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I am submitting herewith a dissertation written by Clarissa E. Tatum entitled "Novel Electrochemical Detections of Biologically and Environmentally Relevant Substances." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

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Novel Electrochemical Detections of Biologically and Environmentally Relevant Substances

A Dissertation Presented for the
Doctor of Philosophy Degree
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Clarissa Eve Tatum

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ABSTRACT

Development and studies of new electrochemical methods for the detection of various biologically and environmentally relevant substances are the focus of this dissertation. A dual amperometric sensor, capable of the simultaneous, real-time determination of NO and O₂, has been developed and optimized. Many tests were performed in order to reduce cross-talking between the two sensors, and an electro-deposited polymer, poly-5-amino-1-naphthol, was shown to reduce the cross-talking to insignificant levels. The use of bismuth-based electrodes in the detection of various metals has been explored. A bismuth bulk electrode has been developed, optimized, and used for the individual and simultaneous determination of Pb(II), Cd(II), and Zn(II). The fundamental electrochemistry of several bismuth-based electrodes in the system used for Cr(VI) analysis has also been explored, and many interactions among the electrode material, ligand, and analyte were observed, particularly the formation of a soluble bismuth-ligand complex. Electrochemical analysis of Cr(VI) was attempted at all of the bismuth-based electrodes, with success at the thin bismuth film electrode. A series of surface modifications were made to the glassy carbon substrate, in an attempt to remove any co-adsorbed contaminants and to understand the sensitivity of the chromium detection process. Inevitably, it was found that the contaminating source was contaminants in the nitrogen gas used for solution deaeration. Upon switching to argon, detection became highly reproducible and showed strong linearity with the Cr(VI) concentration.

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NOMENCLATURE AND ABBREVIATIONS

AA	atomic adsorption
AC	activated carbon
AOP	advanced oxidation process
A_p	peak area
ASV	anodic stripping voltammetry
BiBE	bismuth bulk electrode
BiCNT	bismuth modified carbon nanotube electrode
BiFE	bismuth film electrode
CAdSV	catalytic adsorptive stripping voltammetry
CE	counter electrode
CV	cyclic voltammetry
dia	diameter
DMSO	dimethyl sulfoxide
DPASV	differential pulse anodic stripping voltammetry
DTPA	diethylene triamine pentaacetic acid
E_{peak}	peak potential
EDRF	endothelium-derived relaxing factor
GC	glassy carbon
GCE	glassy carbon electrode
h	hours
HMDE	hanging mercury drop electrode

HOAc	acetic acid
Hz	hertz
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-OES	inductively coupled plasma-optical emission spectroscopy
i_p	peak current
LSV	linear sweep voltammetry
MES	morpholinoethanesulfonic acid
MFE	mercury film electrode
Min	minutes
mL	milliliter
mm	millimeter
mM	millimolar
MS	mass spectrometry
mV	millivolts
mV/s	millivolts per second
MΩ	megaohms
nA	nanoamp
nA/s	nanoamps per second
NaOAc	sodium acetate
nM	nanomolar
PBS	phosphate buffered saline
PIPES	piperazine-N,N-bis(2-ethanesulfonic acid)
ppm	parts per million

ppb	parts per billion
ppt	parts per trillion
PTFE	poly(tetrafluoroethylene)
RE	reference electrode
rpm	revolutions per minute
R_{CT}	resistance to charge transfer
R_{Ω}	solution resistance
s	seconds
SCE	saturated calomel electrode
SWCNT	single wall carbon nanotube
SEM	scanning electron microscopy
SWV	square wave voltammetry
SWASV	square wave anodic stripping voltammetry
t_p	pulse width
V	volts
WE	working electrode
ΔE_p	pulse amplitude
ΔE_s	step height
μA	microamp
μL	microliter
μm	micrometer
μM	micromolar
$^{\circ}C$	degrees celsius

5A1N

5-amino-1-naphthol

Part 1

Introduction and Background

1.1. Introduction

There is currently extensive research in chemical sensors.¹ Interests in their applications can be found in many disciplines, from environmental, to medical and biological, to industrial purposes. While their applications are vast, so are the modes of sensing. Sensors have been developed using electrochemical, spectroelectrochemical, optical, and mass-sensitive modes of operation, just to name a few.

The research presented in this work focuses on the electrochemical detection of various substances relevant to biological and environmental applications. All of the inorganic substances studied can have strict implications upon human health and quality of life; thus, being able to develop their respective levels within the body and in common environmental samples will allow for close monitoring of the levels. After gaining a better understanding of these substances' interactions in the human body, and assessing any positive or negative affects they may have on health, the quality of life can be greatly improved.

In the first section (Part 2), research related to the electrochemical sensing of both NO and O₂ in living tissues is discussed. Preliminary work^{2,3} in this area involved the development of single amperometric sensors for biologically relevant gaseous molecules, primarily NO. Earlier studies focused on the enhancement of sensitivity and selectivity for electrochemical NO detection, while later efforts focused on applying the optimized sensor to living tissues. This dissertation

outlines the development and optimization of a novel dual amperometric NO/O₂ sensor for the simultaneous, real-time detection in living tissues.

In the second, and larger, section, research related to the electrochemical analysis of metals in both biological and environmental matrices is discussed. In Part 3, development of individual and simultaneous determination of lead(II), cadmium(II), and zinc(II) by anodic stripping voltammetry at a bismuth bulk electrode is presented. Subsequent work has been focused on the properties of bismuth electrodes and their impact on electrochemical chromium detection, as discussed in Part 4. Finally, in Part 5, efforts to understand the sensitive nature of chromium detection by catalytic adsorptive stripping voltammetry (CAAdSV) are presented.^{4, 5}

1.2. *Analysis Techniques Used in the Research*

1.2.1. Electrochemical Techniques

A number of electrochemical techniques were used in this research: cyclic voltammetry (CV), linear sweep voltammetry (LSV), amperometric *i-t*, anodic stripping voltammetry (ASV), square wave voltammetry (SWV), catalytic adsorptive stripping voltammetry (CAAdSV), and AC impedance.

In cyclic voltammetry, a potential is linearly varied until it reaches a set potential, the switching potential. At this point, the scan is reversed until the potential returns to the starting value. This is done so that both the oxidation and reduction of the analyte take place within the potential range, and the resulting

faradaic current from the redox reaction is measured. Cyclic voltammetry is often used to provide qualitative information about various electrochemical processes.⁶

Linear sweep voltammetry is simply a CV, but with no return scan. That is, potential is linearly varied until it reaches a set potential, where the scan stops. The faradaic current from either an oxidation or a reduction of the analyte is recorded. Similar to CV, linear sweep voltammetry is used to obtain qualitative information about a redox system.⁷

When using amperometric *i-t*, the potential of the working electrode is held constant, while any current is recorded over a period of time. Current is plotted vs. time, so that a real-time measurement of electrochemical processes can be monitored.⁷

Square wave voltammetry is a type of pulse voltammetry that offers high sensitivity by modulating the potential. The current is sampled twice in each pulse, once in forward half cycle and once in the reverse (Figure 1.1). A series of these pulses is collected through a given potential range. The difference between the forward and reverse currents is plotted against potential to give the voltammogram. Because of the subtraction, background (or non-faradaic) currents are subtracted out, yielding a much higher current-to-background signal. Because of this, sensitivity is greatly enhanced over non-pulse voltammetric methods, and there is no effect of dissolved species that may interfere with the analytical signal.^{6,7}

Anodic stripping voltammetry is a commonly used technique for trace metal analysis.⁷ By holding the electrode at a sufficiently negative potential, the

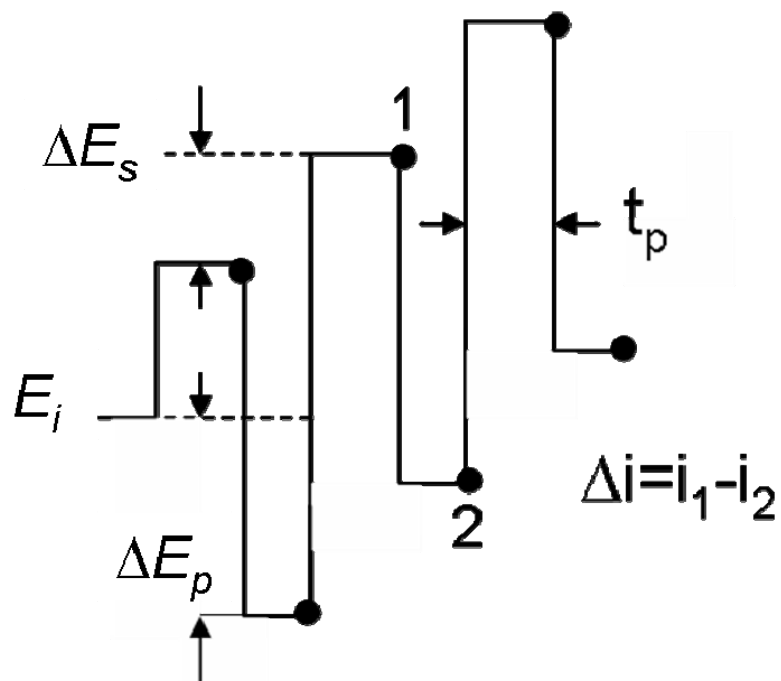


Figure 1.1. Common waveform for square wave voltammetry.⁸ ΔE_s is the step height (the potential increment from one cycle to the next), ΔE_p is the pulse amplitude, and t_p is the pulse width. Heavy dots indicate the time at which current is sampled.

analyte can be reduced to form an amalgam with the electrode, thus preconcentrating the metal onto the electrode surface. Following preconcentration, an anodic scan oxidizes the metals, stripping them off of the electrode and yielding a current response that is linear to the metal concentration.

Catalytic adsorptive stripping voltammetry, another type of stripping technique, utilizes a catalytic cycle to enhance sensitivity. A ligand-metal complex is accumulated at the electrode surface at a certain constant potential for a certain amount of time. Then, a voltammetric scan reduces the complex, stripping it off of the electrode surface, yielding a current response that is proportional to concentration. The presence of an oxidant causes the re-oxidation of the analyte, forming a catalytic cycle, enhancing the signal.⁷

A less commonly used electrochemical technique, AC impedance measurement, gives generalized resistance. An alternating current is used to perturb a system as a means of observing the system at steady state. In particular, valuable information about the electron transfer kinetics can be obtained.⁷

1.2.2. Inductively Coupled Plasma-Optical Emission Spectroscopy

ICP-OES is a common technique primarily used for elemental analysis. First, the molecules in the sample are converted to individual atoms and/or ions using a high temperature radio-frequency-induced Ar plasma. The sample is nebulized and converted to a fine spray, where the optical emission spectroscopy

is then used for analysis. As the atoms make the transition from excited state to a lower energy state, photons are emitted at a wavelength characteristic to each individual element. By observing the wavelengths, the elemental composition of the sample can be determined.⁶

1.2.3. Scanning Electron Microscopy

Scanning electron microscopy is a commonly used imaging technique that was employed to characterize the Bi films reported in this dissertation. A high-energy beam of electrons, produced from a cathode-ray tube, is scanned across a solid sample surface in a raster pattern. When the electron beam hits the sample surface, the electrons lose energy due to scattering and adsorption at the sample surface. Energy is exchanged between the electron beam and the sample surface, resulting in the reflection of high energy electrons, which are detected by specialized detectors. The resulting image is a map of the intensity of the signal being emitted from the sample.⁶ SEM is capable of providing magnifications of 10 to 100,000x and can resolve features down to the nanometer scale.⁹

1.3. *Catalytic Cycle for Electrochemical Cr Determination*

Catalytic adsorptive stripping voltammetry (CAdSV) is the technique used for all Cr(VI) analyses in this dissertation. Due to the enhanced sensitivity it can offer, CAdSV has been widely used for trace detection of numerous heavy metals using a variety of electrodes.¹⁰⁻¹² The mechanistic pathway for the

CAdSV analysis of Cr(VI) is a complex one. Extensive studies by Sander *et al.* and Xue *et al.* lead to a proposed mechanistic pathway for the electrode reaction of Cr(VI) when using diethylene triamine pentaacetic acid (DTPA) as ligand and nitrate (NO_3^-) as oxidant,^{13,14} as shown in Figure 1.2.

The cycle must begin with Cr(VI), as the complexation of Cr with DTPA requires freshly reduced Cr(III). Cr(VI) is accumulated at the electrode surface and electrochemically reduced to Cr(III) by applying a potential of -0.8 V. Freshly reduced Cr(III) then binds to the DTPA ligand at the electrode surface, forming a Cr(III)-DTPA complex. A potential scan from -0.8 to -1.4 V supplies sufficient potential to reduce the Cr(III)-DTPA complex to Cr(II)-DTPA around a potential of -1.1 V. The NO_3^- in the system then oxidizes Cr(II)-DTPA back to Cr(III)-DTPA, forming a catalytic cycle, which enhances the signal.

1.4. Summary of Dissertation Parts

1.4.1. Part Two

The development of an electrochemical dual gas sensor for the direct, real-time, and simultaneous detection of nitric oxide (NO) and oxygen gas (O_2) is reported in Part 2 of this dissertation. The novel sensor consists of dual Pt microelectrodes with planar sensing tips (Pt disk diameter is 25 μm , distance between disks is $\sim 60 \mu\text{m}$) as a working electrode, and a Ag wire reference electrode. To provide selectivity to the sensor, the options of applying a poly(tetrafluoroethylene) (PTFE) gas permeable membrane, or an electro-

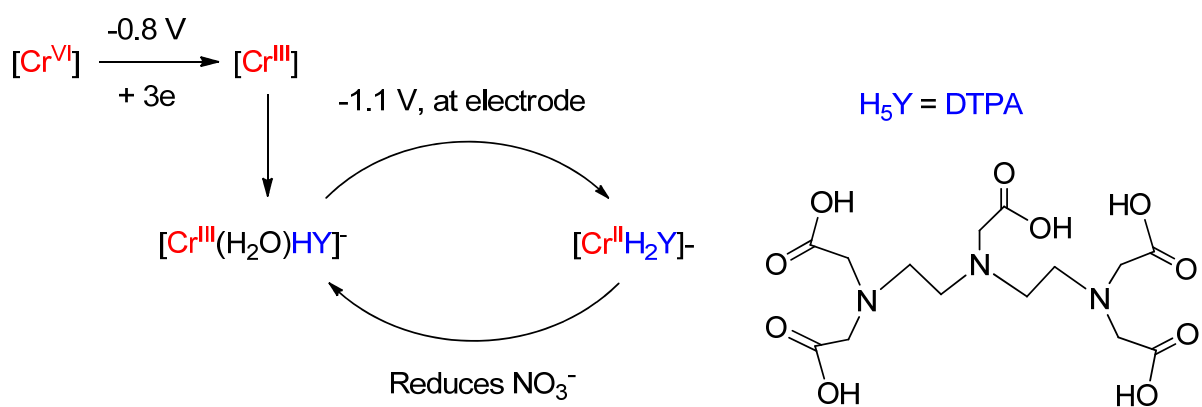


Figure 1.2. Proposed mechanistic pathway for Cr(VI) analysis.

deposited polymer (5-amino-1-naphthol) to the Pt disks were explored and compared. Sensor performance was further optimized by modifying the working electrode surface with a second metal, changing the internal solution composition and concentration, and varying the applied potential to the two working electrodes. Cross-talking between the two disks of the working electrode was also examined. While it was found that the internal solution with the highest pH provided a greater sensitivity ratio of NO sensor to O₂ sensor, the most optimal choice for reducing cross-talking is the electro-deposition of the 5-amino-1-naphthol polymer to the working electrode disks. This work, combined with continued work by a student in Youngmi Lee's research group at Ewha Womans University in Seoul, South Korea, has been published.¹⁵

1.4.2. Part Three

In Part 3, work is reported on the use of a bismuth bulk electrode (BiBE) as an alternative electrode for the anodic stripping voltammetric analysis of Pb(II), Cd(II), and Zn(II). The BiBE, which was fabricated in house, shows results comparable to those of similar analyses at other Bi-based electrodes. Metal accumulation was achieved by holding the electrode potential at -1.40 V (vs. Ag/AgCl) for 180 s followed by a square wave voltammetric stripping scan from -1.40 to -0.35 V. Calibration plots were obtained for all three metals, individually and simultaneously, in the 10-100 ppb range, with a detection limit of 105, 54, and 396 ppt for Pb(II), Cd(II), and Zn(II), respectively. A slight reduction in slope was observed for Cd(II) and Pb(II) when the three metals were calibrated

simultaneously vs. individually. Comparing the sensitivities of the metals when calibrated individually vs. in a mixture revealed that Zn(II) is not affected by stripping in a mixture. However, Pb(II) and Cd(II) experience decreased sensitivities in a mixture. The optimized method was successfully used to test contaminated river water by standard addition. The results demonstrated the ability of the BiBE as an alternative electrode material in heavy metal analysis. The results from this work have been published in an article.¹⁶

1.4.3. Part Four

Part 4 explores electrochemistry of chromium ions in aqueous solution on various bismuth-based electrodes. Among the electrodes employed were a bismuth film electrode (BiFE), bismuth bulk electrode (BiBE), and Bi-modified carbon nanotube electrode (BiCNT). This particular system includes diethylene triamine pentaacetic acid (DTPA) as the ligand, nitrate (NO_3^-) as the oxidant, and sodium acetate (NaOAc) as the buffer. Upon thorough examination, the bulk bismuth electrode was shown to be ineffective for ultra-trace Cr determination, while both of the bismuth-modified electrodes (BiFE and BiCNT) demonstrated success in chromium detection. The interactions between the BiBE and each of the chemicals in the system of interest were assessed using cyclic voltammetry (CV). These tests revealed the basic electrochemical behavior of the BiBE, and showed many interactions among the chemicals in this system. In particular, the formation of a soluble Bi(III)-DTPA complex was observed, showing that the Bi(III) favors the DTPA ligand over the formation of an oxide. At a BiFE, the

thickness of the film was varied, and a correlation between film thickness and chromium analysis was drawn. Impedance measurements were taken for the BiBE, BiFE, and BiCNT and are compared to the impedance of a glassy carbon electrode (GCE), in order to highlight the relative conductivity of each of the electrodes and how it relates to effective Cr analysis. Lastly, scanning electron microscopy images were taken in order to observe the morphology of deposited bismuth on a glassy carbon electrode, which composes the BiFE. These studies yield a better understanding of the fundamental processes that occur in the system for electrochemical Cr detection. Work from this part will be submitted for publication shortly.

1.4.4. Part Five

Electrochemical Cr(VI) analysis was investigated in-depth in Part 5 in order to understand factors that influence the analysis. Cr(VI) analysis was attempted at the BiBE and thick film BiFE with no success. The majority of the work focused on the thin film BiFE, as it had shown the most promise. A number of modifications to the procedure, which used DTPA as the ligand and NO_3^- as the oxidant, were made. Primarily, much study went into the pre-treatment of the GCE. After studying the effects and order of pre-treatment through polishing, ultra-sonication, and the use of piranha solution, other pre-treatments were explored. Different types of polishing on various substrates were studied. Electrochemical pre-treatments of the GCE surface were also investigated, as both anodic and cathodic activations of the GCE were examined. Finally,

modifications were made to the electrochemical protocol for Cr(VI) analysis. The use of two zwitterionic buffers was examined, and the complex accumulation time was altered to see if either modification contributed to an improvement in the Cr(III)-DTPA reduction signal. Inevitably, it was found that gas used for deaeration of the standards was introducing some contaminant(s), as of yet unidentified, onto the electrode surface, fouling it and preventing effective detection.

1.4.6. Part Six

A summary of all of the work in this dissertation is included in Part 6. In addition, concluding remarks about each project are stated, highlighting the important findings of each work. The relevance of the projects and each project's relation to the others are discussed. A central theme is given, unifying all parts of this dissertation.

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Part 2

Development and Optimization of an Amperometric Dual Gas Microsensor for the Simultaneous Detection of Nitric Oxide and Oxygen

2.1. Introduction

A growing amount of research within the medical industry has resulted in a recent increase in the need for improved sensors suitable for biological applications.^{1,2} Sensing systems that are capable of simultaneous measurement of two or more inter-related analytes are especially advantageous.

In particular, both nitric oxide (NO) and molecular oxygen (O₂) are being actively studied, as they both play roles in many significant biological functions. Not only do they both individually play important roles in the body, but also the activities and interactions of both compounds are closely linked and highly inter-related.

In 1987, NO was found to be the endothelium-derived relaxing factor (EDRF), thus confirming its vital role in vasodilation.^{3a,b} In addition to regulating blood pressure via vasodilation, NO has been found to serve as an important neurotransmitter in the brain and peripheral nervous system.⁴ Nitric oxide has also been found to increase filtration in the kidneys by increasing blood flow,^{5a} reduce ischemia/reperfusion injury for organ transplants,^{5b} inhibit platelet adhesion and aggregation,⁶ and act as a mediator in antimicrobial and antitumor activities.^{7,8}

Oxygen is essential for sustaining the lives of most living organisms. Not only is it essential to all respiratory processes, but it is also essential and intimately linked with many enzyme activities.

As NO plays a vital role in vasodilation/vasoconstriction, the body's blood flow, i.e., O₂ supply, is carefully regulated by NO. On the other hand, O₂ is required for the production of NO by NO synthase isozymes.⁹ Additionally, NO decays rapidly in a reaction with O₂.¹⁰ Clearly, the activities of both of these compounds are closely intertwined. By being able to measure both gases simultaneously, and in real time, a better understanding of each compound's individual activities can be obtained, in addition to fully understanding the interactions between the two compounds.

There are many challenges associated with the quantitative detection of NO and O₂ in living tissues. Concentrations of both compounds in biological samples tend to be very low, in the nM to μ M region, so the sensitivity of sensors must be greatly enhanced. Also, NO is a free radical, causing it to rapidly react, usually with O₂ or hemoglobin, immediately after its production.¹⁰ This makes direct detection of NO difficult.

Indeed, due to the short lifetime of NO, most methods for the detection of NO are indirect, and center around the use of spectroscopic methods for the detection of the oxidized product of NO (NO₂⁻ or NO₃⁻). The Greiss assay is an example of one such method, as it measures nitrite levels.¹⁰ More direct methods for the quantification of NO include electron paramagnetic resonance (EPR),^{10,11} mass spectrometry,¹⁰ and chemiluminescence.^{10,12}

In this study, a traditional Clark-type microelectrode¹³ is used for the simultaneous detection of NO and O₂. For the simultaneous quantification of two gases, electrochemical detection with microelectrodes offers many advantages.

Electrochemical methods allow for the direct detection of NO, not any of its reaction products. Since the sensor can be positioned in close proximity to the source of NO and the area of O₂ consumption, a dual microelectrode allows for the detection of both gases at the exact location of interest. A microelectrode produces much smaller currents than larger electrodes; thus, a microelectrode is practically non-destructive to the solution and species being analyzed.

Electrochemical detection methods are capable of detecting smaller concentrations than the above mentioned traditional assays, and it is possible to use much smaller sample volumes. Microelectrodes are capable of much more precise measurements, as well, because steady-state current is rapidly achieved, and there is a significant improvement on spatial resolution.

In this study, our Clark-type dual microelectrode consists of two platinum (Pt) disks (diameter = 25 µm) arranged in a planar sensing tip. The approximate distance between the two disks is around 330 µm. A schematic drawing of the sensor is given in Figure 2.1.

Because the concentration of NO in biological samples is so low (a few hundreds nM), the electrode surface had to be modified to improve detection of the gases. To enhance the performance of the sensor, each Pt disk was platinized. Platinization is a way of modifying the electrode surface by depositing a layer of Pt(s). This increases the surface area of the working electrode, allowing enlarged active sites per unit area. Previous work on electrochemical NO detection has shown that platinization provides the sensor with ~10-fold

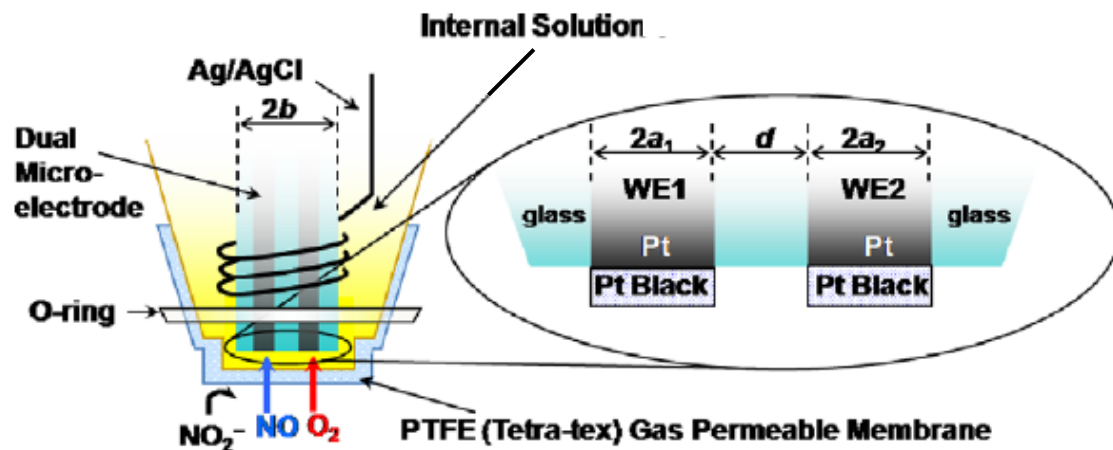


Figure 2.1. Schematic diagram of a dual NO/O₂ gas microsensor. Electrode dimension, $a_1 = a_2 = 12.5 \mu\text{m}$, $2b = \sim 500 \mu\text{m}$, $d = 330 \mu\text{m}$.

higher sensitivity, ~10-fold lower detection limit, and extended stability (at least 3-fold) compared to bare Pt sensors.¹⁴

At the potential applied for NO oxidation, many other species might also be oxidized electrochemically (ascorbic acid, NO_2^-). To obtain accurate analytical measurements of NO and O_2 , it is essential to block all interfering species from the electrode surface. In previous work, involving single electrode amperometric sensors,^{14,15} this was achieved by housing the platinized working electrode behind a microporous poly(tetrafluoroethylene) (PTFE) gas-permeable membrane. However, a significant amount of cross-talking between the NO and O_2 sensors was consistently observed in this work, leading us to explore alternative methods to improve the selectivity of the sensor. Work has recently been done using poly(5-amino-1-naphthol) as a permselective membrane.¹⁶ The monomer, 5-amino-1-naphthol, is electroactive, meaning it can be electrochemically deposited and polymerized onto working electrode surfaces. After the deposition of the polymer on the working electrode surface, cross-talking was greatly diminished, to an almost insignificant level.

2.2 Experimental

2.2.1. Chemicals and Materials

Pt wire (diameter = 25 μm) and Ag wire (diameter = 127 μm) were obtained from Good Fellow. Borosilicate glass theta capillaries (1.5 mm outer diameters) were products of World Precision Instrumentation Inc. Nitric oxide,

oxygen, and argon gases were obtained from Airgas Inc. Sodium chloride and phosphate buffered saline (PBS) were purchased from Fisher Scientific. The platinizing solution, 3% chloroplatinic acid in water, was obtained from LabChem. Hydrochloric acid, sodium nitrite, and dimethyl sulfoxide (DMSO) were purchased from Aldrich, Riedel-de-Haen, and Fisher Scientific, respectively. All solutions, prepared with reagent grade compounds, as obtained without further purification, were dissolved in ultrapure deionized water. Microporous PTFE (50% porosity, thickness $>18\text{ }\mu\text{m}$, pore size $0.07\text{ }\mu\text{m}$) was obtained from Donaldson Co. Inc. 5-amino-1-naphthol was obtained from Aldrich. The polymer solution was prepared with sodium perchlorate and acetonitrile, both obtained from Fisher Scientific. All electrochemical tests were performed on a CHI 900 Electrochemical Workstation (CHI Instruments, Inc.).

All standard NO and O₂ solutions were prepared by purging PBS solution (pH 7.4) with Ar gas for 30 min in order to remove atmospheric oxygen. The solutions were then purged with either NO or O₂ for additional 30 min, allowing for complete saturation. The detailed procedures have been reported.¹⁷⁻¹⁹

All mentioned calibrations were performed in a special two-armed vial, sealed at the ends with rubber septa (Figure 2.2), fabricated in the glass shop in the department. This was done because atmospheric oxygen interfered not only with our ability to obtain accurate values for O₂ calibration, but also with our ability to obtain accurate values for NO calibration, since produced NO reacts rapidly with free O₂.

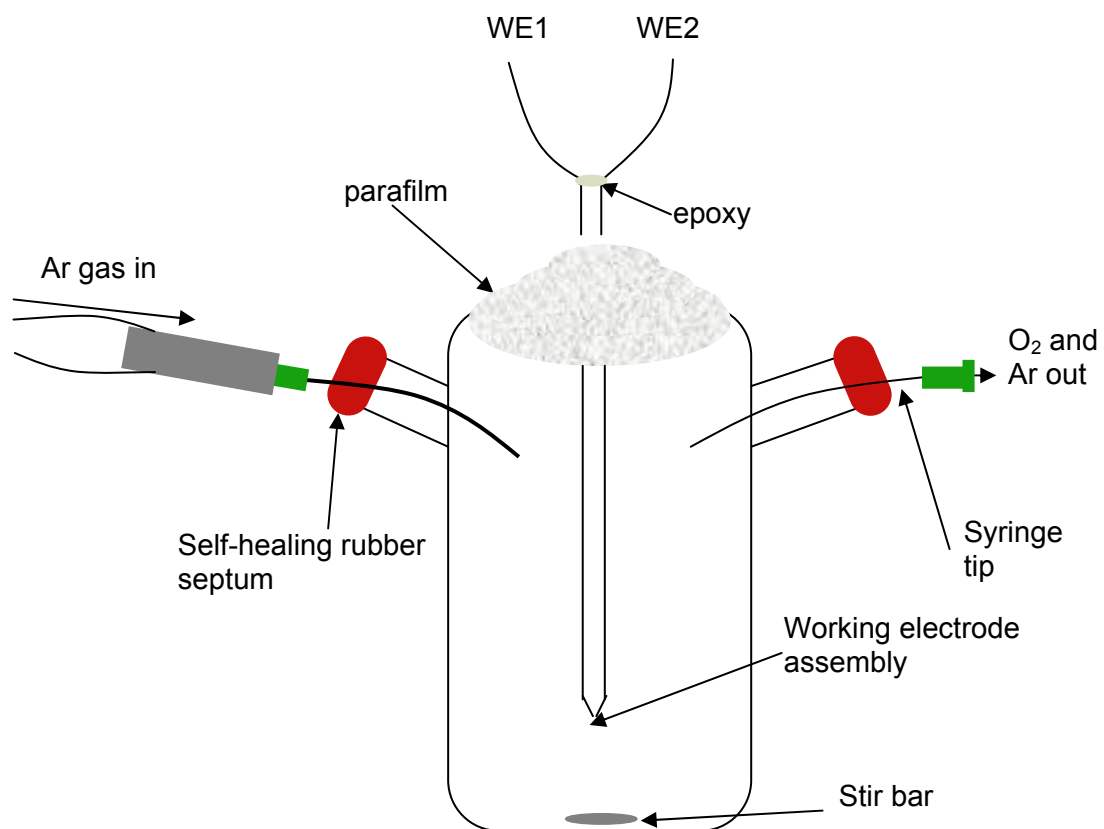


Figure 2.2. Schematic diagram of the electrode assembly encased in a double-armed vial, allowing for removal of atmospheric O₂ prior to testing.

2.2.2. Preparation of Dual NO/O₂ Sensors

Amperometric planar dual NO/O₂ sensors were prepared as depicted in Figure 2.1, using a method similar to the one described for NO/CO dual microsensors.¹⁷ The sensor was composed of a glass-sealed dual platinized Pt working electrode (WE) and a coiled Ag/AgCl wire reference/counter electrode. This ensemble was housed behind a PTFE gas permeable membrane, unless the 5-amino-1-naphthol was used. In these cases, the polymer was electrodeposited on the electrode Pt disks only.

The dual working electrode was prepared according to a published method.²⁰ A Pt wire was inserted into each of the two openings of the theta capillary, and the tapered ends of the capillary were thermally fused around the Pt wire under vacuum. The electrode was then carefully sanded and polished, using sand paper and 3-, 1-, and 0.05- μ m diamond paper successively. After creating a smooth electrode tip by polishing, both Pt disks were platinized. The Pt black layer was deposited onto each Pt disk electrochemically with the platinizing solution (3% chloroplatinic acid in water), using CV, with a sweep from 0.60 to -0.35 V (vs. Ag/AgCl) at a scan rate of 20 mV/s. Ag wire was wrapped around the outside of the capillary. The entire assembly was inserted into a pipet tip (volume 1-200 μ L) (Fisherbrand, Pittsburg, PA), filled with the internal solution of choice, covered at the end by PTFE membrane. The PTFE membrane was secured onto the tip by the placement of an O-ring around the end of the pipet. When the electrode was inserted, it was pushed against the membrane, as to cause a significant amount of stretching, allowing the dual WE to protrude ~0.5-

0.6 mm from the pipet tip. This detailed procedure has been described elsewhere.¹⁴

2.2.3. Testing for Optimal Internal Solution

Three different internal solutions were tested to see which one was the most optimal for NO and O₂ detection: (a) 3 mM HCl/30 mM NaCl, (b) 0.1 M PBS (pH 7.4), and (c) DMSO (1:1 DMSO/H₂O) in 3 mM HCl/30 mM NaCl. For these tests, the same dual electrode was used throughout. The analyte (NO⁻, O₂⁻, or NO₂⁻-saturated PBS solution) was injected, and a linear sweep voltammetry (LSV) was run. LSV for O₂ detection was run from 0.0 to -1.0 V (vs. Ag/AgCl). For NO and NO₂⁻, the sweep was from 0.0 V to 1.0 V. All LSV tests were performed at a scan rate of 50 mV/s. To obtain results about both platinized and unplatinized electrodes, one disk was platinized, while the other was not.

2.2.4. Testing for Optimal HCl/NaCl Internal Solution Concentration

After it was decided that the HCl/NaCl was the optimal choice for internal solution for dual detection of NO and O₂, tests were run to see if the concentration of HCl played any role in reducing cross-talking between the two electrodes while possibly enhancing sensitivity. For this experiment, four different internal solutions were used: 0.3 mM HCl/30 mM NaCl, 3 mM HCl/30 mM NaCl, 30 mM HCl/30 mM NaCl, and 0.1 M PBS (pH 7.4). NO and O₂ calibrations were run separately with the same electrode, in the sensor assembly. Before any mentioned calibrations, electrodes were stabilized for 3 h,

with the potential at the NO electrode set at 0.75 V and the O₂ electrode set at -0.75 V. Calibrations were performed using amperometric *i-t*, applying a constant potential to each WE (specified above) while monitoring the current response after the addition of NO and O₂.

