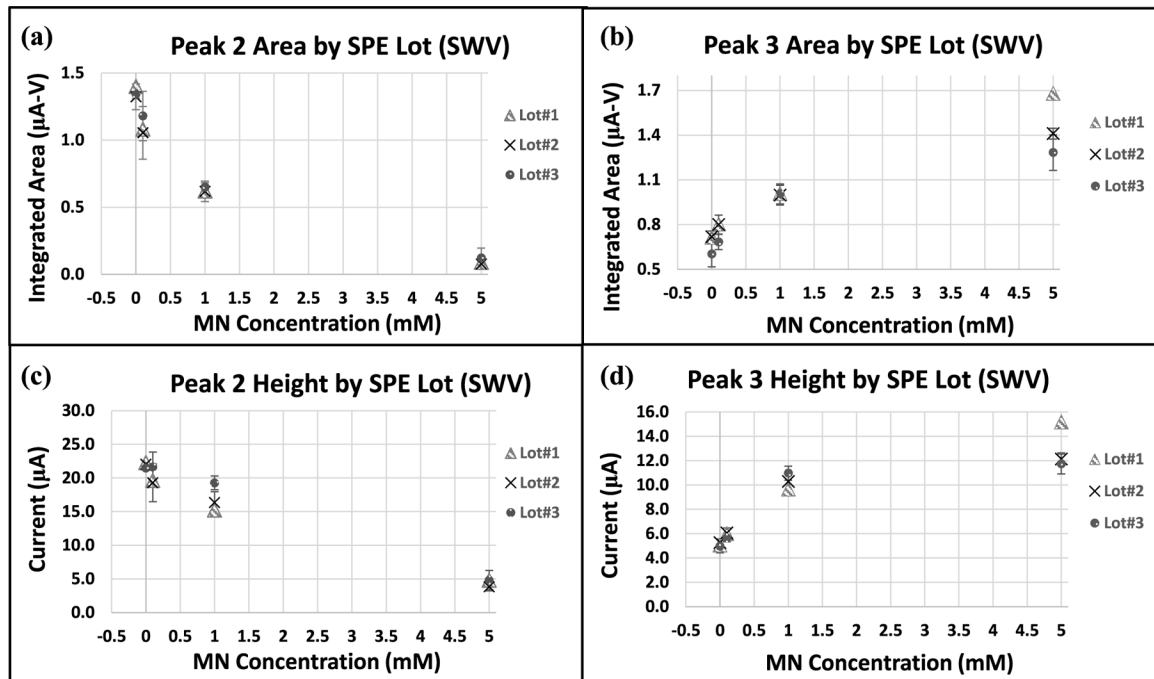
Fig. 5.
The effect of SPE Lot and time on SWV results. Results obtained using diff erent SPE Lot#s on different dates do not indicate significant variability, and trends based on biomarker concentration are consistent. Error bars show nrepresent the standard deviation observed in triplicate measurements.
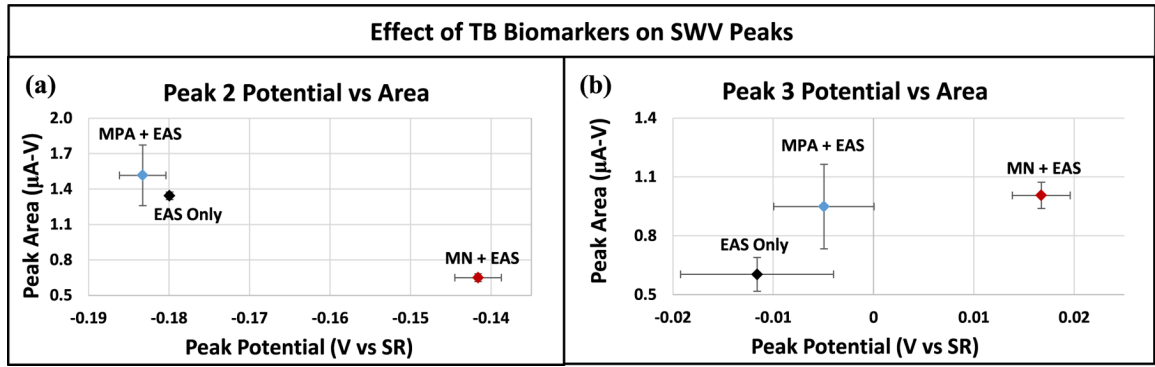
Fig. 6.
The effect of adding 1 mM of TB biomarkers to EAS 2 on the SWV Peaks. Little difference is observed in the potentials of peaks 2 and 3 when MPA (biomarker B) is added, compared to a significant change in both peak potentials and areas observed when MN (biomarker A) is added at the same concentration. Error bars shown represent the standard deviation observed in triplicate measurements. (SPE Lot#3).
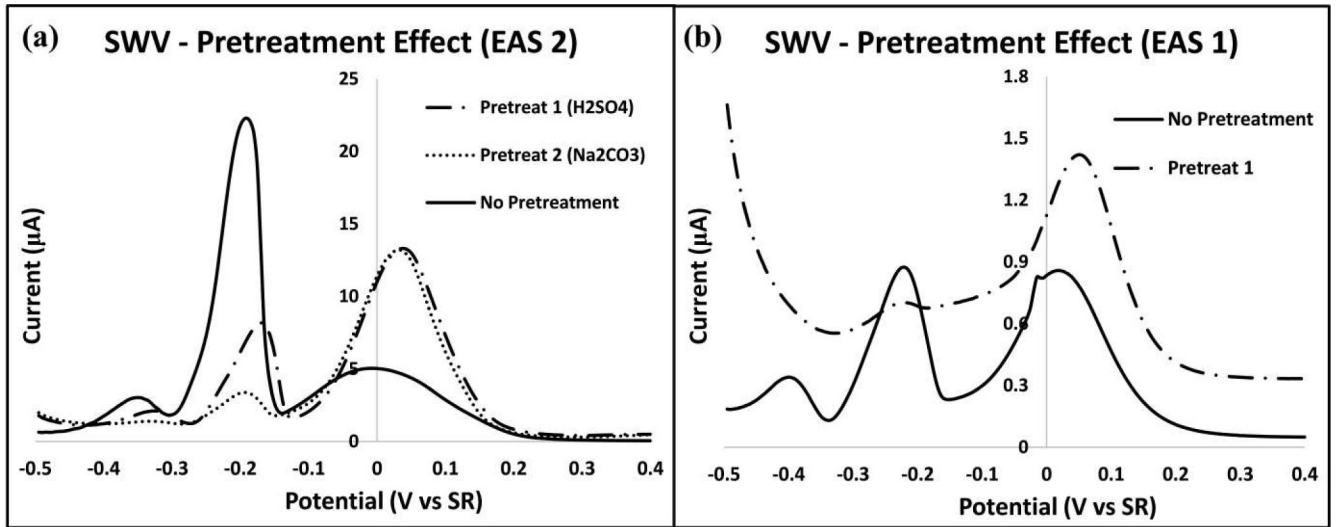
Fig. 7.

