

**New Approaches to Electroanalysis of Metals and
Pretreatment of Biological and
Environmental Samples**

**A Dissertation Presented for
The Doctor of Philosophy
Degree**

The University of Tennessee, Knoxville

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DEDICATION

This dissertation is dedicated to my family and in memory of my grandparents, Patricia Murray, Clara Bragg, and James Elbert Bragg, who have supported and encouraged me throughout my entire academic career.

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As this endeavor comes to a close, I am so humbled by the experience, and there are so many to thank. God has given me more than I could ever deserve, and I know that without Him I would have never made it this far. He has allowed me to make it through this journey, and I will never be able to thank Him enough. I hope that throughout my time here, I made an impact on someone, such that they understand what God has truly done for me.

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ABSTRACT

Pretreatment of biological and environmental samples followed by electrochemical detection are the focus of this dissertation. The Advanced Oxidation Process (AOP) treatment using aqueous hydrogen peroxide and UV irradiation with acid deactivation of the enzyme catalase in blood has been developed and optimized. The kinetics of whole blood AOP in a single-cell reactor was studied, and the effect of pH on the AOP process has been investigated. A new method of dry ashing whole blood has been developed and optimized for trace metal analyses. The new method and subsequent metal analyses in the blood samples has been used to confirm results from both AOP pretreatment and analyses using electrochemical sensors. Chromium(VI) detection is the focus of the electroanalysis with the report of two new electrode systems. The fabrication of a flower-like self-assembly of gold nanoparticles (AuNPs) on a glassy carbon electrode (GCE) displays an unusually large, linear concentration range in the parts per trillion to low parts per billion range. Another novel electrode is fabricated using a modified glassy carbon electrode with physical deposition of single-walled carbon nanotubes followed by electrochemical deposition of a sol-gel using base-catalyzed hydrolysis for the detection of Cr in the low ppb and ppt range.

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NOMENCLATURE AND ABBREVIATIONS

A	absorbance
AA	atomic absorption
AAS	atomic absorption spectroscopy
AOP	Advanced Oxidation Process
ASV	anodic stripping voltammetry
AuNPs	gold nanoparticles
<i>b</i>	pathlength
BiFE	bismuth film electrode
C	concentration
°C	degrees Celsius
CAdSV	catalytic adsorptive stripping voltammetry
CE	counter electrode
CNT	carbon nanotube
CV	cyclic voltammetry
CSSWV	cathodic stripping square wave voltammetry
DI	deionized
DPV	differential pulse voltammetry
DTPA	diethylenetriaminepentaacetic acid
EPA	Environmental Protection Agency
EQCM	electrochemical quartz crystal microbalance

EtOH	ethanol
ϵ	molar absorptivity
g	gram
GCE	glassy carbon electrode
h	hour
h	Planck's constant
H ₂ TMSenCl ₂	acidified n-[3-(trimethoxysilyl)propyl]ethylenediamine
Hg-Bi/SWNT	mercury-bismuth/single-walled carbon nanotubes
HMDE	hanging mercury drop electrode
H ₂ O ₂	hydrogen peroxide
HAc	acetic acid
Hz	hertz
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectroscopy
i_p	peak current
L	liter
m	meter
MeOH	methanol
MFE	mercury film electrode
mM	millimolar
M	molar
M Ω	megaohm

mA	milliamp
min	minutes
mg	milligram
MHz	megahertz
mL	milliliter
MPTS	3-mercaptopropyltrimethoxysilane
MTMOS	methyltrimethoxysilane
MWNT	multi walled carbon nanotube
NaAc	sodium acetate
nM	nanomolar
nm	nanometer
PET	4-pyridineethanethiol hydrochloride
ppb/ $\mu\text{g L}^{-1}$	parts per billion
ppm/ mg L^{-1}	parts per million
ppt/ ng L^{-1}	parts per trillion
R^2	square of correlation coefficient
RE	reference electrode
s	second
SEM	scanning electron microscopy
SWNT	single walled carbon nanotube
SWV	square wave voltammetry
t	time

TMOS	tetramethoxysilane
TMSen	n-[3-(trimethoxysilyl)propyl]ethylenediamine
UV-Vis	ultraviolet-visible
V	volts
WE	working electrode
μA	microamps
μg	microgram
μL	microliter
μm	micrometer
ν	frequency

Part 1

Introduction and Background

1.1. Foreword

Diabetes affects more than 25.8 million people in the United States, or 8.3% of the population. Once thought to be an uncommon disease, an estimated 7 million people are currently undiagnosed. In 2007, the annual medical costs were \$174 billion and are steadily increasing.¹ Chromium(III) has been used as a dietary supplement for diabetic patients in recent years.²⁻⁵ With diabetes reaching epidemic proportions it is imperative to understand the efficiency and safety of all possible therapies. At the same time, chromium(VI) is a well known toxin and carcinogen.⁶ Thus there is an additional interest in the general study of this trace metal.

The study and implementation of chemical sensors are currently on the rise.⁷ Chemical sensors are a popular topic, finding a wide variety of uses and approaches. Biological and environmental sensors that can readily detect either high concentrations or nanomolar quantities are highly desirable, and the techniques they rely on can be optical, electrochemical, mass-sensitive, or spectroelectrochemical in nature. Our research has focused primarily on the development of sensors for monitoring metals, such as chromium, in complex matrices found in biological samples. With the application of the Advanced Oxidation Process (AOP), a sample can be treated using a combination of hydrogen peroxide and ultraviolet light (UV) to decompose organic molecules that often interfere with detection. This dissertation outlines the optimization of

AOP as a technique, the use of novel electrochemical sensors for metal analyses, and the use of these sensors in an AOP modified sample.

1.2. Analysis Techniques Used in the Research Studies

1.2.1. Advanced Oxidation Process

Advanced Oxidation Processes have been studied for many years due to their success in wastewater treatment.⁸⁻¹⁰ AOP is a process that specifically depends on the generation of reactive hydroxyl free radicals ($\bullet\text{OH}$) as the oxidant initiating oxidative degradation.⁸⁻¹¹ It can be used as an alternative to strong oxidants and is more environmentally friendly as it gives water and carbon dioxide as products. The $\bullet\text{OH}$ radicals can be generated by both photochemical and nonphotochemical processes which are discussed below.⁹

1.2.1.1. UV/H₂O₂ Process

As mentioned previously, the primary reaction in AOP is the production of $\bullet\text{OH}$ radicals, which become the oxidant in the decomposition of organic compounds. The most basic generation of $\bullet\text{OH}$ radicals is through the photolytic cleavage of H₂O₂ (Eq. 1.1).⁸⁻¹²



The $\bullet\text{OH}$ radicals are a highly reactive transient that rapidly oxidizes organic substances.¹³ There are three possible reactions that take place between organic molecules and $\bullet\text{OH}$:¹⁰

1) Hydrogen abstraction^{10,13}



2) Electrophilic addition



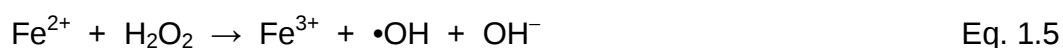
3) Electron transfer

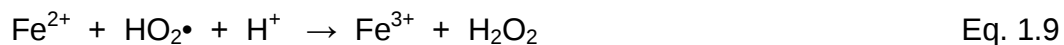
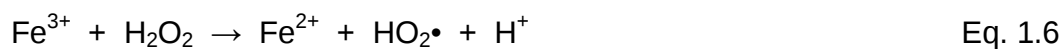


Hydroperoxyl or peroxy radicals are often generated and help further decompose organic compounds.

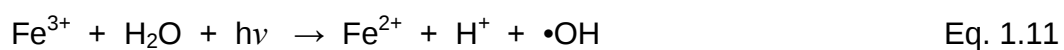
1.2.1.2. Photo-Fenton Process

The Fenton process, a dark process involving no UV irradiation, is the decomposition of H_2O_2 to $\bullet\text{OH}$ using the ferrous ion (Fe^{2+}) or ferric ion (Fe^{3+}) under acidic conditions. The main reactions that take place in the Fenton process are listed below:^{14,15}





Iron operates as a catalyst in the process. The generation of $\bullet\text{OH}$ radicals and rate of decomposition are greatly increased by irradiation with UV light (Eq. 1.11).



When Eqs. 1.5 and 1.11 are combined, the iron is cycled between +2 and +3 oxidation states as a catalyst, and two moles of $\bullet\text{OH}$ are produced per mole of H_2O_2 consumed.⁸

1.2.2. Ultraviolet and Visible Spectrophotometry

When a molecule absorbs energy, specifically UV or visible radiation, an electronic transition occurs in which outer electrons are excited from their ground state to an excited state. The energy is then transferred to nearby molecules or through vibrational relaxation. The concentration of the absorbing species is directly related to the absorbance according to Beer's Law (Eq. 1.12)¹⁶

$$A = \epsilon b C \quad \text{Eq. 1.12}$$

where A is the absorbance, ϵ is the molar absorptivity ($\text{M}^{-1}\text{cm}^{-1}$), b is the pathlength (cm), and C is the concentration (M) of the analyte. UV-Vis spectrophotometry was used in the study of AOP kinetics to monitor the decomposition of biological/organic components in blood as a function of time.

1.2.3. Inductively Coupled Plasma-Optical Emission Spectroscopy

Used in elemental analysis, ICP-OES has a superior sensitivity than other techniques such as atomic absorption. Molecules are initially converted to individual high energy ions and/or atoms using high temperature radio-frequency-induced argon plasma. The sample is nebulized, and the fine spray is analyzed by optical emission spectroscopy. As the atoms relax from the excited state to a lower energy state, photons are emitted at a wavelength characteristic to each individual element, allowing for the sample's composition to be determined.¹⁷

1.2.4. Electrochemical Techniques

A number of electrochemical techniques were used in this research: cyclic voltammetry (CV), anodic stripping voltammetry (ASV), cathodic stripping square wave voltammetry (CSSWV), and AC impedance.

In cyclic voltammetry, the potential at the working electrode (WE) is linearly increased or decreased to a point at which the scan is reversed until it reaches the starting potential. This allows for both oxidation and reduction to occur within the observed range, and the resulting faradaic current from the redox reaction is measured. Cyclic voltammetry is often used for qualitative information about an electrochemical process.¹⁸

Commonly used for the analysis of trace metals, anodic stripping voltammetry allows for the preconcentration of an analyte on the surface by applying a negative potential and allowing it to be reduced. The anodic scan oxidizes the metal and strips it off of the electrode surface. The current response corresponds to the concentration of metal that was accumulated.

Cathodic stripping square wave voltammetry combines the techniques used in both cathodic stripping and square wave voltammetry to provide high sensitivity. Cathodic stripping is a similar process to anodic stripping but requires an accumulation step at a more positive potential. An oxidized analyte can then be reduced off the surface during the scan. As a result, this technique is more commonly utilized for anionic species. Square wave voltammetry is a pulse technique where the potential is modulated; the current is sampled twice per

pulse, once in the forward half cycle and once in the reverse (Figure 1.1). The pulses are collected through a particular range, and the difference between the forward and reverse currents is then used to generate a signal. This allows for the fundamental elimination of background current.

AC impedance is used to measure generalized resistance. An alternating current is used to agitate a system in order to observe the system at a steady state. This technique is especially useful in studying electron transfer kinetics.¹⁸

1.2.5. Scanning Electron Microscopy

Scanning electron microscopy is a high resolution imaging technique often used to characterize solid surfaces. A beam of energetic electrons is scanned over the surface in a raster pattern, which means the beams sweeps the surface in a straight line, returns to the starting position, and shifts downward. This repeatedly occurs until the entire area in question is scanned.¹⁷ A signal is received and converted into an image. SEM is capable of providing magnifications of 10 to 100,000x and can resolve features down to the nanometer scale.²⁰

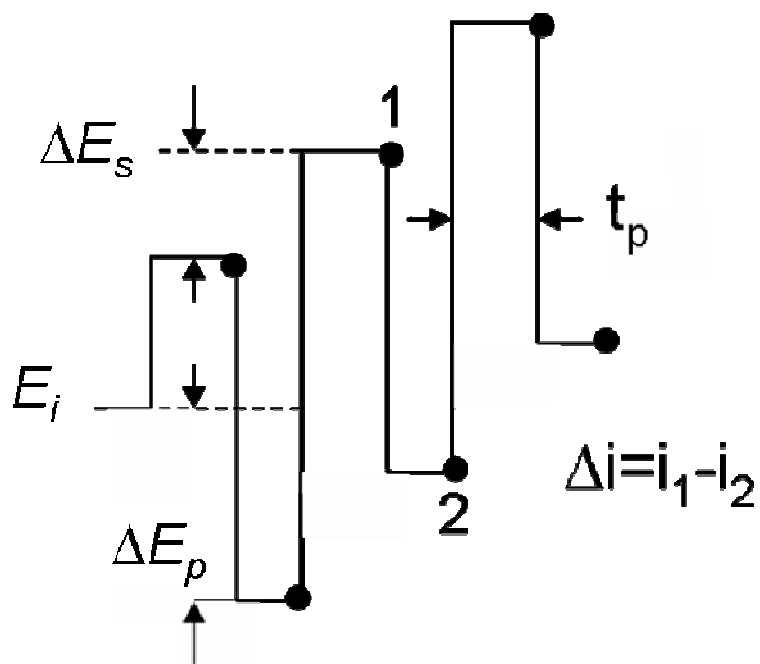


Figure 1.1. Common waveform for square wave voltammetry.¹⁹ ΔE_s is the step height (the potential increment from one cycle to the next), ΔE_p is the pulse amplitude, and t_p is the pulse width. Heavy dots indicate the time at which current is sampled.