2.2.5. Varying Applied Potential on Working Electrodes

Generally, the NO sensor was run at a potential of 0.75 V and the O₂ sensor was run at -0.75 V. However, tests were run where the potentials on the two electrodes were altered to see if it would cut down on the cross-talking between the two electrodes. First, only the O₂ sensor potential was altered. The NO sensor potential was kept constant, at 0.75 V, while the O₂ sensor potential was run at -0.75, -0.70, -0.60, -0.50, and -0.40 V. NO and O₂ calibrations were performed in the routine manner, by adding aliquots of NO- or O₂-saturated PBS while monitoring the current response at a constant potential. Next, the O₂ sensor potential remained constant while the NO sensor potential was run at 0.70, 0.75, 0.80, and 0.85 V. Again, NO and O₂ calibrations were run in the typical manner using amperometric *i-t*.

2.2.6. Electro-deposition of 5-Amino-1-naphthol

When it was found that altering internal solution compositions could not significantly decrease the cross-talking, the PTFE membrane was replaced with the 5-amino-1-naphthol polymer. In such cases, a mixture of 5 mM of the monomer and 0.1 M sodium perchlorate was dissolved in a solution using

acetonitrile as solvent. The monomer was electropolymerized onto the electrode disks via CV, twenty cycles, swept from 0.0 to 1.0 V at a scan rate of 50 mV/s.

2.3. Results and Discussion

2.3.1. Effect of Internal Solution Composition on Sensitivity

Three different internal solutions were tested, using LSV, in order to determine which one was optimal for dual NO and O₂ detection with our amperometric sensor. The three internal solutions of interest were (a) 3 mM HCl/30 mM NaCl, (b) 0.1 M PBS (pH 7.4), and (c) DMSO (1:1 DMSO and H₂O) in 3 mM HCl/30 mM NaCl. The same electrode was used throughout all runs.

The HCl/NaCl mixture in water was reported as an internal solution optimized for favorable kinetics of NO oxidation at Pt by Shibuki and co-workers.²¹ Recently, research has been done using a DMSO/sulfuric acid/water mixture as the internal solution for the detection of certain gaseous analytes.²²⁻²⁴ It was found that the sensitivity almost doubled when DMSO was added to the internal electrolyte. For these reasons, we opted to examine the possible benefits of adding DMSO to our HCl/NaCl internal solution.

It is important to test biological samples in a medium that is similar to the composition of the biological system it is found in. For this reason, most tissues are tested in a PBS buffer (pH 7.4).¹⁵ Thus it was of interest to us to test our sensors with an internal solution similar to that used in tests on biological samples.

Just the dual electrode was used in these tests, not the sensor assembly (which houses the dual electrode in the pipet tip with internal solution). To use the sensor assembly would require that the assembly be taken apart after each test and filled with a new internal solution. In such a process, the membrane would have to be reapplied every time, leading to great inconsistencies among the tests. This is because the thickness of the membrane cannot be mechanically controlled, thus, giving significant differences in sensitivities.

To obtain information about benefits of the platinization, one electrode was platinized, while the other was left unplatinized. The electrode, with a Ag wire wrapped around it, was immersed into the internal solution of interest, and the analyte (NO , O_2 , or NO_2^-) was injected. Nitrite was tested because it is an interfering species at the potential applied for NO detection. To optimize our sensor, it is important to find an internal solution that provides the lowest sensitivity to the interferrant. LSV sweeps for NO , O_2 , and NO_2^- can be observed in Figures 2.3, 2.4, and 2.5, respectively.

For NO detection in the DMSO internal solution, NO oxidation is prominent at approximately 0.80 V for the platinized electrode. However, we are looking for the optimal internal solution for dual detection of NO and O_2 , and O_2 reduction in the DMSO internal solution is not as prominent as O_2 reduction in the HCl/NaCl internal solution. When looking at nitrite oxidation, we are looking at the internal solution which suppresses oxidation the most. PBS shows the lowest response; however, PBS internal solution shows almost no response for NO and O_2 runs. It appears that there is no ideal internal solution which enhances both NO and O_2

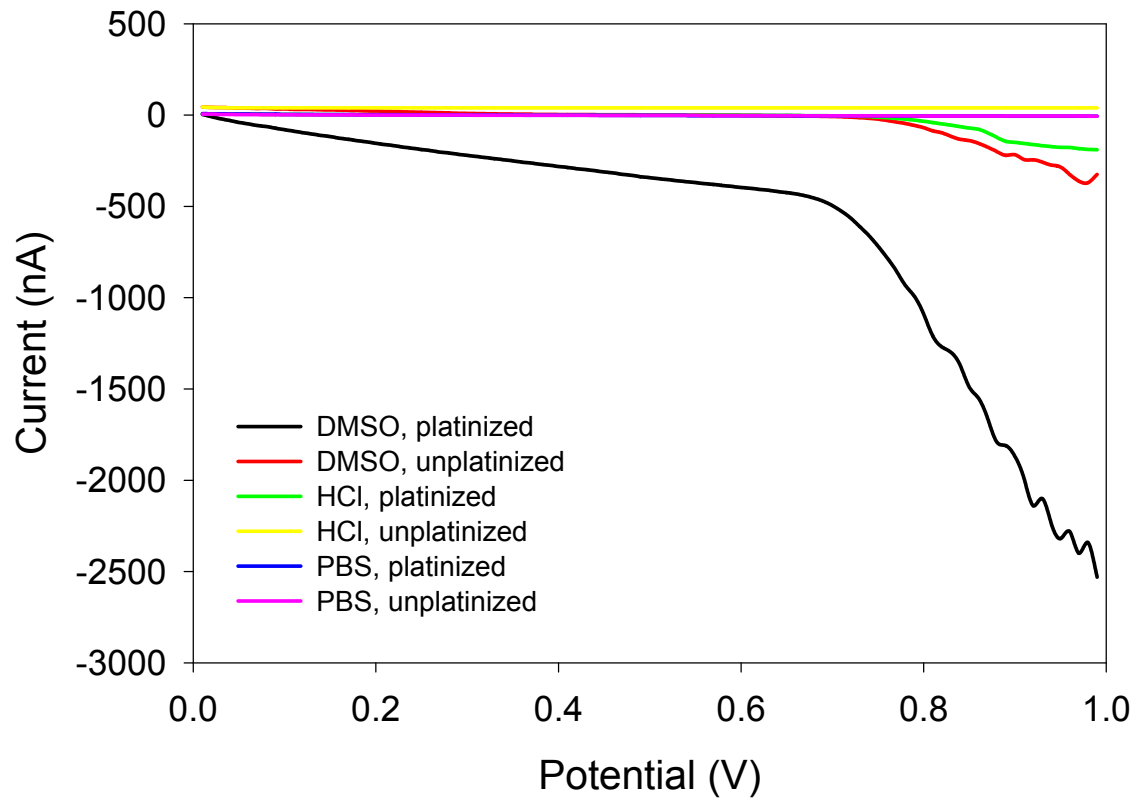


Figure 2.3. LSV obtained in 2.5 μM NO with various internal solutions (DMSO, HCl, and PBS) using both a platinized and unplatinized electrode. All scans were run from 0.0 to 1.0 V at a scan rate of 0.1 V/s, using a 2 s quiet time.

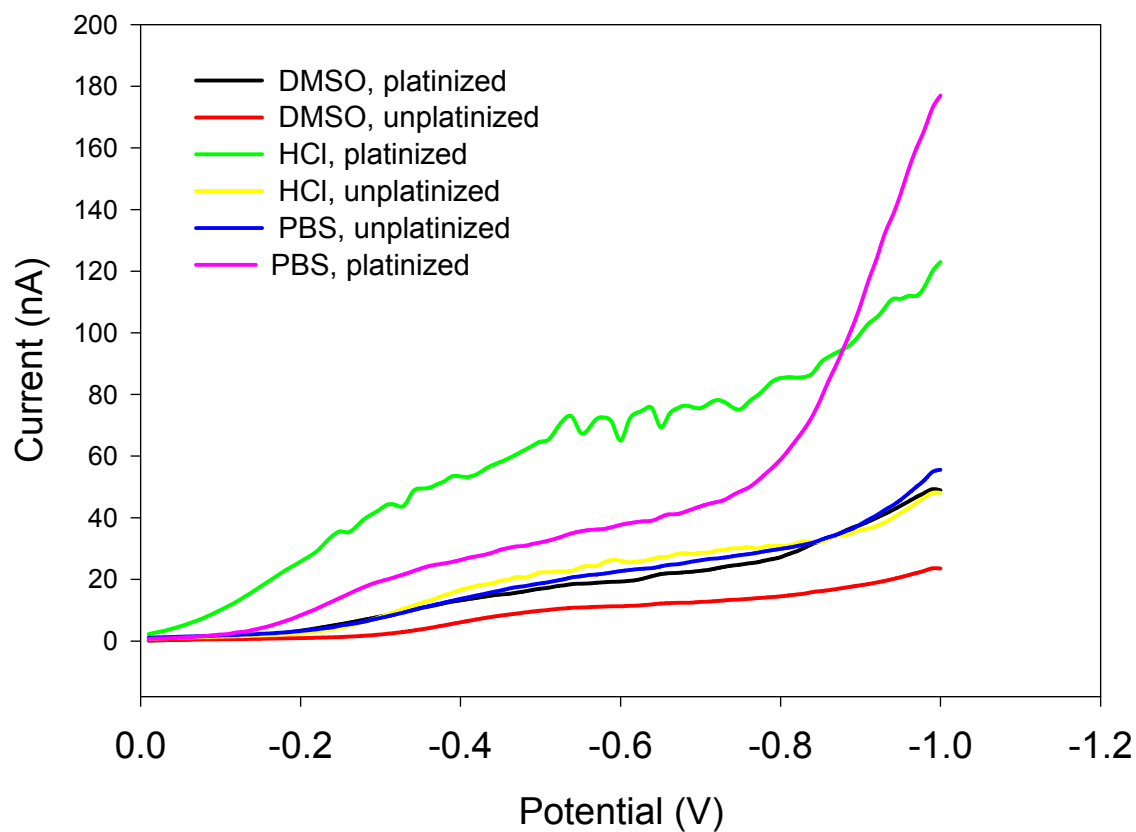


Figure 2.4. LSV obtained in 50 μM O_2 with various internal solutions (DMSO, HCl, and PBS) using both a platinized and unplatinized electrode. All scans were run from 0.0 to 1.0 V at a scan rate of 0.1 V/s, using a 2 s quiet time.

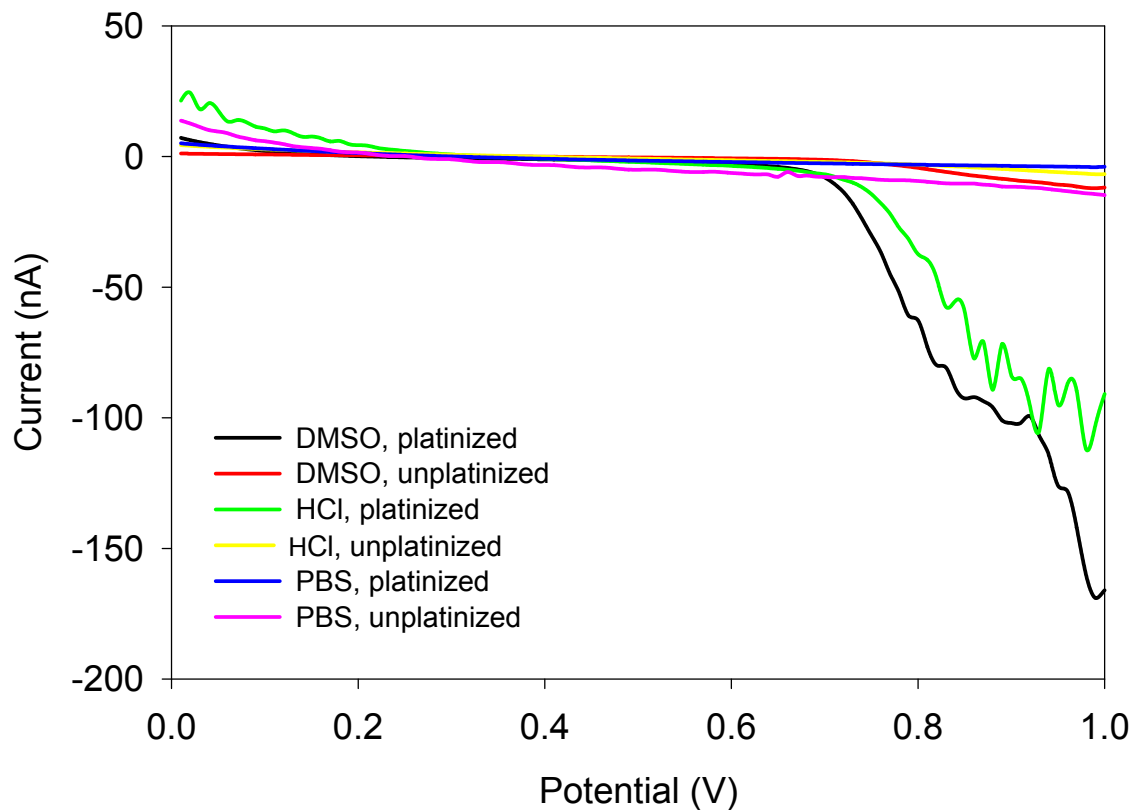


Figure 2.5. LSV obtained in 50 μM NO_2^- with various internal solutions (DMSO, HCl, and PBS) using both a platinized and unplatinized electrode. All scans were run from 0.0 to 1.0 V at a scan rate of 0.1 V/s, using a 2 s quiet time.

responses, while suppressing the NO_2^- response. Inevitably, it was decided that the HCl/NaCl solution was optimum for dual NO and O_2 detection, while suppressing nitrite, the interferrant. By using the PTFE membrane covering in the sensor assembly, practically all NO_2^- in solution is blocked from reaching the electrodes.

2.3.2. Effect of HCl/NaCl Concentration on Cross-talking

Further testing was done using the HCl/NaCl internal solution. However, a great amount of cross-talking between the WE1 and WE2 was observed. The possibility of electrical causes for the cross-talking (i.e., electrical signals being passed between the clamps on both WEs) was observed and investigated. Inevitably, electrical causes were ruled out. The cause for the cross-talking is chemically related. The product of the reaction at one electrode is migrating to the second electrode, where it then reacts, producing an additional current response on top of the target analyte. Cross-talking is a much bigger issue for NO detection than O_2 detection. During an NO calibration, the WE2 sensor response is actually greater in magnitude than the WE1 response. During O_2 calibration, the WE1 has a minimal response. Figures 2.6 and 2.7 are dynamic range plots that show the simultaneous WE1 and WE2 responses during a calibration for NO and O_2 , respectively.

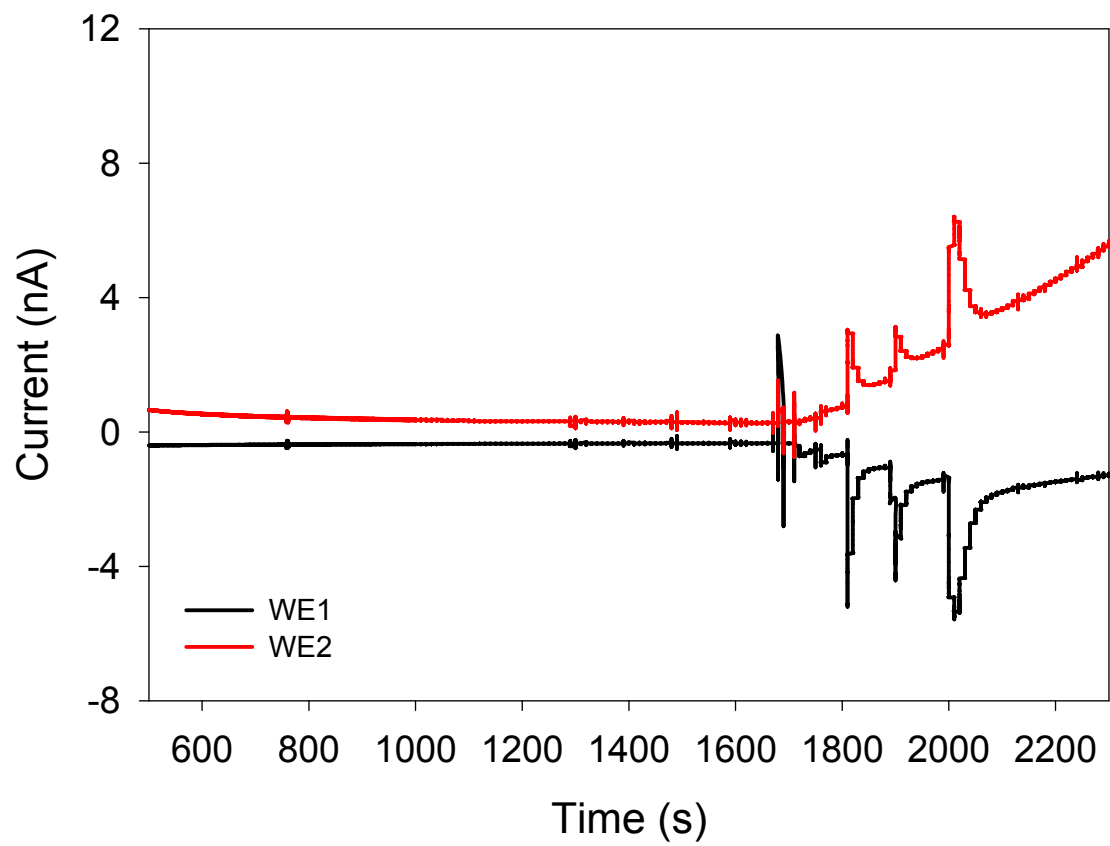


Figure 2.6. Dynamic response curves monitoring the current response of both the WE1 and WE2 during an NO calibration. Amperometric i - t was used with the potential of WE1 set at 0.75 V and a potential of -0.75 V for WE2. The concentration of NO additions ranged from 0 to 5.7 μ M.

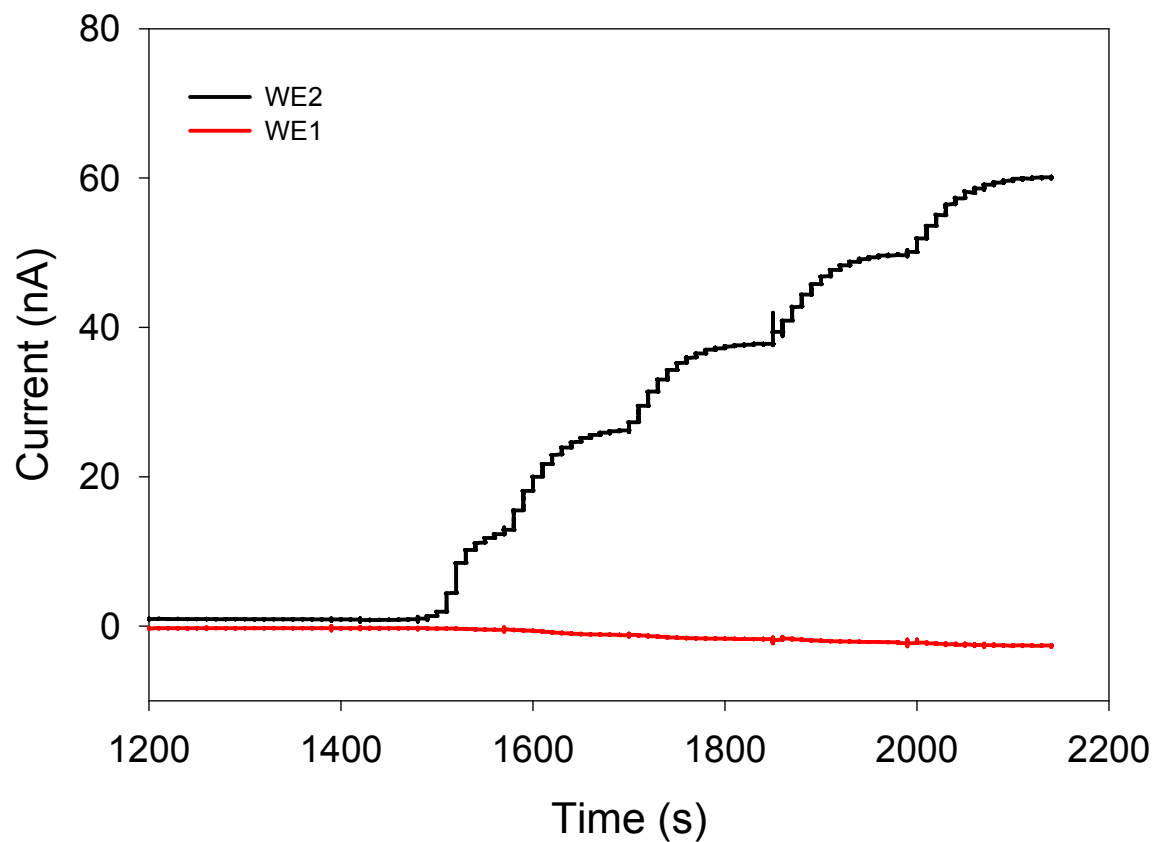
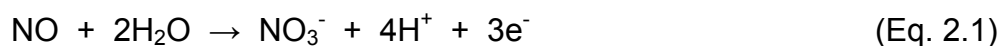


Figure 2.7. Dynamic response curves monitoring the current response of both the WE1 and WE2 during an O₂ calibration. Amperometric *i-t* was used with the potential of WE2 set at -0.75 V and a potential of 0.75 V for WE1. The concentration of O₂ additions ranged from 0 to 120 μ M.

At the WE1 surface, the following oxidation reaction is believed to occur:



The oxidation of NO to NO_3^- occurs at approximately 0.75 V for a platinized platinum electrode. In addition, NO is reduced at the WE2 as follows:



This produces a current response at WE2 that is added onto the current response for actual O_2 reduction. In fact, much favorable NO reduction could make the WE2 response much larger than the WE1 response during NO calibration (Figure 2.6).

At the WE2 surface, the following reduction reactions are believed to occur in acidic condition:



On the other hand, O_2 proceeds the following reduction in alkaline condition.



An applied potential of -0.75 V (vs. Ag/AgCl) is sufficient for the O₂ reduction at a platinized Pt electrode. It is probable that the H₂O₂ or OH⁻ produced in the above reactions are migrating to WE1 and is being re-oxidized, producing a small current response at WE1. However, for O₂ calibration, the response at the WE1 is much less in magnitude than the response at WE2 (Figure 2.7) under the conditions we tested. This can be considered negligible.

Some of the products of WE2 (H₂O₂ or OH⁻) are reactants for the reaction at WE1. The PTFE membrane is capable of keeping interferrants in the samples away from the electrode disks. However, interferrants are being produced in the reactions at the disks. Once produced inside the membrane, any products are free to migrate to opposing electrodes and react.

Because chemical cross-talking between the two WEs was suspected, additional tests were performed in order to investigate alternative methods for reducing or eliminating the cross-talking between the two WEs. The HCl concentration in the HCl/NaCl internal solution was varied to see how it might affect any cross-talking.

For this test, we used the HCl/NaCl internal solution with four different HCl concentrations: (a) 30 mM HCl/30 mM NaCl (pH 1.52), (b) 3 mM HCl/30 mM NaCl (pH 2.52), (c) 0.3 mM HCl/30 mM NaCl (pH 3.52), and (d) 0.1 M PBS (pH 7.4). NO and O₂ calibrations were run, using the same electrode, in the sensor assembly, housing the internal solution of choice behind the PTFE membrane. After calibrations, a comparison was drawn between (a) the WE1 sensitivity to

the WE2 sensitivity during NO calibration and (b) the WE2 sensitivity to the WE1 sensitivity during O₂ calibration. Results are given in Table 2.1.

As pH decreased, the WE2 response during NO calibration became much greater in magnitude than the WE1 response. This is probably because of much favored NO reduction at WE2.

2.3.3. Effect of Varying Applied Potentials at Working Electrodes

Even the use of the internal solution with the highest pH could not reduce cross-talking to an acceptable level. By altering the potentials of both the WE1 and WE2, effective oxidization or reduction of the target analyte might be possible, while managing to cut down on cross-talking by applying a potential at which the interfering products are not capable of being oxidized or reduced.

First, the potential on the WE2 (E_{app}) was altered, while the potential on the WE1 remained at 0.75 V. NO and O₂ calibrations were run at each of five different potentials for the WE2: $E_1 = -0.40$ V, $E_2 = -0.50$ V, $E_3 = -0.60$ V, $E_4 = -0.70$ V, and $E_5 = -0.75$ V. The same electrode, in the sensor assembly, was used throughout, and the same internal solution was used throughout (0.3 mM HCl/30 mM NaCl). Thus, the sensor was never disassembled throughout testing, and there are no inconsistencies due to altering membrane thicknesses. Results are given in Tables 2.2 and 2.3.

While all potentials for O₂ calibration were found to be optimal, changing the potential of WE2 never created optimal conditions for NO calibration. No

Table 2.1. Comparison of WE1 and WE2 responses during NO and O₂ calibrations using internal solutions of varying pH values. Ratios were obtained by comparing the sensitivities obtained from a calibration plot of each WE during each calibration.

Internal solution composition	NO calibration (WE1/WE2)	O₂ calibration (WE2/WE1)
0.1 M PBS (pH 7.4)	0.0075x	237x
0.3 mM HCl/30 mM NaCl (pH 3.52)	0.45x	22.7x
3 mM HCl/30 mM NaCl (pH 2.52)	0.273x	42.2x
30 mM HCl/30 mM NaCl (pH 1.52)	0.174x	454x

Table 2.2. WE1 and WE2 sensitivities during NO calibration while varying E_{app} for WE2.

E_{app} for WE2	WE2 sensitivity	WE2 sensitivity	WE1/WE2 sensitivity ratio
$E_1 = -0.40 \text{ V}$	0.1726	0.9088	0.19
$E_2 = -0.50 \text{ V}$	0.174	0.4094	0.43
$E_3 = -0.60 \text{ V}$	0.0528	0.1054	0.50
$E_4 = -0.70 \text{ V}$	0.0368	0.0905	0.41
$E_{\text{normal}} = -0.75 \text{ V}$	0.0658	0.1896	0.344

Table 2.3. WE1 and WE2 sensitivities during O₂ calibration while varying E_{app} for the WE1.

E_{app} for WE1	WE2 sensitivity	WE1 sensitivity	WE2/WE1 sensitivity ratio
$E_1 = -0.40 \text{ V}$	0.2231	0.0456	4.89
$E_2 = -0.50 \text{ V}$	0.2473	0.0593	4.17
$E_3 = -0.60 \text{ V}$	0.0735	0.0015	49
$E_4 = -0.70 \text{ V}$	0.2187	0.0332	6.59
$E_{\text{normal}} = -0.75 \text{ V}$	0.1676	0.0129	13

matter what potential was applied to WE2, it still showed a greater response than WE1 during NO calibration.

It is also of interest that the WE2 sensitivity is, in most cases, greater during NO calibration than it is during O₂ calibration. This is because NO is also reduced at WE2. In addition, the products of NO oxidation may possibly feed WE2 with more reactant than in the case of an actual O₂ calibration.

Further evidence for chemical cross-talking is observed in that the WE1 sensitivity during NO calibration varies as the applied potential on WE2 varies. Theoretically, if there were no chemical cross-talking and the WEs responded independently, WE1 sensitivity should remain constant during NO calibration, no matter what potential is applied to WE2. The fact that the WE1 sensitivity changes shows that the WE's responses are linked.

The potential on WE1 was altered next, while the potential on WE2 remained constant. This was done in the same manner as the previous study, with the electrode in the sensor assembly and the same electrode and internal solution throughout. Results are given in Table 2.3.

This test gave us similar results to that of the previous study. While sensitivities at both electrodes altered, depending on the WE1 potential, no optimal condition was ever observed for NO calibration, that was able to suppress the large response at WE2.

2.3.4. Electro-deposition of 5-Amino-1-naphthol

It is apparent that, for our particular dual sensor, a PTFE membrane cannot sufficiently block interferrants from the sensors, since a great amount of the interferrants are produced at one WE within the membrane, and are capable of reacting at the opposing WE. It is necessary to produce an individual permselective layer at the site of the electrode disks in order to block the product interferrants. Research has been done using electro-deposited polymers onto electrode surfaces as a means of providing selectivity to sensors.¹⁶

We chose to investigate the potential benefits of using the monomer 5-amino-1-naphthol. Since it is electro-polymerizable, it is easily deposited onto the electrode disks. After its deposition, it should “trap” the products of the reaction at each electrode, inhibiting their migration to the opposing disk.

Aside from offering the benefits of selectivity, the electro-deposited polymer is also beneficial because the thickness can be controlled, unlike the manually applied PTFE membrane. This can reduce any inconsistencies in the measured sensitivities due to membrane thickness.

To test the polymer’s ability to enhance selectivity, three calibrations were performed on a single electrode: (a) NO, (b) O₂, and (c) deaerated PBS (pH 7.4). The electrode was first platinized, according to standard protocol, and then the polymer was deposited onto each disk. A Ag wire was dipped in 0.1 M FeCl₃ to allow Ag to form Ag/AgCl and then to serve as the counter/reference electrode. Calibrations were run in PBS (pH 7.4). Results are given in Figures 2.8, 2.9, and 2.10.

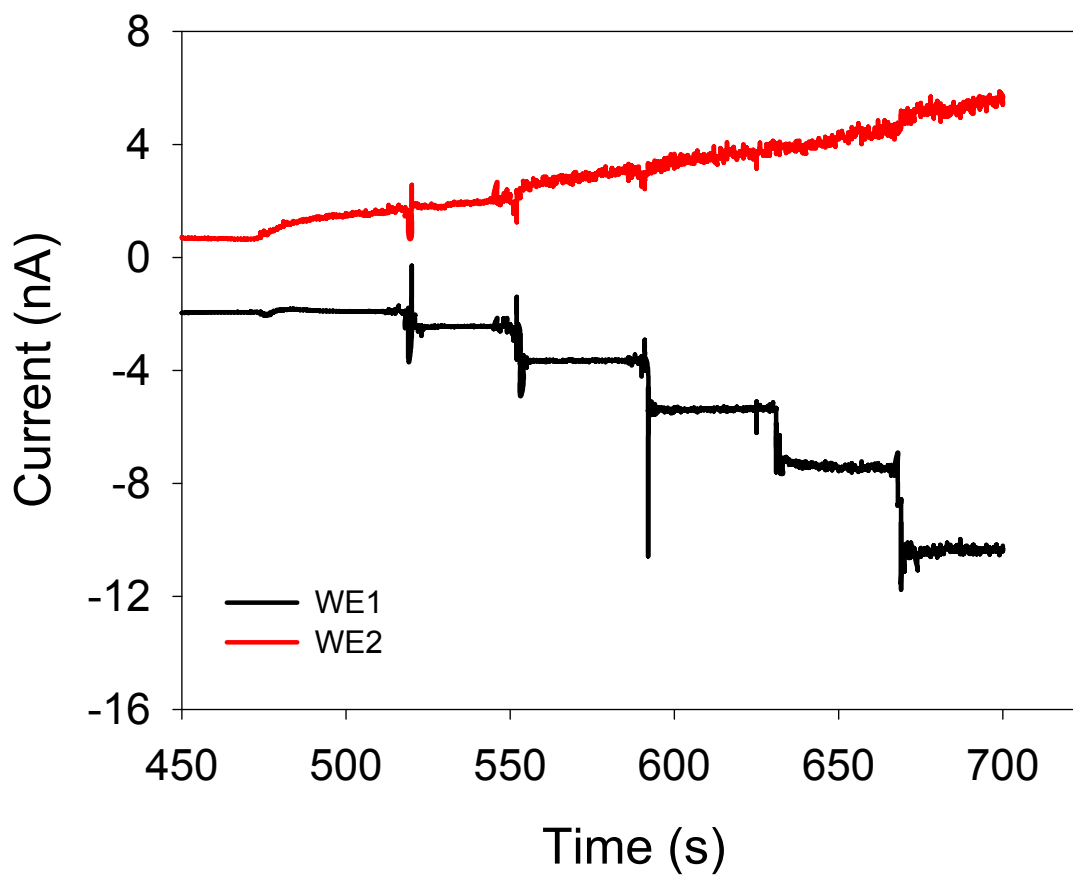


Figure 2.8. Dynamic response curves for both WE1 and WE2 during a NO calibration. [NO] ranged from 0-6 μM . Both electrode disks were first platinized, and then coated in the electro-deposited 5-amino-1-naphthol.

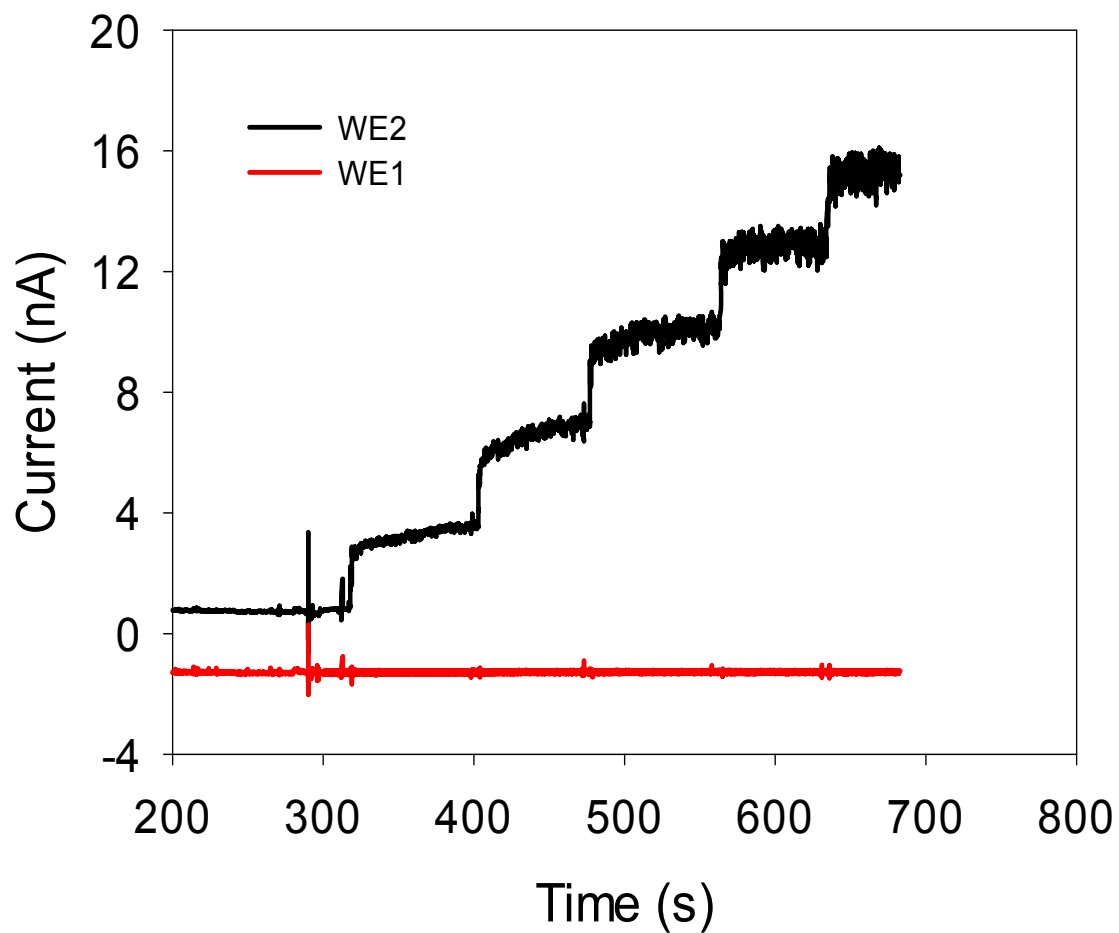


Figure 2.9. Dynamic response curves for both WE1 and WE2 during an O₂ calibration. [O₂] ranged from 0-120 μ M. Both electrode disks were first platinized, and then coated in the electro-deposited 5-amino-1-naphthol.