Comparison of the square wave voltammograms of (a) EAS 2 and (b) EAS 1 using pretreated and untreated SPEs. For EAS 1, both treated SPEs result in lower current magnitudes for two of the three redox peaks, including an undefined peak 1 for pretreatment 2. For EAS 2, pretreatment results in lack of resolution between peaks, making it unsuitable for fingerprint identification of biomarkers. (SPE Lot#1).
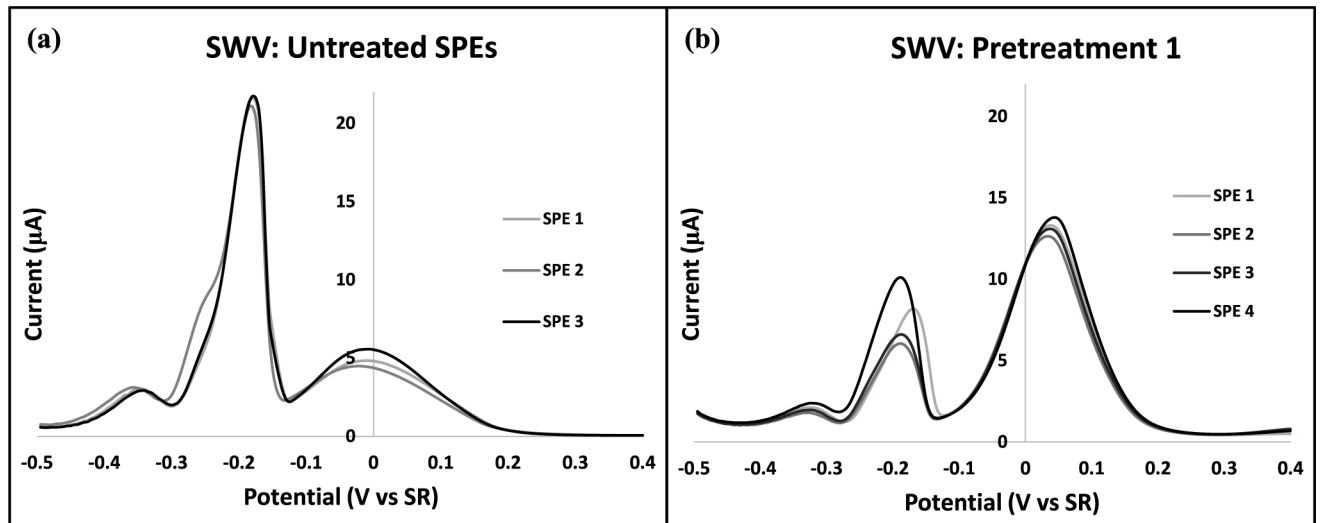
Fig. 8.

Comparison of square wave voltammograms of EAS 2 using (a) three new, untreated SPEs, and (b) four different SPEs pretreated using 1 M H_2SO_4. Under these conditions pretreatment 1 does not appear to improve reproducibility of the voltammograms. (SPE Lot#1).

TABLE I

Standard Electrode Potentials in Aqueous Solutions at 25°C

Reduction Reaction	Potential, V (vs SHE) [25]	Potential, V (vs Ag/AgCl)
$Cu^{2+} + e^- \rightarrow Cu^+$	0.159	−0.063
$Cu^{2+} + 2e^- \rightarrow Cu^0$	0.340	0.118
$Cu^+ + e^- \rightarrow Cu^0$	0.520	0.298
$AgCl + e^- \rightarrow Ag^0 + Cl^-$	0.222	0