1.2.6. Fourier Transform Infrared Spectroscopy

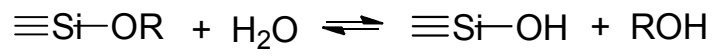
Another form of spectroscopy, molecules can be excited to higher energy states by exposing them to infrared radiation. Since infrared radiation has lower energy, the resulting absorption corresponds to the stretching and vibrational frequencies of most covalent bonds. Only vibrations that change the dipole moment in a molecule are capable of absorbing infrared radiation giving an IR spectrum. Thus stretchings of symmetric bonds such as H_2 or Cl_2 do not absorb. A typical Fourier transform infrared spectrometer uses a beam splitter to split the source light into two equal beams. One beam passes through the beamsplitter to an adjustable mirror, while the other is reflected to a stationary mirror and reflected back where the two beams are superimposed before going through the sample and to the detector. The distance between the mirrors is different, therefore the two waves are not in phase when they reach the detector. Fourier analysis decomposes an interferogram from the time to frequency domain to obtain the spectrum. The interferogram contains the spectrum of the source minus the spectrum of the sample.¹⁷

1.3. Overview of Sol-Gels for Sensing Applications

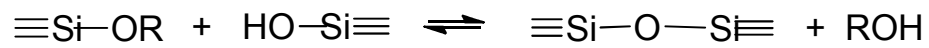
The term sol-gel refers to reactions that use alkoxide precursors to prepare solid glass and ceramic oxide materials.²¹ First developed over 150 years ago, these techniques are attractive due to their lower processing

temperatures and high purity and homogeneity in comparison to traditional glass melting and ceramic methods at the time.²² Initially, an alkoxide precursor, such as $\text{Si}(\text{OR})_4$, is hydrolyzed, followed by the condensation of the resulting silanols which form a cross-linked inorganic matrix. Upon drying, this forms a three-dimensional porous glass. These reactions, summarized in Figure 1.2, are strongly influenced by many factors, including ligand size, concentration, temperature, solution pH, solvents, and catalysts.^{21,23-28} As a result these factors impact the properties of the sol-gel structure and can be tailored for a specific instance. For example, catalysts are often used to enhance the hydrolysis rate of the sol-gel reaction. Acid-catalyzed reactions result in dense gels, while base-catalyzed reactions give less dense, more porous gels.^{21,29-31} By carefully adjusting these factors, sol-gel materials can be used for numerous applications, such as electronics, optics, separation technology, catalysis, and sensing.^{21,29-31}

Hydrolysis



Alcohol Condensation



Water Condensation

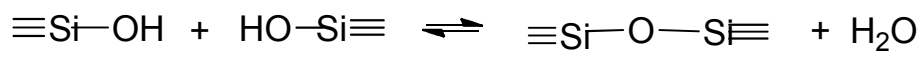


Figure 1.2. The basic sol-gel reactions.

Chemical sensing through sol-gels has been a central idea in our research group.³²⁻³⁷ Sol-gels have several physical properties that make them suitable for sensing applications. They are extremely inert and highly compatible with many chemical agents, making the fairly straightforward incorporation of sensing elements to be fairly straightforward. Because little to no heating is required, even thermal sensitive organic molecules can be encapsulated within sol-gel without decomposition.³⁸ This is typically done by doping the molecules inside the sol-gel or grafting them to the backbone of the matrix. While doping is easier and involves the physical entrapment of the reagent, leaching can often become an issue. Grafting provides a more reproducible, stable product, but it is far more tedious.³⁸⁻⁴¹ This particular technique requires that compounds contain $-\text{Si}(\text{OR})_3$ groups, thus limiting the available reagents for the process. With their porous nature, sol-gels easily allow the transport of analyte to the encapsulated reagent. Sol-gels have found much use in optical sensing as they are transparent in the visible region.⁴² These sensors are now in many applications such as monitoring of pH,^{23,25,27,28,39,40,42-47} metal ions,⁴⁸⁻⁵¹ and various other analytes.⁵²⁻⁵⁴ Sol-gels have also found use in electrochemical^{32,55-61} and spectroelectrochemical sensing applications.⁶²

1.4. Summary of Dissertation Parts

1.4.1. Part Two

A new process to pretreat blood samples is reported in Part 2 of this dissertation. This process combines the Advanced Oxidation Process using H_2O_2 and UV irradiation with acid deactivation of the enzyme catalase in blood. A four-cell reactor has been designed and built in house. The effect of pH on the AOP process has been investigated. The kinetics of the pretreatment process shows that at high $C_{\text{H}_2\text{O}_2, t=0}$, the reaction is zeroth order with respect to $C_{\text{H}_2\text{O}_2}$ and first order with respect to C_{blood} . The rate limiting process is photon flux from the UV lamp. Degradation of whole blood has been compared with that of pure hemoglobin samples. The AOP pretreatment of the blood samples has led to the subsequent determination of chromium and zinc concentrations in the samples using electrochemical methods. An article detailing this work has been published.⁶³

1.4.2. Part Three

Part 3 describes the use of dry ashing of whole blood samples. Ashing these samples are difficult to carry out with significant sample loss, and the procedure is not well documented. A new procedure has been developed and optimized to dry ash whole blood samples for trace metal analyses. The procedure reduces both the sample loss and dry ashing time by more than two

thirds. The ashed sample can be readily used in subsequent, simultaneous or individual analysis of several metals by ICP-OES, as demonstrated in the analysis of a whole blood sample. The new procedure is simple, inexpensive, and faster than the established method. An article containing the results discussed in this section has been published.⁶⁴

1.4.3. Part Four

We report here the fabrication of a flower-like self-assembly of gold nanoparticles (AuNPs) on a glassy carbon electrode (GCE) as a highly sensitive platform for ultratrace Cr(VI) detection. Two AuNP layers are used in the current approach, in which the first is electroplated on the GCE surface as anchors for the binding of an overcoated thiol sol-gel film derived from a 3-mercaptopropyltrimethoxysilane (MPTS). The second AuNP layer is then self-assembled on the surface of the sol-gel film, forming flower-like gold nanoelectrodes enlarging the electrode surface. When functionalized by the thiol pyridinium, the fabricated electrode displays a well-defined peak for selective Cr(VI) reduction with an unusually large, linear concentration range of 10-1200 ng L⁻¹ and a low detection limit of 2.9 ng L⁻¹. In comparison to previous approaches using MPTS and AuNPs on Au electrodes, the current work expands the use of AuNPs to the GCE. Subsequent functionalization of the secondary AuNPs by a thiol pyridinium and adsorption/preconcentration of Cr(VI) lead to the unusually large detection range and high sensitivity. The stepwise preparation of

the electrode has been characterized by electrochemical impedance spectroscopy (EIS), scanning electronic microscopy (SEM), and IR. The newly designed electrode exhibits good stability, and has been successfully employed to measure chromium in a pretreated blood sample. The method demonstrates acceptable fabrication reproducibility and accuracy. The results described in this section have been published.⁶⁵

1.4.4. Part Five

Part 5 of this dissertation reports work on the novel method of modifying a glassy carbon electrode with physical deposition of single-walled carbon nanotubes (SWNTs) followed by electrochemical deposition of a sol-gel using base-catalyzed hydrolysis. The thin sol-gel film fabricated from a new ethylenediamine derivative is believed to preconcentrate Cr(VI) anions. The SWNTs allow for greater conductivity and increased surface area on the electrode surface which in turn gives greater sensitivity. Our studies have shown detection of Cr in the low ppb and ppt range.

1.4.5. Part Six

Error analysis for each part is reported in Part 6. Sources of error and standard deviation measurements are included.

1.4.6. Part Seven

A summary of all of the work in this dissertation is included in Part 7. Concluding remarks are given, highlighting the important findings of each work. The relevance of the projects and each project's relation to the others is discussed. A central theme is given, unifying all parts of this dissertation.

References

1. Centers for Disease Control and Prevention. National Diabetes Fact Sheet: National Estimates and General Information on Diabetes and Prediabetes in the United States, 2011. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2011. http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf Accessed 1/28/2012.
2. Anderson, R.A. *Nutr. Res. Rev.* **2003**, 16, 267-275.
3. Anderson, R.A. *J. Am. Coll. Nutr.* **1998**, 17, 548-555.
4. Katz, S.A.; Salem, H. *The Biological and Environmental Chemistry of Chromium*; VCH: Weinheim, 1994.
5. Ryan, G.J.; Wanko, N.S.; Redman, A.R.; Cook, C.B. *Ann. Pharmacother.* **2003**, 37, 876-885.
6. Iyengar, G.V. *Elemental Analysis of Biological Systems. Volume I: Biomedical, Environmental, Compositional, and Methodological Aspects of Trace Elements*, CRC Press: Boca Raton, 1989.
7. A SciFinder (Chemical Abstracts) search in January 2012 using the term “sensors” and refined by the year “2011” yielded 72,618 hits. The same search in October 2010 for the year 2009 yielded 69,214 hits.
8. Parsons, S., Ed. *Advanced Oxidation Processes for Water and Wastewater Treatment*; IWA Publishing: London, 2004.

9. *Handbook on Advanced Photochemical Oxidation Processes*. EPA, 1998.
10. Legrini, O.; Oliveros, E.; Braun, A.M. *Chem. Rev.* **1993**, 93, 671-698.
11. Symons, J.M.; Worley, K.L. *J. Amer. Wat. Works Assn.* **1995**, 87, 66-75.
12. Schulte, P.; Bayer, A.; Kuhn, F.; Luy, T.; Volkmer, M. *Ozone Sci. Eng.* **1995**, 17, 119-134.
13. Zepp, R.G.; Faust, B.C.; Holgne, J. *Environ. Sci. Technol.* **1992**, 26, 313-319.
14. Sigman, M.E.; Buchanan III, A.C.; Smith, S.M. *J. Adv. Oxid. Technol.* **1997**, 2, 415-423.
15. De Laat, J.; Gallard, H. *Environ. Sci. Technol.* **1999**, 33, 2726-2732
16. Harris, D.C. *Quantitative Chemical Analysis*, 8th ed.; W. H. Freeman, 2010.
17. Skoog, D.A.; Holler, F.J.; Nieman, T.A. *Principles of Instrumental Analysis*, 5th ed.; Brooks Cole, 1998.
18. Bard, A.J.; Faulkner, L.R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons, Inc.: New York, 2001.
19. Osteryoung, J. *Acc. Chem. Res.* **1993**, 26, 77-83.
20. Whan, R.E. *Metals Handbook – Materials Characterization*, 9th ed.; American Society for Metals: Ohio, 1986, Vol. 10.
21. Brinker, C.J.; Scherer, G.W. *Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing*; 1st ed.; Academic Press: Boston, 1990.
22. Hench, L.L.; West, J.K.; *Chem. Rev.* **1990**, 90, 33-72.

23. Allain, L.R.; Sorasaene, K.; Xue, Z.-L. *Anal. Chem.* **1997**, 69, 3076-3080.
24. Allain, L.R.; Xue, Z.-L. *Anal. Chem.* **2000**, 72, 1078-1083.
25. Allain, L.R.; Xue, Z.-L. *Anal. Chim. Acta* **2001**, 433, 97-102.
26. Schubert, U. *Chem. Mater.* **1995**, 7, 2010-2027.
27. Carey, W.P.; Jorgensen, B.S.; *Appl. Spectrosc.* **1991**, 45, 834-838.
28. Carey, W.P.; DeGrandpre, M.D.; Jorgensen, B.S. *Anal. Chem.* **1989**, 61, 1674-1678.
29. Klein, L.C. Solgel coatings in Coatings Technology Handbook, 3rd ed.; Tracton, A.A. Ed. CRC Press: Boca Raton, Florida 2006, pp. 96/1-96/4.
30. Sanchez, C.; Julian, B.; Belleville, P.; Popall, M. *J. Mater. Chem.* **2005**, 15, 3559-3592.
31. McDonagh, C.; MacCraith, B.D.; McEvoy, A.K. *Anal. Chem.* **1998**, 70, 45-50.
32. Carrington, N.A.; Yong, L.; Xue, Z.-L. *Anal. Chim. Acta* **2006**, 572, 17-24.
33. Carrington, N.A.; Xue, Z.-L. *Acc. Chem. Res.* **2007**, 40, 343-50.
34. Carrington, N.A.; Thomas, G.H.; Rodman, D.L.; Beach, D.B.; Xue, Z.-L. *Anal. Chim. Acta* **2007**, 581, 232-40.
35. Carrington, N.A.; Qiu, H.; Xue, Z.-L. *Am. Lab.* **2007**, 39, 37-41.
36. Dansby-Sparks, R.N.; Ouyang, R.; Xue, Z.-L. *Sci. China Ser. B Chem.* **2009**, 52, 1777-1788.

37. Dansby-Sparks, R.N.; Jin, J.; Mechery, S.J.; Sampathkumaran, U.; Owen, T.W.; Yu, B.D.; Goswami, K.; Hong, K.; Grant, J.; Xue, Z.-L. *Anal. Chem.* **2010**, 82, 593-600.
38. Lin, J.; Brown, C.W. *Trends Anal. Chem.* **1997**, 16, 200-211.
39. MacCraith, B.D.; Ruddy, V.; Potter, C.; O'Kelly, B.; McGilp, J.F. *Electron. Lett.* **1991**, 27, 1247-1248.
40. Lobnik, A.; Oehme, I.; Murkovic, I.; Wolfbeis, O.S. *Anal. Chim. Acta* **1998**, 367, 159-165.
41. Avnir, D. *Acc. Chem. Res.* **1995**, 28, 328-334.
42. Allain, L.R.; Canada, T.A.; Xue, Z.-L. *Anal. Chem.* **2001**, 73, 4592-4598.
43. Canada, T.A.; Allain, L.R.; Beach, D.B.; Xue, Z.-L. *Anal. Chem.* **2002**, 74, 2535-2540.
44. Shamsipur, M.; Gholamhassan, A. *Anal. Lett.* **2001**, 34, 1603-1616.
45. Wang, E.; Chow, K.F.; Wang, W.; Wong, C.; Yee, C.; Persad, A.; Mann, J.; Bocarsly, A. *Anal. Chim. Acta* **2005**, 534, 301-306.
46. Canada, T.A.; Xue, Z.-L. *Anal. Chem.* **2002**, 74, 6073-6079.
47. Canada, T.A.; Beach, D.B.; Xue, Z.-L. *Anal. Chem.* **2005**, 77, 2842-2851.
48. Rodman, D.L.; Pan, H.; Clavier, C.W.; Feng, X.; Xue, Z.-L. *Anal. Chem.* **2005**, 77, 3231-3237.
49. Clavier, C.W.; Rodman, D.L.; Sinski, J.F.; Allain, L.R.; Im, H.-J.; Yang, Y.; Clark, J.C.; Xue, Z.-L. *J. Mater. Chem.* **2005**, 15, 2356-2361.