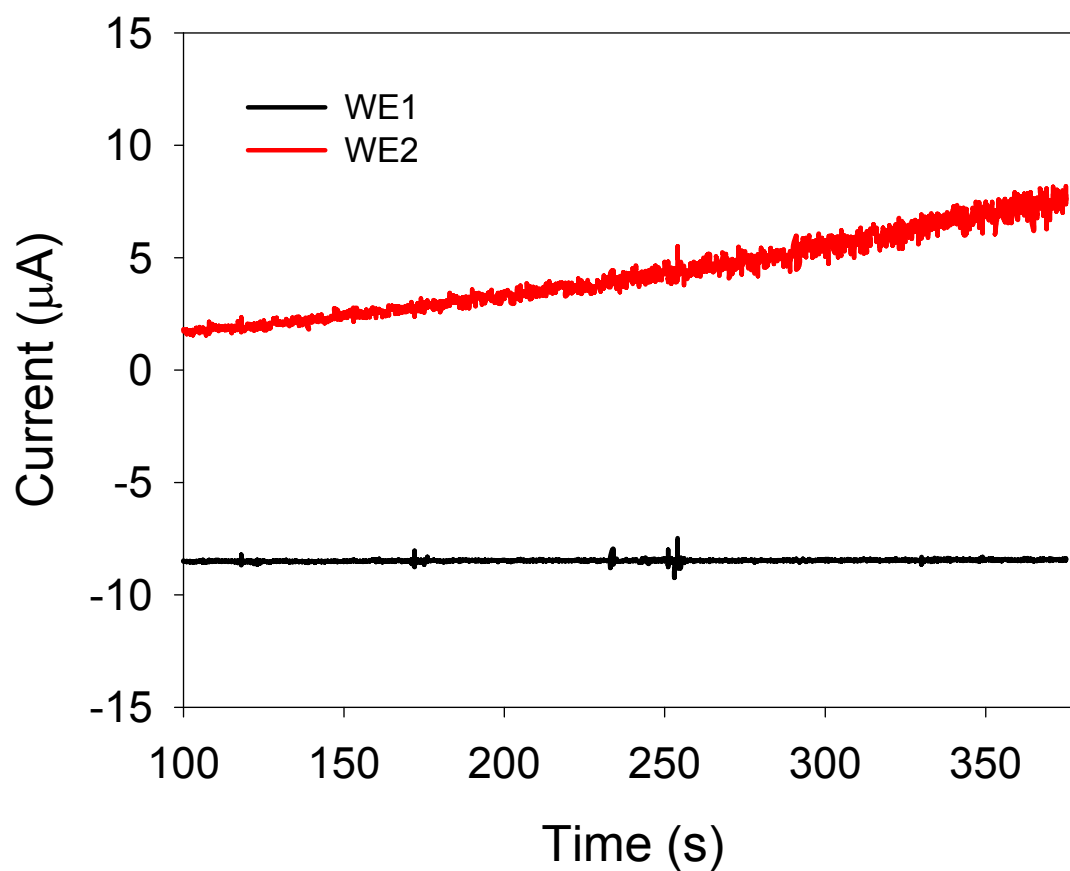


Figure 2.10. Dynamic response curves for both WE1 and WE2 during deaerated PBS injections. Both electrode disks were first platinized, and then coated in the electro-deposited 5-amino-1-naphthol.

It was apparent that, during NO calibration, the response from WE2 greatly decreased. However, there is still a slight response. The deaerated PBS calibration was run as a means of testing a blank. When comparing the WE2 slope during NO calibration (0.02035 nA/s), it is only slightly greater than the WE2 slope during PBS calibration (0.0148 nA/s) due to the NO reduction occurring at WE2. Thus, we can assume that the polymer is preventing a significant amount of cross-talking between the electrodes, and that the majority of the response observed at WE2 during NO calibration is due to atmospheric oxygen leaking. A similar result was observed for WE1 during O₂ calibration, vs. WE1 during PBS calibration.

Next, we compared the performance of an electrode housed within a PTFE membrane versus an electrode with the polymer coating. The same sensor was used throughout, first in an electrode assembly with the PTFE membrane, and then with the polymer electro-deposited. NO, O₂, and deaerated PBS calibrations were performed. Responses at each sensor were compared. Results are given in Figure 2.11. It is apparent that the polymer successfully diminishes cross-talking.

2.4. Conclusions

An amperometric, planar dual microsensor with improved analytical performances has been prepared and characterized. The sensor is composed of two glass-sealed 25 μm Pt working electrodes and a Ag/AgCl reference/counter

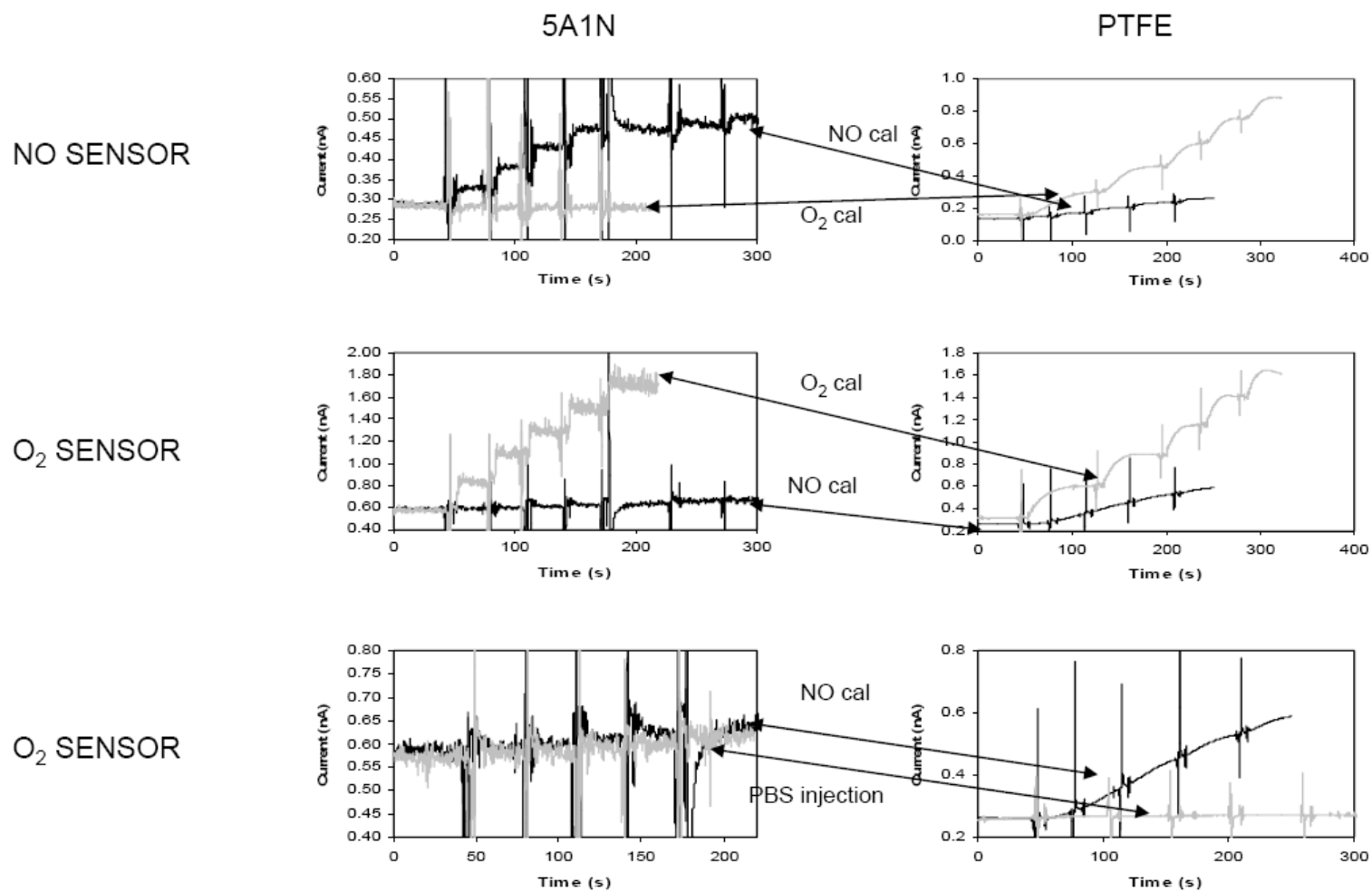


Figure 2.11. Dynamic response curves of a dual sensor based on poly- 5-amino-1-naphthol coated vs. PTFE-encased sensor.

electrode. Initially, an internal solution of 3 mM HCl/30 mM NaCl was housed behind a PTFE gas permeable membrane.

It was found that an internal solution consisting of a HCl/NaCl mixture exhibits the greatest sensitivity for the dual detection of NO and O₂. The concentration of HCl was examined to see if altering the concentration could reduce the observed cross-talking between the two electrode disks. It was found that as [HCl] decreased (pH increased), response at the opposing electrode decreased and cross-talking was slightly diminished.

However, no matter what concentration of HCl was used, cross-talking was still a prominent issue, particularly for NO detection and the WE2 response. The WE2 potential was altered first, keeping the WE1 potential constant. However, at no potential were there any significant improvements upon cross-talking. The WE1 potential was altered also, while keeping the WE2 potential constant. While some sensitivities changed, based upon the potential change, once again, no great benefits of altering the applied potential were observed.

Alternate means of providing selectivity to the sensor were examined, and the electro-polymerized 5-amino-1-naphthol was chosen to be deposited onto each individual electrode disk. After running calibrations, it was evident that the polymer greatly reduced cross-talking between the two electrode disks. Any response seen at WE2 was merely that of atmospheric oxygen leaking.

2.5. Additional Information

Due to Dr. Youngmi Lee's departure from the University of Tennessee in July 2007, this research was continued at Ewha Womans University in Seoul, South Korea. The work presented in this dissertation covers only the work conducted at the University of Tennessee. This work, paired with continuing work at Ewha, led to a publication in 2009 in *Electrochemistry Communications*.²⁵

The main focus of the continuing work at Ewha centered around continual efforts to reduce chemical cross-talking between the two working electrodes. LSV studies showed that, not only was O₂ being reduced at WE2, but the reduction of NO also occurred. In addition to this, the cross-talking occurring at WE1 was further confirmed.

The internal solution composition and concentration were further studied, all containing NaCl. The pH was adjusted, using either NaOH or HCl, from 2.52 to 12.24. In both alkaline and acidic conditions, WE1 showed an increase in current as [O₂] increased, but without linearity. It was confirmed that the cross-talking occurring at this electrode was due to the re-oxidation of the O₂ reduction product at WE1.

At a Pt electrode, in an acidic solution, as was the case for most of the work presented in this dissertation, the reduction of O₂ should occur according to the 4 electron process shown in Eq. 2.2, as previously stated, producing H₂O. In this situation, it is unclear why cross-talking would be observed.

However, prior work showed that, in a system with increased mass-transport, it is possible that O₂ reduction could occur via a 2/2-electron transfer

process.²⁶ In the situation with the platinized electrodes utilized in these studies, porous microstructures were made, resulting in a high mass-transport.

It is likely that an intermediate of the 2/2-electron transfer involved in O₂ reduction, probably H₂O₂, is getting re-oxidized at the WE1, serving as the source for any cross-talking. It was found that a slightly basic internal solution (pH 7.5) was optimal for decreasing this cross-talking.

Even with an optimized internal solution pH, significant cross-talking was still observed. This was remedied by making the dual sensor such that there was a greater distance between the two WEs (>330 μm). In doing this, all cross-talking was eliminated, and WE1 showed only a response for NO oxidation.

Concerning the WE2, linearity was observed in correlation with both the [O₂] and [NO]. Regardless of the WE1 potential, WE2 showed similar current responses to NO, confirming that the currents were caused by NO reduction and were not due to any cross-talking.

In the presence of both NO and O₂, the dual sensor currents can be represented by the following equations:

$$I_{WE1(+0.75\text{ V})} = (S_{WE1,NO} \times [NO]) \quad (\text{Eq. 2.6})$$

$$I_{WE2(-0.40\text{ V})} = (S_{WE2,NO} \times [NO]) + (S_{WE2,O_2} \times [O_2]) \quad (\text{Eq. 2.7})$$

where *I* is the current (A), *S* is the sensitivity (A/M), [] is the concentration (M), and the subscripts represent kinds of the corresponding electrodes or gases.

[NO] can be determined using Eq. 2.6, since the WE1 sensitivity for NO can be obtained from a prior sensor calibration. In Eq. 2.7, WE2 sensitivities for O₂ and NO can be obtained from prior sensor calibrations, and [NO] was determined from Eq. 2.6. Therefore, by using Eq. 2.7, it is possible to determine [O₂].

Using the optimized sensor, detection limits for NO and O₂ were determined to be <5 nM and <500 nM, respectively. A calibration stability of $\pm 10\%$ over 50 h was observed if the sensor was stored in PBS buffer. WE1 showed 20 times greater selectivity to NO over carbon monoxide (CO) and showed no responses from biological interferrants (nitrite, uric acid, ascorbic acid, acetaminophen, and dopamine).

Due to the continuing work at Ewha, the dual NO/O₂ microsensor is now optimized and suitable for the quantification of the gases in biological samples.

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Part 3

Individual and Simultaneous Determination of Lead, Cadmium, and Zinc by Anodic Stripping Voltammetry at a Bismuth Bulk Electrode

3.1. Introduction

Anodic stripping voltammetry is a commonly used technique for trace metal analysis.¹ By holding the electrode at a sufficiently negative potential, the analyte can be reduced to form an amalgam with the electrode, thus preconcentrating the metal onto the electrode surface. Following preconcentration, an anodic scan oxidizes the metals, stripping them off of the electrode and yielding a current response that is linear to the metal concentration.

In the past, mercury(Hg)-based electrodes have been the material of choice for stripping analyses due to its attractive analytical properties.^{1b} Among these, the extended negative potential window and its ability to form alloys with other metals¹ make mercury an ideal candidate for heavy metal analysis.

Due to the toxicity and inconvenient disposal of Hg, however, alternative electrode materials are being explored. Gold-coated diamond thin film electrodes² and boron-doped diamond electrodes³ have been developed and used in ASV analyses. Among alternative electrode materials, bismuth-based electrodes have been growing in popularity. Bismuth-modified carbon paste electrodes,⁴ bismuth-modified carbon nanotubes,⁵ and the widely-used bismuth film electrode (BiFE)⁶⁻¹¹ initially developed by Wang and coworkers, share many of the same desirable electrochemical characteristics as Hg-based electrodes. Similar to Hg, Bi is capable of forming fused alloys with heavy metals,¹² and exhibits high hydrogen overpotential.¹³ In addition to the attractive

electrochemical properties, Bi is more environmentally friendly and less toxic.

Among the bismuth-based electrode options, little work has been done using a bismuth bulk electrode (BiBE). Work with a BiBE can, however, offer many advantages. Other Bi-based electrodes can be difficult and time-consuming to fabricate. BiFE films have been shown to be very fragile.¹⁴ The BiBE is easily fabricated, cost-efficient, stable and robust, and is capable of yielding more reproducible results than other previously examined Bi-based electrodes.

Past work with a BiBE has been limited. The use of a BiBE as a substrate for self-assembled monolayers (SAMs) has been examined, allowing for a more negative potential window than SAMs on gold substrates.¹⁵ In addition, a simple method for the creation of Bi nanoparticles has been developed by cathodic dispersion of a BiBE.¹⁶ To date, the BiBE has only been used twice for quantitative analyses. Bučková and coworkers demonstrated the use of the BiBE as a means of enhancing daunomycin detection.¹⁷ Pauliukaite and coworkers characterized the BiBE, and showed its potential use for heavy metal detection.¹³

The pioneering studies performed by Pauliukaite and coworkers characterized the electrode, highlighting both its cathodic electrochemical behavior and its behavior under anodic conditions, which causes the formation of a thin, conductive Bi_2O_3 film. The electrochemistry of various well-known redox couples was inspected and both inorganic and organic compounds were tested at the electrode. The feasibility of the BiBE for use in anodic stripping

voltammetry of Pb(II), Cd(II), and Zn(II) was qualitatively shown, thus demonstrating the capability of the BiBE for heavy metal stripping analysis. The only quantitative stripping measurement was that for Pb(II), which resulted in a limit of detection of 3.2 ppb. This work provided a basis of understanding and laid the foundation for further studies using the BiBE, as it provided the inspiration for the current studies.

Pb(II), Cd(II), and Zn(II) detection has been performed in the current work using ASV at the BiBE. The BiBE is made in-house by melting bismuth needles into a hand-blown glass casing. All three metals have been quantitatively measured, both individually and in mixtures, with detection limits in the ppt range, upon optimization. Extensive studies, with a focus on examining the interactions of the three metals when in a mixture, reveal that, in a mixture, the sensitivity of the metals always drops after the first metal has been stripped. As consecutive metals are stripped, the sensitivity of any remaining metals continues to drop. This shows that, as each metal is stripped, it inadvertently removes portions of any remaining metals adsorbed to the electrode. The potential of this method for use in environmental detection is demonstrated by testing for these metals in river water samples. The method is validated by comparing the results of the samples to those obtained using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

3.2. *Experimental*

3.2.1. Chemicals and Materials

All voltammetric measurements were performed using a modulated potentiostat (CHI 440, CH Instruments). A three-electrode configuration consisted of a BiBE disk (1.5 mm diameter), Ag/AgCl, and a platinum wire as working, reference, and counter electrodes, respectively. The BiBE was made in-house by melting bismuth needles (99.998% purity, Alfa Aesar) into a hand-blown glass casing. The melting process involved placing several rods of bismuth needles into the glass casing. A tin-coated copper wire was inserted partially into the glass casing such that the bismuth would melt around it and would act as the electrical lead. This ensemble was vertically inserted into a glass tube, which was then sealed and connected to a vacuum line. Air from the tube was removed by vacuum and replaced with nitrogen repeatedly to ensure complete removal of the air. The tube was held over a Bunsen burner until the bismuth was melted completely (melting point: 271.5 °C).¹⁸ This process was done under continuous vacuum to ensure that no air bubbles developed in the sealed tube. After cooling, the electrode was removed from the tubing and the end was cut with a diamond band saw, serving as the surface of the electrode. The freshly exposed electrode surface was polished with a series of emery paper of 200, 400, 600, and 800 grit. Further polishing was done using a standard electrode polishing kit (CH Instruments) that includes a 1200 grit Carbimet disk, 1.0 µm alumina slurry on a nylon cloth, 0.3 µm alumina slurry on a nylon cloth,

and 0.05 μm alumina slurry on a microcloth polishing pad. The images in Figure 3.1 are a schematic and pictures of the BiBE and its surface. Using a digital ohm meter, the resistance of the fabricated BiBE was measured to be 0.3 Ω .

Acetic acid (glacial, Fisher), sodium acetate (anhydrous, Certified ACS, Fisher), cadmium acetate (Certified ACS, Fisher), and lead nitrate (Certified ACS, Fisher) were used as received. Zn(II) AA standard solution (1000 ppm, Aldrich) was diluted prior to use. The buffer solution contained 0.1 M sodium acetate and its pH was adjusted to 5.0 with CH_3COOH . Pb(II), Cd(II), and Zn(II) standards (10-100 ppb) were prepared by diluting the appropriate amount of stock solution in electrolytes. All aqueous solutions were prepared in 18 M Ω deionized H_2O .

3.2.2. Procedure

For ASV experiments, 20 mL of standard solutions were used. No deaeration of the samples was required. Square wave voltammetry (SWV) was used for both the accumulation and stripping steps with the following parameters: initial E , -1.4 V; final E , -0.35 V; increasing E , 4 mV; amplitude, 25 mV; frequency, 15 Hz; and quiet time, 180 s. The quiet time was used for the accumulation step, during which the solution was stirred at approximately 1200 rpm. The potential was held constant at the initial potential during the entire quiet time. The SWV scan starts immediately upon completion of the quiet time. No resting period was used between the accumulation and stripping steps. Stirring

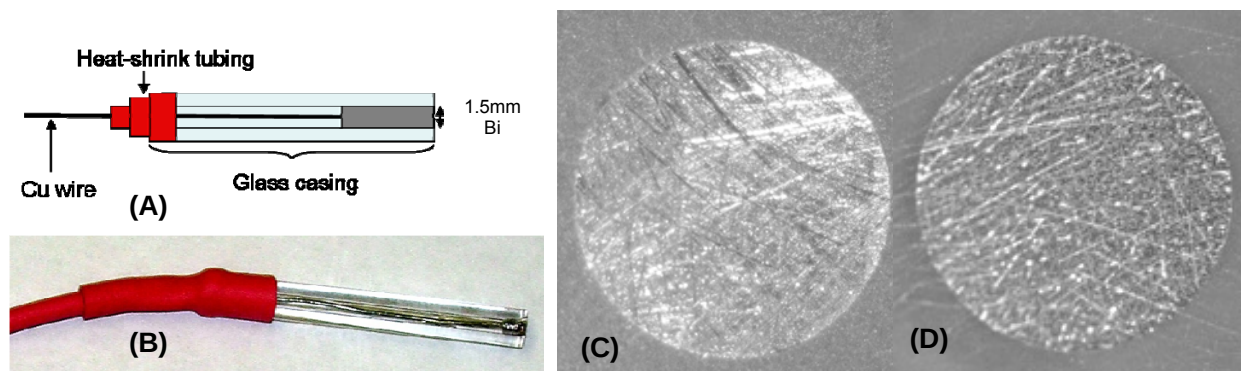


Figure 3.1. Bismuth bulk electrode: schematic diagram (A), electrode picture (B), an unpolished electrode surface (C), and a polished electrode surface (D). Images were taken using a Canon PowerShot A630 digital camera and a Cambridge Instruments StereoZoom 7 optical microscope, zoom range of 1.0-7.0 $\times$.¹⁹

at high speed was required during the accumulation process but was stopped at the end of the quiet period for the stripping step.

3.3. Results and Discussion

3.3.1. Individual Determination of Pb(II), Cd(II), and Zn(II)

Determination of all three metals was done by making standards containing each of the individual metals. ASV resulted in voltammograms containing peaks corresponding to the oxidation of Pb(0), Cd(0), and Zn(0). The peaks were assigned to each metal by spiking. The peaks for Pb(II) (-0.50 V), Cd(II) (-0.75 V), and Zn(II) (-1.10 V) are in agreement to values found in literature.¹³ Figures 3.2, 3.4, and 3.6 are the voltammograms for 10-100 ppb metals. Calibration plots for the three individual metal solutions (Figures 3.3, 3.5, and 3.7) give slopes and values of the correlation coefficient R^2 in Table 3.1.

It is worth noting that, for Cd(II) and Zn(II) analyses, in particular, there appears to be a peak potential dependence on concentration. For the Zn(II) analysis, as concentration increases, the peak potential appears to shift in the positive direction. The trend for Cd(II) appears to be the opposite of that observed for Zn(II) – as concentration increases, the peak shifts to a more negative potential. This result suggests that the shift is not due to an IR drop effect.

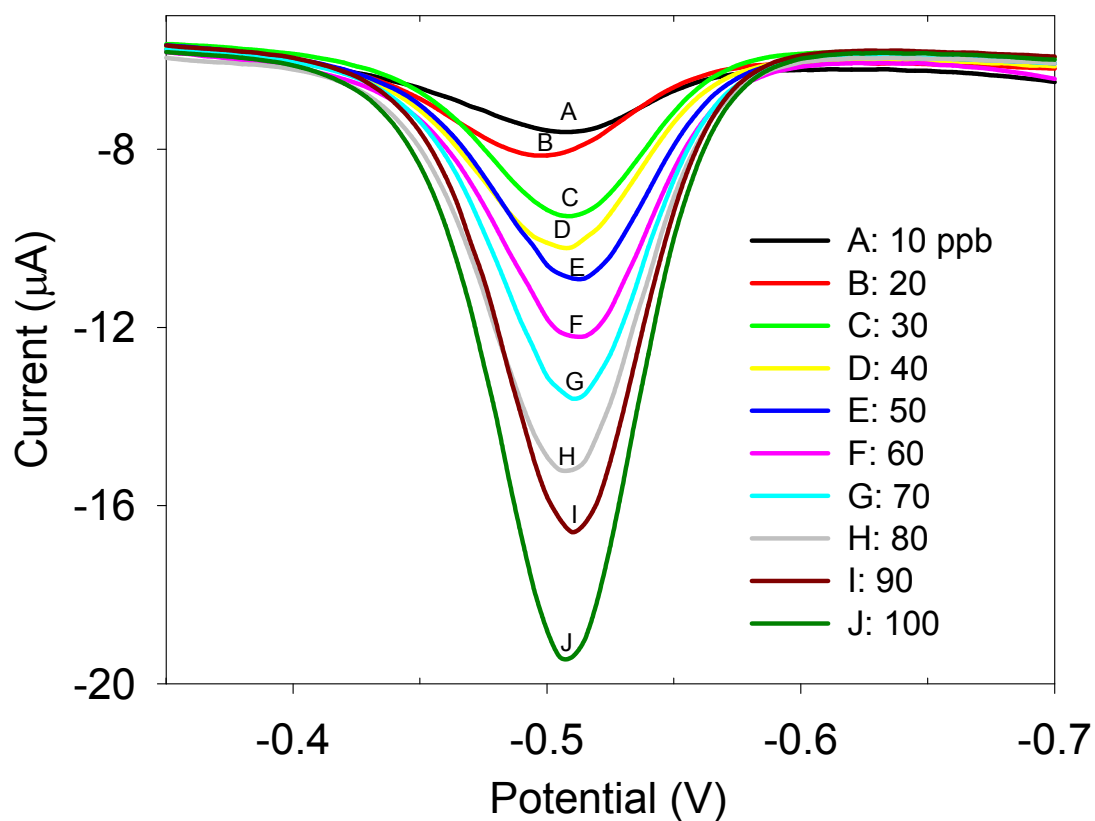


Figure 3.2. ASV calibration of Pb(II) detected at the BiBE. Calibrations were conducted using a 1.5 mm BiBE in standards of 10 to 100 ppb of metal ions in 0.1 M sodium acetate at pH 5.0. An accumulation period of 180 s at -1.4 V was used, with stirring at 1200 rpm. Immediately after accumulation, metals were stripped using a SWV scan from -1.4 to -0.35 V.

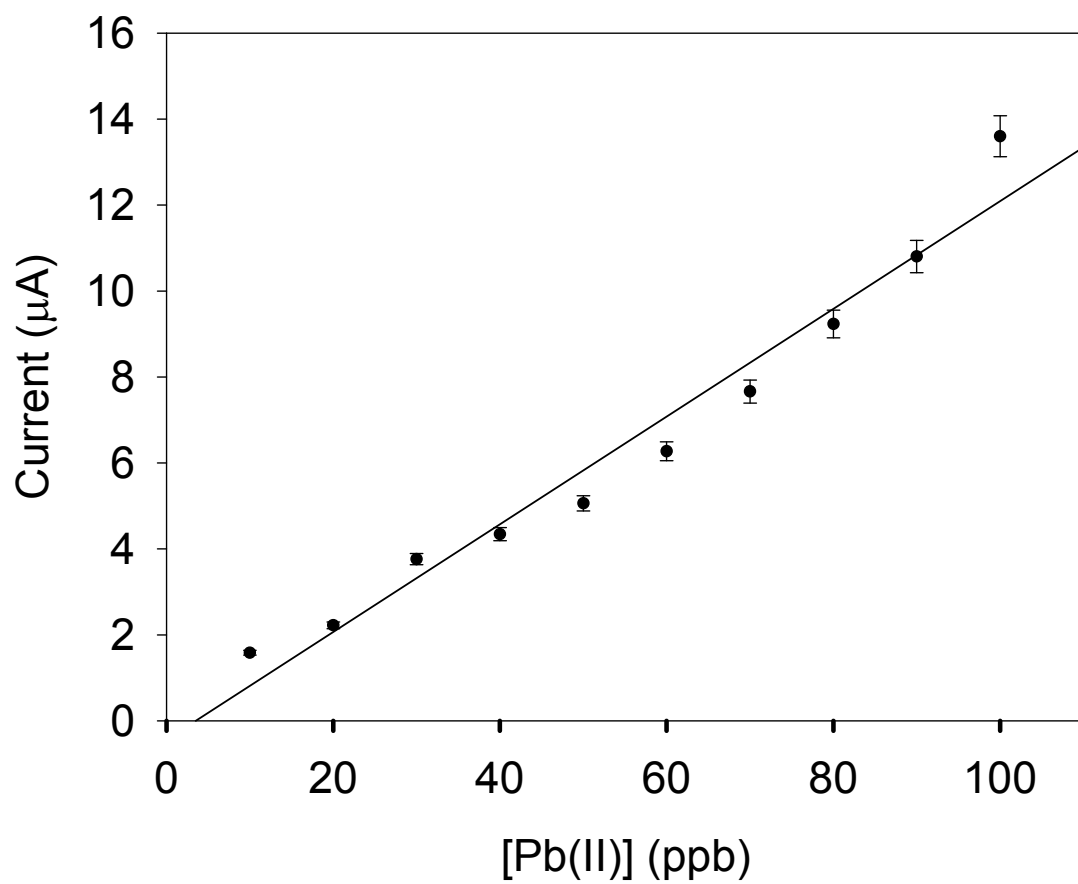


Figure 3.3. Calibration plot for Pb(II) detected at the BiBE.

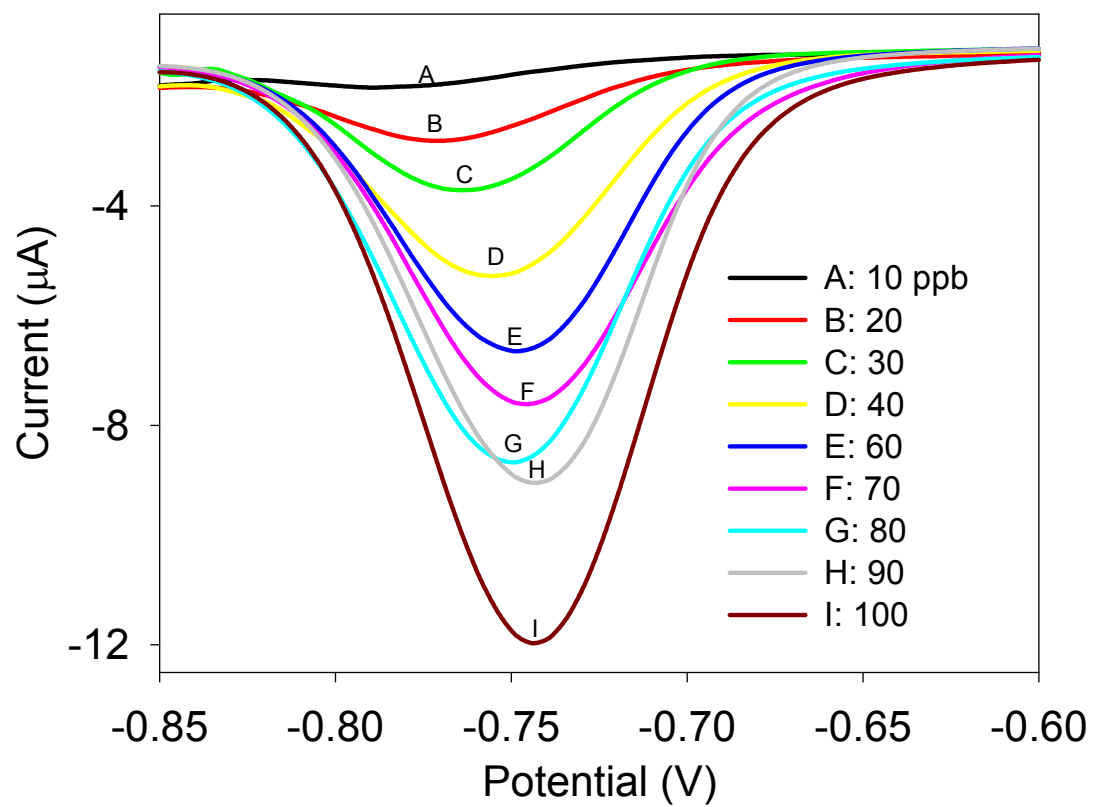


Figure 3.4. ASV calibration of Cd(II) detected at the BiBE.