50. Zevin, M.; Reisfeld, R.; Oehme, I.; Wolfbeis, O.S. *Sensor Actuat. B-Chem.* **1997**, *B39*, 235-238.
51. Oehme, I.; Wolfbeis, O.S. *Mikrochim. Acta* **1997**, *126*, 177-192.
52. Lan, E.H.; Dave, B.C.; Fukuto, J.M.; Dunn, B.; Zink, J.I.; Valentine, J.S. *J. Mater. Chem.* **1999**, *9*, 45-53.
53. Narang, U.; Prasad, P.N.; Bright, F.V.; Ramanathan, K.; Kumar, N.D.; Malhotra, B.D.; Kamalasanan, M.N.; Chandra, S. *Anal. Chem.* **1994**, *66*, 3139-3144.
54. Rao, M.; B.C. Dave *Adv. Mater.* **2002**, *14*, 443-447.
55. Jena, B.K.; Raj, C.R. *Talanta* **2008**, *76*, 161-165.
56. Walcarius, A.; Mandler, D.; Cox, J.A.; Collinson, M.; Lev, O. *J. Mater. Chem.* **2005**, *15*, 3663-3689.
57. Lev, O.; Wu, Z.; Bharathi, S.; Glezer, V.; Modestov, A.; Gun, J.; Rabinovich, L.; Sampath, S. *Chem. Mater.* **1997**, *9*, 2354-2375.
58. Alber, K.S.; Cox, J.A. *Microchim. Acta* **1997**, *127*, 131-147.
59. Deepa, P.N.; Kanungo, M.; Claycomb, G.; Sherwood, P.M.A.; Collinson, M.M. *Anal. Chem.* **2003**, *75*, 5399-5405.
60. Walcarius, A.; Sibottier, E. *Electroanal.* **2005**, *17*, 1716-1726.
61. Shacham, R.; Avnir, D.; Mandler, D. *Adv. Mater.* **1999**, *11*, 384-388.
62. Shi, Y.; Slaterbeck, A.F.; Seliskar, C.J.; Heineman, W.R. *Anal. Chem.* **1997**, *69*, 3679-3686.
63. Bragg, S.A.; Armstrong, K.C.; Xue, Z.-L. *Talanta* **2012**, *97*, 118-123.

64. Bragg, S.A.; Xue, Z.-L. *Am. J. Analyt. Chem.* **2011**, 2, 979-983.
65. Ouyang, R.; Bragg, S.A.; Chambers, J.Q.; Xue, Z.-L. *Anal. Chim. Acta.* **2012**, 722, 1-7.

Part 2

Pretreatment of Whole Blood Using Hydrogen Peroxide and UV Irradiation. Design of the Advanced Oxidation Process and Kinetic Studies

This chapter is revised based on a paper by Stefanie A. Bragg, Kristie C. Armstrong, and Zi-Ling Xue. Only minor revisions were made.

S.A. Bragg, K.C. Armstrong, and Z.-L. Xue. Pretreatment of whole blood using hydrogen peroxide and UV irradiation. Design of the advanced oxidation process. *Talanta*, **2012**, 97, 118-123.

Abstract

A new process to pretreat blood samples is reported. This process combines the Advanced Oxidation Process using H_2O_2 and UV irradiation with acid deactivation of the enzyme catalase in blood. A four-cell reactor has been designed and built in house. The effect of pH on the AOP process has been investigated. The kinetics of the pretreatment process shows that at high $C_{\text{H}_2\text{O}_2, t=0}$, the reaction is zeroth order with respect to $C_{\text{H}_2\text{O}_2}$ and first order with respect to C_{blood} . The rate limiting process is photon flux from the UV lamp. Degradation of whole blood has been compared with that of pure hemoglobin samples. The AOP pretreatment of the blood samples has led to the subsequent determination of chromium and zinc concentrations in the samples using electrochemical methods.

2.1. Introduction

Chemical analysis of metals in blood samples is often used and actively studied.¹⁻⁴ The analysis of some metals in whole blood such as lead and cadmium by established methods require minimal preconcentration or pretreatment prior to analysis.⁵ Some metals such as chromium, however, are often bound or complex with macromolecules and pretreatment of the sample is required prior to their detection and analysis.⁶⁻⁷ The pretreatment process can vary in complexity, ranging from simple extractions⁸ to online separations.⁹ Many pretreatment processes such as microwave¹⁰⁻¹¹ and HPLC⁹ are effective yet require costly instrumentation. Depending on the matrix and the specific metal to be analyzed, pretreatment can take multiple steps, requiring hours and even days to complete. There is an increasing need for fast and convenient pretreatment and detection of heavy metals in various samples.^{6,12-23}

The Advanced Oxidation Process (AOP) is a combination of hydrogen peroxide (H_2O_2) and ultraviolet (UV) radiation (or ozone) to generate hydroxyl radicals ($\bullet OH$), and it has been used both for the decomposition of organic compounds²⁴⁻²⁷ and in sample pretreatments.²⁸⁻³⁰ This process decomposes organic matter in environmental and biological samples prior to chemical analysis.^{6,31-35} It has also been demonstrated in the pretreatment of water samples.^{27,36-39}

There have been few studies of pretreatment of blood samples by AOP for

chemical analysis. In a paper that reported pretreating a blood sample through oxidation processes, potassium persulfate ($K_2S_2O_8$) and UV irradiation were used after plasma and erythrocytes were separated and “de-proteinized.”³⁵ Another paper recommended the use of long UV irradiation and H_2O_2 in blood pretreatment but did not disclose an exact time or elaborate on details.²⁸ Blood is a complicated matrix and is often separated into its serum and cellular components. This, however, requires more tedious effort and time. Examination of whole blood is faster and easier. Catalase is an enzyme that decomposes hydrogen peroxide and protects the body from hydroxyl radicals.⁴⁰ The enzyme is, however, detrimental to the AOP treatment of blood samples and leads to vigorous foaming of the sample. Our group has recently used for the first time H_2O_2 and UV irradiation in an AOP process to pretreat whole blood in the detection of trace biological chromium.⁷

AOP of blood may offer a new pretreatment method, simplifying the detection process and reducing cost for the analysis of metals in blood. Our earlier study of blood pretreatment was targeted toward chromium detection and limited in scope.⁷ We have studied the pretreatment of whole blood by the AOP process in detail. A four-cell reactor has been designed and built to optimize the AOP process that uses 80% less blood in comparison to a single cell reactor.⁷ Finally, the pretreated blood sample was examined using electrochemical analysis to detect the metals chromium and zinc. We report here our results and observations.

2.2. Experimental

2.2.1. Reagents and Materials

Sodium hydroxide (NaOH, Certified ACS, Fisher), potassium oxalate ($K_2C_2O_4$, Certified ACS, Fisher), ferrous sulfate hydrate ($FeSO_4 \cdot 7H_2O$, Acros), acetic acid (HAc, glacial, 99.9%, Fisher), ethanol (95%, Decon Laboratories, Inc.), sodium acetate (NaAc, anhydrous, Certified ACS, Sigma–Aldrich), potassium chloride (KCl, 100%, Mallinckrodt), porcine hemoglobin (Sigma), and nitric acid (HNO_3 , 70%, Trace Metal Grade, Fisher) were used as received. Hg(II), Bi(III) and Zn(II) AA standard solutions (1000 mg L^{-1} , Aldrich) were diluted prior to use. Single walled carbon nanotubes (SWNTs) were obtained from Shenzhen Nanotech Port Co., Ltd., China, (<http://www.nanotubes.com.cn/doce/default.html>). Porcine blood samples were obtained from Wampler's Farm (Lenoir City, Tennessee). The blood was collected in 1-L bottles each containing 2 g of $K_2C_2O_4$ to prevent coagulation. Deionized water ($18\text{ M}\Omega\text{ cm}$) was used in the preparation of aqueous solutions. All glassware was soaked in 1 M nitric acid and rinsed several times with deionized water prior to use. The buffer solution (pH 6.0) contained 0.1 M KCl and 0.1 M NaAc/HAc. The buffer solution (pH 4.5) was prepared by mixing 0.1 M NaAc and 0.1 M HAc.

A three-electrode configuration consisted of a Hg–Bi/SWNTs modified (GCE), Ag/AgCl, and a platinum wire (CH Instruments, Inc.) as working,

reference and counter electrodes, respectively. Zn(II) standards with different concentrations were prepared by diluting the appropriate amount of stock solution in electrolytes.

2.2.1.1. Preparation of Carboxylated SWNTs

SWNTs of a certain mass (40 mg) were dispersed in 30% HNO₃ and then refluxed for 24 h at 140 °C to obtain carboxylic group-functionalized SWNTs. The resulting suspension was centrifuged, and the sediment was washed with deionized water until the pH reached 7.0. Then, the oxidized SWNTs were dispersed in deionized water to a concentration of 0.5 mg mL⁻¹.

2.2.2. Instrumentation

UV-Visible spectra were collected using an Agilent 8453 photodiode array spectrophotometer and a 2.0- or 10.0-mm quartz cuvette. Blank spectra of deionized water were recorded and subtracted from those of the samples. pH measurements were carried out with a pH meter (Accumet Basic, Fisher Scientific). **Caution:** UV light can be dangerous. Wear eye protection.

Anodic stripping voltammetry (ASV) measurements were conducted using an Electrochemical Workstation 440/650A (CH Instruments).

2.2.3. Electrode Modification with Hg-Bi/SWNTs

A GCE (3 mm diameter, CH Instruments.) was polished to a mirror like surface using a standard electrode polishing kit (CH Instruments) including a 1200 grit Carbimet disk, 1.0 and 0.3 μm alumina slurry on a nylon cloth, and 0.05 μm alumina slurry on a microcloth polishing pad. After successive sonication in deionized water, ethanol, and deionized water, 3.2 μL of 0.5 mg mL^{-1} carboxylic group-functionalized SWNTs solution was dropped on the pretreated GCE and dried in N_2 flow. Before the formation of the Hg-Bi film, the SWNTs-coated GCE was then immersed in 0.1 M NaAc/HAc (pH 4.5) solution containing 100 mg/L of a mixture of Hg and Bi (4:1) ions for 4 min under stirring. Right after that, the ex-situ codeposition of Hg and Bi was performed at -1.0 V for 2 min under stirring. After carefully rinsing with deionized water, the obtained Hg-Bi/SWNTs/GCE was used for subsequent assays.

2.2.4. Experimental Procedures

2.2.4.1. Exp. 1 - Pretreatment of the Blood Samples by the Single-Sample, 5.5-W AOP Reactor

Studies of the pretreatment of blood samples by AOP were carried out in a 20-mL photochemical reactor shown in Figure 2.1. The photoreactor consisted of an outer vessel containing the sample, water-jacketed quartz immersion well, and a UV lamp. The reactor was designed and built in house, except for the

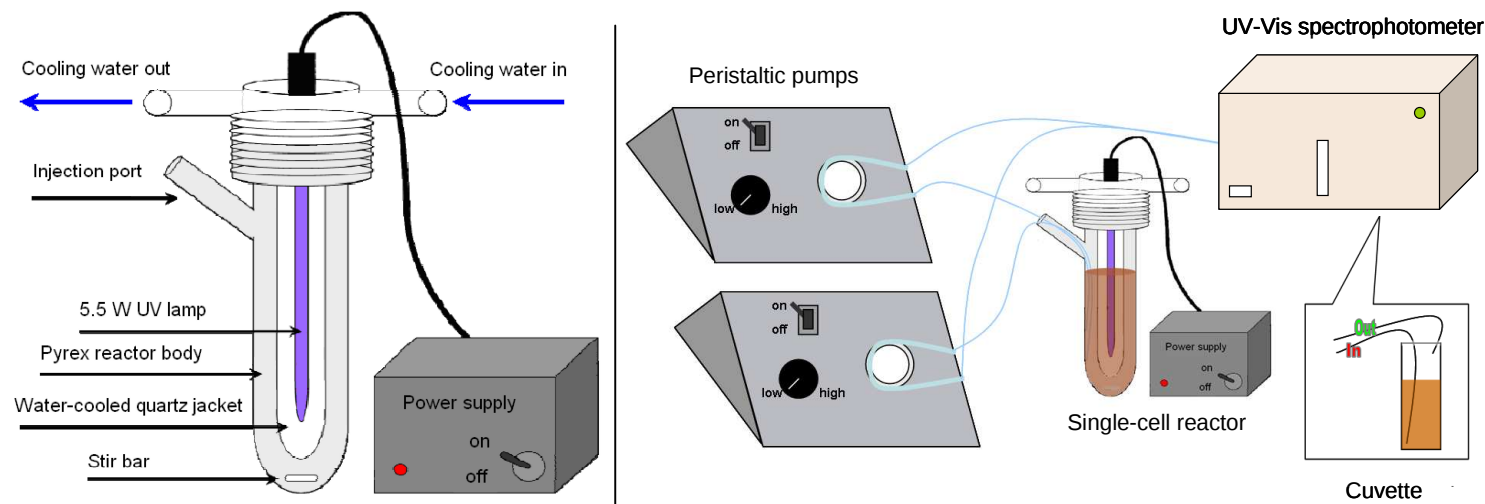


Figure 2.1. Schematic of: (a) The single-cell AOP reactor for Exps. 1-6. (b) The setup using a UV-visible spectrophotometer to conduct the kinetic studies.

quartz immersion well, which was purchased from Ace Glass. The UV lamp was a 5.5-W quartz low-pressure cold cathode mercury gaseous discharge lamp (Ace Glass/Pen-Ray 12132-08).

The UV-Vis absorbance of the AOP treated blood samples was measured at different time intervals during the process. For an individual sample, 500 μL was added to 15 mL of deionized H_2O and adjusted to pH 3 with one drop of concentrated HNO_3 . A 1-L batch of blood was prepared so that its composition would be the same for all experiments. For this blood sample, 32.5 mL of blood was diluted with 975 mL of deionized H_2O in a large beaker. The pH was adjusted to 3.0 with concentrated HNO_3 . It was transferred into a plastic 1 L bottle and stored in the refrigerator until use. For each individual AOP reaction, 15 mL of the diluted, pH-adjusted blood solution was transferred into the AOP reactor. An appropriate amount of 30% H_2O_2 was then added so that the final concentration was in the range of 0.5 to 15.0 g L^{-1} H_2O_2 . The immersion well was inserted and the stirring and UV lamp were turned on. A dual peristaltic pump system was used to continuously monitor the degradation of the sample. The UV-Vis spectrum was recorded every minute for a total of 60 min.

Exp. 2-4, Fenton processes with or without UV irradiation, are given in Appendix A.

2.2.4.2. Exp. 5 - The Effect of Irradiation on the Sample with No Added H₂O₂ --A Control Study

The AOP process was compared to a control process in which the sample was only exposed to UV irradiation without hydrogen peroxide. The data was collected using the apparatus shown in Figure 2.1 using UV light exposure alone. The procedure was performed as described previously for a single sample using 15 mL of solution.

2.2.4.3. Exp. 6 - Pretreatment of Pure Hemoglobin by AOP

AOP of a pure hemoglobin solution was also studied. The solution was produced based on calculations of how much hemoglobin is in whole blood and therefore in a diluted AOP sample. Porcine hemoglobin (0.12 g) was dissolved in 15 mL deionized water and the pH was adjusted to 3 using concentrated nitric acid. AOP was performed as described previously for a single sample using 15 mL of solution.

2.2.4.4. Kinetic Studies of the Pretreatment of Blood Samples

The kinetics of AOP with respect to H₂O₂ concentrations were studied using the same batch stock solution as described in Exp. 1. The following concentrations were studied in triplicate: 0.50, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 7.5, 10.0, 13.0, and 15.0 g L⁻¹.⁴¹ When studying AOP kinetics with respect

to the blood solution, the concentration of blood in the sample was varied while keeping the hydrogen peroxide concentration constant. In such cases, 250 μL , 500 μL , or 750 μL of whole blood in 15 mL of deionized water were used.

2.2.4.5. *Four-Cell Reactor*⁴¹

The reactor is made of a base that contains four stirrers fashioned from DC motors (Jameco Electronics). The stirrers were placed in a custom-made box and controls were added. The top of the box was designed to allow a water-cooled quartz immersion well (Ace Glass) to be inserted horizontally over the sample stirrers. A 100-W UV lamp (Hanovia) was then inserted into the immersion well that was placed inside an elliptical shaped cylinder. A wooden box was used to host the setup to contain any dangerous UV light. A power supply for the 100-W UV lamp was kept outside of the box. Cells for the reactor were made from square borosilicate tubing. The volume of each cell is 5 mL with a surface area of 6.45 cm^2 . A photo and details about the four-cell reactor are provided in Appendix A.

In a typical test, 3 mL of the 1-L batch stock solution was placed into each of the four cells. A stir bar was placed in each cell and all cells were transferred carefully into the reactor.

2.2.4.6. Zn Analysis

For ASV experiments, 20 mL of standard solutions were used without deaeration. Convective accumulation of Zn(II) on the Hg–Bi/SWNTs/GCE was carried out at a deposition potential of -1.3 V under stirring for 5 min. After the accumulation time, the anodic voltammetric measurements were registered in the quiescent solution by square wave voltammetry (SWV) with frequency of 15 Hz, step potential of 4 mV and amplitude of 25 mV. The potential sweep was ended at -0.3 V. The SWV scan started immediately upon completion of the accumulation. No resting period was used between the accumulation and stripping steps. Stirring at a high speed was required during the accumulation process but was stopped at the end of the quiet period for the stripping step. Between measurements, an electrochemical pre-cleaning step was applied in 0.1 M NaAc/HAc solution containing 0.1 M KCl at -0.3 V for 60 s, to guarantee the efficient removal of Zn(II).

After AOP 10 mL of the sample was added to 10 mL of a buffer solution (pH 6.0) containing 0.1 M KCl and 0.1 M NaAc/HAc. ASV experiments were performed as described previously.

2.3. Results and Discussion

The AOP reaction produces $\bullet\text{OH}$ radicals, a very potent oxidant, by Eq. 1.1.²⁴ Subsequent attack of organic/biological species by the radicals gives

water, CO₂, and free metal ions. A combination of the following processes contribute to the generation of •OH radicals and the overall efficiency of the method.⁴³⁻⁴⁹ If Fe^{2/3+} ions are present, they may catalyze the generation of the •OH radicals by the Fenton process as well as the photo-Fenton process.⁴¹



2.3.1. Pretreatment of Blood – A Comparison of Different Processes

In blood, iron is part of proteins and there are generally no free Fe^{2/3+} ions as catalyst.⁴⁰ UV irradiation of a blood solution containing H₂O₂ leads to decomposition of the proteins using the •OH radicals generated in Eq. 1.1. This initial step is a non-catalytic process. The decomposed proteins, however, release Fe^{2/3+} ions that may then catalyze the subsequent reactions through the photo-Fenton reactions.⁴¹ In other words, the AOP pretreatment of blood is an auto-catalytic process.

We have conducted studies to compare a direct photodissociation, Fenton process conducted without photodissociation, and the photo-Fenton process with an added iron catalyst. In Exp. 1, H₂O₂ was added to the sample and irradiated in a photochemical reactor designed in house (Figure 2.1a) for a total of 60 min. A Pen-Ray 5.5-W UV lamp was used in the reactor. This lamp gives an intense peak at 254 nm that accounts for ca. 45.6% of the total intensity of the lamp.⁴¹ This irradiation and possibly others in the 200-300 nm range leads to direct

dissociation of H_2O_2 into $\bullet\text{OH}$ radicals.^{46,51-53} In Exp. 2, the same amount of H_2O_2 was added, and the sample was kept in dark in the reactor for 60 min.⁴¹ Exp. 3 was similar to Exp. 2 except that a small amount of FeSO_4 was used.⁴¹

The results of Exp. 1 are shown in Figure 2.2. The 300-500 nm range of the spectra reveals the peak of hemoglobins (ca. 380 nm) which essentially disappeared in 60 min. When the UV irradiation was removed in Exp. 2, little decomposition of the blood in dark was observed (Figure A.1)⁴¹ indicating that auto-catalytic Fenton process relying on the release of iron from decomposed proteins was very slow. Subsequently, FeSO_4 was added to the sample to provide Fe^{2+} ions (Exp. 3), with no UV irradiation. Over a period of 60 min, the degree of decomposition in Exp. 3 was very small (Figure A.2)⁴¹ in comparison to that in Exp. 1 (Figure 2.2).

Exp. 4, with UV irradiation, was conducted as in Exp. 1 except that a small amount of FeSO_4 was added as catalyst. To our surprise, this process was slower than that in Exp. 1.⁴¹ After 60 min, the peak at 380 nm was still significant (Figures 2.3 and A.3). Perhaps the added Fe^{2+} ions bind to the organic/biological species in blood, reducing their catalytic capacity. A control test (Exp. 5) using direct UV irradiation without H_2O_2 (Figure A.4) showed that the decomposition of the blood by UV alone is negligible.⁴¹

The results from Exps. 1-5 clearly show that Exp. 1 is the best process. The observation that the added Fe^{2+} ions in Exp. 4 did not increase the rate suggests that, except at very high $C_{\text{H}_2\text{O}_2, t=0}$ discussed below, Fenton or photo-

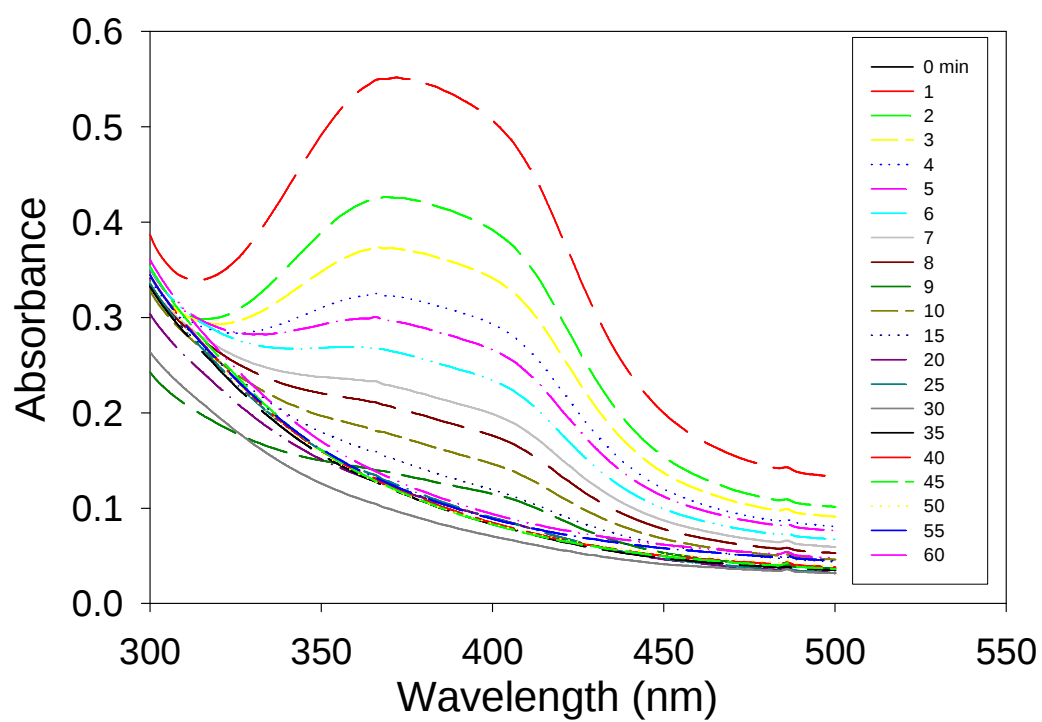


Figure 2.2. UV-Vis spectra of blood in the AOP pretreatment in Exp. 1, as shown by the peak at 380 nm. $C_{H_2O_2} = 1.5 \text{ g L}^{-1}$.

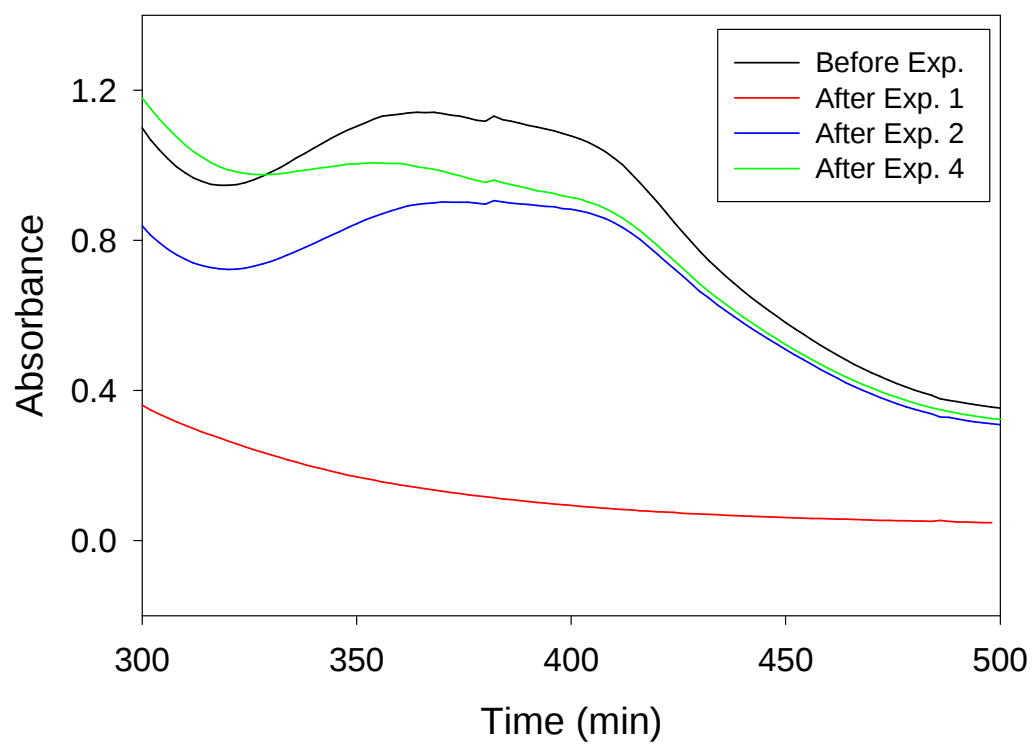


Figure 2.3. A comparison of the results in Exps. 1, 2 and 4. $C_{H_2O_2} = 1.5 \text{ g L}^{-1}$.