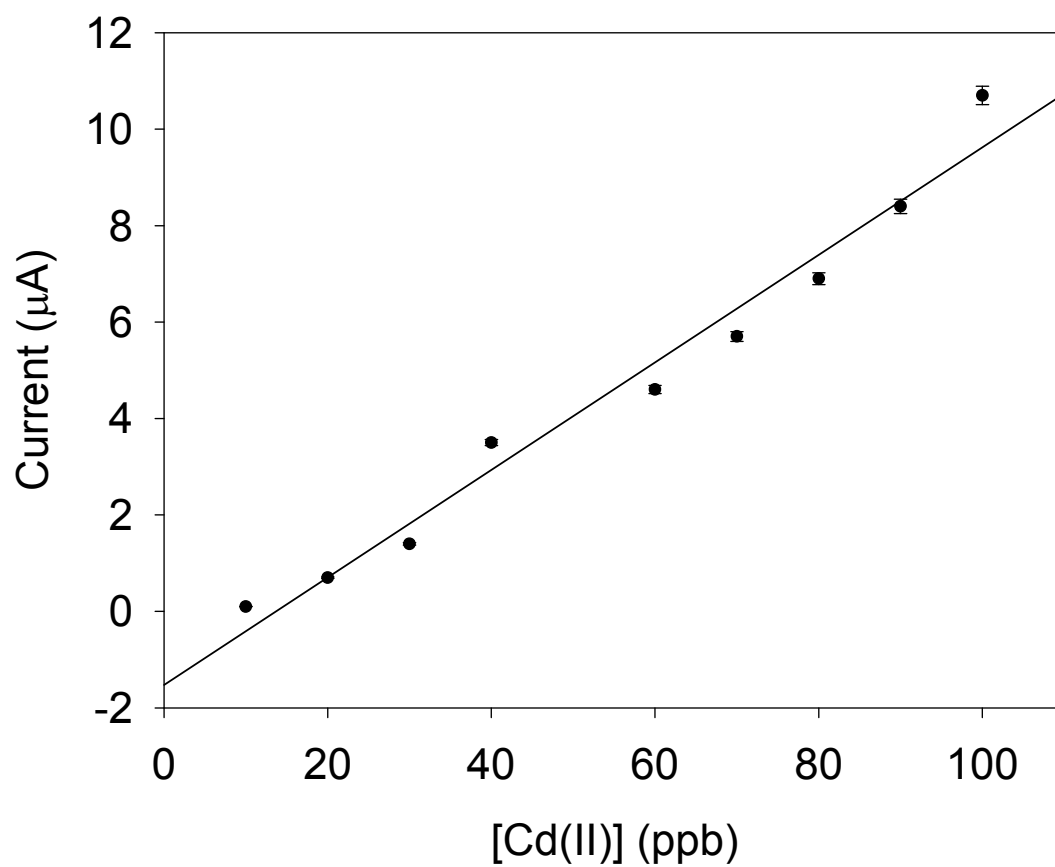


Figure 3.5. Calibration plot for Cd(II) detected at the BiBE.

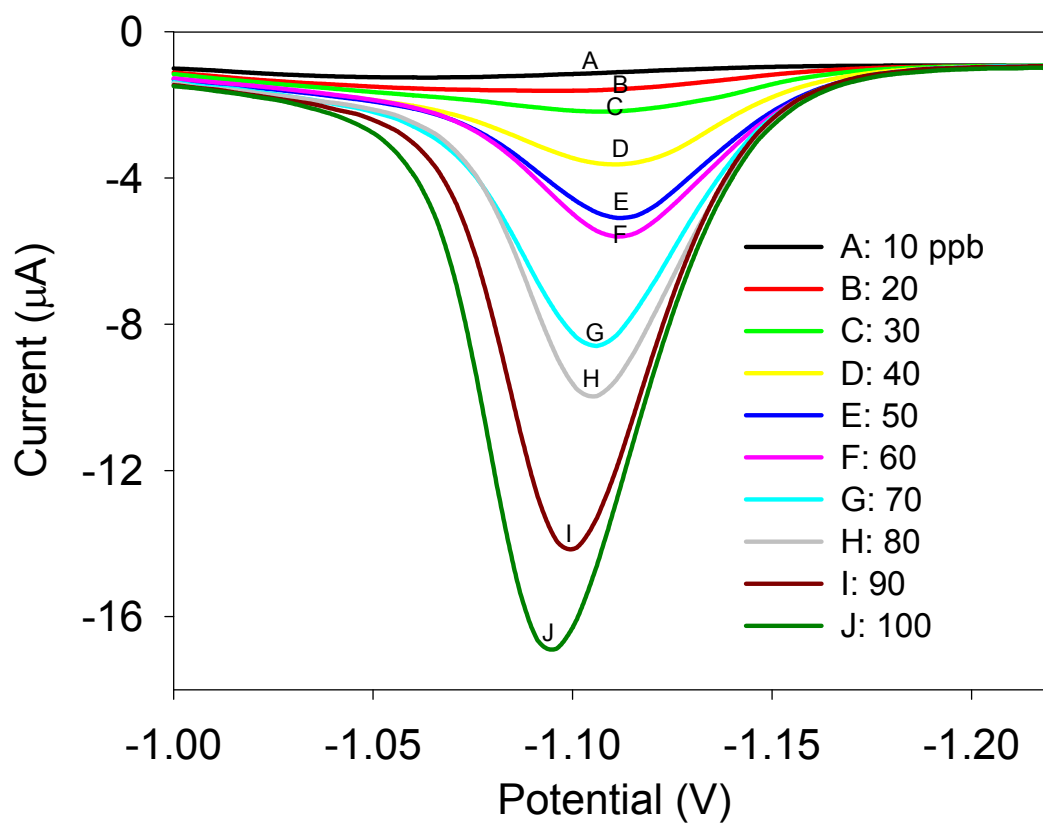


Figure 3.6. ASV calibration of Zn(II) detected at the BiBE.

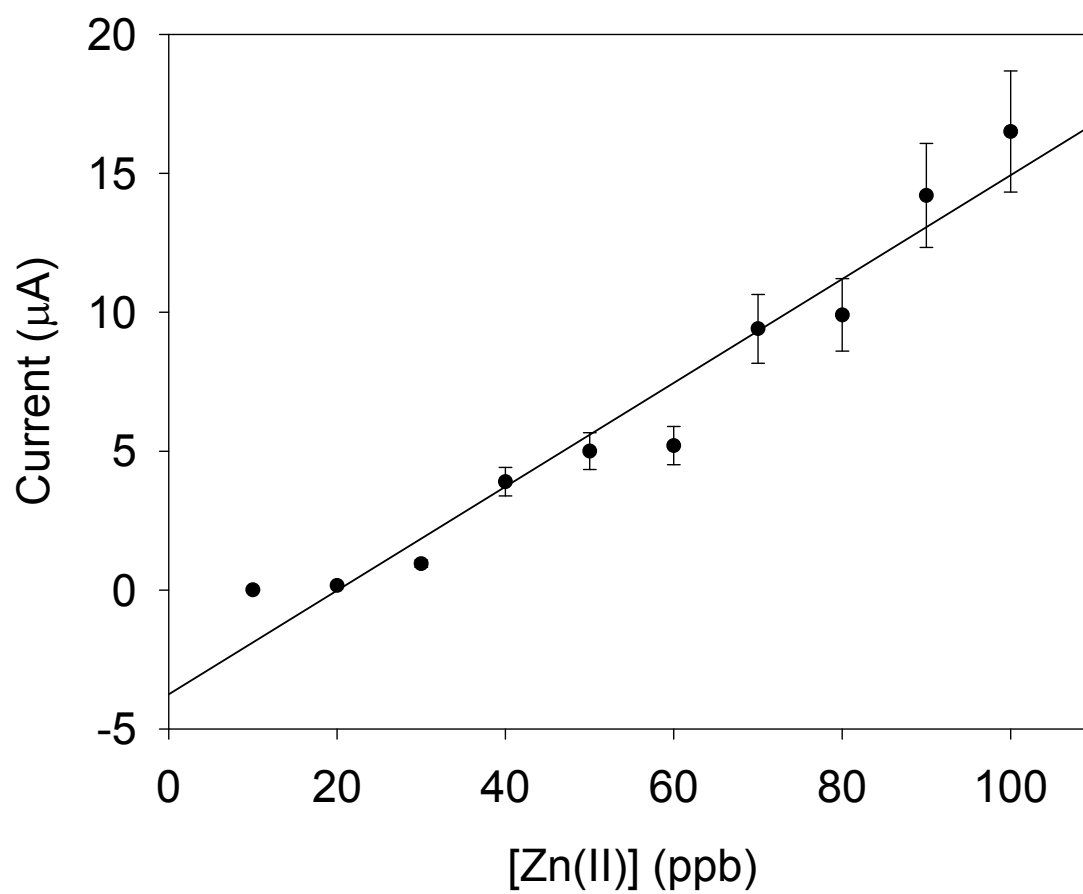


Figure 3.7. Calibration plot for Zn(II) detected at the BiBE.

3.3.2. Simultaneous Determination of Pb(II), Cd(II), and Zn(II)

Simultaneous determination of the metals was performed according to the same parameters determined for the individual experiments. Mixtures containing 10-100 ppb of each of the three metals were tested. Figure 3.8 is a voltammogram of mixtures of Pb(II), Cd(II), and Zn(II). Calibration plots for the three metals in the mixtures give slopes and values of the correlation coefficient R^2 (Table 3.1).

3.3.3. Comparison of Individual and Simultaneous Determination

Table 3.1 lists the R^2 values and slopes for the calibration plots of both individual and simultaneous analyses. For both determinations, the E_{peak} values occur at the same potential and R^2 values are similar.

When comparing individual to simultaneous analyses, it is worth noting the sensitivities of each metal calibrated individually vs. in the mixture. The sensitivity of the Zn(II) determination remains relatively unchanged. However, both Cd(II) and Pb(II) sensitivities experience significant drop when in a mixture. This finding prompted further studies, aimed at examining the interactions among the metals in a mixture.

Thus, a set of experiments was designed such that solutions containing individual metals were compared against solutions containing all three metals, with varying accumulation potentials. Solutions containing the individual metals were tested with an accumulation potential slightly more negative than their respective stripping peak potentials. The accumulation potentials were held at

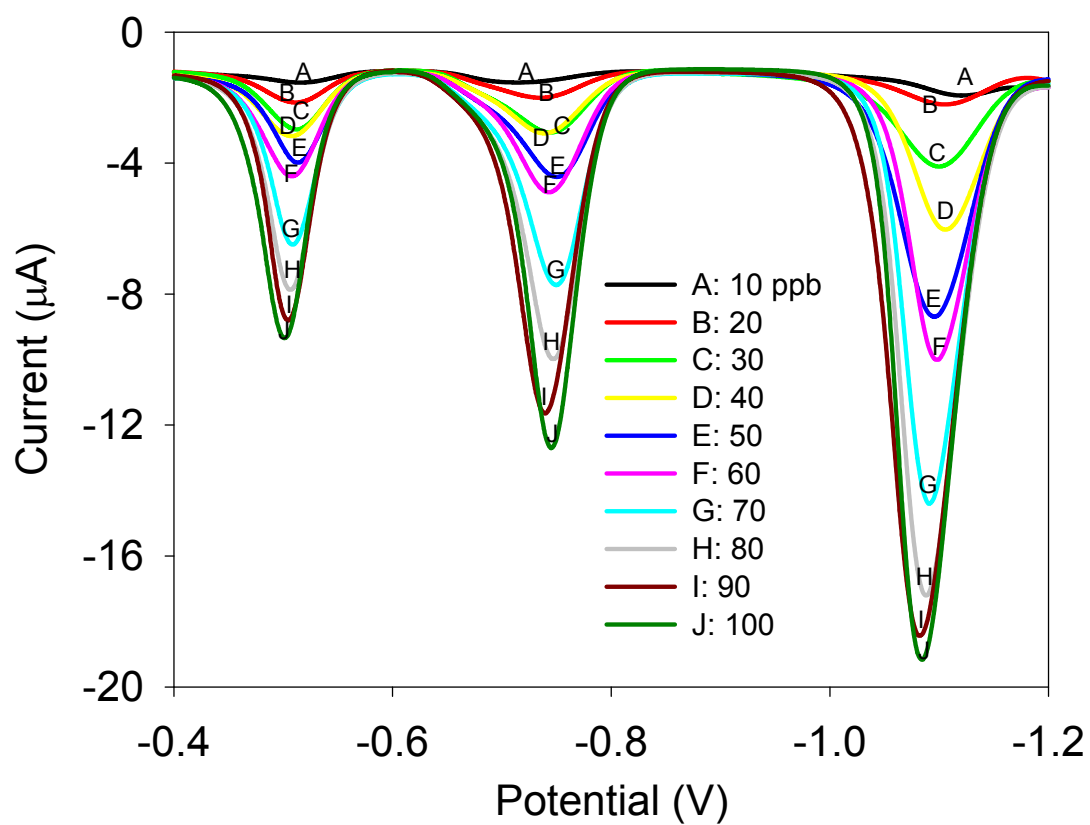


Figure 3.8. ASV calibration of mixed metals. The solutions contained Pb(II) (-0.50 V), Cd(II) (-0.75 V), and Zn(II) (-1.10 V).

Table 3.1. R^2 and slope values for Pb(II), Cd(II), and Zn(II) detected simultaneously and individually at the BiBE.

Individual	R^2	Sensitivity ($\mu\text{A/ppb}$)
Pb(II)	0.963	0.125
Cd(II)	0.973	0.112
Zn(II)	0.950	0.187
Mixture	R^2	Sensitivity ($\mu\text{A/ppb}$)
Pb(II)	0.975	0.071
Cd(II)	0.987	0.099
Zn(II)	0.953	0.185

-0.6, -0.9, and -1.2 V for Pb(II), Cd(II) and Zn(II) solutions, respectively.

Solutions containing all three metals (20-100 ppb each) were also tested at accumulation potentials of -0.6, -0.9, and -1.2 V, and compared to the individual metals. Aside from varying accumulation potentials, all electrochemical parameters for testing remained the same as was used for the SWV calibrations mentioned earlier in this paper.

Results of these tests are shown in Table 3.2. When comparing the sensitivities of individual metal calibrations to the sensitivities of mixed metal calibrations, a significant change in slope is only observed in a certain metal if another metal was stripped prior to that particular metal. If the accumulation potential of a metal in a mixture was such that the metal of interest was stripped first, then its sensitivity remains roughly the same as compared to an individual calibration.

For instance, the stripping sensitivity of Pb(II) calibrated individually, with an accumulation potential of -0.6 V, is 0.030 ± 0.003 $\mu\text{A/ppb}$. In a mixture containing Pb(II), Cd(II), and Zn(II), with an accumulation potential of -0.6 V, the stripping sensitivity for Pb(II) is 0.026 ± 0.001 $\mu\text{A/ppb}$. This sensitivity is within the range of deviation of the Pb(II) stripping sensitivity in the individual calibration. This is because, at an accumulation potential of -0.6 V, no Cd(II) or Zn(II) are being accumulated; thus no Cd(0) or Zn(0) are being stripped. However, when the accumulation potential is set to -1.4 V, the stripping sensitivity of Pb(II) individually is 0.125 $\mu\text{A/ppb}$, vs. a sensitivity of 0.071 $\mu\text{A/ppb}$ in a mixture. At an accumulation potential of -1.4 V, all three metals, Pb(II),

Table 3.2. Deposition potential and stripping sensitivities of Pb(II), Cd(II), and Zn(II) individually vs. in a mixture.

Individual	Deposition potential (V)	Sensitivity ($\mu\text{A/ppb}$)
Pb(II)	-1.4	0.125 ± 0.008
Pb(II)	-0.6	0.030 ± 0.003
Cd(II)	-1.4	0.112 ± 0.005
Cd(II)	-0.9	0.0055 ± 0.0005
Zn(II)	-1.4	0.187 ± 0.017
Zn(II)	-1.2	0.038 ± 0.008
Mixture	Deposition potential (V)	Sensitivity ($\mu\text{A/ppb}$)
Pb(II)	-1.4	0.071 ± 0.007
Pb(II)	-0.6	0.026 ± 0.001
Cd(II)	-1.4	0.099 ± 0.010
Cd(II)	-0.9	0.0042 ± 0.0008
Zn(II)	-1.4	0.185 ± 0.012
Zn(II)	-1.2	0.037 ± 0.005

Cd(II), and Zn(II), are accumulated and stripped. Zn(0) and Cd(0) are stripped off before Pb(0), and in the process of stripping, are also removing a significant portion of the accumulated Pb(0), as well, causing a 43% drop in sensitivity for Pb(II), in this particular case. A similar trend for Cd(II) can be observed, and the sensitivity for Zn(II) stripping remains relatively unchanged when calibrated individually vs. in a mixture.

It can be noted that, as is well known for ASV, the sensitivity is a strong function of the deposition potential. Accordingly, the river water analyses described below were carried out using a standard addition protocol and a deposition potential of -1.4 V.

Another factor that could account for the differences in slopes between simultaneous and individual calibrations is the interactions among the heavy metals when placed together in a mixture. Work by Manivannan and coworkers showed that, in the presence of Pb(II), peak currents for Cd(II) were cut in half, as compared to a calibration of Cd(II) alone.²⁰ Thus, it can be assumed that, in a mixture, interactions among various heavy metals can diminish the current response for each metal.

3.3.4. Optimization of the Analytical Signal

Different accumulation times were tested for optimal peak formation. An accumulation time of 180 s was found to be best. At times lower than this, peak heights were lower; at longer accumulation times, the peaks became distorted, possibly due to saturation of the electrode.

Stirring was another important issue that was studied. Slow stirring resulted in much smaller peaks than stirring at full speed. This is due to the increased convective mass transport to the electrode surface.

3.3.5. Electrode Stability

After extensive use of the BiBE for multiple runs, misshapen and broad peaks were observed, as shown in Figure 3.9. Upon examination, the BiBE surface, which, upon fresh polishing has a metallic luster, appeared dull with some black spots after multiple runs. This is believed to be the formation of a bismuth oxide.^{16,21,22} A Pb(II) calibration run at a freshly polished BiBE (Figure 3.2) shows a single, well-defined peak. The presence of the double peak for Pb(II), which is observed only after extensive use of the BiBE, indicates that the BiBE surface was altered by the suspected oxide formation. The double peak suggests that Pb(II) exists in two different adsorption states, perhaps associated with the formation of the bismuth oxide. From these data, it is estimated that 30 samples could be run consecutively before the altered state of the electrode distorts the ASV response. At this point, an additional polishing step would be needed to regenerate the electrode surface. This is comparable to Pb(II), Cd(II), and Zn(II) analyses done at other electrodes. Kefala et al. stated that only small drifts in peak height were observed after twenty consecutive measurements at a Nafion-coated BiFE, indicating satisfactory stability. Similar results were seen by Lee, et al. Twenty consecutive measurements of 30 ppb of Pb(II) and Cd(II) at a Bi nano-powder electrode resulted in very low RSDs.

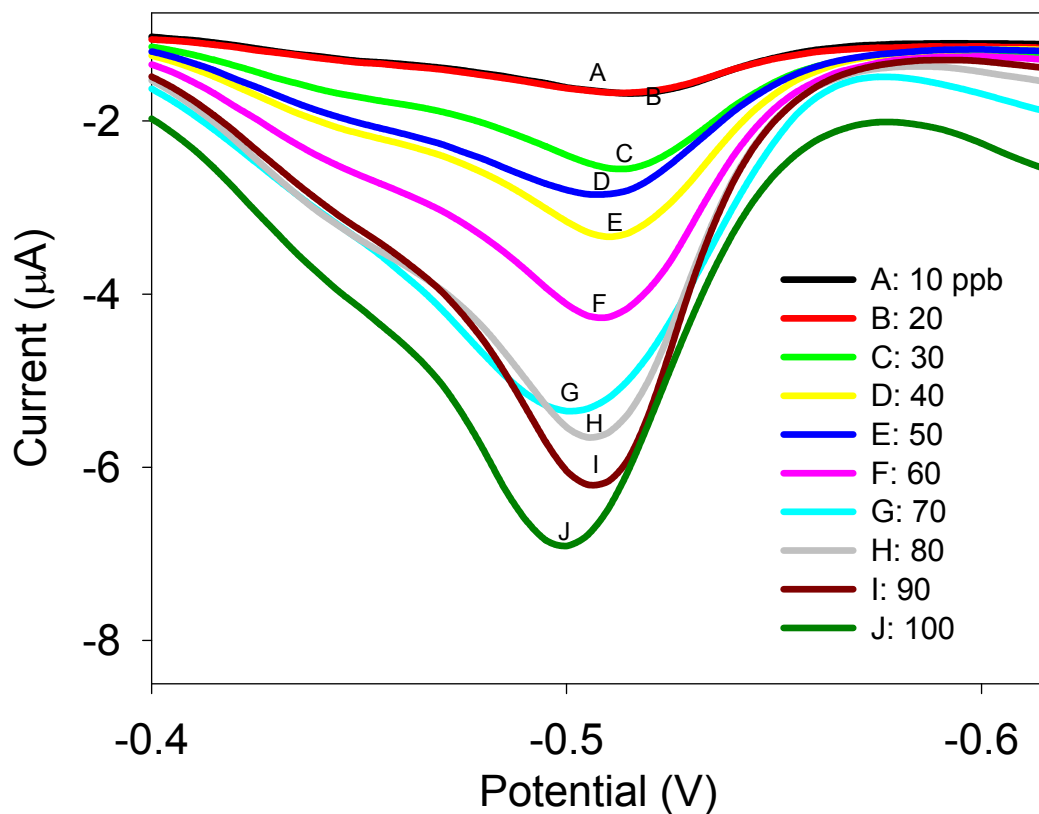


Figure 3.9. ASV calibration of Pb(II) at the BiBE after 30 consecutive runs. A 1.5 mm BiBE was used in standards ranging from 10 to 100 ppb Pb(II) in 0.1 M sodium acetate at pH 5.0. An accumulation period of 180 s at -1.40 V was used, with a stirring at 1200 rpm. Immediately after accumulation, metals were stripped using a SWV scan from -1.40 to -0.35 V.

3.3.6. Dynamic Range and Detection Limits

Using a 180 s accumulation time, the limit of detection calculated for each metal (3σ) is 105 ppt for Pb(II), 54 ppt for Cd(II), and 396 ppt for Zn(II) for the 1.5 mm diameter electrode. The limit of quantification (10σ) was calculated to be 350 ppt for Pb(II), 180 ppt for Cd(II), and 1.32 ppb for Zn(II). The dynamic range was 10-100 ppb for all metals.

When comparing this work to Pb(II), Cd(II), and Zn(II) analyses done in the past by others, it appears that the BiBE offers one of the lower detection limits for these metals. Table 3.3 summarizes past work on ASV of Pb(II), Cd(II), and Zn(II) at various electrodes.

Some recent research efforts have focused on the on-line combination of electrochemistry for pre-concentration with detection by, e.g., ICP-MS for various heavy metals as a means of enhancing sensitivity over traditional spectroscopic techniques.^{32,33} ASV, generally at a thin film mercury electrode, serves to enhance the signal by pre-concentrating the metals onto the electrode surface. Immediately following pre-concentration, an oxidative scan is performed, stripping the metals from the surface. This solution is diverted out of the electrochemical flow cell and into a cell used for MS or ICP-MS detection. The coupling of electrochemical pre-concentration on a mercury electrode with MS or ICP-MS has been shown to greatly enhance the sensitivity over traditional spectroscopic methods, improving the signal-to-noise ratio by factors up to 11.³² In comparison with our results here, it seems that this pairing does appear to enhance the detection limit over traditional electrochemical techniques alone. A

Table 3.3. Summary of past efforts on ASV detection of Pb(II), Cd(II), and/or Zn(II) at various electrodes. *Denotes potential vs. SCE.

Author	Detected Metals	Electrode Type	Accumulation Time and Potential	Stripping Technique	Detection Limit (ppb)	Ref.
Rico et al.	Pb(II), Cd(II), Zn(II)	Bi nanoparticles on screen-printed C	-1.4 V, 120 s	SWASV	Pb(II) = 0.9 Cd(II) = 1.3 Zn(II) = 2.6	23
El Tall et al.	Pb(II), Cd(II), Zn(II), Cu(II)	B-doped diamond	-1.7 V*, 60 s	DPASV	Pb(II) = 1.15 Cd(II) = 0.36 Zn(II) = 1.6	3
Rehacek et al.	Pb(II), Cd(II), Zn(II)	Disc graphite BiFE	-1.3 V, 120 s	SWASV	Pb(II) = 0.497 Cd(II) = 0.325 Zn(II) = 0.785	24
Lee et al.	Pb(II), Cd(II)	Bi nanopowder on C	-1.2 V, 3 min	SWASV	Pb(II) = 0.15 Cd(II) = 0.07	25
Svancara et al.	Pb(II), Cd(II)	Bi film-C paste	-0.95 V, 120 s	SWASV	Pb(II) = 0.8 Cd(II) = 1.0	4
Hwang et al.	Pb(II), Cd(II), Zn(II)	Bi-C nanotube	-1.4 V*, 300 s	SWASV	Pb(II) = 1.3 Cd(II) = 0.7 Zn(II) = 12	5
Wu et al.	Pb(II), Cd(II), Zn(II)	Bi/poly(p-ABSA)	-1.4 V, 240 s	DPASV	Pb(II) = 0.80 Cd(II) = 0.63 Zn(II) = 0.62	26
McGraw et al.	Pb(II), Cd(II), Zn(II), Cu(II), Ag(I)	B-doped diamond	-0.8 V (Pb), -1.0 V (Cd), -1.3 V (Zn)	DPASV	Pb(II) = 5.0 Cd(II) = 1.0 Zn(II) = 50	27
McGraw et al.	Pb(II), Cd(II), Zn(II), Cu(II), Ag(I)	MFE	-0.8 V (Pb), -1.0 V (Cd), -1.3 V (Zn)	DPASV	Pb(II) = 5.0 Cd(II) = 1.0 Zn(II) = 10	27
Siriangkhwut	Pb(II), Cd(II)	BiFE	-1.10 V, 90 s	SWASV	Pb(II) = 6.9 Cd(II) = 1.4	28
Torma et al.	Pb(II), Cd(II), Zn(II)	NC(Bpy) BiFE	-1.4 V, 2 min	SWASV	Pb(II) = 0.077 Cd(II) = 0.12 Zn(II) = 0.56	29
Kefala et al.	Pb(II), Cd(II), Zn(II)	NC BiFE	-1.40 V	SWASV	Pb(II) = 2 Cd(II) = 2 Zn(II) = 6	30
Tesarova et al.	Pb(II), Cd(II)	Sb-CPE	-1.2 V, 120 s	SWASV	Pb(II) = 0.2 Cd(II) = 0.8	31

detection limit of 0.16 ppt was obtained for Cd(II) using this combination,³² which is lower than any electrochemical technique currently known, and far lower than the detection limit (1 ppb) attainable by ICP-MS alone. It should be noted that this technique used a pre-concentration time of 340 s vs. 180 s in the current work. Also, sophisticated instrumentation, plus flow cells and high efficiency nebulizers to connect the two different instruments, are needed to achieve the detection limit of 0.16 ppt. In comparison, the current work requires a simple standard electrochemical cell.

3.3.7. Analytical Application of the BiBE in River Water Samples

The BiBE was used to determine the Pb(II), Cd(II), and Zn(II) levels in two separate river water samples. The first sample was obtained from the Tennessee River in Knoxville, TN. The second sample was from the Emory River, located near Harriman, TN, obtained shortly after contamination from a massive coal fly ash spill.³³ Prior to analysis, the river water was acidified by nitric acid and filtered, with standard filter paper, to remove non-dissolved solids. Sodium acetate buffer was made by dilution in the treated river water. The pH was then adjusted to 5.0 with ammonium hydroxide. Standard additions of Pb(II), Cd(II), and Zn(II) were added, individually, to the river water, to a total volume of 25 mL. Pb(II) additions ranged from 0 to 12 ppb, Cd(II) from 0 to 4 ppb, and Zn(II) from 0 to 120 ppb. After a 240 s accumulation period, SWV was used for a stripping analysis as described above.

For the sample obtained from the Tennessee River, no Pb(II), Cd(II), or Zn(II) was found. It can be assumed that the quantity of each of these three metals in the sample was below the detection limit of the BiBE.

For the contaminated river water, obtained from the Emory River, peaks corresponding to Pb(II) and Zn(II) were observed, but no peak for Cd(II) was seen. A Cd(II) standard addition was run, again, on the same sample of water, using a larger concentration range of standards (0 to 100 ppb), obtaining an estimated Cd(II) concentration of 3.0 ± 6.0 ppb (Figure 3.10). Thus, it can be assumed that no Cd(II) is present in this particular river water sample. For the Zn(II) standard addition (Figure 3.11), the peak was misshapen and broad, resulting in poor linearity ($R^2 = 0.5297$). It can be assumed that, since the water was contaminated with a number of heavy metals and various other impurities, the presence of an interfering species prevented effective detection of Zn(II). Standard addition of Pb(II) (Figure 3.12) yielded an estimated concentration of 8.69 ± 0.72 ppb.

Electrochemical results for Pb(II) were validated using ICP-OES, which gave a concentration of 7.79 ± 0.48 ppb (Table 3.4). In the range from 0 to 4 ppb, linearity of Cd(II) analysis was poor. The detection limit of the instruments is not sensitive enough for reliable analysis in that particular range. Thus, this does match electrochemical results, and confirms that a negligible amount of Cd(II) is present in this particular water sample.

These results show that the potential applications of this electrode will depend on the nature of the sample being analyzed. In a normal river water

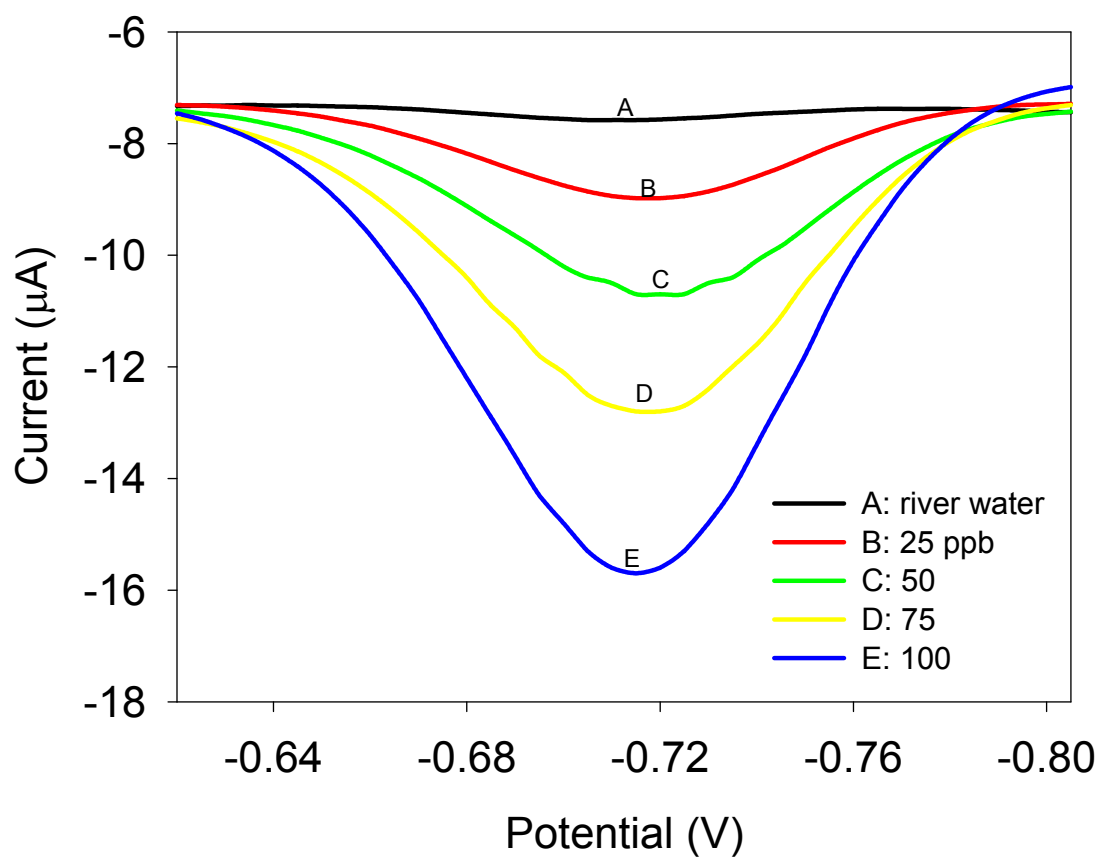


Figure 3.10. ASV of Cd(II) standard addition at the BiBE. Concentration of the Cd(II) additions ranged from 0 to 100 ppb.

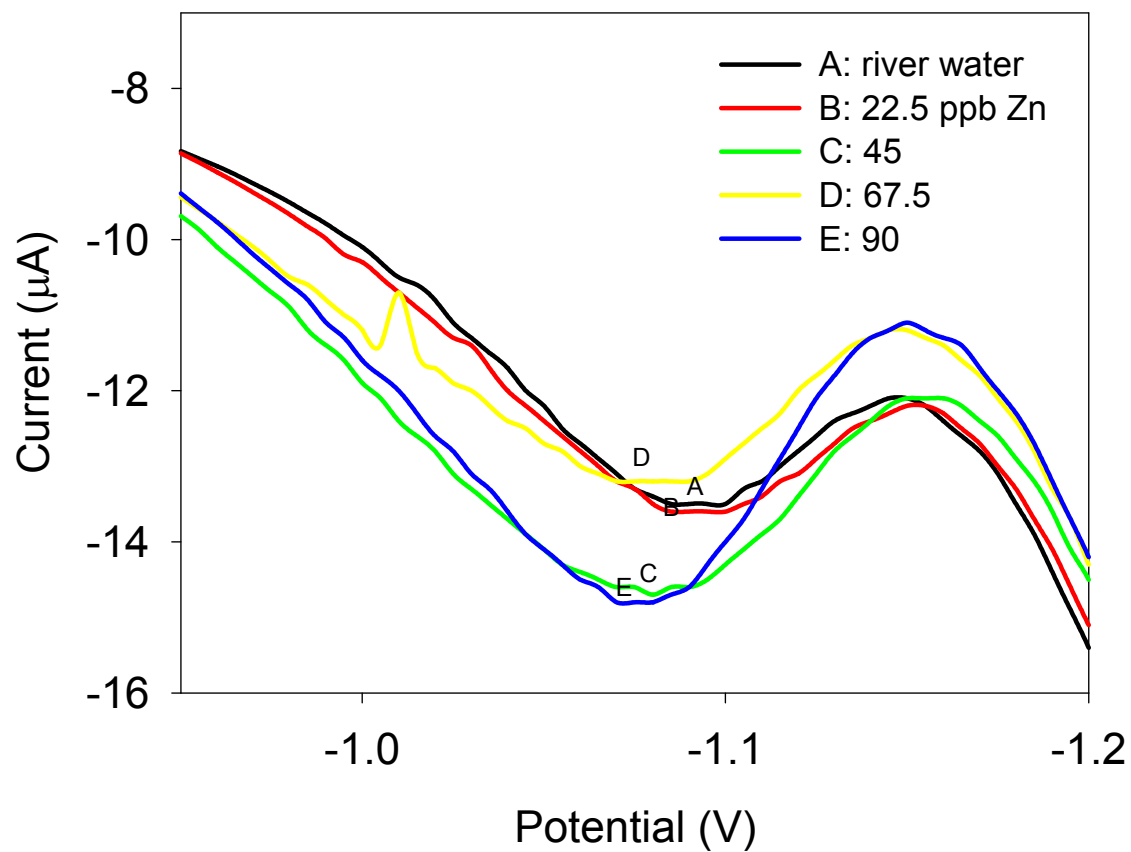


Figure 3.11. ASV of Zn(II) standard addition at the BiBE. Concentration of the Zn(II) additions ranged from 0 to 90 ppb.

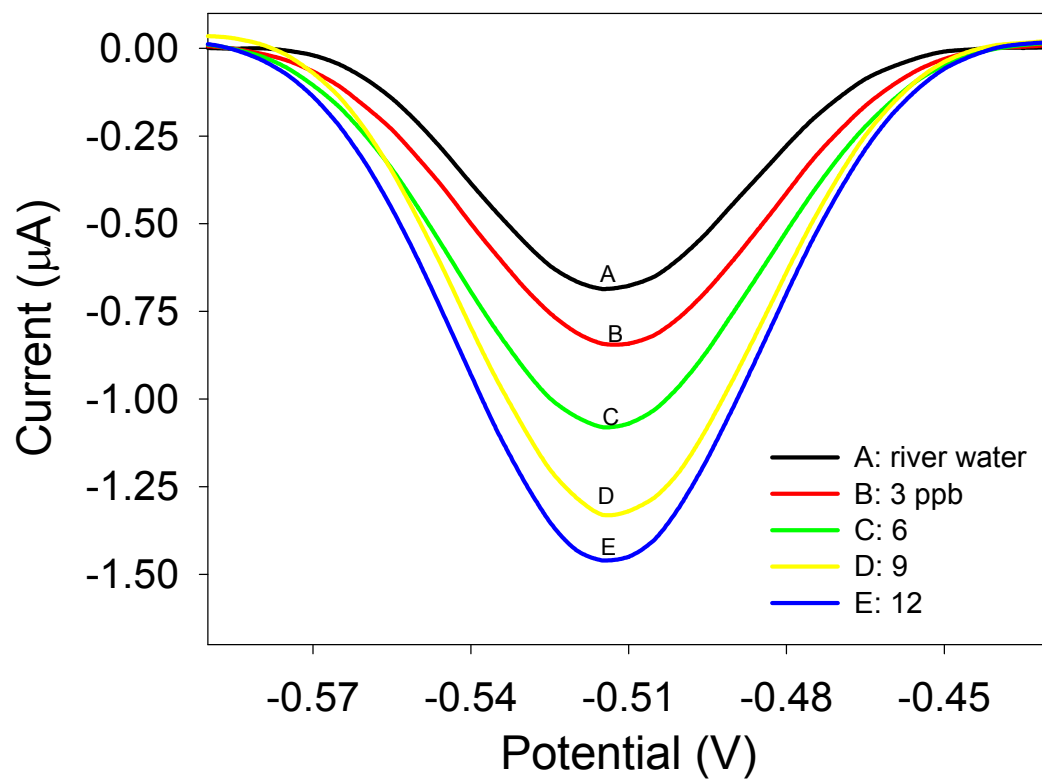


Figure 3.12. ASV of Pb(II) standard addition at the BiBE. Concentration of the Pb(II) additions ranged from 0 to 12 ppb.

Table 3.4. Comparison of ICP-OES and ASV analysis of Pb(II) using standard addition.

	[Pb(II)] (ppb)	R^2
ASV Analysis	8.69 ± 0.72	0.9665
ICP-OES	7.79 ± 0.48	0.9628

sample, such as that obtained from the uncontaminated Tennessee River, the levels of Pb(II), Cd(II), and Zn(II) are lower than the detection limits for both this electrode and ICP-OES. For analyses of this type of sample, where only ultra-trace levels of the analyte exist, a more sensitive technique will need to be employed, perhaps the online-combination of ASV with MS mentioned earlier.^{32,33} The BiBE is better suited for analyses in samples containing higher concentrations of the metal ions, such as water from the heavily-contaminated Emory River. Even in cases such as these, the possibility of interfering species, as with Zn(II) in our contaminated water sample, should be considered and evaluated prior to analyses. By using a standard addition method for sample quantification, matrix effects are significantly diminished, allowing for the potential application of the BiBE to the detection of many other metal ions.

3.4. Conclusions

The BiBE has been shown as a useful sensor for Pb(II), Cd(II), and Zn(II), with detection limits in the ppt range. The parameters for accumulation time and stirring were optimized. Calibrations resulted in good linearity for solutions containing not only the individual metals, but also all three metals at once, demonstrating the potential use of the BiBE as a multi-sensor for heavy metals. By analyzing river water samples, the capability of the BiBE for use in environmental analysis was demonstrated. Due to its inexpensive and easy fabrication, and extended electrode stability, the electrode is a desirable option

for heavy metal sensing, while avoiding the toxicity associated with mercury-based electrodes.

3.5. Additional Information

Kristie C. Armstrong, a previous member of our group, worked on this project.³⁵ Subsequent studies in this Part have completed the project. The results are reported in an article in *Talanta*.³⁶

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Part 4

Properties of Bismuth Electrodes and Their Impact on Electrochemical Chromium Detection

4.1. Introduction

While Cr(VI) is particularly well-known for being a carcinogenic agent and toxic pollutant, Cr(III) complexes have actually been used as dietary supplements for the treatment of diabetes and its complications.^{1,2} With over 16 million cases in the U.S. alone, and an estimated 800,000 new cases being diagnosed each year, diabetes is one of the most prevalent and fast-growing diseases in the U.S.³⁻⁶ In addition, Cr deficiency has been linked to increased risk of nonfatal myocardial infarction and cardiovascular disease.⁷⁻⁹

The frequency of actual chromium deficiency in the population is unknown in part because reliable measures for assessing chromium status in humans are limited.¹⁰ Biological levels of chromium in mammals are low, having been measured at 3-10 ppb (in blood).¹¹⁻¹⁷ There are currently three techniques that are capable of detecting Cr at levels sensitive enough for detection in biological samples from humans: neutron activation,⁷⁻⁹ mass spectrometry,¹⁰ and graphite furnace atomic absorption spectroscopy (GF-AAS).^{18,19} However, these techniques require costly instrumentation, are susceptible to interferences, and/or require a lengthy pre-treatment/analysis time. There is currently a need to develop a sensor that is capable of detecting Cr in the range required for human biological samples. In order to better understand the role of Cr(III) in diabetes and its treatment, the development of a robust, reproducible, inexpensive, and easily fabricated Cr(III) sensor for use in biological fluids is essential.

Electrochemical detection of Cr has traditionally been conducted at either a hanging mercury drop electrode (HMDE)²⁰ or mercury film electrode (MFE).²¹ While the detection is reproducible, and can be attained in the lower ppb/upper ppt range, the toxicity of the mercury makes it an undesirable choice for use in the medical industry. Thus, alternative electrode materials are being explored.

Among mercury alternatives, bismuth-based electrodes have emerged as a promising alternative for Cr analysis. Bi-based electrodes share many of the same electrochemical properties that make Hg so desirable for Cr detection. Bi is capable of forming fused alloys with heavy metals,²² and exhibits a high hydrogen overpotential,²³ making it an ideal candidate for this analysis.

Recent efforts in this research group have centered around this same objective. Among the electrode materials examined, we have studied electrochemical Cr analysis at the already well-established bismuth film electrode (BiFE)^{24,25} and bismuth bulk electrode. While the bulk electrode has yet to be shown effective for ultra-trace Cr analysis, we have found that the film electrode has shown success, to some degree. However, often times, attaining reproducible detection and Cr peaks that correlate directly to Cr concentration is difficult.

A correlation has been drawn between the thickness of the Bi film and the capability of obtaining a Cr peak. Bi film thickness has also been shown to directly influence oxidant (NO_3^-) activity during analysis. The failure of the BiBE as compared to the success of the Bi-modified electrodes has introduced

fundamental inquiries pertaining to the behavior and interactions of each electrode within the system of interest.

The fundamental electrochemical activity of the BiBE has been investigated, using cyclic voltammetry. Each species in the system of interest was added to acetate buffer, revealing many interactions among the species. By varying the thickness of the Bi film on a glassy carbon substrate, the effect of Bi quantity on both the oxidant activity and the resulting Cr analysis was observed. Lastly, impedance measurements and scanning electron microscopy (SEM) images were taken in order to assess basic physical characteristics of the electrodes. Impedance measurements were taken on a number of various Bi-based electrodes and compared to the impedance of a glassy carbon electrode. SEM images were taken of Bi deposited on glassy carbon plates in order to understand the morphology of the resulting film. Part of the research was conducted by a previous graduate student.²⁶ More in-depth studies in the current work provide an understanding of the properties of bismuth electrodes and their impact on electrochemical chromium detection.

4.2. *Experimental*

4.2.1. Chemicals and Materials

All voltammetric measurements were performed using a modulated CHI 440 potentiostat, and all impedance measurements were conducted using a CHI 650 (CH Instruments). A three-electrode configuration consisted of the working

electrode of choice (BiBE, GCE, BiFE), a Ag/AgCl reference electrode, and a platinum wire counter electrode.

Potassium nitrate (Certified ACS, Fisher), acetic acid (glacial, Fisher), sodium acetate (anhydrous, Certified ACS, Fisher), cadmium acetate (Certified ACS, Fisher), and diethylenetriaminepentaacetic acid (DTPA, $\geq 99\%$, Fluka) were used as received. Cr(VI) AA standard solution (1000 ppm, Aldrich) was diluted prior to use. The buffer solution contained 0.1 M sodium acetate and 0.25 M KNO_3 and its pH was adjusted to 6.0 with CH_3COOH . The DTPA solution (0.1 M) was prepared by dissolving DTPA in water while slowly adjusting pH to 6.0 with 25% NH_4OH (Fisher Scientific). Cr(VI) standards were prepared by diluting the appropriate amount of stock solution in electrolytes. All aqueous solutions were prepared in 18 M Ω deionized H_2O .

4.2.2. Electrodes

All glassy carbon electrodes used were purchased from Cypress Systems. Prior to use, GCEs were polished on a standard electrode polishing kit (CH Instruments). This included polishing on a 1200 grit Carbimet disk, 1.0 μm alumina slurry on a nylon cloth, 0.3 μm alumina slurry on a nylon cloth, and 0.05 μm alumina slurry on a microcloth polishing pad. After polishing, GCEs were sonicated, for 10 min each, in acetone (Certified ACS, Fisher), then ethanol (Decon Lab, Inc.), and finally deionized water.

Thin films were prepared according to the procedure outlined in the literature.²⁵ Basically, metallic Bi(s) was electrodeposited onto a GCE substrate

using a 1.245 ppm Bi(III) AA standard solution (1000 ppm, Fluka) in 0.1 M sodium acetate buffer (pH 4.5). The electrode potential was held at -1.2 V for 120 s while stirring the Bi(III) plating solution.

To make the thicker film electrodes, an appropriate weight of Bi(s) needles was dissolved in concentrated HCl (Certified ACS, Fisher) and HNO₃ (Certified ACS, Fisher). After the Bi needles dissolved, KBr was added as an electrolyte, and the solution was dissolved in deionized water to give a concentration of 0.02 M Bi and 0.5 M KBr. Photographs of the films were taken on a standard optical microscope using a 5x zoom and a Cannon Powershot 640A digital camera.

The BiBE was fabricated according to the procedure outlined in Section 3.2.2. Between uses, it was polished on the last polishing pad with the alumina slurry and rinsed with DI H₂O.

4.2.3. Procedure

All CAdSV measurements of Cr were done using the electrochemical procedure proposed by Wang and coworkers for the BiFE.²⁷ This procedure, which was adopted for Cr(VI) detection in blood by this research group,²⁵ uses a 0.1 M acetate buffer at pH 6. In the procedure, DTPA is added to a solution containing Cr(VI) immediately prior to analysis. During a 120 s quiet time, the Cr(VI) is reduced to Cr(III). Cr(III) then complexes with DTPA, and the complex is pre-concentrated onto the electrode surface. A square wave voltammetric scan starts immediately after complex accumulation, from -0.8 to -1.4 V. This scan reduces the Cr(III)-DTPA complex to a Cr(II)-DTPA complex. Nitrate, the

oxidant in this case, re-oxidizes the Cr(II)-DTPA to Cr(III)-DTPA, thus instituting a catalytic cycle, which amplifies the current response, providing optimal sensitivity.

4.2.4. Fundamental Electrochemical Activity of the Bi Bulk Electrode

The BiBE was polished before each use following the method outlined in the previous section to ensure a fresh bismuth surface. A conditioning step was developed and found to provide more reproducible results when used immediately after polishing. The electrode was cycled in a 0.1 M acetate buffer solution containing 0.25 M KNO₃ (pH 6.0). The conditioning step included cycling 0 to -1.4 V (50 scans) with a scan rate of 50 mV/s. A solution of cadmium was measured by CV to determine if the electrode was well conditioned and ready for experiments.

Cyclic voltammetry (CV) experiments were performed to characterize the BiBE and to study its interactions with DTPA, Cr(VI), and Cr(III). The CVs were performed under the same conditions as the conditioning step.

4.2.5. Physical Properties of Bi-Based Electrodes

AC impedance measurements were taken on six different types of electrodes, each in the presence of two different redox couples. Both the ferrocyanide couple [Fe(CN)₆^{3-/4-}] and the copper bipyridine couple [Cu(bpy)₂^{2+/1+}] were used at a concentration of 5 mM, with 0.1 M KCl (Mallinkrodt) as the electrolyte.

To make the ferrocyanide couple, an appropriate amount of both potassium ferrocyanide (ACS Certified, Baker) and potassium ferricyanide (ACS Certified, Baker) were dissolved in deionized water and electrolyte. The ratio of Fe^{2+} to Fe^{3+} was 1:1. Prior to testing the impedance, the open circuit potential of the couple was tested. This potential was recorded and used as the potential for the impedance measurements.

To make the copper bipyridine couple, the procedure was followed as outlined in previous work by Heineman and co-workers.²⁸ An appropriate amount of CuSO_4 (ACS Certified, Baker) and a 4-fold excess of the ligand, 2,2'-dipyridyl (bpy) ($\geq 99\%$, Aldrich), were dissolved in deionized water and electrolyte. Prior to testing the impedance, a cyclic voltammogram was run for the couple, and the oxidation and reduction potentials were used to calculate an open circuit potential, which was used for impedance measurements.

Images were taken using a Canon PowerShot A630 digital camera and a Cambridge Instruments StereoZoom 7 optical microscope (zoom range of 1.0-7.0 $\times$). SEM images were taken of a thin Bi film deposited onto a glassy carbon plate (1 mm thick, 50 x 50 mm², Alfa Aesar) prepared in the same manner as a thin film BiFE on a GCE substrate. All SEM images were taken at the Center for Nanophase Materials Science (CNMS) at Oak Ridge National Laboratory (ORNL). Images were obtained using a Hitachi 4700 Scanning Electron Microscope.

4.3. Results and Discussion

4.3.1. Fundamental Electrochemical Activity of the Bismuth Bulk Electrode

A bismuth bulk electrode was developed as an alternative to a bismuth film electrode for electrochemical Cr analysis. Since a Bi film has been shown to be very fragile,²⁹ the BiBE was thought to offer a more robust alternative for Cr detection. Little work has been done using a BiBE^{30,31} but some characteristic studies of the electrode have been performed.³² Pauliukaite and coworkers introduced the BiBE as a possible alternative electrode material for heavy metal sensing. Studies were conducted which highlighted both the cathodic electrochemical behavior and the behavior under anodic conditions. In addition to testing the electrochemistry of various well-known redox couples, both inorganic and organic compounds were also tested at the BiBE.

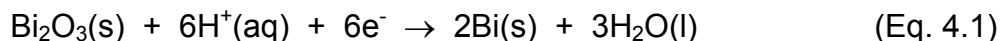
Due to the poor reproducibility and fragility of the BiFE, efforts in this research group included detection of Cr at the BiBE. However, repeated attempts consistently showed that, for trace amounts of Cr(VI), only a NO_3^- peak was seen and no Cr peak was observed (Section 4.3.3.3 and Figure 4.5). It was deduced that the BiBE was not a viable material for electrochemical Cr detection.

These results prompted further characteristic studies of the BiBE. Employing cyclic voltammetry, the behavior of BiBE in various solutions was investigated. Results are given below with a discussion providing an understanding of the differences between the BiBE and BiFE in the Cr detection.

4.3.1.1. BiBE in Acetate Buffer

A multi-sweep CV obtained at a BiBE in 0.1 M acetate buffer is shown in Figure 4.1. This behavior is consistent with previous work. The presence of two anodic peaks in the oxide formation region has been previously attributed to a complex nucleation phenomenon by Williams and Wright.³³

During the forward sweep in the negative direction, there is a very large peak occurring at -0.40 V, due to the reduction of bismuth oxide to bismuth. According to Williams and Wright, this cathodic reduction of the bismuth oxide causes the surface to roughen, leaving a porous surface consisting of metallic bismuth. The reduction of the anodically formed bismuth oxide to metallic bismuth is given in the following reaction:



There is a small cathodic tail that follows the reduction of bismuth oxide which can be observed at approximately -0.9 V. This cathodic tail is due to bismuth oxide reduction at a pitted or porous surface, which is formed by the oxidation and dissolution of the bismuth on the electrode. Electron micrographs have confirmed the formation of a porous Bi(s) layer upon reduction of the bismuth oxide.³¹ The current begins to increase around -1.35 V as a result of hydrogen evolution, which can cause additional roughening of the surface.³³⁻³⁵ Should the potential sweep be reversed before any occurrence of hydrogen

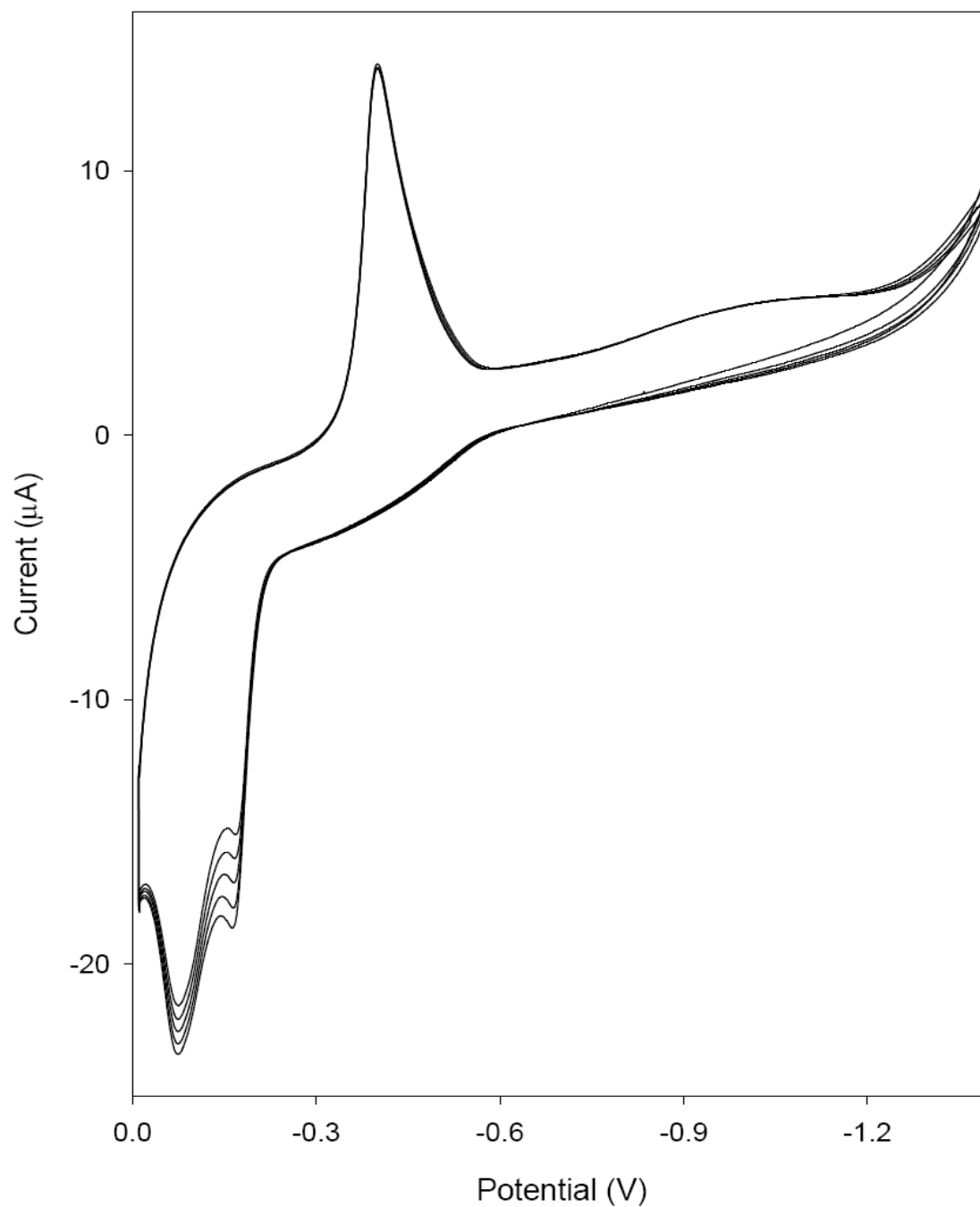


Figure 4.1. CV of BiBE in 0.1 M acetate buffer at pH 6.0. A scan rate of 50 mV/s was used.

evolution, there will be a gradual disappearance of the first of the two anodic peaks, while the current of the second anodic peak increases.³³

The working potential window for this electrode is from 0.0 to -1.4 V. At potentials above 0.0 V, a thick bismuth oxide layer is formed. The shiny surface of the bismuth turns black as a result of the oxidation. The potential window can be expanded to more negative potentials, up to -1.7 V, if the pH of the electrolyte solution is increased to approximately 13.³²

During the return sweep, there are large anodic peaks in the range from -0.2 to 0.0 V. As stated by Williams and Wright,³³ two anodic peaks in this region are common, and any variations are due to the differences in the nature of the surface. The formation of these anodic peaks is the result of a diffusion-controlled process resulting in the nucleation and spreading of the oxide nuclei over the surface.³⁵⁻³⁷ If the potential sweep were to continue beyond 0.0 V, a thickening of the anodic oxide film would occur.³⁵ The formation and nucleation of bismuth oxide is represented by the reverse reaction in Eq. 4.1.

There is a small, broad peak around -0.45 V which appears to be coupled to the cathodic tail present at -0.9 V. This is simply the oxidation of the produced porous bismuth.

4.3.1.2. Cyclic Voltammetry of BiBE in Presence of DTPA

The exact role of DTPA in the mechanism of electrochemical Cr(VI) detection is not fully understood. It is unclear if DTPA is bound to the electrode surface by physical adsorption or by covalent bonding. The following

experiments were performed in order to gain a better understanding of the interactions of the BiBE with the chemicals in the system of interest.

Experiments similar to those of Sander and coworkers were conducted, using CV.³⁸ Sander and coworkers examined DTPA binding with Cr(VI) and Cr(III) at a HMDE. In this series of experiments, CV was used to study the electrochemistry of solutions of acetate buffer, Cr(III), and Cr(VI) both in the absence and presence of DTPA. Cr(III) and Cr(VI) (0.25 mM) in 0.1 M acetate buffer was used.

DTPA at the BiBE

In Figure 4.2, CVs of acetate buffer without (blue) and with (red) DTPA are shown. The CVs show that a soluble Bi(III)-DTPA complex is formed when the BiBE is oxidized.

The CV of only acetate buffer and no DTPA is in accordance with what is expected. The familiar peak for bismuth oxide reduction is present at -0.4 V, as well as that for the oxidation/nucleation of bismuth around -0.2 to 0 V.

When DTPA is added to the solution, the reduction peak at -0.4 V disappears, and the oxidation peak from -0.2 to 0.0 V increases significantly. Also, the oxidation peak around -0.45 V is attenuated in the presence of DTPA.

The absence of the reduction peak for bismuth oxide suggests that no bismuth oxide is being formed in the presence of DTPA. When the BiBE is immersed into a solution containing DTPA, the oxidized Bi(III) is complexed by the ligand, forming a soluble Bi(III)-DTPA complex (Eq. 4.2).^{39,40} The formation

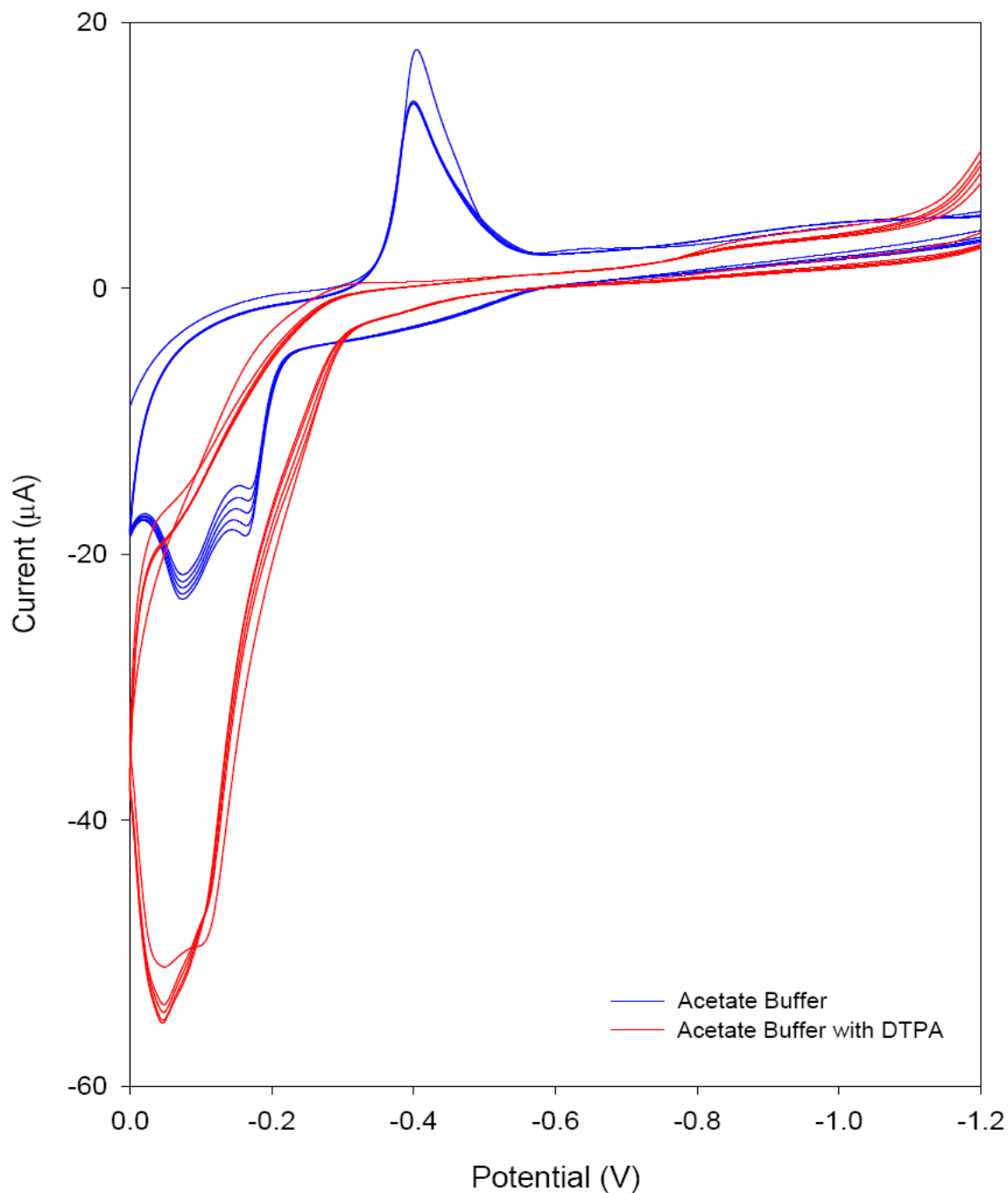
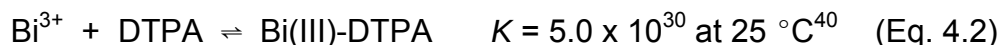
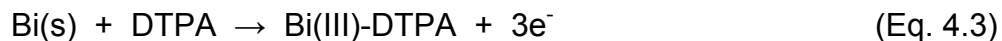


Figure 4.2. CVs of acetate buffer with and without DTPA at the BiBE. The solution consisted of 0.1 M NaOAc at pH 6. Upon addition to the acetate buffer, DTPA concentration was 5 mM. A scan rate of 50 mV/s was used.

of this complex is thermodynamically favored, with a large stability constant of $K = 5.0 \times 10^{30}$ at 25 °C, explaining the disappearance of the reduction peak for bismuth oxide.



During the anodic sweep, the increase in peak current from -0.2 to 0 V is also due to the oxidation of Bi(0) to Bi(III) and subsequent binding of DTPA to Bi(III) to form Bi(III)-DTPA. Because of the formation of the complex Bi(III)-DTPA, it is thermodynamically more favorable to oxidize Bi(0) to Bi(III) (Eq. 4.3) in comparison to the oxidization of Bi(0) to Bi₂O₃ in absence of DTPA, leading to the increased current in this region. In other words, the presence of DTPA promotes the oxidation of Bi(0) metal to Bi(III) through the formation of the Bi(III)-DTPA complex. Compared to just the BiBE in acetate buffer in the absence of DTPA, this peak shifts slightly in the more negative direction, and increases in current.



An increase in current is observed at -1.1 V, prior to hydrogen evolution, upon the addition of DTPA. This current is due to the reduction of the newly formed Bi(III)-DTPA complex.

Cr(III) at the BiBE

Behavior of Cr(III) at the BiBE without (blue) and with (red) DTPA are shown in Figure 4.3. In the solution containing Cr(III) and no DTPA, the expected peaks attributed to the BiBE behavior are, again, observed. We see the reduction of bismuth oxide at -0.4 V and the oxidation/nucleation of bismuth from -0.2 to 0 V.

When DTPA is added, the reduction peak for bismuth oxide at -0.4 V is absent, as expected. The peak current for bismuth oxidation from -0.2 to 0 V also increases again, suggesting the oxidation of Bi(0) and the formation and oxidation of the Bi-DTPA complex. The peak around -0.8 V is due to the reduction of Cr(III)-DTPA. The electroactive form of this complex is a kinetic intermediate. Thus there may be no correlation with concentration. This reduction process has been shown to be irreversible at GCEs.³⁸

Also, a peak appears at -1.2 V upon the addition of DTPA. This is attributed to the reduction of the Bi(III)-DTPA complex, which is the reverse reaction in Eq. 4.3.

Cr(VI) at the BiBE

CV experiments gave an insight into the interaction of Cr(VI) with DTPA and the BiBE. Figure 4.4 includes voltammograms of Cr(VI) solutions without (blue) and with (red) DTPA. In the scans without DTPA, the expected peaks associated with bismuth oxidation/nucleation and bismuth oxide reduction were

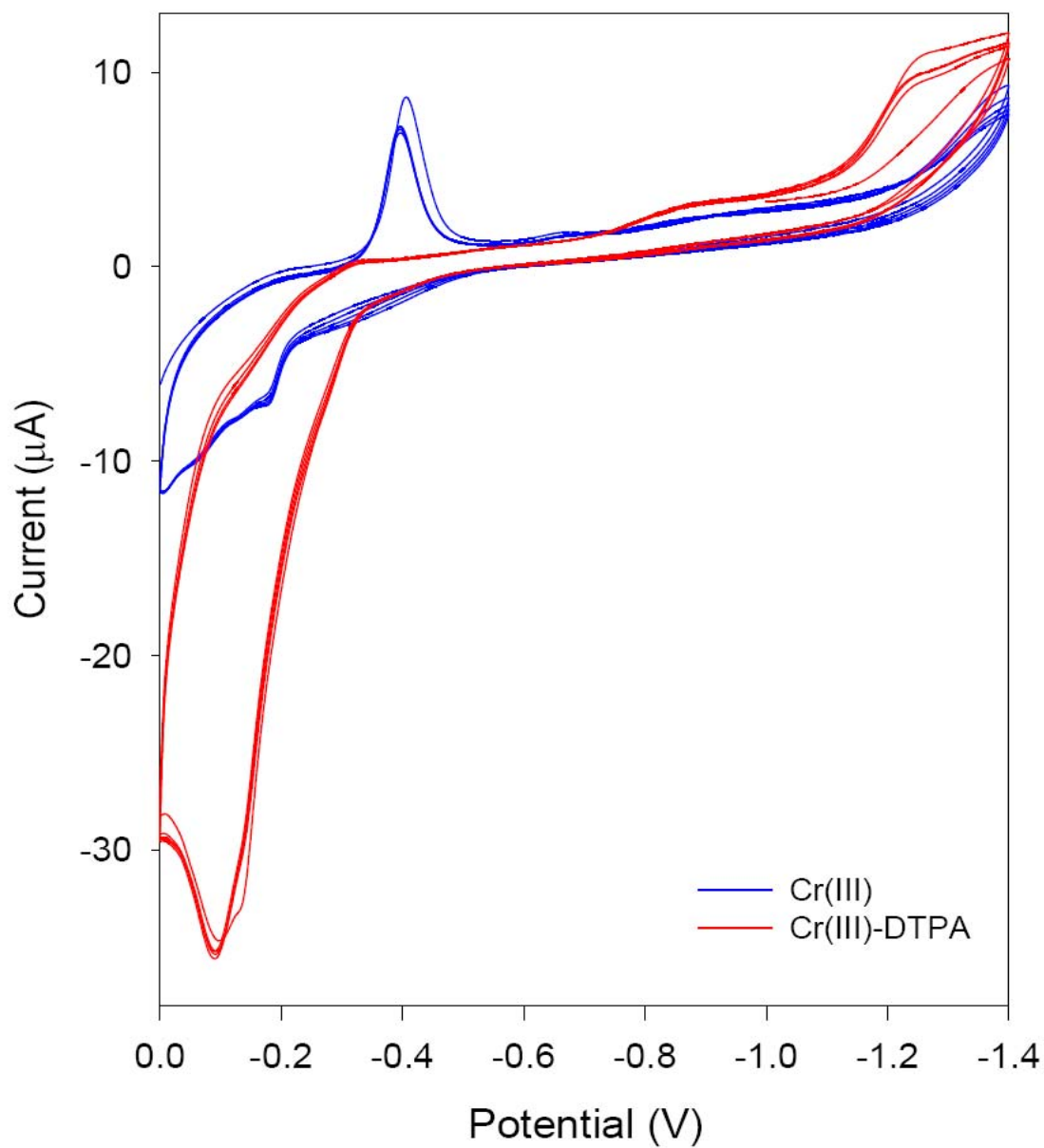


Figure 4.3. CVs of Cr(III) solutions with and without DTPA at the BiBE. The solutions consisted of 0.1 M NaOAc at pH 6 and 0.25 mM Cr(III). Upon addition, DTPA concentration was 5 mM. A sweep rate of 50 mV/s was used.

observed at their expected potentials. At approximately -0.65 V, there is a very small peak, which is due to Cr(VI) reduction to Cr(III).