Fenton process does not play a leading role in the AOP of blood using UV irradiation at room temperature. The principal pathway is apparently the generation of $\bullet\text{OH}$ radicals from H_2O_2 in the mixture by Eq. 1.1 which then decompose the blood. The reasons for the lack of the Fenton catalysis at the early stage of the AOP process in the current work are not clear. We speculate that the chemical and biological species in fresh blood bind to the $\text{Fe}^{2/3+}$ ions, preventing them from catalyzing the conversion of H_2O_2 to $\bullet\text{OH}$ radicals. At this early stage, the AOP process perhaps relies on the $\bullet\text{OH}$ radicals generated by the direct photolysis of H_2O_2 in Eq. 1.1. After the AOP process has proceeded for a while, the chemical and biological species in the blood have significantly decomposed, and their abilities to bind to $\text{Fe}^{2/3+}$ ions have weakened. The newly released $\text{Fe}^{2/3+}$ ions will then catalyze subsequent blood decomposition.

In all the tests, HNO_3 was added to the samples to lower pH to 3 prior to the addition of H_2O_2 .⁷ This step inhibited the enzyme catalase so that, when H_2O_2 was added to a diluted sample, no vigorous foaming due to the decomposition of H_2O_2 would occur. The selection of HNO_3 over other compounds to inhibit catalase has been discussed previously.⁷ H_2SO_4 and HCl have been studied as well, and both performed similarly to HNO_3 , although it appeared that HCl was not quite as effective. Some initial foaming was observed.

Studies were conducted to confirm that the peak at 380 nm which was monitored in Exps. 1-5 was indeed that of hemoglobin.⁵⁴ The confirmation was

also important to the kinetic studies below. In Exp. 6, a solution of solely porcine hemoglobin was treated with H_2O_2 and UV irradiation, as in Exp. 1. The results (Figure A.5) show that the 380 nm peak is indeed essentially that of hemoglobin, and its decomposition is similar to that of whole blood in Figure 2.2.⁴¹ Blood is a complex mixture of many chemical and biological components, and there is no good method to follow the decomposition of every component in blood. Hemoglobin is a major component of blood, and its decomposition provides a simple, direct means to follow the blood decomposition.

2.3.2. Kinetics of Blood Pretreatment by AOP

An understanding of the kinetics of the pretreatment process would provide basic kinetic parameters such as rates and orders with respect to reactants, and help to better design the process itself. The kinetics of AOP processes for numerous organic compounds has been studied, including the development of a model by Crittenden and coworkers.³⁶ Zeroth-order,^{24,27} first-order,⁵⁵⁻⁵⁷ pseudo-first order,⁵⁸ and second-order^{43,59-61} kinetics have been reported.

The H_2O_2 photolysis itself is the initial step in an AOP process with a quantum efficiency of 0.98 at 254 nm.^{46,59-62} The analysis in Appendix A shows that, if the concentration of H_2O_2 is high and the absorption by water in the UV region is ignored, the limiting reagent is the photon flux of the UV light source (rate of photon emission). Such photochemical dissociation of H_2O_2 follows

zeroth-order kinetics. If the concentration of H_2O_2 ($C_{\text{H}_2\text{O}_2}$) is not high, such photochemical dissociation of H_2O_2 follows first-order kinetics.⁴¹ Thus the limiting kinetic orders for the H_2O_2 photolysis itself are zero when $C_{\text{H}_2\text{O}_2}$ is high and first when $C_{\text{H}_2\text{O}_2}$ is not high.

There are challenges in studying the kinetics of the AOP pretreatment of blood by H_2O_2 . Blood is a very complex matrix. At any given time of the AOP process, numerous species within the blood could be decomposing. For the sake of simplicity, hemoglobin is used to represent the components in blood (C_{blood}). A decrease in absorbance of hemoglobin at 380 nm indicates the blood decomposition, as shown in Exp. 6. However, using kinetic laws designed for specific reactants for a complex mixture of numerous species in blood may lead to significant deviation from the kinetic laws. In addition, blood absorbs in the UV range (Figures 2.2 and 2.3). Although Exp. 5 revealed that the blood decomposition by direct UV irradiation is negligible,⁴¹ the absorption of blood in the UV range reduces the number of photons for the photolysis of H_2O_2 , affecting the kinetics of the process.

In order to monitor the process, the solution in the photochemical reactor was circulated through a cuvette in a UV-visible spectrophotometer by a dual peristaltic pump system (Figure 2.1b). When $C_{\text{H}_2\text{O}_2}$ is sufficiently high, the rate law can be expressed as

$$-(dC_{\text{blood}} / dt) = \text{Rate} = k C_{\text{blood}}^y \quad \text{Eq. 2.1}$$

where y = order with respect C_{blood} ; k = rate constant.

In order to determine y , $\ln A$ vs. t are plotted (Figure 2.4). When $C_{\text{H}_2\text{O}_2, t=0} = 1.0\text{-}2.5 \text{ g L}^{-1}$, the plots significantly deviate from being linear, suggesting that $C_{\text{H}_2\text{O}_2, t=0}$ here is perhaps too low to make the reaction zeroth-order with respect to the concentration of H_2O_2 . At $C_{\text{H}_2\text{O}_2, t=0} = 3.0, 4.0, \text{ and } 5.0 \text{ g L}^{-1}$, the $\ln A$ vs. t plots are essentially linear with nearly identical slopes of -0.0230 ($R^2 = 0.996$), -0.0241 ($R^2 = 0.996$), and -0.0235 ($R^2 = 0.990$), respectively. These results suggest that the reactions have reached a rate-limiting stage at these high $C_{\text{H}_2\text{O}_2, t=0}$. In addition, at this rate-limiting stage, the blood decomposition itself follows first-order kinetics. In other words, $y = 1$ and the rate law in Eq. 2.1 becomes

$$-(dC_{\text{blood}} / dt) = \text{Rate} = kC_{\text{blood}} \quad \text{Eq. 2.2}$$

where the observed rate constant $k = 0.0230\text{-}0.0241 \text{ min}^{-1}$.

Additional kinetic studies were performed in which the $C_{\text{blood}, t=0}$ were changed from 250, 500, and 750 μL in 15 mL of solution, respectively, while $C_{\text{H}_2\text{O}_2, t=0}$ was changed from 3.0, 5.0, to 7.0 g L^{-1} . First-order kinetic relationships were again observed. k from these studies (Table 2.1), with the average $k = 0.0216(18) \text{ min}^{-1}$, are close to those obtained in Figure 2.4. The fact that the rate

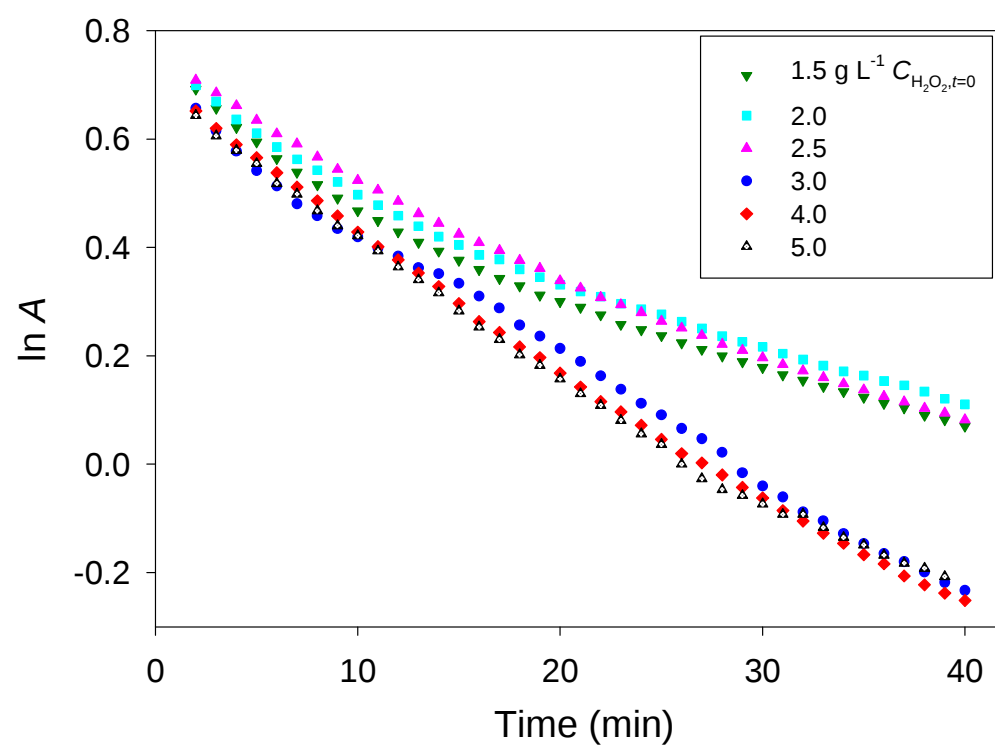


Figure 2.4. Plots of $\ln A$ vs. t for the AOP pretreatment of the blood samples.

Table 2.1. First-order rate constants for $C_{H_2O_2,t=0}$

$C_{H_2O_2,t=0}$ (g L ⁻¹)	k_{obs}		
	$C_{blood,t=0} = 250 \mu\text{L}:15 \text{ mL H}_2\text{O}$	$C_{blood,t=0} = 500 \mu\text{L}:15 \text{ mL H}_2\text{O}$	$C_{blood,t=0} = 750 \mu\text{L}:15 \text{ mL H}_2\text{O}$
3.0	0.0203 ± 0.0018	0.0221 ± 0.0007	0.0204 ± 0.0012
5.0	0.0232 ± 0.0065	0.0236 ± 0.0011	0.0200 ± 0.0004
7.0	0.0201 ± 0.0023	0.0195 ± 0.0021	0.0222 ± 0.0007

* $C_{blood,t=0}$ here refers to the volume of the blood in 15 mL of solution.

reaches a rate-limiting stage at $C_{H_2O_2,t=0} = 3.0-7.0 \text{ g L}^{-1}$ indicates that, at the high concentrations of H_2O_2 , the reaction rate is limited by the photon flux reaching the solution that decomposes H_2O_2 to the $\bullet OH$ radicals. In other words, the decomposition of H_2O_2 has reached a steady-state with a constant rate of the formation of the $\bullet OH$ radicals such that Eq. 2.1 appropriates to Eq. 2.2.

Additional tests using $C_{H_2O_2,t=0} = 7.5-15 \text{ g L}^{-1}$ show that the treatment by Exp. 1 is faster than that at $C_{H_2O_2,t=0} = 3.0-7.0 \text{ g L}^{-1}$. At such high concentrations of H_2O_2 , the catalytic role played by the iron ions released from the decomposition of proteins is perhaps more significant. In other words, both direct AOP and the photo-Fenton process are involved in the decomposition of the blood here. It should be pointed out, however, too high H_2O_2 concentrations tend to scavenge the $\bullet OH$ radicals, rendering the process less effective.⁶³

2.3.3. Four-Cell Reactor

After extensive studies using the original reactor, our group designed and built a four-cell reactor, well suited for small sample volumes. Additionally multiple samples can be run simultaneously.⁴¹ The reactor uses a 100-W UV lamp and four 5-mL cells. An aluminum mirror in the shape of an elliptical cylinder was made with a portion of its bottom open to focus the light from the lamp to the four cells underneath.⁴¹

The spectrum of the lamp reveals several emission lines in the 200-300 nm range that together account for 14.2% of the total intensity.⁴¹ Thus each cell

on average receives ca. 3.6 W irradiation in 200-300 nm, the range leading to direct H_2O_2 dissociation into the $\bullet\text{OH}$ radicals.^{45,50-52} In comparison, UV irradiation of the 5.5-W lamp in the single-cell reactor is centered at 254 nm,⁴¹ accounting for ca. 45.6% (2.5 W) of the total irradiation with 100% quantum efficiency. [The irradiation in the 200-300 nm range accounts for 46.3% (or 2.5 W) of the total.] Thus the reaction rates in the single- and four-cell reactors are not expected to be significantly different.

Results of tests using the four-cell reactor are given in Figure 2.5. First, only 97 μL of blood was needed per cell, and the solution volume was 3 mL. In comparison, 500 μL of the blood was needed for the single-cell reactor, and the solution volume was 15 mL. Second, the reactions in the four cells (Figure 2.5) proceeded at rates comparable to that in the single-cell reactor, and the process was nearly complete in 40 min, as expected from the analysis of the irradiation from both lamps in the 200-300 nm range. Third, the reactions in each of the four cells proceeded at similar rates (Figure 2.5).