When DTPA was added to the Cr(VI) solution, the peak at -0.4 V, which was previously only present in the absence of DTPA, returned. This peak is, indeed, due to bismuth oxide reduction. Bi_2O_3 is present, even in the presence of DTPA, in this case, due to the potent oxidant activity of Cr(VI). Cr(VI) oxidizes Bi, allowing bismuth oxide to form, resulting in an oxide reduction wave.

The reduction peak for Cr(VI), at -0.65 V, disappears in the presence of DTPA. This is due to the fact that the Cr(VI) is consumed by the chemical oxidation of the Bi to Bi oxide. As Cr(VI) is the oxidant in this case, it is chemically reduced to Cr(III) during the oxidation of the metallic Bi.

A large cathodic wave appears around -1.2 V in the presence of Cr(VI) both with and without DTPA. This is due to the reduction of NO_3^- .

4.3.2. CAdSV Detection of Cr at the BiBE

CAdSV detection of Cr at the BiBE was attempted, using the electrochemical procedure outlined in the experimental portion of this paper. The detection of Cr(VI) at ppt or lower ppb levels was unsuccessful at the BiBE.

Two explanations have been developed to account for this trend. One is discussed below. The other relates to variations in Bi quantity; thus, it will be discussed in the section in this part pertaining to thick film vs. thin film BiFEs (Section 4.3.3.3). The first explanation for the unsuccessful determination of Cr

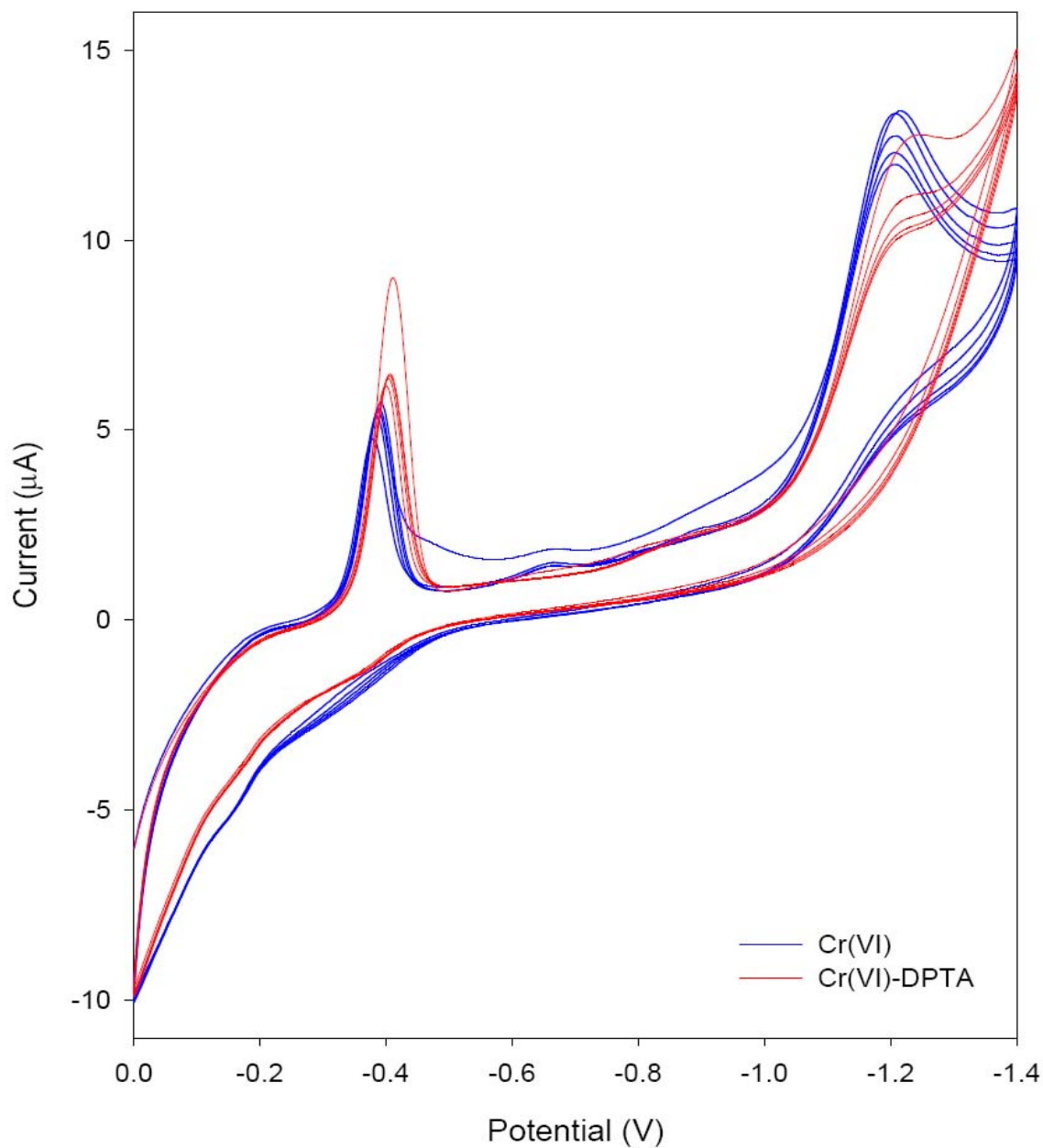


Figure 4.4. CVs of Cr(VI) solutions without and with DTPA at the BiBE. The solutions consisted of 0.1 M NaOAc at pH 6 and 0.25 mM Cr(VI). Upon addition, DTPA concentration was 5 mM. A sweep rate of 50 mV/s was used.

at the BiBE is due to the usage of nitrate as an oxidant, which supposedly enhance the signal.

CAdSV was used to compare the activity of a system with and without Cr at the BiBE (Figure 4.5). A broad peak appears at -1.28 V, even in the system without Cr. This peak is due to the reduction of nitrate. This nitrate reduction peak occurs near the potential where Cr(III) is reduced to Cr(II). The peak is much larger than, and dominates, the Cr(III) reduction peak (Figure 4.5), thus hindering trace Cr(VI) detection.

4.3.3. Film Quality of BiFE

While studying CAdSV detection of Cr at the BiFE, it became evident that the film quality and quantity had important implications upon the resulting Cr peak. Although film quality is difficult to control, a strong correlation between the physical appearance of the film and the resulting Cr peak was observed and is discussed below.

4.3.3.1. Thin Film BiFEs

Thus far, all success in obtaining a Cr peak has been observed at a “thin film” BiFE. By calling it a “thin film,” we are implying that large amounts of glassy carbon, the substrate, can be observed, with a certain “starry night” pattern of metallic Bi dispersed throughout. A typical thin film BiFE is shown in Figure 4.6.

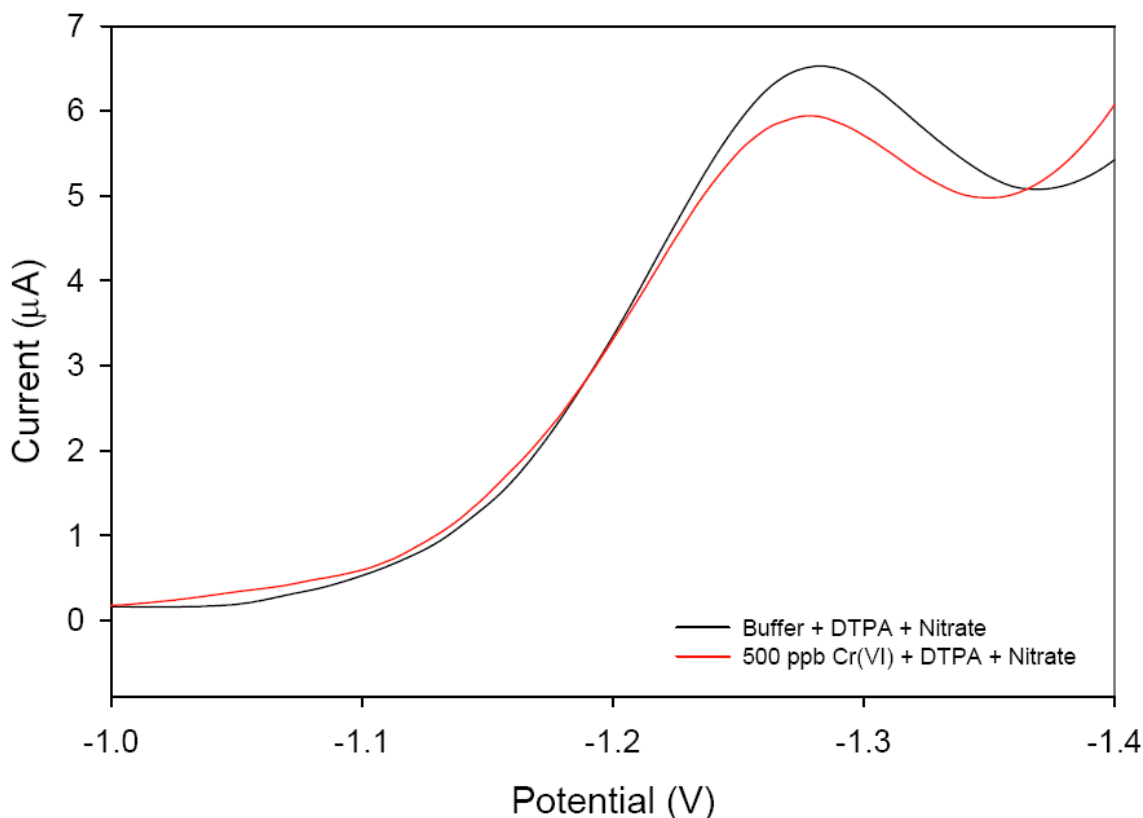


Figure 4.5. SWV of 500 ppb Cr(VI) in 0.1 M acetate buffer with 0.25 M nitrate and 5 mM DTPA (red) as compared to SWV of buffer, nitrate, and DTPA (same concentrations) with no Cr(VI) (black) at a BiBE. DTPA was added immediately prior to testing. The DTPA complexes were accumulated at the BiBE surface by holding the electrode at a potential of -0.8 V for 120 s while stirring the solution. Stirring was stopped as a SWV scan was run from -0.8 to -1.4 V. SWV parameters were as follows: 4 mV potential increment, 25 mV amplitude, and a 15 Hz frequency.

Using a film such as that shown in Figure 4.6 results in an observable Cr peak (Figure 4.7). The reduction peak for Cr(III)-DTPA to Cr(II)-DTPA can be observed at -1.14 V.

4.3.3.2. Thick Film BiFEs

By increasing the deposition time and/or Bi(III) concentration, thicker films can be obtained. By referring to a film as “thick,” it implies that there can be very little to no glassy carbon substrate observed. A typical thick film is shown in Figure 4.8.

To date, no Cr peak has been observed using a thick film electrode for trace Cr detection. Using a thick film BiFE, voltammograms similar to that in Figure 4.9 are reproducible. A large peak corresponding to nitrate reduction is observed at -1.28 V.

4.3.3.3. Comparison of Thin and Thick Film BiFEs

Repeated tests helped establish a firm correlation between the quantity and quality of metallic Bi on a glassy carbon substrate and the ability to obtain a Cr peak. If no Bi is present, Cr SWV peaks for the ppt to ppb range cannot be observed. Yet, if the GC substrate is completely covered by a Bi film, no Cr peak is observed either.

By studying the fundamental electrochemical behavior of the BiBE and the thin and thick film BiFE, a better understanding of the system of interest has been formulated. The CV experiments on the BiBE showed that, in the presence

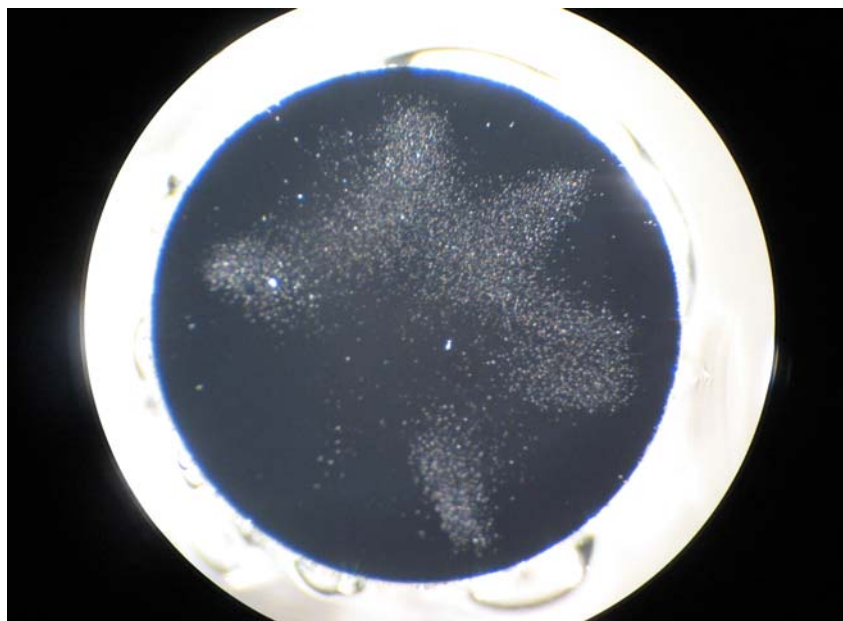


Figure 4.6. A picture of a typical thin film BiFE on a glassy carbon substrate. The white outer ring is glass wall surrounding the GCE substrate. The darker inner circle is the GCE substrate, and the silver specks on this are the electrodeposited Bi(s).

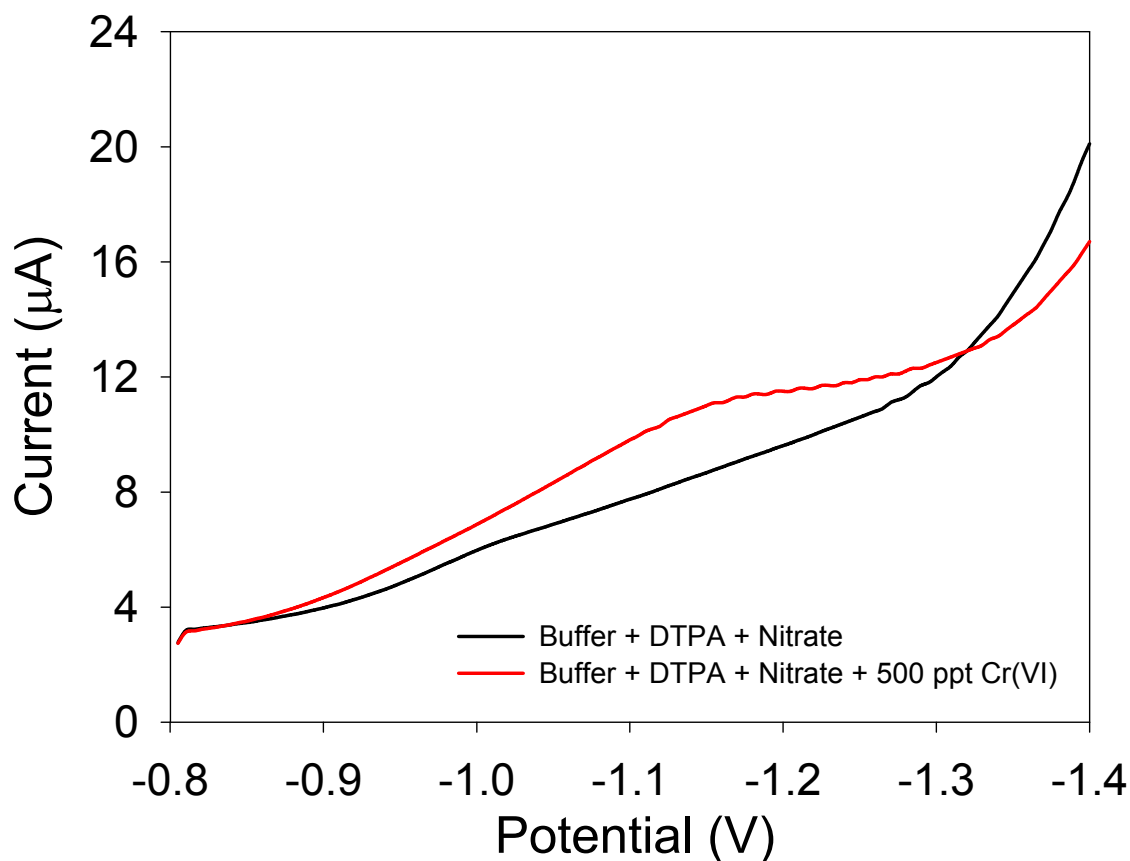


Figure 4.7. SWV at a thin film BiFE. Solutions consisted of 0.1 M acetate buffer with 0.25 M nitrate and 5 mM DTPA (black) as compared to SWV of buffer, nitrate, and DTPA (same concentrations) with 500 ppt Cr(VI) present (red). DTPA was added immediately prior to testing. The DTPA complexes were accumulated at the BiBE surface by holding the electrode at a potential of -0.8 V for 120 s while stirring the solution. Stirring was stopped as a SWV scan was run from -0.8 to -1.4 V. SWV parameters were as follows: 4 mV potential increment, 25 mV amplitude, and a 15 Hz frequency.

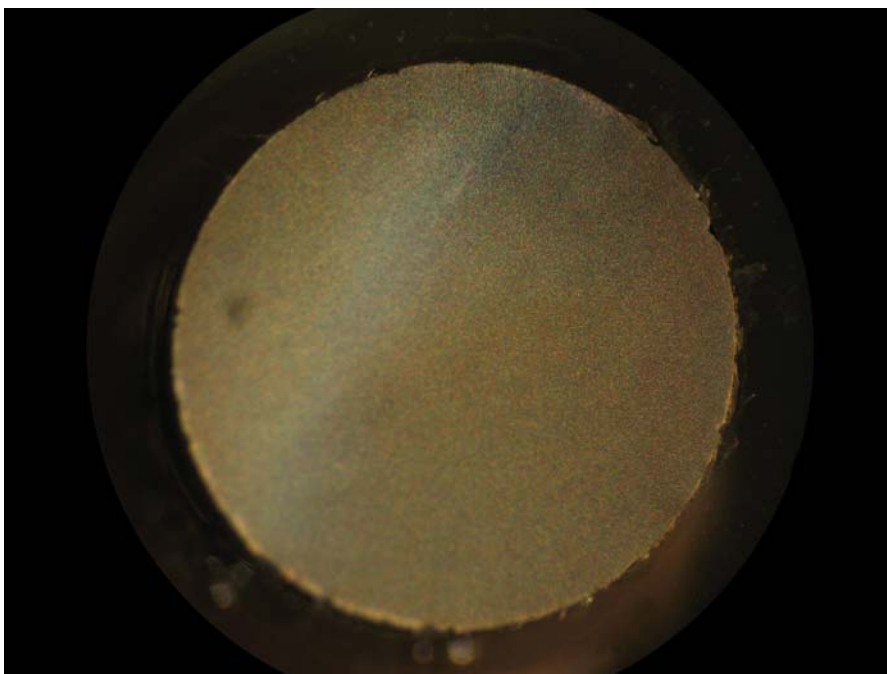


Figure 4.8. A picture of a typical thick film BiFE on a glassy carbon substrate.

The translucent outer ring is a glass casing, and the metallic inner ring is the glassy carbon substrate, completely covered in electrodeposited metallic Bi(s).

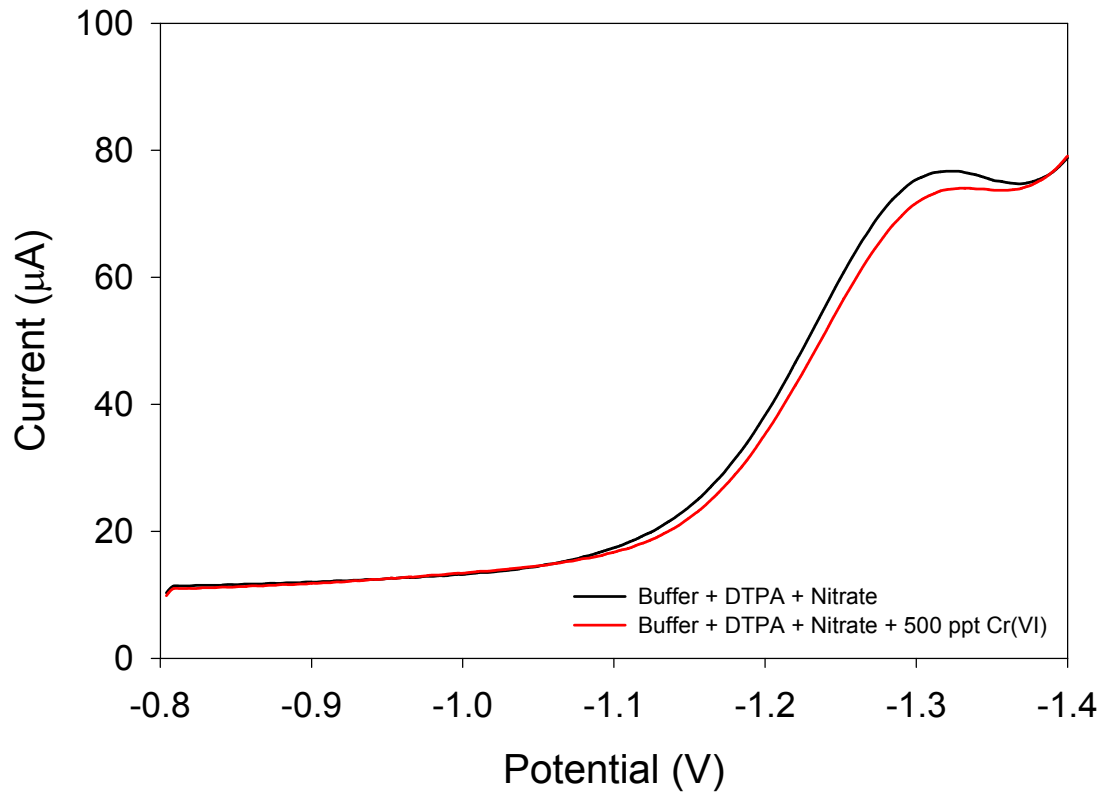


Figure 4.9. Voltammogram showing unsuccessful Cr determination at a thick film BiFE. Parameters are the same as listed in Figure 4.7.

of DTPA, Bi is oxidized to a soluble Bi(III)-DTPA complex. In the presence of an excess of Bi, such as work done at the BiBE or thick film BiFE, no Cr(III) to Cr(II) reduction peak is observed. It is likely that Bi(III) and Cr(III) actually compete for the DTPA ligand. In an excess of Bi(III), a majority of the DTPA in solution could be used up in complexation with Bi(III) instead of Cr(III), leaving little or no Cr(III)-DTPA complex in solution. This may explain the absence of the reduction of the Cr(III)-DTPA complex at -1.10 V when BiBE or thick film BiFE was used. The reduction of the Cr(III)-DTPA complex is a key step in the catalytic reduction of NO_3^- (Figure 1.2). It should also be pointed out that the reduction of NO_3^- itself is observed at -1.28 V, and this peak, which is quite large and broad, may engulf the peak of the Cr(III)-DTPA reduction, making it difficult to observe. The overlapping of the peaks of the Cr(III)-DTPA reduction and direct reduction of NO_3^- may occur in both thin and thick film BiFEs.

4.3.4. Physical Properties of Bi-based Electrodes

4.3.4.1. Impedance Measurements

AC impedance measurements were taken of various Bi-based electrodes. The impedance of a thin film BiFE, thick film BiFE, Bi-CNT, freshly reduced BiBE, and heavily oxidized BiBE were compared to that of an un-modified glassy carbon electrode.

A measure of AC impedance is similar to a measure of resistance, using an alternating current to perturb a system as a means of observing the system at

steady state. In particular, valuable information about the electron transfer kinetics can be obtained.⁴¹

All measurements were taken in either 5 mM $[\text{Fe}(\text{CN})_4]^{3-/4-}$ or $[\text{Cu}(\text{bpy})]^{2+/1+}$ in 0.1 M KCl. The “fresh” BiBE was run immediately after extensive polishing, assuming a higher portion of metallic Bi was present over bismuth oxide. The “oxidized” BiBE was heavily conditioned, cycling from 0 to -1.4 V fifty times, insuring a higher bismuth oxide content. All other electrodes were prepared according to the protocol outlined in the experimental section. Results obtained using $[\text{Fe}(\text{CN})_4]^{1-/2-}$ and $[\text{Cu}(\text{bpy})_2]^{2+/1+}$ are given in Figures 4.10 and 4.11, respectively.

The GCE shows an expected Nyquist plot for the $[\text{Fe}(\text{CN})_6]^{3-/4-}$. For all of the other electrodes, the Bi/Bi₂O₃ couple interferes with the measurement of the $E_{1/2}$ of the ferri/ferrocyanide couple.

For the more positive $[\text{Cu}(\text{bpy})_2]^{2+/1+}$ couple, the GCE, thick film BiFE, and thin film BiFE gave similar impedance spectra. The BiBE results showed an impedance that was drastically larger than the aforementioned electrodes.

The solution resistance is within reasonable limits for all four electrodes – about 80 Ω for the GCE, thin film BiFE, and fresh BiBE, and approximately 220 Ω for the oxidized BiBE.

Using the Nquist plots, quantitative kinetic information was estimated for all electrodes here. Using the solution resistance, R_Ω , the resistance to charge transfer, R_{CT} was calculated, which reflects the rate of e^- transfer at each electrode. Data from these calculations are shown in Table 4.1.

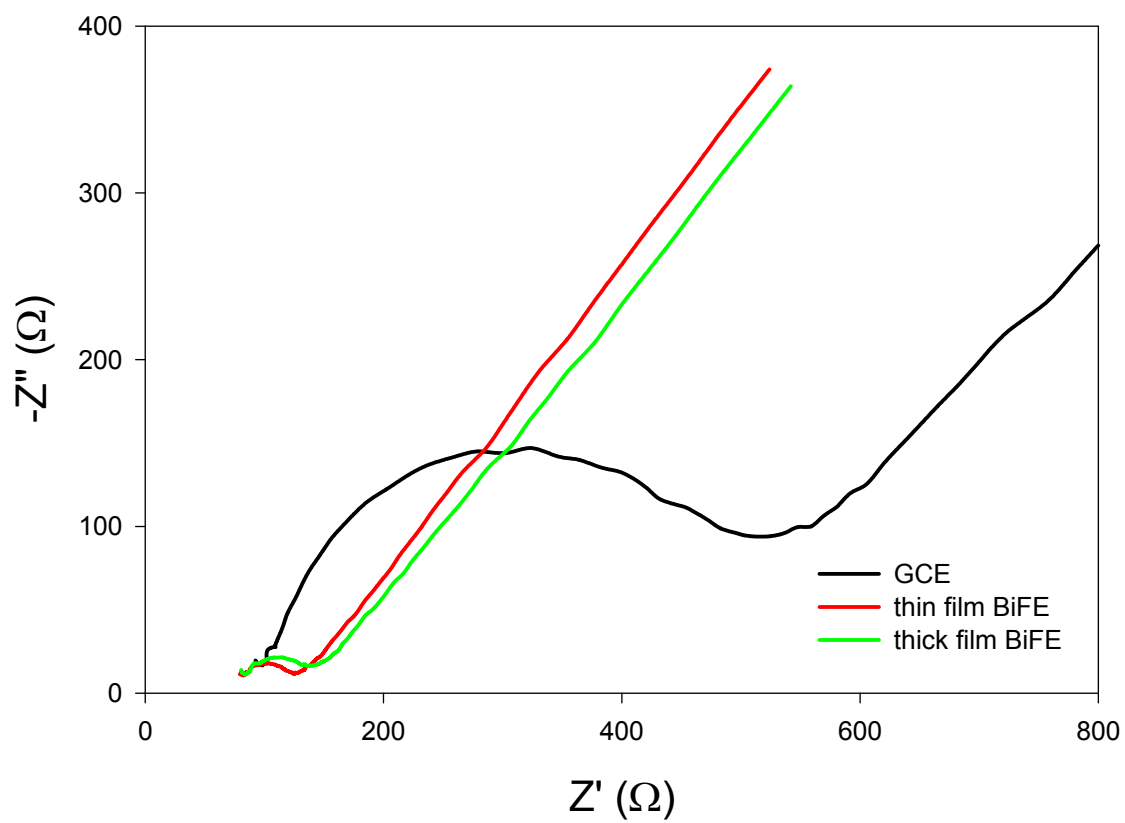


Figure 4.10. Impedance measurements for a glassy carbon electrode, thin film BiFE, and thick film BiFE in $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

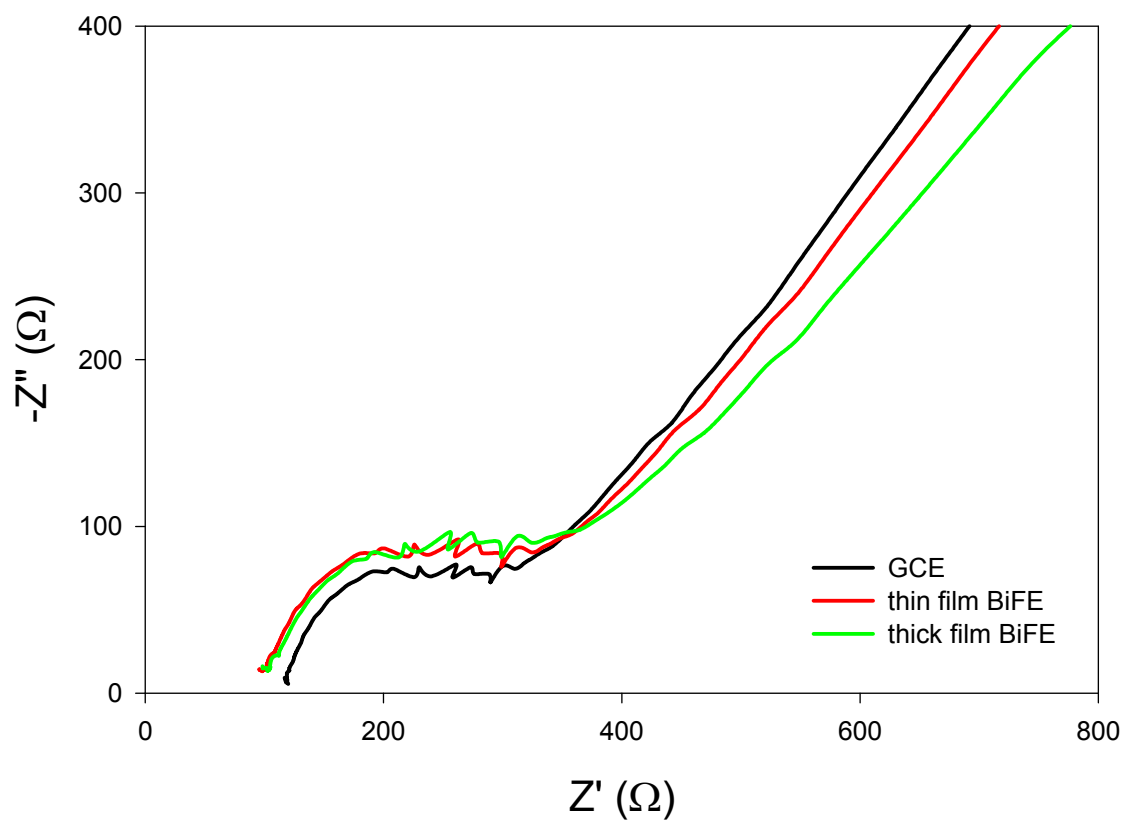


Figure 4.11. Impedance measurements for a glassy carbon electrode, thin film BiFE, and thick film BiFE in $[\text{Cu}(\text{bpy})_2]^{2+/1+}$.

From the data, it is evident that e^- transfer at either BiBE is extremely impeded. For the BiBEs, there was no mass transfer “tail” portion in the Nyquist plot, and the kinetically related semi-circle never fully formed. Thus, quantitative data for the BiBEs was estimated from the incomplete kinetically related semi-circle. The Nyquist plots and calculated data show that, while both BiBEs exhibit an impedance magnitude higher than those of the other electrodes, the oxidized BiBE has a slightly lower impedance than the fresh BiBE.

These results highlight an important point concerning usage of the thick film BiFE for SWV Cr detection. While the thick film BiFE and BiBE give similar results for SWV determination of Cr, their impedances are quite different. This demonstrates that failure of Cr detection at the thick film is not due to issues with electron transfer.

4.3.4.2. SEM Images of Bi on a GC Substrate

While difficult to determine with a simple optical microscope, it is easy to see that the morphology of the “film” on a BiFE is not like a “blanket.” However, a higher resolution image was needed, in order to truly observe the morphology of a Bi film on a GC substrate.

GC plates, from Alfa Aesar, were used as a substrate. Bi(III), at a concentration of 1.245 ppm, was deposited for 240 s in a NaOAc buffer at pH 4.5. Plates were adhered to the SEM specimen stage, and images were obtained. Figure 4.12 shows a few SEM images.

Table 4.1. Calculated values for R_{CT} using the Nyquist plots in various redox couples.

	R_{CT} in $[\text{Fe}(\text{CN})_6]^{3-/4-}$	R_{CT} in $[\text{Cu}(\text{bpy})_2]^{2+/1+}$
Bare GCE	442	231
Thin film BiFE	68	291
Thick film BiFE	64	270
Fresh BiBE	$> 10^5$	$> 10^4$
Oxidized BiBE	$> 10^4$	$> 10^3$

Instead of depositing in a “blanket” style, the Bi appears to nucleate, continually growing to form a sort of mound. The Bi deposition curve, obtained using an amperometric *i-t* test, is also indicative of Bi nucleation.

4.4. Conclusions

Several Bi-based electrodes were tested for their use for Cr detection. Both the Bi-modified, C-containing electrodes (Bi-CNT and thin film BiFE) showed success in electrochemical Cr detection. However, electrodes with no exposed C (BiBE and thick film BiFE) repeatedly failed at detecting Cr. Extensive studies were performed to account for the differences in behaviors among these electrodes. Using cyclic voltammetry, the fundamental behavior of a BiBE, in the presence of DTPA, Cr(VI), and Cr(III) was observed. These results showed that DTPA forms a complex with Bi(III) that was oxidized from the BiBE, preventing the formation of bismuth oxide in the potential range studied here. It also showed that the potentials and peak currents of both Cr(VI) and Cr(III) varied, depending on if they were complexed with DTPA. In addition, the highly oxidizing potential of Cr(VI) was shown to oxidize Bi(0) on the BiBE, causing the formation of bismuth oxide.

The thickness of the Bi film in a BiFE was shown to heavily influence the outcome of Cr detection. A thin film was shown to be the most successful for Cr detection. However, film quality was shown to vary among GCE substrates. The number and arrangement of active sites accounts for film variations, as metallic

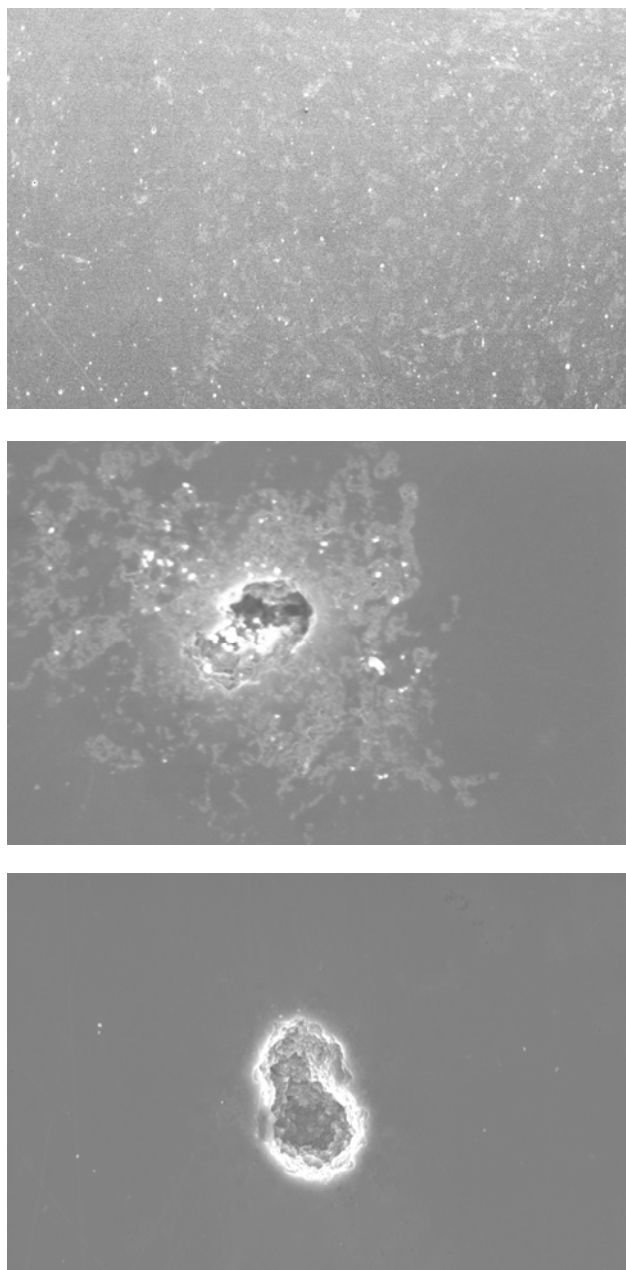


Figure 4.12. SEM images of a Bi film deposited onto a GC substrate. All three images were obtained using a beam energy of 10.0 kV. The horizontal fields of view are 5 mm, 12.5 μm , and 75 μm , respectively.

Bi deposits at exposed active sites. No success was observed at a thick film BiFE.

Impedance measurements were used to calculate the resistances to charge transfer for each of the electrodes. Results showed that the BiBEs had impedance values magnitudes greater than all of the C-based Bi-based electrodes, and they demonstrated poor e^- transfer. While a thick film BiFE shows results similar to that obtained at a BiBE for SWV Cr detection, the impedance of a thick film BiFE was much lower than the BiBE, and similar to a thin film BiFE. This result shows that the inability to achieve successful SWV Cr detection at a thick film BiFE is not due to any issues with e^- transfer and conductivity.

SEM images confirmed the nucleating formation of the Bi film on a glassy carbon substrate. The amperometric $i-t$ curve reflecting Bi deposition was indicative of Bi nucleation.

From all of the data obtained from these various tests, a better understanding of electrochemical Cr detection at Bi-based electrodes has been obtained. CVs of the BiBE showed that Bi binds tightly to DTPA, forming a Bi-DTPA complex that is electrochemically active. The formation of this complex could be problematic in an excess of Bi (as in the case with a thick film BiFE and BiBE) for two reasons. First, it is likely that the Bi competes with the Cr for the ligand. In an excess of Bi, it is possible that Bi(III) complexes with the DTPA present, leaving little or no DTPA present for complexation with Cr(III). This could hinder any Cr(III)-DTPA reduction, resulting in a lack of its peak in a SWV

voltammogram. However, as a NO_3^- peak is prominent in the voltammograms with the BiBE and thick film BiFE, it is difficult to observe the underlying Cr(III)-DTPA reduction peak.

These results and analyses have helped formulate a picture of how the electrochemical process of Cr detection occurs in this system, allowing for future modifications, and enhancement and optimization of electrochemical Cr detection.

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Part 5

Electrochemical Determination of Chromium(VI) at Bismuth-based Electrodes. Understanding the Sensitivity of the Process

5.1. Introduction

As discussed previously, Cr(III) has found use as a dietary supplement for the treatment of diabetes and its complications.^{1,2} Yet, the frequency of actual chromium deficiency in the population is unknown in part because reliable measures for assessing chromium status in humans are limited.³ Biological levels of chromium in mammals are extremely low, at ca. 3-10 ppb (in blood).⁴⁻¹⁰ There are currently three techniques that are capable of detecting Cr at levels sensitive enough for biological samples: neutron activation,¹¹⁻¹³ mass spectrometry,³ and graphite furnace atomic absorption spectroscopy (GF-AAS).^{14,15} However, these techniques either require costly instrumentation, are susceptible to interferences, and/or require a lengthy pre-treatment/analysis time. There is currently a need to develop a sensor that is capable of detecting Cr in the range required for human biological samples. In order to better understand the role of Cr(III) in diabetes and its treatment, the development of a robust, reproducible, inexpensive, and easily fabricated Cr(III) sensor for use in biological fluids is essential.

Electrochemical Cr analysis has recently attracted much interest, due to its high sensitivity, portability, and ability to distinguish Cr(VI) from Cr(III). Electrochemical detection of Cr has traditionally been done at either a hanging mercury drop electrode (HMDE)¹⁶ or mercury film electrode (MFE).¹⁷ While detection is reproducible, and can be attained in the lower ppb/upper ppt range,

the toxicity of the mercury makes it an undesirable choice for use in the medical industry. Thus, alternative electrode materials are being explored.

Among mercury alternatives, bismuth-based electrodes have emerged as a promising alternative for Cr analysis. Bi-based electrodes share many of the same electrochemical properties that make Hg so desirable for Cr detection. Bi is capable of forming fused alloys with heavy metals,¹⁸ and exhibits a high hydrogen overpotential,¹⁹ making it an ideal candidate for this analysis.

It should be noted that, while Cr(III) is the substance of interest, due to its biological implications, electrochemical analyses generally focus on the detection of Cr(VI). This is due to the complicated nature of the analysis and the catalytic cycle discussed in Part 1 (see Section 1.3 and Figure 1.2). For electrochemical analysis of Cr species when using a complexing ligand (DTPA, in this case), the procedure must start with Cr(VI), which is reduced to Cr(III) at a certain applied potential. This is because, in aqueous solution, Cr(III) will form a stable and relatively electrochemically inactive hexaqua complex. In order for Cr(III) to complex with the DTPA, it must be freshly reduced from Cr(VI).^{20, 21} Only when Cr(III) is freshly reduced will it complex with the DTPA to form the electrochemically active complex, as shown in Figure 1.2. When analyzing biological samples, such as blood, the conversion of Cr(III) to Cr(VI) is achieved through a procedure known as the Advanced Oxidation Process, or AOP.²² AOP uses H₂O₂ and UV light as a means of breaking down all of the organic matter making up the matrix. In the process, any Cr(III) in the blood is oxidized to Cr(VI), which is then suitable for use in an electrochemical analysis.

In this work, three Bi-based electrodes were tested. Several modifications were made to the protocol in hopes of attaining reproducible Cr(VI) analysis. Most modifications focused on the pre-treatment and activation of the glassy carbon electrode (GCE) substrate. These tests were performed because, based on previous studies,²²⁻³³ there were suspicions that the surface condition of the GCE had strict implications upon the electro-plated Bi film and the resulting Cr(VI) analysis.

5.2. Experimental

5.2.1. Chemicals and Materials

All CAdSV tests were performed in a 0.1 M sodium acetate (NaOAc) buffer with 0.25 M KNO₃, both purchased from Sigma-Aldrich, as the oxidant, dissolved in ultra-pure deionized (18 MΩ) water. The pH was adjusted to 6 using CH₃COOH (glacial, Fisher Scientific). Cr(VI) atomic adsorption standard (1000 ppm, Fluka) was diluted with the NaOAc/KNO₃ buffer to make Cr(VI) standards. Diethylenetriaminepentaacetic acid (DTPA) (Fluka) was dissolved in ultra-pure deionized water and the pH of the solution was adjusted to 6 using NH₄OH (29%, Fisher Scientific).

In the event that piranha solution was used for GCE pre-treatment, it consisted of a 3:1 ratio of H₂SO₄ to H₂O₂ (30%), both purchased from Fisher Scientific. Ethanol (190 proof), acetone, and isopropyl alcohol used for GCE pre-treatment, were obtained from Deacon Laboratories, and Fisher Scientific,

respectively. Images of the BiFEs were taken using a Canon PowerShot A630 digital camera and a Cambridge Instruments StereoZoom 7 optical microscope (zoom range of 1.0-7.0 $\times$). The zwitterionic buffers (both Biotech Performance Certified) and activated carbon (DLC Supra 50) were obtained from Sigma-Aldrich and Norit, respectively.

All voltammetric measurements were performed using a modulated CHI 440 potentiostat (CH Instruments). A three-electrode configuration consisted of a Ag/AgCl reference electrode (RE), a platinum wire counter electrode (CE), and either a BiBE, thick film BiFE, or thin film BiFE as the working electrode (WE).

5.2.2. Electrodes

The BiBE was prepared according to the protocol outlined in Section 3.2.1. A conditioning step, as described in Section 3.2.2, was required after polishing, prior to any analysis. The BiBE was used for analysis immediately after conditioning.

The thin and thick film BiFEs were prepared as described in Section 4.2.2, according to a protocol previously developed and published by this group.²² Unless otherwise stated, GCEs were purchased from Cypress. A polishing/sonication procedure as that described in Section 4.2.2 was used, unless otherwise stated.

5.2.2. Procedure

For all CAdSV measurements, 20 mL of the standard were used. All Cr(VI) solutions were deaerated with house N₂, unless otherwise stated, for 5 min prior to testing in order to remove any atmospheric O₂.

Unless otherwise stated, CAdSV measurements of Cr were done using the electrochemical procedure proposed by Wang and coworkers for the BiFE.³⁵ This procedure was used for Cr(VI) detection in blood by this research group,³⁴ and it uses a 0.1 M acetate buffer at pH 6. In the procedure, DTPA is added to a solution containing Cr(VI) immediately prior to analysis, to a final concentration of 5 mM. During a 120 s quiet time, during which the potential is held at -0.8 V, the Cr(VI) is electrochemically reduced to Cr(III). Cr(III) then complexes with the DTPA, and the complex is pre-concentrated onto the electrode surface. A square wave voltammetric scan starts immediately after the complex accumulation, from -0.8 to -1.4 V. This scan reduces the Cr(III)-DTPA complex to Cr(II)-DTPA. Nitrate, the oxidant in this case, re-oxidizes the Cr(II)-DTPA to Cr(III)-DTPA, thus instituting a cycle as shown in Figure 1.2, which amplifies the current response, providing optimal sensitivity. Any deviations from this protocol will be noted in the appropriate sections.

5.3. Results and Discussion

5.3.1. Cr(VI) Determination at a Bi-bulk Electrode

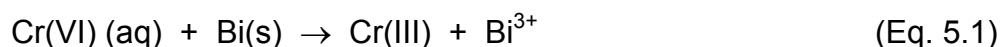
The BiBE was explored as a possible alternative electrode material for the CAdSV determination of Cr(VI). While past work at the BiFE from Wang and coworkers and our group was successful,^{22,34} we have found that reproducibility is not desirable and the process is highly sensitive. In addition, a Bi film on GCE has been shown to be very fragile.³⁵ The BiBE has been studied in the current work to see if it would provide a more robust alternative electrode for Cr(VI) analysis.

Former group members have attempted Cr(VI) detection at a BiBE in the past, using the same protocol developed by Wang, described above in Section 5.2.3, for the detection of Cr(VI) at a BiBE.³⁶ No peak correlating to Cr(III)-DTPA reduction was observed, and Cr(VI) detection was found to be unsuccessful. A NO_3^- peak, however, was repeatedly observed at -1.28 V (Figure 4.6).

The protocol for Cr(VI) determination at the BiBE was slightly modified. Previous attempts used an applied voltage of -0.8 V for 120 s to electrochemically convert the Cr(VI) to Cr(III). In this set of experiments, the protocol allowed for the chemical conversion of Cr(VI) to Cr(III).

First, the BiBE was cycled from 0.0 to -0.7 V in a HOAc/NaOAc buffer containing KNO_3 . This allowed for the repeated reduction and oxidation of the BiBE surface, which resulted in a roughened surface. Immediately after the conditioning, aliquots of appropriate amounts of Cr(VI) and DTPA were added to

the buffer-KNO₃ solution used for conditioning, in which the BiBE was already immersed. The solution was stirred for 10 s to ensure sufficient mixing, and was then allowed to quiet for varying amounts of time (30 s, 1 min, or 2 min), with no potential applied. Because Cr(VI) is a potent oxidant, the quiet time provided a period for the chemical conversion of Cr(VI) to Cr(III). In this time, Cr(VI) oxidizes the metallic Bi to Bi(III), and the Cr(VI) will, itself, get chemically reduced to Cr(III), as represented in the equation below:



After the chemical conversion of Cr(VI) to Cr(III), a SWV scan was run from -0.70 to -1.35 V, electrochemically reducing Cr(III)-DTPA to Cr(II)-DTPA and instituting the catalytic cycle with NO₃⁻.

Unfortunately, a peak corresponding to Cr(III)-DTPA reduction was only observed at high concentrations (100 ppm), as shown in Figure 5.1. When lower concentrations were run (1 ppm or lower), only a NO₃⁻ peak was observed, at the expected potential of -1.28 V, showing results similar to previous attempts at a BiBE (Figure 4.6). Thus, the revised protocol for Cr(VI) determination at a BiBE was not successful.

Cr(VI) determination was also attempted at a thick film BiFE, as discussed in Section 4.3.3.2., with results shown in Figure 4.10. Results at a thick film are similar to results at a BiBE. An in-depth discussion of these results can be found in Part 4.

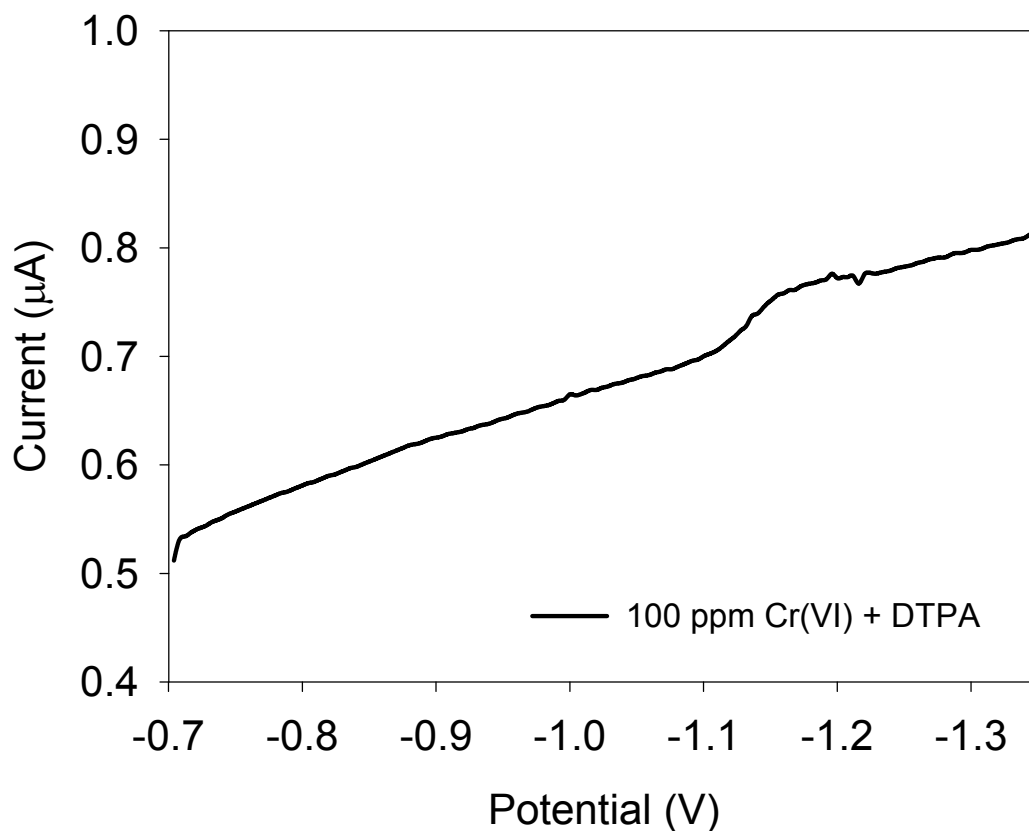


Figure 5.1. SWV scan of 100 ppm Cr(VI) at a BiBE. DTPA was added immediately prior to testing. The DTPA complexes were accumulated at the BiBE surface by holding the electrode at a potential of -0.80 V for 120 s while stirring the solution. Stirring was stopped as a SWV scan was run from -0.80 to -1.40 V. SWV parameters were as follows: 4 mV potential increment, 25 mV amplitude, and a 15 Hz frequency.

5.3.2. Cr(VI) Determination at a Bismuth Thin Film Electrode

The majority of the studies pertaining to Cr(VI) determination employed a thin Bi film, as it has shown the most promising results to date. In general, the protocol developed by Wang³⁴ was used. However, modifications were made, with intentions of improving and optimizing the procedure to obtain more reproducible Cr(VI) analysis.

5.3.2.1. Effects of GCE Pre-treatment on Bi Films

One of the main shortcomings of the protocol for thin film BiFEs is the poor reproducibility they show. As stated in Part 4, the quality of the Bi film has strict implications on the resulting Cr analysis. If a film is too thick, it behaves like a BiBE, showing only NO_3^- peaks. If a film is too thin, however, no Cr(III)-DTPA peak can be observed.

Numerous studies have been performed, showing that the electrochemical behaviors of certain redox systems at a GCE are largely dependent upon GCE pre-treatment.²²⁻³³ Thus, a series of experiments were designed in order to assess whether the pre-treatment of a GCE by various methods had any effect on the amount and reproducibility of deposited Bi.

Typical GCE pre-treatment is achieved through the use of polishing on a standard electrode polishing kit, using a series of alumina slurries (as mentioned in Section 4.2.2). Previous work in this group also explored treating the GCE with piranha solution prior to the polishing step,²² as a means of exposing a fresh layer of glassy carbon. When treating GCEs, polishing is generally followed by

ultra-sonication, in either ultra-pure deionized H₂O, an organic solvent, such as acetone or ethanol, or a combination of these.

Thus, various pre-treatment methods were explored, using a combination of GCE exposure to piranha solution, polishing, and ultra-sonication, in a number of different orders. The same Cypress GCE was used for all of these experiments, and the same Bi plating technique was used as described in Section 4.2.2, other than the pre-treatment procedures. When piranha solution was used, the GCE was first rinsed with DI H₂O, and then exposed to the piranha solution for 5 min. Afterwards, the electrode was rinsed with DI H₂O. Polishing was done on all four polishing pads, with the alumina slurries, for 30 s on each pad. Between each polishing steps, the electrode was rinsed with DI H₂O. When sonicating, the GCEs were immersed in DI H₂O and placed in the sonicator for 5 min.

Films were qualitatively observed under an optical microscope and images were taken. To obtain quantitative information about the Bi film, a linear sweep voltammetric (LSV) scan was used to oxidize the electro-deposited Bi off of the GCE. Since Bi(s) is oxidized at ~ -0.2 V, the scan was from 0.2 to -0.9 V, at a scan rate of 50 mV/s. The peak current (i_p) and peak area (A_p) were obtained from the Bi oxidation peak.

No strong correlation was observed among the order of pre-treatment methods and the quality and quantity of the Bi film. However, the best combination of pre-treatment methods appeared to be one without any exposure to piranha solution, using the standard polishing procedure, followed by ultra-

sonication. This method gave the most reproducible film, as shown in Figure 5.2. This combination resulted in a thicker Bi film than desired, but simple modifications can be made to reduce the film thickness, such as decreasing Bi deposition time and Bi deposition solution concentration.

5.3.2.2. Glassy Carbon Electrode Activation

A great amount of past research dealing with glassy carbon electrodes has addressed the high sensitivity of redox reaction rates towards the state of the electrode surface.²²⁻³³ The results of many electrochemical tests heavily depend upon the nature and reproducibility of the method of electrode pre-treatment.²⁸ A number of different pre-treatment procedures have been studied using many varying redox systems as a means of assessing the effects of pre-treatment on electron transfer. However, the optimal pre-treatment method varies, depending on the type of electron transfer process involved.²²

The effect of GCE pre-treatment on Bi electro-deposition has not yet been studied. Thus, a number of different electrode activation procedures were tested. After the GCE pre-treatment of choice, the Bi(III) was electro-plated, according to the standard procedure outlined in Section 4.2.2. Cr(VI) SWV analysis was done in order to observe any effect of the pre-treatment. Cr(VI) analysis was conducted by the procedure in Section 5.2.2.

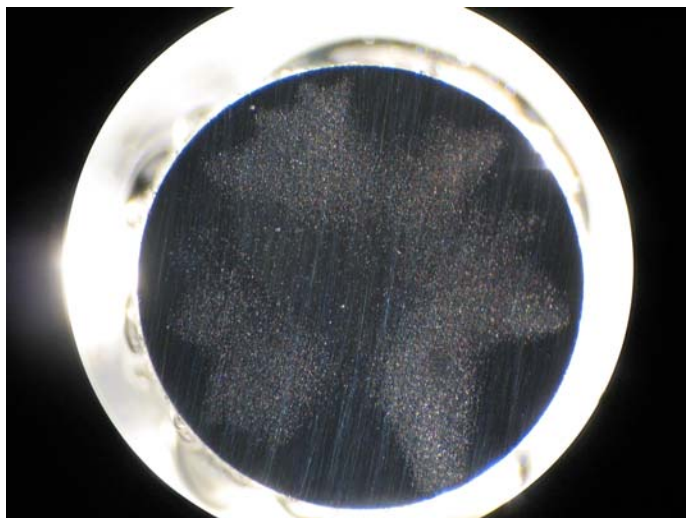


Figure 5.2. Image of the Bi film produced after standard polishing followed by ultra-sonication in DI H₂O. The outer white ring is the glass casing of the Cypress GCE, while the black areas are the GC substrate. The metallic pattern of spots are the electro-deposited Bi(s) film.

Polishing on Glass Plates

Previous studies have shown the potential drawbacks of using polishing pads in polishing GCEs before electrochemical use.^{22,24,25} As the GCE is a highly adsorbent material, any potential contaminants present can, and often do, adsorb to the GCE, blocking active sites. Polishing can serve as one of many ways to introduce contaminants to the GCE surface. Hu and co-workers explored the potential advantages of polishing with the alumina slurries without the use of polishing pads. Instead, glass plates were used, with the alumina slurries on top, as a way of pre-treating the GCE. After polishing, electrode activity was assessed using the ferri-/ferro-cyanide redox couple, mostly because its heterogeneous electron transfer has been extensively studied, and also because its electron transfer rate has been shown to be very sensitive to the state of the GCE surface. Results showed that, by polishing on the glass plates, the activity of the GCE was greatly increased, to a level similar to that observed at a Pt electrode.

The polishing procedure described by Hu and co-workers was used for these studies.²² All glass plates were cleaned by soaking in a 1 M HNO₃ acid bath overnight prior to use. The GCE was polished for 30 s on each plate with the alumina slurry. After polishing, the GCE was sonicated for 5 min in DI H₂O. Bi(III) was electro-plated and the Cr(VI) analysis was performed immediately after electro-plating. The resulting Bi film is shown in Figure 5.3.

The resulting Cr(VI) SWV scan showed no peak corresponding to Cr(III)-DTPA reduction. Thus, while this pre-treatment procedure showed

improvements in GCE activity in the ferri-/ferro-cyanide redox system, no benefits were observed pertaining to its use with the Bi electro-plating procedure.

GCE Sanding

Upon the suggestion of Dr. Richard McCreery, an expert in electrochemical studies of GCEs, a GCE was thoroughly sanded in order to remove the top layer of glassy carbon. According to Dr. McCreery, the glassy carbon contains active sites, which is where the majority of any electrochemistry occurs. Through time, the top layer of the glassy carbon can become fouled and contaminated,³⁰ and studies have shown that GCE surfaces will undergo oxidation in air and experience irreversible changes in solutions under both open- and closed-circuit conditions.^{28,37,38} Through thorough sanding on emery paper, the potentially deactivated top layer of GC can be removed, exposing fresh active sites.

The GCE was sanded on both 600 and 800 grit emery paper for 3 min each. Due to the heavy amount of surface scratching and roughening caused by sanding, the following polishing procedure was much more extensive. The GCE was polished on all four polishing pads and their respective alumina slurries for 3 min each, followed by ultra-sonication in DI H₂O for 10 min. This same polishing and sonication procedure was repeated a second time, followed with a final polishing step on the final polishing pad with the alumina slurry for additional 3 min. A final ultra-sonication was done, using ethanol, for 10 min. This extensive procedure ensured that the smooth, mirror-like finish of a standard GCE was

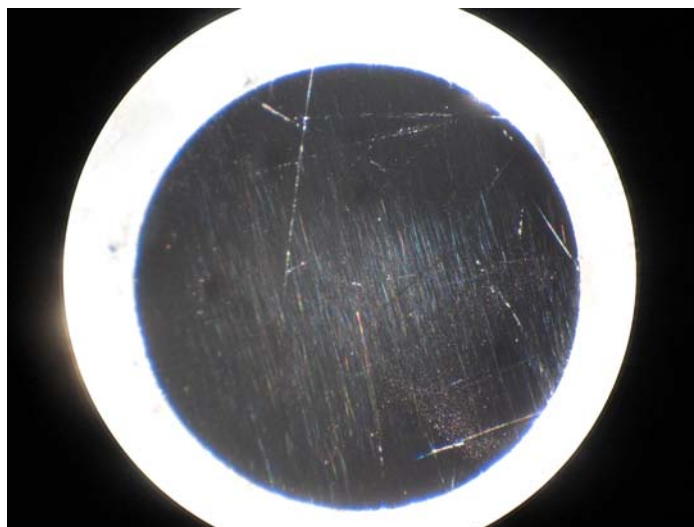


Figure 5.3. Image of the Bi film produced after polishing on glass plates with alumina slurries.

obtained and any loose matter was removed from the GCE surface. After sanding, polishing, and sonicating, the Bi film was electro-deposited using the typical procedure, and Cr(VI) analysis was performed, also using the typical procedure. Images of the GCE after sanding, polishing/sonication, and film deposition are shown in Figure 5.4.

Cr(VI) SWV analysis showed no significant improvements resulting from this technique. A much larger background current was observed, but no Cr(III)-DTPA peak or NO_3^- peak was observed.

Anodic Activation of the GCE

Deliberate modification of the electrode surface is often performed with the aim of introducing some surface functionality that can aid in the electron transfer process involved with the analyte. Work performed by Cabaniss and co-workers showed that deliberate oxidation of the GCE prior to its use leads to a notable increase in the oxygen-to-carbon ratio by the introduction of mostly alcoholic and ketonic groups.³¹ By applying a strongly oxidative potential to a GCE prior to use, these oxidized carbon-based groups are formed, which may help provide pathways for more complex electron transfer processes, notably increasing the rate of electron transfer.

A series of anodic activation processes were performed between the polishing/sonication steps and the film deposition. A potential of 1.8 V was applied to the GCE in either 0.1 M NaOAc (pH 6), 0.1 M H_2SO_4 , or 0.1 M HNO_3 immediately prior to the Bi electro-plating procedure for times varying from 30 to

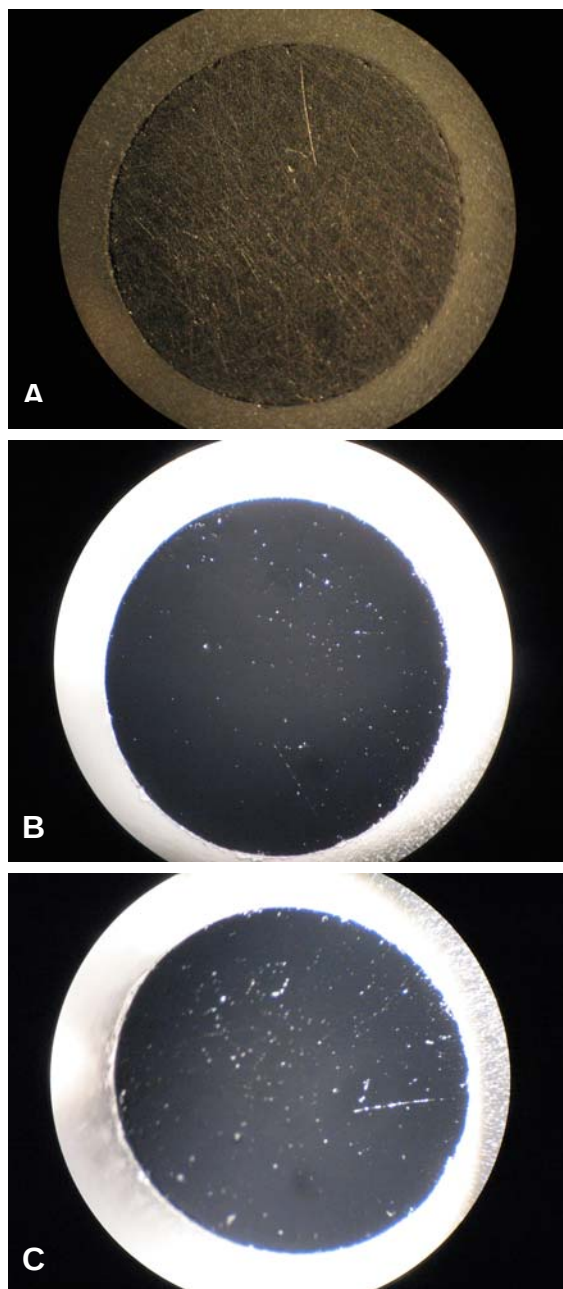


Figure 5.4. Images of the GCE after sanding (A), polishing/sonication (B), and Bi film deposition (C).

300 s. The standard polishing and sonication procedures were used, including the use of the polishing pads with the alumina slurries before activation. After film deposition, Cr(VI) analysis was performed according to the standard protocol in order to observe any effects the activation procedure may have had on Cr(III)-DTPA reduction.

Results were similar for the activation in any of the three solutions. A prominent reductive peak was present at -0.95 V (Figure 5.5), and this peak linearly increased as activation time increased. Generally, however, the Cr(III)-DTPA reduction peak is observed at approximately -1.1 V when using the standard electrode pre-treatment procedures. It was uncertain whether this shift in peak potential was due to surface modification as a result of the activation procedure or if the reduction peak simply was not that of Cr(III)-DTPA reduction.

Because of the uncertainty of the reductive peak at -0.95 V, a Cr(VI) calibration was run, using Cr(VI) concentrations ranging from 1 to 50 ppb. The polishing and sonication procedures were standard, as was the film deposition and Cr(VI) analysis. Between the polishing/sonication and film deposition, anodic activation of the GCE was performed in HNO₃ for 5 min, as described above. Results are given in Figure 5.5.

If the peak at -0.95 V were due to Cr(III)-DTPA reduction, it would increase as the Cr(VI) concentration increased. However, this trend is not observed. It appears that the peak is diminishing with consecutive scans, suggesting that the peak is not due to Cr(III)-DTPA reduction. While the origin of the peak is uncertain, it is more than likely due to the reduction of an O-

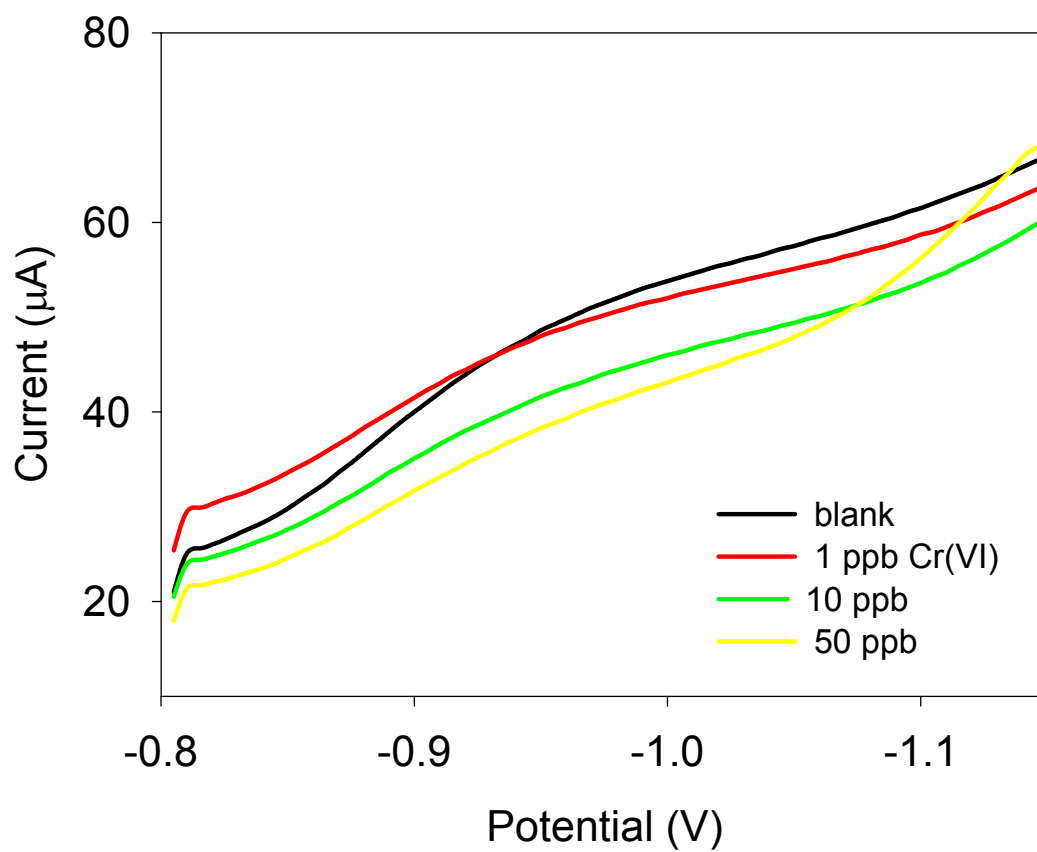


Figure 5.5. SWV calibration of Cr(VI) after a 5 min anodic activation pre-treatment in 0.1 M HNO₃. SWV parameters are the same as those listed in Figure 5.1.

containing group that was introduced onto the GCE surface by the anodic activation procedure. The peak diminishes through consecutive runs as the O-containing group is being fully reduced over time.