2.3.4. Effect of pH

The AOP of blood needs to start at pH 3 in order to inhibit catalase in blood.⁴¹ Our earlier studies show that AOP is optimal at higher pH.⁷ The AOP treatment of chromium(III) propionate, for example, produces protons from both

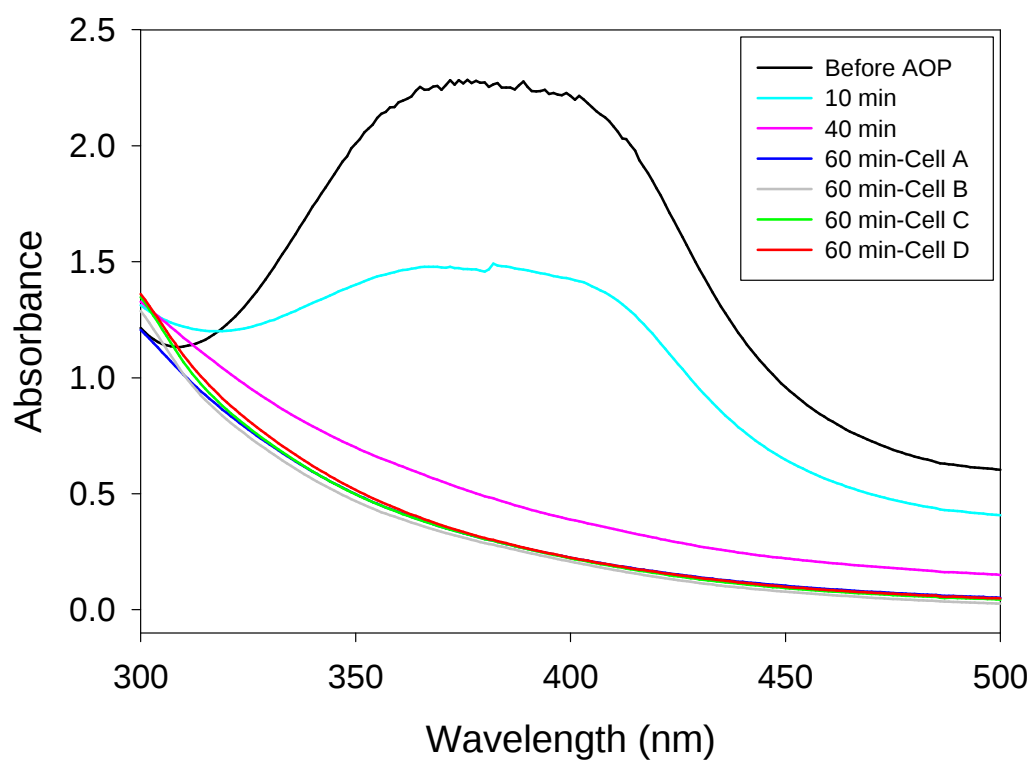


Figure 2.5. A comparison of the four cells in the reactor. NaOH as a base was added at 40 min.

decomposition of the ligand and the Cr(III) to Cr(VI) conversion.^{6,41} It is expected that degradation of biological/organic species in blood, generating CO₂ and protons, also lowers pH.⁴¹ Thus, after the AOP process has proceeded sufficiently to decompose catalase, raising pH of the solution at this point to neutralize the acids speeds up the process. After reactions had proceeded for 40 min, raising pH to 9.5 led to additional degradation of the sample (Figure A.6).⁴¹ If NaOH is added before 40 min, foaming occurs indicating that catalase has not fully decomposed.

2.3.5. Electrochemical Analysis of Cr and Zn in AOP-Treated Blood

Samples

Chromium has been studied for its use as a dietary supplement for the treatment of diabetes and its complications, but since levels are extremely low in biological tissues and fluids (<10 ppb in blood of mammals), many difficulties arise during detection. Current analytical techniques with the adequate sensitivity require expensive instrumentation and are thus not widely available. Our group developed a method to use adsorptive stripping voltammetry (CA_{AdSV}) for Cr analysis in the AOP-treated blood sample. In the porcine blood sample, the Cr concentration was 6.0(0.3) ppb.⁷

It was well established over 100 years ago that zinc is an essential nutrient.⁶⁴ Zinc deficiency has been found detrimental in physical growth and bodily function.⁶⁴ Low zinc dietary intake has been shown to effect one-third to

one-half of the world's population.⁶⁴ High zinc levels, however, can be toxic.⁶⁴ Zinc level has been analyzed by various methods including electrochemistry which has been shown to be highly sensitive.^{42,65}

Zn concentration in our AOP-treated blood sample was found to be 282(8) ppb, and the result was consistent with that from the standard dry ashing, followed by an ICP analysis which has been published elsewhere.⁶⁶ The results of the Zn detection using ASV are shown in Figure 2.6. A calibration range of 5-10 ppb (correlation coefficient $R^2 = 0.995$) in a buffer solution (pH 6.0) containing 0.1 M KCl and 0.1 M NaAc/HAc was used to obtain the Zn concentration in the AOP sample.⁴² The Zn peak shifted slightly more positive, most likely due to matrix effects of the residues in the AOP-treated sample which has been witnessed other detections.^{7,67} While other metals are found in blood they are either at a much lower concentration or are reduced at a different potential and therefore not interfering with zinc.^{42,66} Additionally, the electrode used in this analysis found minimal interference regarding zinc detection.⁴² While Ru(III) and Rh(III) showed slight interference, neither of these metals are normally found in whole blood.⁶⁶

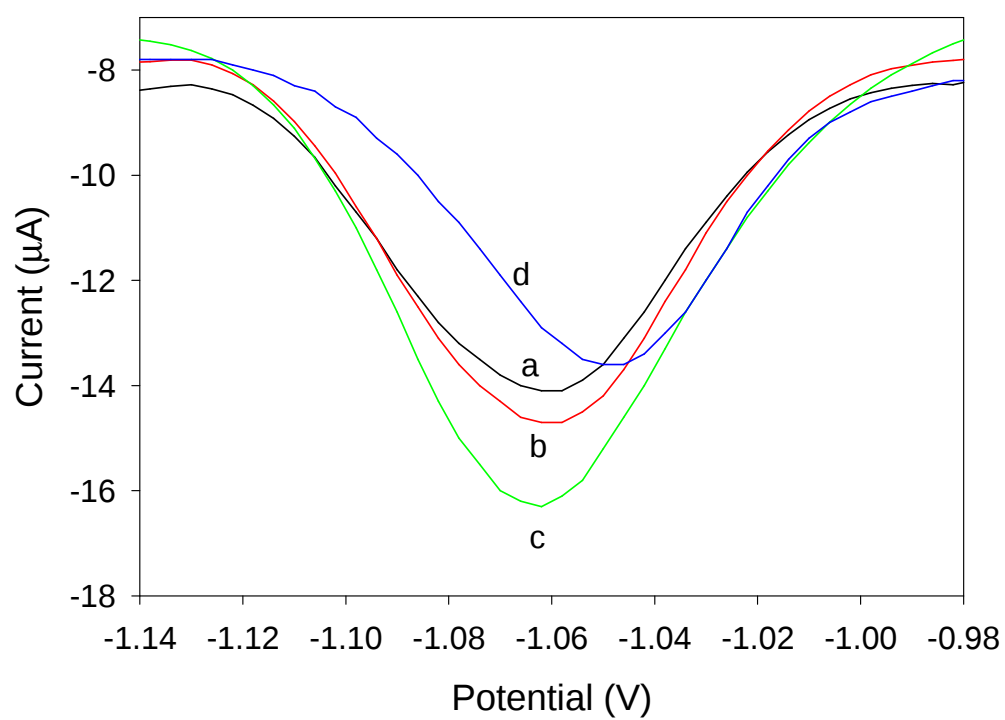


Figure 2.6. ASV of Zn in standard solutions: (a) 5; (b) 7; and (c) 10 ppb; (d) represents detection in a blood sample after AOP.

2.4. Conclusions

The decomposition of blood by AOP has been studied in detail. The studies give an insight into the processes taking place during the pretreatment. The rate limiting process is photon flux from the lamp when $C_{H_2O_2, t=0}$ is sufficiently high. The reaction is zeroth-order with respect to $C_{H_2O_2}$ and first-order with respect to C_{blood} . The four-cell reactor uses much less blood and allows several samples to be treated simultaneously. The blood pretreatment is interdisciplinary in nature involving, e.g., photochemistry of the inorganic chemical H_2O_2 , degradation of biological/organic species in blood by $\bullet OH$ radicals, kinetics of the reactions, and the design of new reactors for the pretreatment. To confirm the success of the procedure, Cr and Zn in blood samples were detected. This procedure developed in the current work leads to the successful decomposition of biological/organic species in blood.

References

1. Miller, N.J.; Rice-Evans, C.; Davies, M.J.; Gopinathan, V.; Milner, A. *Clin. Sci.* **1993**, *84*, 407-412.
2. Knahr, K.; Karamat, L.; Pinggera, O. *Ceramics in Orthopaedics (Bioceramics and Alternative Bearings in Joint Arthroplasty)*, Springer, 2005; pp. 39-44.
3. Lewis, S.A.; O'Haver, T.C.; Harnly, J.M. *Anal. Chem.* **1984**, *56*, 1066-1070.
4. Wang, J. *J. Electroanal. Chem.* **1982**, *139*, 225-232.
5. Eller, P.M.; Haartz, J.C. *Am. Ind. Hyg. Assoc. J.* **1977**, *38*, 116-124.
6. Rodman, D.L.; Carrington, N.A.; Xue, Z.-L. *Talanta* **2006**, *70*, 668-675.
7. Yong, L.; Armstrong, K.C.; Dansby-Sparks, R.N.; Carrington, N.A.; Chambers, J.Q.; Xue, Z.-L. *Anal. Chem.* **2006**, *78*, 7582-7587.
8. Silvestre, C.I.C.; Santos, J.L.M.; Lima, J.L.F.C.; Zagatto, E.A.G. *Anal. Chim. Acta.* **2009**, *652*, 54-65.
9. Pyrzynska, K. *Crit. Rev. Anal. Chem.* **2007**, *37*, 39-49.
10. Guo, T.; Baasner, J. *Talanta* **1993**, *40*, 1927-1936.
11. Huang, C.; Yang, M.; Shih, T. *Anal. Chem.* **1997**, *69*, 3930-3939.
12. Ibeto, C.N.; Okoye, C.O.B. *Res. J. Environ. Sci.* **2010**, *4*, 371-382.
13. Hoenig, M. *Talanta* **2001**, *54*, 1021-1038.
14. Mushtakova, V.M.; Fomina, V.A.; Rogovin, V.V. *Biol. Bull.* **2005**, *32*, 276-

278.

15. Vereda Alonso, E.; de Torres, A.G.; Cano Pavon, J.M. *Talanta* **1996**, *43*, 493-501.
16. Rodman, D.L.; Carrington, N.A.; Xue, Z.-L. *Talanta* **2006**, *70*, 426-431.
17. Carrington, N.A.; Thomas, G.H.; Rodman, D.L.; Beach, D.B.; Xue, Z.-L. *Anal. Chim. Acta* **2007**, *581*, 232-240.
18. Carrington, N.A.; Yong, L.; Xue, Z.-L. *Anal. Chim. Acta* **2006**, *572*, 17-24.
19. Rodman, D.L.; Pan, H.; Clavier, C.W.; Feng, X.; Xue, Z.-L. *Anal. Chem.* **2005**, *77*, 3231-3237.
20. Carrington, N. A.; Qiu, H.; Xue, Z.-L. *Am. Lab.* **2007**, *39*, 37-41.
21. Dansby-Sparks, R.N.; Chambers, J.Q.; Xue, Z.-L. *Anal. Chim. Acta* **2009**, *643*, 19-25.
22. Carrington, N.A.; Xue, Z.-L. *Acc. Chem. Res.* **2007**, *40*, 343-350.
23. Dansby-Sparks, R.N.; Ouyang, R.; Xue Z.-L. *Sci. China Ser. B.* **2009**, *52*, 1777-1788.
24. Schulte, P.; Bayer, A.; Kuhn, F.; Luy, T.; Volkmer, M. *Ozone-Sci. Eng.* **1995**, *17*, 119-134.
25. Safarzadeh-Amiri, A. *Water Res.* **2001**, *35*, 3706-3714.
26. Beltran, F.J.; Gonzalez, M.; Rivas, J.; Marin, M. *Ind. Eng. Chem.* **1994**, *33*, 125-136.
27. Sigman, M.E.; Buchanan III, A.C.; Smith, S.M. *J. Adv. Oxid. Technol.* **1997**, *2*, 415-423.

28. Golimowski, J.; Golimowska, K. *Anal. Chim. Acta* **1996**, 325, 111-133.
29. Capelo-Martinez, J.; Ximénez-Embún, P.; Madrid Y.; Cámara, C. *TrAC-Trends Anal. Chem.* **2004**, 23, 331-340.
30. Apostoli, P. *J. Chromatogr. B.* **2002**, 778, 63-97.
31. Crittenden, J.C.; Hu, S.; Hand, D.W.; Green, S.A. *Water Res.* **1999**, 33, 2315-2328.
32. Malaiyandi, M.; Sadar, H.; Lee, P.; O' Grady, R. *Water Res.* **1980**, 14, 1131-1135.
33. Mandal, T.; Maity, S.; Dasgupta, D.; Datta, S. *Desalination* **2010**, 250, 87-94.
34. Stasinakis, A.S. *Global NEST J.* **2008** 10, 376-385.
35. Ping, L.; Dasgupta, P.K. *Anal. Chem.* **1989**, 61, 1230-1235.
36. Atallah, R.H.; Kalman, D.A. *Talanta* **1991**, 38, 167-173.
37. Guéguen, C.; Belin, C.; Thomas, B.A.; Monna, F.; Favarger, P.Y.; Dominik, J. *Anal. Chim. Acta* **1999**, 386, 155-159.
38. Begerow, J.; Turfeld, M.; Dunemann, L. *Fresen. J. Anal. Chem.* **1997**, 359, 427-429.
39. He, B.; Jiang, G.; Xu, X. *Fresen. J. Anal. Chem.* **2000**, 368, 803-808.
40. Berg, J.M.; Tymoczko, J.L.; Stryer, L. in *Biochemistry*, Freeman: New York, 2002; p. 974.
41. See Appendix A.
42. Ouyang, R.; Zhu, Z.; Tatum, C.E.; Chambers, J.Q.; Xue, Z.-L. *J.*