Anodic and Cathodic Activation of the GCE

Work by Engstrom and co-workers showed that application of a substantial oxidative potential followed by a brief reductive potential resulted in voltammetric waves that were more sharply defined.^{24,25} A brief cathodic activation step was found to reduce the functional groups introduced by anodic activation, resulting in a surface with increased activity.

The electrochemical pre-treatment of the GCE was modified, such that an anodic activation procedure was followed by a short cathodic step. Standard polishing procedure was performed, followed by an anodic oxidation of the GCE. This was done by applying 1.8 V for 30 s in 0.1 M HNO₃. Immediately following the anodic oxidation was a brief cathodic reduction, achieved by holding the potential at -0.2 V for 15 s in the HNO₃ solution. Afterwards the Bi film was deposited onto the GCE and Cr(VI) SWV analysis was performed.

Results were very similar to that observed at a GCE with just the anodic activation. The electro-plated Bi films for both electrochemical pre-treatment techniques were quite similar in appearance. A prominent peak was seen at -0.95 V (Figure 5.5), which was due to the reduction of an oxygen-containing C group on the electrode surface. No Cr(III)-DTPA reduction peak was observed.

Activated Carbon for Impurity Removal

As GCE surface modifications by pre-treatment were not showing promising results, there were concerns that that lack of electron transfer involved in the Cr(VI) system was simply due to a contaminated GCE surface. Carbon surfaces have a propensity towards adsorbing and oxidizing impurities, and this can often lead to fouled and deactivated surfaces.

Upon further communications with Dr. McCreery, it was suggested that activated carbon (AC) was present in the solvents used for electrode pre-treatment, similar to published work in his research group.³⁰ Reagent solvents contain impurities which adsorb to a GC surface. However, AC, because of its strong adsorptive properties and large surface area, will compete with the GC to adsorb the impurities, leading to a contaminant-free GCE with more active sites exposed.

All parameters for the tests with AC remained the same except for the GCE pre-treatment. Before AC treatment, the GCE was polished on all four polishing pads, using the alumina slurries, and then sonicated for 10 min in DI H₂O. The freshly-sonicated GCE was placed in a 1:3 (v/v) mixture of AC to isopropyl alcohol and stirred for 10 min at a rate of 500 rpm. In order to remove any AC from the surface, the GCE was sonicated for an additional 5 min in DI H₂O.

The resulting film quality was notably different, as the film was considerably thicker. The film is shown in Figure 5.6. SWV Cr(VI) analysis was performed, showing a large peak at -1.15 V. However, the peak was also

observed in a blank solution containing just the NaOAc buffer (Figure 5.7). This result is similar to that observed after the electrochemical pre-treatment of the GCE. The peak is related to the reduction of some O-containing C-based group that was introduced during the procedure, probably from remaining pieces of activated carbon itself.

5.3.2.3. Zwitterionic Buffers

In 1966, Norman E. Good, a botanist at Michigan State University, prepared and tested twelve new hydrogen ion buffers, which later became known as Good's Buffers.³⁹ Ten of the twelve buffers were zwitterionic amino acids, and their use in biological and chemical applications has taken off since Good's initial work with them. Their success can be largely attributed to the unique physical and chemical properties that zwitterions offer. While a zwitterion carries a net charge of zero, there are formal positive and negative charges on different atoms. This makes zwitterions highly polar and water soluble, and their high ionic conductivity makes their applications in electrochemistry highly desirable. Zwitterionic buffers have also been shown to be highly useful as non-coordinating buffers in the use of metal-ion chemistry. Due to both their high ionic conductivity and non-coordinating behavior, the use of zwitterionic buffers in electrochemical Cr(VI) analysis could offer potential benefits over the use of NaOAc buffer, as it has actually been shown to complex with Cr.⁴⁰

Work by Korolczuk and co-workers explored the use of one such zwitterionic buffer for the electrochemical determination of Cr(VI).⁴² By replacing

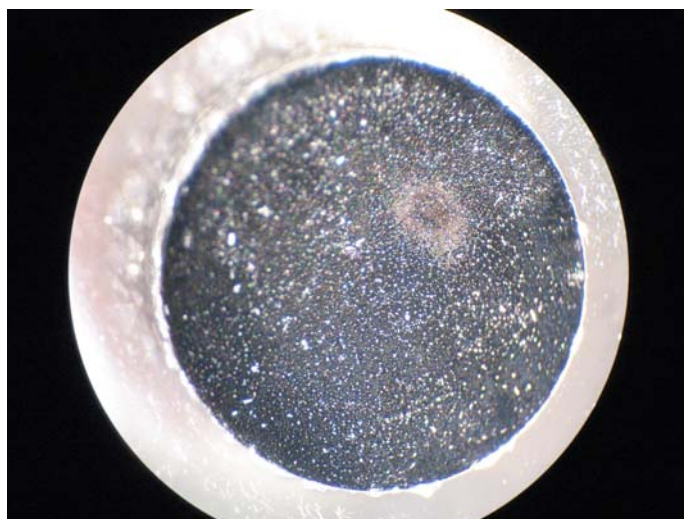


Figure 5.6. Image of the Bi film produced after treating the GCE with an AC/IPA mixture.

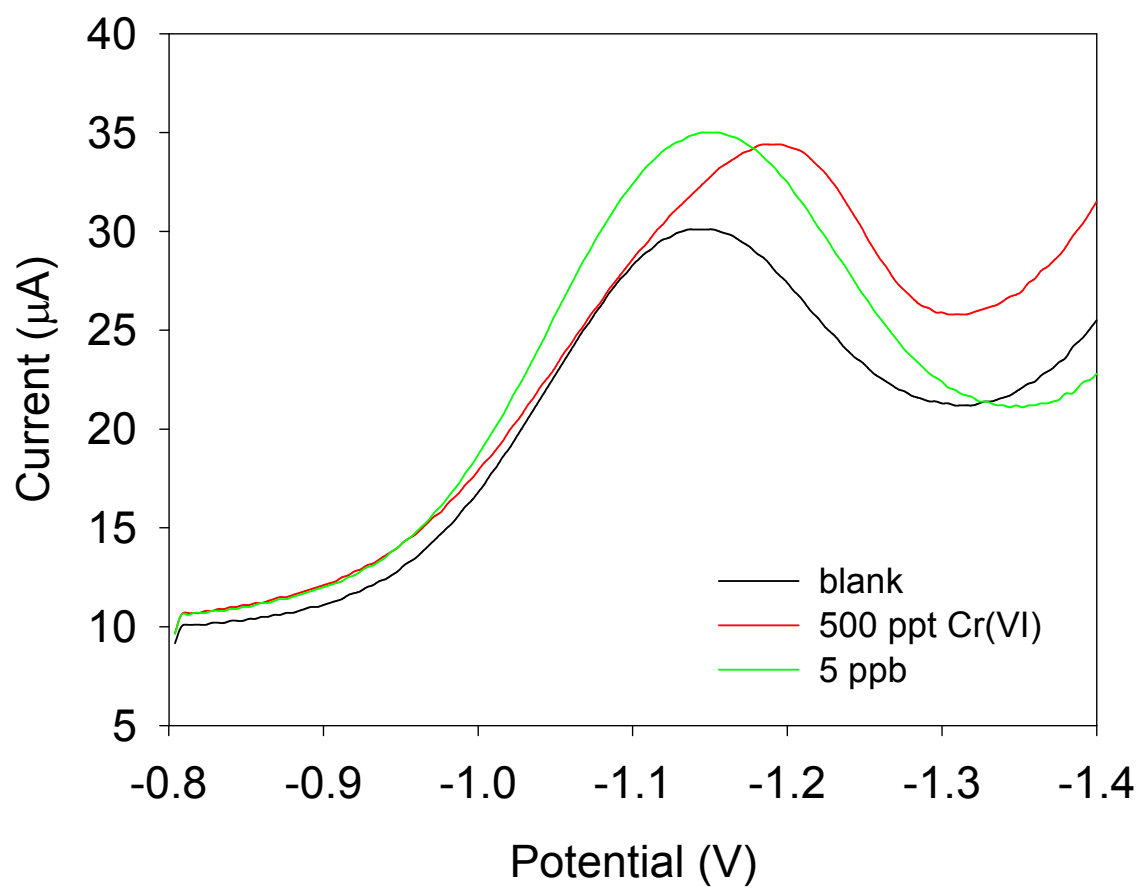


Figure 5.7. SWV calibration of Cr(VI) after treatment of the GCE in an AC/ Pr^iOH mixture prior to Bi film deposition. SWV parameters are the same as listed in Figure 5.1.

NaOAc with morpholinoethanesulfonic acid (MES) and its sodium salt, successful Cr(VI) analysis was observed, with no decrease in sensitivity over the traditional method using NaOAc. In addition, the presence of dissolved O₂ seemed to have no effect on the analysis, meaning analysis time could be significantly reduced since solutions did not need to be purged for the removal of O₂.

Two different zwitterionic buffers were tested in the system for Cr(VI) analysis: MES and piperazine-N,N-bis(2-ethanesulfonic acid) (PIPES). Every part of the standard electrode pre-treatment, Bi electro-plating, and Cr(VI) analysis procedure remained the same. The only difference was that all Cr(VI) standards contained 0.1 M PIPES in place of 0.1 M NaOAc. The pH of the PIPES was adjusted to 6.05 using NH₄OH. The chemical structures of MES and PIPES are given in Figure 5.8.

MES was tested in the Cr(VI) system in the exact same manner as shown by Korolczuk and co-workers.⁴¹ The only difference was that a thin film Bi electrode (BiFE), prepared according to standard protocol, replaced the mercury film electrode (MFE) that Korolczuk and co-workers used.

However, results for both buffers showed no peak correlating to Cr(III)-DTPA reduction. A variety of different GCEs were tested, to ensure that the GCE was not fouled, but no electrochemical activity was observed.

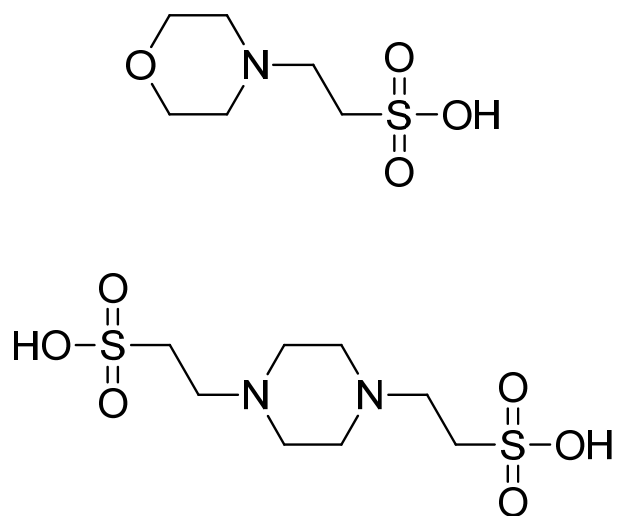


Figure 5.8. The chemical structures of 2-(N-morpholino)ethanesulfonic acid (upper) and 1,4-piperazinebis(ethanesulfonic acid) (bottom).

5.3.2.4. Accumulation Time for the Cr-DTPA Complex

Kinetic issues were explored after the pre-treatment of the GCE and use of a different buffer were shown to be unsuccessful in obtaining a Cr(III)-DTPA reduction peak. A critical part of the Cr(VI) analysis cycle is the accumulation of the Cr(III)-DTPA complex onto the BiFE. In previous studies, a 120 or 140 s accumulation was used.^{22,34} However, the lack of Cr(III)-DTPA peak raised concerns that the Cr(III)-DTPA complex was either not completely forming or was not efficiently accumulating onto the BiFE.

All experimental parameters for the electrochemical determination of Cr(VI) were standard. The only variation was the accumulation time, which was tested at 2, 5, and 7 min. Cr(VI) analysis after each accumulation time showed the same results. No Cr(III)-DTPA or NO_3^- peaks were observed.

5.3.2.5. Effects of Deaeration and Gas Purity

In previous publications addressing Cr(VI) analysis, all solutions were deaerated with N_2 .^{22,34} Thus, all deaeration for this work was done using house N_2 . Throughout time, Cr(VI) analysis seemed to be, at best, sporadic. Oftentimes, a Cr(III)-DTPA reduction peak could be attained for a substantial amount of time (up to a few months). Yet, after a certain amount of time, the peak would disappear, without any changes to the protocol. A number of factors were considered – namely contamination by a number of different sources. Glassware was baked and thoroughly cleansed, the lab was cleaned a number of

times, and the experiments were performed in different rooms. Yet, reproducible Cr(VI) analysis could not be achieved.

As all solutions are deaerated prior to analysis, it was possible that the contaminant source lie in the deaeration gas. Thus, a N₂ tank, purchased from Air Gas, was used, as opposed to the house N₂, which is replenished monthly by Lindy. Still, no Cr(III)-DTPA reduction peak was observed.

Deaeration was then performed using an Ar tank, purchased from Air Gas. Results showed, not only a reproducible Cr(III)-DTPA peak, but a series of peaks corresponding to concentration, as shown in Figure 5.9.

Results similar to that obtained in Figure 5.9 were repeatedly observed when using Ar gas for deaeration. Figure 5.10 shows the analysis of 2 ppb Cr(VI), performed when deaerating with house N₂, as compared to an analysis of the same concentration of Cr(VI) using Ar for deaeration. These results clearly show that the deaeration gas used was the source of contamination. Since a Cr(III)-DTPA reduction peak was attainable when using Ar gas, a Cr(VI) calibration was run, resulting in linear Cr(III)-DTPA reduction peaks, correlating to concentration, as shown in Figure 5.9.

While the source of the contamination is now known, the identity of the specific contaminant remains unknown, at the current time. Since both N₂ and Ar are inert gases, it is not possible that they are the contaminants, as inert gases are both chemically and electrochemically inactive. There is trace impurity present in both the house and tank N₂ that, while not shown to affect any other chemistry in the department, is fouling the GCE surface.

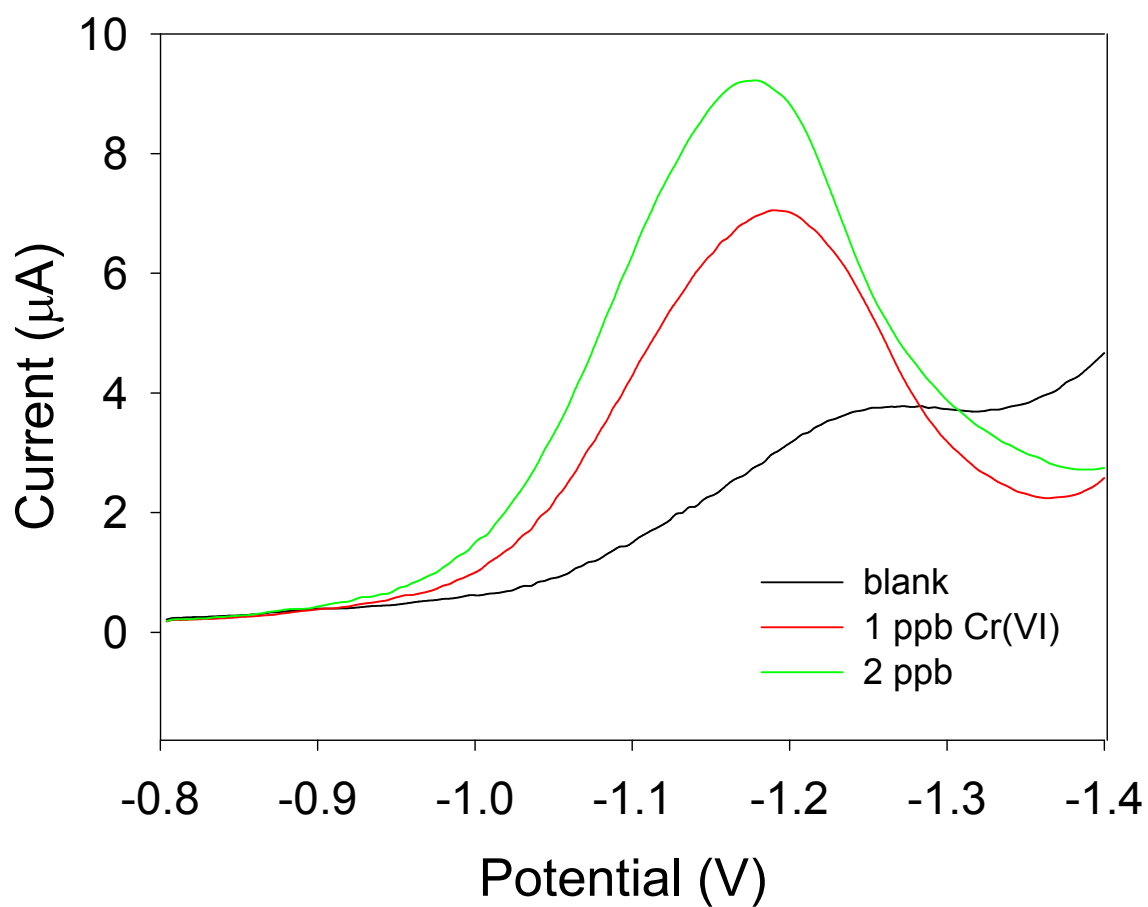


Figure 5.9. SWV Calibration of Cr(VI) after all solutions were deaerated with Ar instead of N₂. SWV parameters are the same as those listed in Figure 5.1.

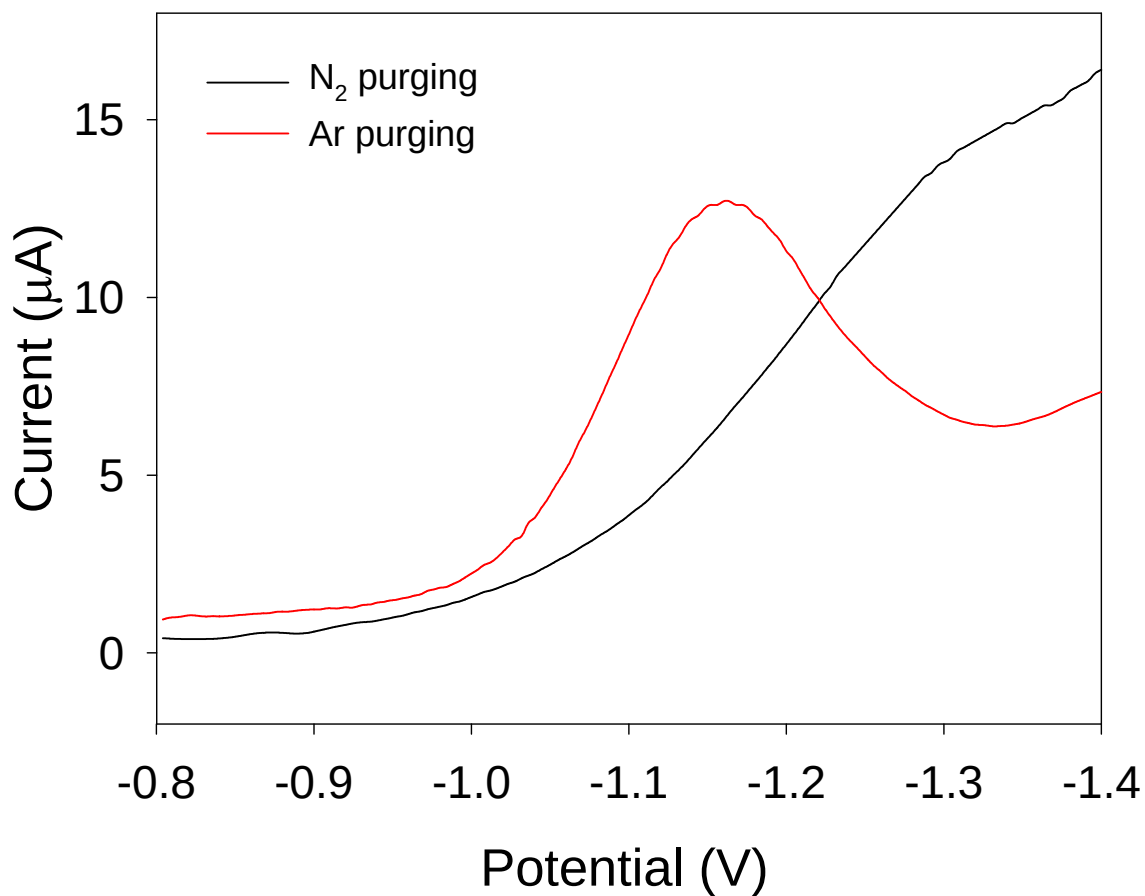


Figure 5.10. Comparison of SWV analysis of 2 ppb Cr(VI) after deaerating solutions with N₂ vs. Ar. SWV parameters are the same as those listed in Figure 5.1.

5.4. Conclusions

The Cr(VI) analysis at the low ppb to ppt level by CAdSV on BiFE proved to be very sensitive to trace impurities. The use of the BiBE and thick film BiFE was not successful for Cr(VI) analysis, as only a large NO_3^- peak was visible. Extensive studies were performed at a thin film BiFE, which has been used for trace Cr analysis by CAdSV, to understand the factors influencing the electrochemical detection.

A series of modifications were made to the procedure, in hopes of optimizing Cr(VI) analysis and making the detection process less sensitive to impurities. The main goal of the majority of the modifications aimed to treat the surface of the GCE in order to remove any adsorbed impurities, which could have prevented effective Cr(VI) analysis. Significant work was focused on modifying the GCE surface for pre-treatment prior to Bi film deposition. The best film quality was observed after standard polishing, followed by sonication in DI H_2O , without the use of any piranha solution to treat the GCE. The GCE was polished on glass plates, in hopes of avoiding common contaminants associated with the use of polishing pads. Also, the top layer of the GCE was sanded down, in hopes of removing any upper fouled layer, exposing fresh active sites. Lastly, the GCE was treated with a solution of AC/IPA, in order to remove any contaminants from the treatment solvent. However, these modifications did not give the Cr(III)-DTPA reduction peak.

Electrochemical pre-treatment of the GCE by anodic activation (followed by cathodic activation, in some cases) did not offer any benefits for the Cr(VI) analysis either. Instead, an O-containing carbon group was introduced onto the surface of the GCE, which was electroactive in the potential range for Cr(III)-DTPA reduction.

After the modifications of the pre-treatment of the GCE were not able to give the Cr(III)-DTPA reduction peak, new buffers were tested. Neither of the zwitterionic buffers, MES and PIPES, gave Cr(III)-DTPA reduction peak either.

Kinetic factors were studied as well. By altering the accumulation time, an ample period was given for Cr(III)-DTPA complex formation and accumulation to the BiFE. However, up to an accumulation time of even 7 min, no Cr(III)-DTPA reduction peak was observed.

Lastly, the deaeration gas was changed from N₂ to Ar. Not only were Cr(III)-DTPA reduction peaks consistently observed, but they also correlated to concentration of Cr(VI). It appears to be that the purity of the inert gas is critical, as any impurities can adsorb to the electrode surface and hinder electron transfer. Since the deaeration by house and tank nitrogen was used *after* polishing and sonication of the GCE and Bi plating, any prior effort to remove the impurities, while important, could not prevent the fouling of the BiFE surface by the impurities in the gas. The impurities block either the adsorption of the Cr-DTPA complex or its electron transfer with the surface.

While the exact contaminant is unknown at the current time, future work will focus on identifying and removing the contaminant(s). Also, some of the

studies performed earlier, before the deaeration gas issue was addressed, can be repeated, possibly showing more optimal results with the use of a contaminant-free gas.

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Part 6

Concluding Remarks

This work centered around the development and optimization of electrochemical sensors and their applications in both biological and environmental matrices. Most of the inorganic substances studied can be found in the human body. As NO, O₂, Cr, and Zn have all been shown to play important roles in biological processes, developing a sensor which can detect them will greatly aid in medical research. The purpose of studying the remaining inorganic substances, Pb(II) and Cd(II), is not directly biologically related, as these substances are found in environmental samples. However, the purpose for developing a sensor for their detection lies in the fact that levels of these pollutants must be monitored, due to their toxicity and potentially negative affects to human health. Through developing a sensor capable of achieving reliable detection of these substances, studies and the routine monitoring of their levels in biological and/or environmental samples will ensure that human health can be at an optimum, through the regulation of toxic pollutants and study of biologically relevant substances and their implications to diseases and their treatments.

The first portion of this dissertation discusses the development and optimization of a dual amperometric gas microsensor, which is capable of achieving real-time, simultaneous measurements of NO and O₂ in living biological samples. Not only have NO and O₂ been shown to be individually relevant, but also their interactions with each other within the human body have strict implications upon human health. As such, it is of great advantage to be able to develop a sensor that is capable of studying them simultaneously, at the location of their production, in real-time.

A Clark-type microelectrode sensor assembly was developed, including two working electrodes (one for NO and one for O₂), and a quasi-reference and counter electrode. This assembly was housed behind a PTFE membrane, providing selectivity to the sensor, and the internal solution of choice was studied. After finding that an internal solution consisting of HCl/NaCl yielded the most optimal response, the concentration of HCl was altered to see if there was any reduction/elimination of cross-talking between the two WEs. While varying concentrations showed slight changes in cross-talking, no concentration was shown to effectively reduce the response at the O₂ sensor during NO calibration.

As further means of reducing cross-talking, the applied potential at each WE was altered during both NO and O₂ calibrations. However, again, no significant reduction in cross-talking was observed. Thus, further means were taken to reduce the cross-talking. Efficient reduction of the cross-talking was achieved, finally, through the use of electro-polymerization of the 5-amino-1-naphthol monomer onto both WE surfaces. The electro-deposited polymer coating effectively trapped the reaction products at each WE at the location of their production, preventing the products from migrating to the other WE. The response at the O₂ sensor during NO calibration was diminished to an insignificant level, and nice linearity was observed at each WE during their respective calibrations.

The remainder of the dissertation focused on the detection of various metals, both in biologically and environmentally related samples. First, the development of a BiBE was performed, and the electrode was optimized for the

detection, both simultaneously and individually, of Pb(II), Cd(II), and Zn(II). The Bi-based electrode was chosen as an alternative to the common, and toxic, mercury-based electrodes. The bulk nature of the electrode served to improve the robustness of the electrode material, as other Bi-based electrodes (BiFE, BiCNT) have been shown to be quite fragile.

Both individual and simultaneous calibrations of the metals showed nice linearity in relation to metal concentration. However, a significant drop in sensitivity was observed in Pb(II), and a smaller drop in Cd(II), when the electrode was placed in a mixture. After a series of tests were performed, stripping the metals in both mixtures and individually, at varying potentials, it was found that the sensitivity only decreased for a certain metal if another metal was stripped prior to that metal. As such, it can be deduced that each time a metal is stripped, it inadvertently removes small portions of any remaining metals with it, accounting for the reduction in sensitivity for Pb(II) and Cd(II) in mixtures.

To demonstrate the capability of the BiBE as an environmental sensor, standard additions of contaminated river water were tested. Results showed that an insignificant amount of Cd(II) was present in the contaminated water, and this result was also verified by ICP-OES analysis of the same sample. Using the BiBE, Pb(II) levels were found to be 8.69 ± 0.72 ppb in the river water. This result was verified by ICP-OES analysis, which gave a concentration of 7.79 ± 0.48 ppb. While ICP-OES analysis of the Zn(II) was possible, the ASV analysis was not. Broad peaks were observed and the standard addition showed poor linearity. Thus, no estimation could be made for Zn(II) levels in the water.

The analysis of these metals with the BiBE was shown to be one of the most sensitive to date, resulting in a limit of detection of 105 ppt, 54 ppt, and 396 ppt for the Pb(II), Cd(II), and Zn(II), respectively. The limit of quantification for each metal, which provides a more realistic assessment of the sensitivity of the technique, was found to be 350 ppt, 180 ppt, and 1.3 ppb for Pb(II), Cd(II), and Zn(II), respectively.

The remaining portion of the work focused on the electrochemical detection system of chromium. Low levels of Cr(III), which is found in the human body, have been linked to diabetes. Thus, a new method for its determination in biological fluids is necessary in order to study it in the human body.

Since levels of Cr(III) in the human body are so low, any technique used for its analysis must be very sensitive. Thus, CAdSV was used, which can enhance detection. First, Cr(III) is complexed with a ligand, DTPA in this case, so that the complex can adsorb and accumulate at the electrode surface. Second, the presence of an oxidant, NO_3^- in this case, causes the oxidation of the reduced product, instituting a catalytic cycle.

The CAdSV technique used for Cr(III), while offering excellent sensitivity, was found to lack reproducibility. Many interactions among the chemicals in the system of interest exist. Thus, a detailed study of the interactions and mechanisms involved in the detection system was necessary, in order to gain a better understanding of the system, leading towards future optimization of the technique.

Electrochemical detection of Cr at the BiBE was repeatedly shown to be unsuccessful, resulting only in a prominent peak corresponding to NO_3^- reduction. Thus, fundamental studies of the BiBE in the detection system were performed. Results showed that the BiBE, itself, exhibited prominent electrochemical activity, exhibiting both a reduction and oxidation peak. Upon the addition of DTPA, results indicate the formation of the Bi(III)-DTPA complex. The failure of the BiBE for Cr detection can be attributed to the complexation of Bi(III) to the DTPA. The excessive Bi in the BiBE, upon oxidation, uses up most or all of the DTPA in solution, leaving little or none to complex with Cr(III). Since no Cr(III)-DTPA complex is formed, little to no Cr(III) can accumulate at the electrode surface, meaning no Cr detection is possible.

Similar to the BiBE, the thick film BiFE was shown to be unsuccessful for Cr determination, for the same reasons as those for the BiBE. However, by reducing the quantity of Bi in the film, Cr determination was possible, as more DTPA was present to complex with the Cr(III) ions.

Impedance measurements showed that electron transfer at the BiBE was very poor, further explaining its failure in Cr determination. The impedances of both the thick and thin film BiFE were found to be quite similar, demonstrating a low resistance to charge transfer.

After obtaining a better understanding of the fundamental behavior and interactions of the detection system, electrochemical detection of chromium was attempted. Since the thin film BiFE showed the most promising results, most of the work centered around its use and optimization.

A large portion of the work focused on the removal of impurities from the system, particularly from the GCE substrate. Numerous studies have shown that the pre-treatment of a GCE substrate can strongly affect the behavior of some redox systems. A series of different activation techniques for the GCE were explored, including anodic and/or cathodic activation techniques, in addition to sanding and polishing the GCE on glass plates. While the sanding and polishing showed no variations in results, the anodic and cathodic activations introduced a large O-containing organic group(s) onto the GCE surface, which was reduced in the same potential range as Cr(III)-DTPA. Pre-treatment of the GCE surface with activated carbon also yielded similar results.

After GCE pre-treatment showed no significant improvements for detection, the focus shifted towards changes to the experimental protocol. First, two new buffers were tested, replacing the NaOAc typically used for the analysis. The zwitterionic buffers PIPES and MES, which do not complex with Cr(III), did not offer any significant improvements to the system, as no Cr(III)-DTPA reduction peak was observed. Kinetic factors were also examined, as the complex accumulation time was altered, in order to allow for complete formation of the Cr(III)-DTPA complex. However, this change also resulted in no improvements, and no Cr(III)-DTPA reduction peak was still observed.

Lastly, the deaeration gas used to purge solutions prior to experiments was changed. House N₂ had been used, but was switched to tank Ar. This change showed immediate results, as the Cr(III)-DTPA reduction peak appeared. The peak was observed consistently, and calibrations resulted in a good linear

correlation between the Cr(VI) concentration and the peak current of the reduction peak.

After an extensive series of tests, mostly aimed at removing impurities, it was found that the critical factor was the purity of the gas used for purging the solutions. As the GCE substrate used for the BiFE is an adsorbent, it is crucial that measures be taken to ensure the removal and avoidance of any contaminants in the system. In this case, any measures taken to remove impurities were negated by the presence of the impurity present in the purging gas.

Future work will focus on identifying the contaminant present in the gas, and repeating earlier tests aimed at further removing impurities. Now that a reproducible and linear Cr calibration can be obtained, future work will address the detection of Cr in biological matrices (e.g., blood and urine).

The aim of the work in this dissertation focused on developing reliable sensors capable of detecting biologically and environmentally relevant substances. The detection of all of these inorganic substances was achieved, eventually, with good linearity and reproducibility. Through optimizing the detection methods, a clear understanding of the detection systems has been obtained. The focus of any future work can center around more extensive studies of the detection of these substances in their biological and environmental matrices. Once this has been shown to be successful, the methods can be used to aid in relevant medical and environmental research.

VITA

Clarissa Eve Tatum was born on January 8, 1983 in Cedar Bluff, VA. She spent all of her childhood and adolescent years with her family in Cedar Bluff, graduating from Richlands High School in May 2001. The following fall, Clarissa enrolled at Emory and Henry College in Emory, VA. She graduated in May 2005 with a major in chemistry and a minor in biology.

Clarissa began her graduate studies at the University of Tennessee Knoxville in August 2005. She joined Dr. Youngmi Lee's research group in the chemistry department, where her studies focused on amperometric gas dual microsenors and their potential applications to biological matrices. Upon Dr. Lee's departure in July of 2007, Clarissa joined Dr. Ziling (Ben) Xue's research group, where she studied the electrochemical detection of heavy metals for applications in biological and environmental matrices. Her work resulted in three publications by the time she received her PhD in the fall of 2010, with other publications to follow.