- Electroanal. Chem.* **2011**, 656, 78-84.
43. Benitez, F.J.; Real, F. J.; Acero, J.L.; Leal, A.I.; Cotilla, S. *J. Environ. Sci. Heal. A.* **2005**, 40, 2153-2169.
44. Zheng, K.; Lai, C.Y.; He, L.P.; Li, F. *Sci. China Chem.* **2010**, 53, 1398-1405.
45. Ntampegliotis, K.; Riga, A.; Karayannis, V.; Bontozoglou, V.; Papapolymerou, G. *J. Hazard. Mater.* **2006**, 136, 75-84.
46. Benitez, F.J.; Real, F.J.; Acero, J.L.; Garcia, C.; Llanos, E.M. *J. Chem. Technol. Biot.* **2007**, 82, 65-73.
47. Hunt, J.P.; Taube, H. *J. Am. Chem. Soc.* **1952**, 74, 5999-6002.
48. Bauer, R.; Fallmann, H. *Res. Chem. Intermediat.* **1997**, 23, 341-354.
49. Liang, A.; Zhang, N.N.; Jiang, Z.L.; Liu, R.J. *Sci. China Ser. B: Chem.* **2008**, 51, 226-232.
50. Litter, M. In *Environmental Photochemistry Part II*, P. Boule, D.W. Bahnemann, P.K.J. Robertson, Eds.; Springer-Verlag: Berlin/Heidelberg, 2005; Vol. 2M, pp. 325-366.
51. Schumb, W.C.; Satterfield, C.N.; Wentworth, R.L. *Hydrogen Peroxide*, Reinhold Publishing: New York, 1955; p. 291.
52. Holt, R.B.; McLane, C.K.; Oldenberg, O. *J. Chem. Phys.* **1948**, 16, 225-229.
53. Wang, F.; Haag, W. 1999 Proceedings of the 4th International Conference on Atomic and Molecular Pulsed Lasers, Tomsk, Russia, September 13-

- 17, 1999; UCRL-JC-135610.
54. Atamna, H.; Ginsburg, H. *J. Biol. Chem.* **1995**, *270*, 24876-24883.
 55. Kavitha, V.; Palanivelu, K. *J. Photoch. Photobio. A.* **2005**, *170*, 83-95.
 56. Zhang, W.; Xiao, X.; An, T.; Song, Z.; Fu, J.; Sheng, G.; Cui, M. *J. Chem. Technol. Biot.* **2003**, *78*, 788-794.
 57. Shemer, H.; Kunukcu, Y.K.; Linden, K.G. *Chemosphere* **2006**, *63*, 269-276.
 58. Zimbron, J.A.; Reardon, K.F. *Water Res.* **2005**, *39*, 865-869.
 59. Zepp, R.G.; Cline, D.M. *Environ. Sci. Technol.* **1977**, *11*, 359-366.
 60. Baxendale, J.H.; Wilson, J.A. *T. Faraday Soc.* **1957**, *53*, 344-356.
 61. Weeks, J.L.; Matheson, M.S. *J. Am. Chem. Soc.* **1956**, *78*, 1273-1278.
 62. Alshamsi, F.S.; Albadwawi, A.S.; Alnuaimi, M.M.; Rauf, M.A.; Ashraf, S.S. *Dyes Pigments* **2007**, *74*, 283-287.
 63. Stephan, M.I. in *Advanced Oxidation Processes for Water and Wastewater Treatment*; S. Parsons, Ed.; IWA Publishing: London, 2004, p. 7.
 64. Brown, K.H.; Wuehler, S.E.; Peerson, J.M. *Food Nutr. Bull.* **2001**, *22*, 113-125.
 65. See Armstrong, K.C.; Tatum, C.E.; Dansby-Sparks, R.N.; Chambers, J.Q.; Xue, Z.-L. *Talanta* **2010**, *82*, 675-680 and references therein.
 66. Bragg, S.A.; Xue, Z.-L. *Am. J. Analyt. Chem.* **2011**, *2*, 979-983.
 67. Ouyang, R.; Bragg, S.A.; Chambers, J.Q.; Xue, Z.-L. *Anal. Chim. Acta*

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Part 3

Optimization of Dry Ashing of Whole Blood Samples for Trace Metal Analysis

This chapter is revised based on a paper published by Stefanie A. Bragg and Zi-Ling Xue. Only minor revisions were made.

S.A. Bragg and Z.-L. Xue. Optimization of dry ashing of whole blood samples for trace metal analysis. *American Journal of Analytical Chemistry*, **2011**, 2, 979-983.

Abstract

The use of dry ashing of whole blood samples is described. Ashing these samples are difficult to carry out with significant sample loss, and the procedure is not well documented. A new procedure has been developed and optimized to dry ash whole blood samples for trace metal analyses. The procedure reduces both the sample loss and dry ashing time by more than two thirds. The ashed sample can be readily used in subsequent, simultaneous or individual analysis of several metals by ICP-OES, as demonstrated in the analysis of a whole blood sample. The new procedure is simple, inexpensive, and faster than the established method.

3.1. Introduction

Initial elemental analysis was first recorded by Antoine Lavoisier when he attempted to determine the composition of organic compounds in the eighteenth

century.¹ Since then much progress has been made in this area including optimization of spectroscopic techniques, invention of new instrumentation, and collaboration between isolation and analysis so that ultra trace amounts of elements can be detected.¹⁻³ Biological samples such as tissues, blood, serum, and urine have been studied in multiple instruments for the detection of various molecules and elements. Due to their complexity, it is often necessary to pretreat biological samples prior to injection into an instrument. Depending on the sample and the experimental conditions, interferences may become problematic or residues may be left behind on the inner surface.⁴ Ashing procedures are often implemented prior to elemental or molecular analysis. Many studies have incorporated ashing techniques for the study of abundant minerals as well as evidence of trace metals using methods such as atomic absorption (AA),^{5,6} flameless atomic absorption (FAA),⁷ and inductively coupled plasma optical emission spectrometry (ICP-OES).⁸ Further, many researchers have used ashing prior to studying radioactive isotopes due to matrix interferences.^{4,9,10} More importantly, dry ashing has been a benchmark in the analysis of many biological samples and the development of new analytical methods^{9,11-14} with many citations in the past year.¹⁵⁻²⁰

The process of dry ashing is a versatile technique for sample preparation. Simply defined, dry ashing is the process of heating a sample to high temperatures to remove all organic matter with or without an ashing aid.²¹ A muffle furnace may be used to perform the actual ashing as it allows for a

temperature gradient.^{4,6,9} These are, however, quite expensive and are not a practical purchase for intermittent use. There are other pretreatment methods for samples prior to chemical analysis. Wet digestion, also called wet ashing, has been widely used, but it introduces possible background interference from the addition of reagents. Furthermore, wet digestion is typically used for isolated specific elemental detection and is usually only necessary for very volatile elements like boron, fluorine, mercury and selenium.^{5,8,10,22} Microwave digestion has recently found use for pretreatment of samples but has costly instrumentation, and, like wet digestion, sample size is small, usually ten grams or less.^{23,24} In comparison to wet or microwave digestion, dry ashing is an inexpensive method that allows for multiple elemental analyses in one sample without the addition of chemical interferents. Its most attractive feature, though, is the ability to preconcentrate samples for detection of trace metals.

Although dry ashing has been used for a long time,¹ there is unfortunately little standardization of this technique, particularly for biological samples.²¹ For dry ashing with respect to the determination of inorganic matter in organic matter, Bock offers several materials for the crucible itself, including silica, porcelain, and platinum, and discusses options of sample size to be ashed. Further discussion of ashing temperature options follows and time is only mentioned with respect to air flow over the sample. No discussion of an approximate time is given.²¹ Various articles have used dry ashing as a standard for decomposition of organic samples but there are still inconsistencies among methods and procedures.

Tidehag and co-workers dry-ashed blood samples for a total of 48 h but did not mention the total ash obtained.²⁵ Temperatures play a critical role in both the initial removal of water and gradual ashing of organic and biological samples into ashes by flames. However, few have reported the use of thermocouples for temperature measurements and control in dry ashing.

During our recent studies of new analyses for the trace metal chromium in blood samples,^{13,26} we have found that the lack of standardization and temperature monitoring/control for dry ashing of blood samples leads to a long process (often several days) with significant sample loss. We have developed an improved dry ashing procedure with the use of thermocouples. The temperature of the process is elevated gradually but without the need of a muffle furnace. This new, optimized process significantly reduces the dry ashing time and sample loss. By using an inductively coupled plasma-optical emission spectroscopy (ICP-OES) analyzer, we have used the dry ashing for the analyses of abundant and trace metals in blood samples. The development of this new process for dry ashing of whole blood samples is reported here.

3.2. *Experimental*

3.2.1. Materials and Reagents

CoorsTek Ceramic evaporating dishes (120 mL), Pyrex watch glasses (100 mm), high purity grade nitric acid, and potassium oxalate were purchased

from Fisher Scientific, Pittsburgh, PA. Chromium and iron AA standards were purchased from Sigma-Aldrich, St. Louis, MO. Whole blood samples (porcine) were obtained from Wampler's Farm Sausage, Lenoir City, TN. Samples were collected in Nalgene bottles using potassium oxalate as anti-coagulant. Trace metal detection was performed using a Perkin Elmer ICP-OES Optima 2100 DV. Type K (chromel–alumel) thermocouples were used to monitor/control the temperatures.

3.2.2. New Method

Whole blood (40.1-71.3 g) was placed in an evaporating dish, covered with a watch glass, and placed on a hot plate at 80-100 °C in order to evaporate water in the sample *slowly without bumping or boiling*. Upon evaporation of water, the dish was transferred to a Bunsen burner and heated slowly with a cool flame (250-300 °C) initially in order to avoid boiling and bumping. After 30 min, the temperature of the flame was increased to 500 °C and the sample was heated continuously for several hours. Intermittent stirring allowed for even ashing and monitoring of the sample. Ashing was determined complete when the sample was a rust red-brown color, typical for iron oxides, and no black substance, indicating carbon, remained.^{13,26} Total ashing time was dependent on the volume of sample in the evaporating dish. This procedure was performed 5 times using 223 g of whole blood in order to obtain sufficient ash (2.44 g).

3.2.3. Reported Method as Control

In the current work, the reported method was slightly modified by using parallel steps as in the new method. This is not the recommended procedure.

Whole blood (40.1 g) was placed in a crucible and placed on a hot plate at 80-100 °C in order to evaporate water in the sample. After 8 h, the crucible was transferred to a Bunsen burner and heated slowly with a cool flame. After 90 min, the temperature of the flame was increased to 500 °C and the sample was heated continuously for several hours. Ashing was determined complete when the sample was a rust red-brown color, typical for iron oxides, and no black substance, indicating carbon, remained.^{13,26}

3.2.4. Elemental Analysis Using ICP-OES

The ash (2.44 g) was dissolved in high purity concentrated nitric acid (50 mL) and heated. This solution was divided into 10 mL aliquots and each diluted to 100 mL. Samples were spiked with chromium and standard addition was performed.²⁷ For the determination of iron, a 1 mL aliquot of the diluted sample was taken and diluted further to 100 mL using a 5% nitric acid solution. Calibration standards were used for the determination of iron, copper, zinc, manganese, and molybdenum. The concentrations of iron, copper, zinc, chromium, manganese, vanadium, and molybdenum in the ash samples were determined using ICP-OES. Each sample was repeated three times in order to obtain standard deviations.

3.3. Results and Discussion

Dry ashing has been used for sample preparation for over 200 years. It is often used to demonstrate quantitative analysis techniques. The equipment is inexpensive, and almost if not all laboratories have the tools needed. Dry ashing has been under scrutiny, but one cannot deny its simplicity and cost effective qualities. Arguments such as possible sample loss, reaction with the surface of a container, and incomplete ashing have all been presented.^{15,28} Despite this, many still regard dry ashing to be a viable practice provided care is taken when preparing samples.^{9,11,13} While there are a few volatile compounds that cannot be preconcentrated and detected after dry ashing, this technique allows for many metals to be identified simultaneously or individually.

In the last century, attempts have been made to establish a uniform process for the treatment of samples prior to metal detection. Despite efforts, only general guidelines were formulated with specific details for various samples. Middleton and Stuckey summarized previous studies using either dry or wet methods for some metals and recommended a temperature range of 500-550 °C.²⁹ Gorsuch and Thiers elaborated on this method, adding more comprehensive information for additional metals based on ashing of numerous samples.^{30,31} In order to form a collective view of these methods, the Analytical Methods Committee published "Methods for the Destruction of Organic Matter" discussing wet and dry decomposition.²² In this article, guidelines still in practice

today were outlined for dry ashing, and advantages of the technique over wet digestion were established. Dry ashing allows for most common metals to be analyzed. The lack of additional reagents prevents interferences in a blank during analysis. A larger quantity of sample can be analyzed, but perhaps the most attractive trait of all is the small amount of attention required for ash samples to be completed.²²

In our past experience with ashing by the reported procedures using either an open dish or a crucible (and lid),²¹ temperature monitoring and significant sample loss are concerns. Ashing blood samples in an open dish was found to lead to significant sample loss from smoke and ash particles. We then focused on the common practice of using a crucible and lid. This procedure, used here as control for comparison, was also found to lead to significant sample loss. The sample has a tendency to flow out of the crucible even under close monitoring. In addition, while the procedure is simple and requires little attention, it may take several days to complete. No temperature control was established in either procedure.

After much experience with ashing blood, we have developed a model method that uses a ceramic evaporating dish and Pyrex watch glass cover rather than a standard crucible and lid. Such a design allows for increased air exposure to the sample to increase the ashing rate. With the use of a thermocouple, the ashing temperature has been closely controlled, leading to minimum sample loss and much faster ashing time in comparison to the control process that uses a

reported crucible system. All other variables remain consistent and are given in detail, including initial evaporation time, ashing times, temperatures, and overall yields (Table 3.1). The volume of blood one can ash is dependent on the size of the evaporating dish. With our equipment, as much as 50-100 mL of blood could be dry-ashed in one procedure.

Closely controlling the ashing temperature has also been found to prevent sample loss and cross reaction with the container. Additionally, the temperature was elevated gradually, as is commonly practiced in a muffle furnace. We have found that the literature on this point has been inconsistent. Thus, careful monitoring of the temperature is recorded in association with the ashing time at a specific temperature. Maximum temperature was 500 °C in order to prevent any possible volatilization of metals as well as reaction with the crucible container.²⁹

Another key improvement in the current procedure is the use of a ceramic evaporating dish with a Pyrex watch glass as a cover rather than a ceramic crucible and lid. This modification leads to an increase in the air flow over the sample, allowing for a significantly shorter total ashing time. Sufficient supply of O₂ in the air flow is believed to increase the rate of the oxidization of organic materials in the heated sample, leading to their conversion to CO₂ and reducing the ashing time. We have conducted control studies to better demonstrate the improvement of this system in comparison to the literature procedure using a crucible (and lid) and Bunsen burner. Two experiments were performed using the same burner and the same mass of whole blood, except that one used a

ceramic evaporating dish with a Pyrex watch glass, and another used a crucible and lid as control. To the dish and the crucible, whole blood (40.1 g) was added to each. Each apparatus were set on the hot plate and allowed to evaporate water for 8 h. Initial heating on the burner was allowed. The sample in the crucible, however, required both an extra hour of low heat and close monitoring, as its sample under the lid, spilled over from the inside. After 30 min of low heat (250-300 °C), the evaporating dish/watch glass system was increased to 500 °C. With intermittent stirring the total ashing time was 18 h for the improved system. Under the same conditions the crucible system required a total of 55 h of ashing. Furthermore, a slight increase in final product was found in the dish versus that of the crucible. A comparison of both methods is summarized in Table 3.1. Our studies here show that the control using the reported procedure leads to at least 11% loss of the ash product.

The alterations implemented in the new method allows for the sample to be completely ashed in less than one-third of the time used in the control. It also involves less work than with a crucible system. We have also used the new method to ash, in one procedure, more blood samples than the reported procedure using a crucible. This is a very attractive feature, especially when examining trace metals. If a smaller amount of blood is ashed in, e.g., the analysis of abundant metals, the new procedure using an evaporating dish can be completed in shorter time than 18 h.

Table 3.1. Comparison of ashing times and yields of the new method and control.

	<i>Evaporation period (h)</i> 80-100 °C	<i>Initial heating period (h)</i> 250-300 °C	<i>Ashing period (h)</i> 500 °C	<i>Mass of ash (g)</i>
Evaporating dish (New method)	8	0.5	18	0.412
Crucible (Control)	8	1.5	55	0.371

To demonstrate both abundant and trace analysis, the concentrations of iron, copper, zinc, chromium, manganese, vanadium, and molybdenum were determined from the same blood sample using ICP-OES. Results are shown in Table 3.2.

3.4. Conclusions

Dry ashing is a versatile technique allowing for trace metal detection due to the potential to decompose larger sample quantities. It is still a valid technique that helps set a standard for other methods. The current work demonstrates the optimization of the previously established dry ashing technique such that the method can be implemented in any lab setting for sporadic use. Many metals can be detected at once or individually. The method is simple, inexpensive and faster than the established crucible system. The dry ashing of blood samples using the new method developed in the current work here helps set a precedent for dry ashing of other samples.

Table 3.2. Analysis of metals in dry-ashed whole blood.

<i>Element</i>	<i>Concentration</i>
Fe	298.1 ± 5.8 ppm
Zn	272 ± 14 ppb
Cu	169.4 ± 1.7 ppb
Cr	7.71 ± 1.7 ppb
Mn	858 ± 16 ppt
V	below limit of detection
Mo	below limit of detection

References

1. Burns, D.T. *Anal. Proc.* **1993**, 30, 272-275.
2. Cholak, J.; Hubbard, D.M. *Anal. Chem.* **1948**, 20, 73-76.
3. Sysalova, J.; Spevackova, V. *Cent. Eur. J. Chem.* **2003**, 1, 108-120.
4. Versieck, J.; Vanballenberghe, L. *Anal. Chem.* **1991**, 63, 1143-1146.
5. Cobo, G.; Gomez, M.; Camara, C.; Palacios, M.A. *Microchim. Acta* **1993**, 110, 103-110.
6. Vesterberg, L.; Bergstrom, T. *Clin. Chem.* **1977**, 23, 555-559.
7. Nise, G.; Vesterberg, O. *Clin. Chim. Acta* **1978**, 84, 129-136.
8. Price, C.J.; Strong, P.L.; Murray, F.J.; Goldberg, M.M. *Reprod. Toxicol.* **1997**, 11, 833-842.
9. Bukhave, K.; Sørensen, A.; Hansen, M. *J. Trace Elem. Med. Bio.* **2001**, 15, 56-58.
10. Drábek, K.; Kalousková, J. *J. Radioanal. Nucl. Chem.* **1987**, 119, 119-129.
11. Titze, J.; Rittweger, J.; Dietsch, P.; Krause, H.; Schwind, K.H.; Engelke, K.; Lang, R.; Kirsch, K.A.; Luft, F.C.; Hilgers, K.F.J. *Hypertens.* **2004**, 22, 803-810.
12. Titze, J.; Krause, H.; Hecht, H.; Dietsch, P.; Rittweger, J.; Lang, R.; Kirsch, K.A.; Hilgers, K.F. *Am. J. Physiol. Renal. Physiol.* **2002**, 283, F134-141.
13. Yong, L.; Armstrong, K.C.; Dansby-Sparks, R.N.; Carrington, N.A.; Chambers, J.Q.; Xue, Z.-L. *Anal. Chem.* **2006**, 78, 7582-7587.

14. Jiang, W.; Tong, H.; Liu, Z.-C.; Wang, K.; Chen, B.-C.; Li, C.-H. *Chinese J. Health Lab. Technol.* **2008**, *18*, 464-465.
15. Bian, J.; Zhang, X.; Ni, W. *Chem. Res. (Huaxue Yanjiu)*, **2011**, *22*, 61-64.
16. Tomovic, V.M.; Petrovic, L.S.; Tomovic, M.S.; Kevresan, Z.S.; Jokanovic, M.R.; Dzinic, N.R.; Despotovic, A.R. *Food Chem.* **2011**, *129*, 100-103.
17. Brown, C.R.; Haynes, K.G.; Moore, M.; Pavek, M.J.; Hane, D.C.; Love, S.L.; Novy, R.G.; Miller, J.C.Jr. *Am. J. Potato Res.* **2011**, *88*, 238-244.
18. Sogabe, N.; Maruyama, R.; Baba, O.; Hosoi, T.; Goseki-Sone, M. *Bone*, **2011**, *48*, 1036-1042.
19. Rossete, A.L.R.M.; Carneiro, J.M.T.; Batagello, H.H.; Oliveira, J.G.G.; Bendassolli, J.A. *Quim. Nova.* **2011**, *34*, 341-343.
20. Vogl, J.; Rosner, M.; Pritzkow, W. *J. Anal. Atom. Spectrom.* **2011**, *26*, 861-869.
21. Rudolf Bock *A Handbook of Decomposition Methods in Analytical Chemistry*; International Textbook Co.: Glasgow, 1979.
22. Analytical Methods Committee. *Analyst* **1960**, *85*, 643.
23. Van Montfort, P.F.E.; Agterdenbos, J.; Juetten, B.A.H.G. *Anal. Chem.* **1979**, *51*, 1553-1557.
24. Ferrando, A.; Green, N.; Barnes, K.; Woodward, B. *Biol. Trace Elem. Res.* **1993**, *37*, 17-25.
25. Tidehag, P.; Hallmans, G.; Wing, K.; Sjöström, R.; Ågren, G.; Lundin, E.; Zhang, J. *Brit. J. Nutr.* **1996**, *75*, 281-289.

26. Dansby-Sparks, R.N.; Ouyang, R.; Xue, Z.-L. *Sci. China Ser. B.* **2009**, *52*, 1777-1788.
27. Harris, D.C. *Quantitative Chemical Analysis*; Macmillan, 2003.
28. Mader, P.; Száková, J.; Curdová, E. *Talanta* **1996**, *43*, 521-534.
29. Middleton, G.; Stuckey, R.E. *Analyst* **1953**, *78*, 532.
30. Gorsuch, T.T. *Analyst* **1959**, *84*, 135.
31. Thiers, R.E. In *Methods of Biochemical Analysis*; Interscience: New York, 1957; Vol. 5.

Part 4

Flower-Like Self-Assembly of Gold Nanoparticles for Highly Sensitive Electrochemical Detection of Chromium(VI)

This chapter is revised based on a paper by Ruizhuo Ouyang, Stefanie A. Bragg, James Q. Chambers, and Zi-Ling Xue. Only minor revisions were made.

R. Ouyang, S.A. Bragg, J.Q. Chambers, Z.-L. Xue. Flower-like self-assembly of gold nanoparticles for highly sensitive electrochemical detection of chromium(VI). *Analytica Chimica Acta*, **2012**, 722, 1-7.

Abstract

We report here the fabrication of a flower-like self-assembly of gold nanoparticles (AuNPs) on a glassy carbon electrode (GCE) as a highly sensitive platform for ultratrace Cr(VI) detection. Two AuNP layers are used in the current approach, in which the first is electroplated on the GCE surface as anchors for the binding of an overcoated thiol sol-gel film derived from a 3-mercaptopropyltrimethoxysilane (MPTS). The second AuNP layer is then self-assembled on the surface of the sol-gel film, forming flower-like gold nanoelectrodes enlarging the electrode surface. When functionalized by the thiol pyridinium, the fabricated electrode displays a well-defined peak for selective Cr(VI) reduction with an unusually large, linear concentration range of 10-1200 ng L⁻¹ and a low detection limit of 2.9 ng L⁻¹. In comparison to previous approaches using MPTS and AuNPs on Au electrodes, the current work expands the use of AuNPs to the GCE. Subsequent functionalization of the secondary AuNPs by a thiol pyridinium and adsorption/preconcentration of Cr(VI) lead to the

unusually large detection range and high sensitivity. The stepwise preparation of the electrode has been characterized by electrochemical impedance spectroscopy (EIS), scanning electronic microscopy (SEM), and IR. The newly designed electrode exhibits good stability, and has been successfully employed to measure chromium in a pretreated blood sample. The method demonstrates acceptable fabrication reproducibility and accuracy.

4.1. Introduction

Hexavalent chromium is a toxic, strong oxidizing agent. The wide use of chromium in industry has inevitably led to ecological impacts from Cr(VI) contamination, causing allergic dermatitis, as well as toxic, carcinogenic effects and mutagenesis in animals and humans.¹⁻⁴ There has thus been a growing interest in highly sensitive and selective assays for trace Cr determination. A number of sensitive techniques have been employed, such as atomic absorption spectrophotometry,⁵ plasma mass spectrometry,⁶ spectrofluorimetry,⁷ spectrophotometry,⁸ and chemiluminescence,⁹ but each of these processes is time consuming and necessitates expensive equipments. Various sensor systems have also been reported.¹⁰⁻¹⁶ Most of these systems, however, have either limitations in sensitivity, simplicity or the high cost of equipments as well.

Electrochemical methods have been considered desirable for the Cr(VI) determination, and they have demonstrated advantages including speed of

analysis, good selectivity, and sensitivity.¹⁷⁻³¹ A poly(4-vinylpyridinium)-coated platinum electrode was developed by Cox and Kulesza for Cr(VI) preconcentration in the polymer films, followed by determination with the lack of interference by metal cations.¹⁷ In a flow injection analysis system, a polyaniline/polystyrene composite electrode was used as a detector to monitor Cr(VI) with a detection limit of 4 ng L⁻¹.¹⁸ A self-assembled monolayer of pyridinium on the Au electrode surface for sensitive Cr(VI) detection has been developed by Turyan and Mandler.¹⁹ We have developed 2- and 4-pyridinium-functionalized sol-gel films through electrodeposition at a glassy carbon electrode (GCE) surface for Cr(VI) detection in aqueous solution.²⁰⁻²³ The narrow concentration ranges, however, significantly limit the practical applications. Mercury electrodes have also been used for the electrochemical detection of Cr(VI).^{24,25} While extremely sensitive, their applications are limited due to the toxicity of mercury. In an effort to develop alternative electrodes to mercury, bismuth-film electrodes (BiFEs) have been developed for Cr(VI) detection through catalytic adsorption stripping voltammetry (CAAdSV).²⁶⁻²⁹ However, the poor reproducibility of BiFEs is challenging for Cr detection at a low concentration, and a complexing ligand is needed for the process, adding to the complexity of the detection process. It is thus highly desirable to develop a system that is not only sensitive, selective, and reliable but also simple, practical, and economical in its operation.

Nanosized particles of noble metals, especially gold nanoparticles

(AuNPs), have been studied extensively due to their attractive electronic, optical, and thermal properties as well as catalytic properties and potential applications over numerous science and medical fields.³⁰⁻³³ AuNPs have been used in electrochemistry to develop highly sensitive nanodevices for analytical and bioanalytical applications because of their superior biological compatibility, excellent conducting capability, high surface-to-volume ratio and high surface energy. The introduction of AuNPs onto the electrochemical interfaces has infused a new vigor into electrochemistry, as AuNPs have successfully functioned as electron-conducting pathways between the electrochemically active targets and the electrode surface.³⁴⁻⁴¹ The amperometric detection of ultratrace amount of Cr(VI) has been developed based on AuNPs growing on a conducting substrate modified with sol-gel-derived thiol functionalized silicate network, but the detection limit was not low enough for Cr(VI) detection at the ng L⁻¹ level.⁴²

With the aim of developing a sensitive platform for voltammetric Cr(VI) detection without a complexing ligand, two layers of AuNPs have been introduced to design a pyridinium-functionalized flower-like self-assembly of AuNPs for sensitive detection of ultratrace Cr(VI) (Figures 4.1). The electroplating of gold formed the first AuNPs layer on the GCE surface, followed by the chemisorption of a sol-gel coating derived from 3-mercaptopropyl-trimethoxysilane (MPTS). Chemically produced AuNPs were then infiltrated into the three dimensional (3D) matrix by forming Au←S linkages, forming a flower-

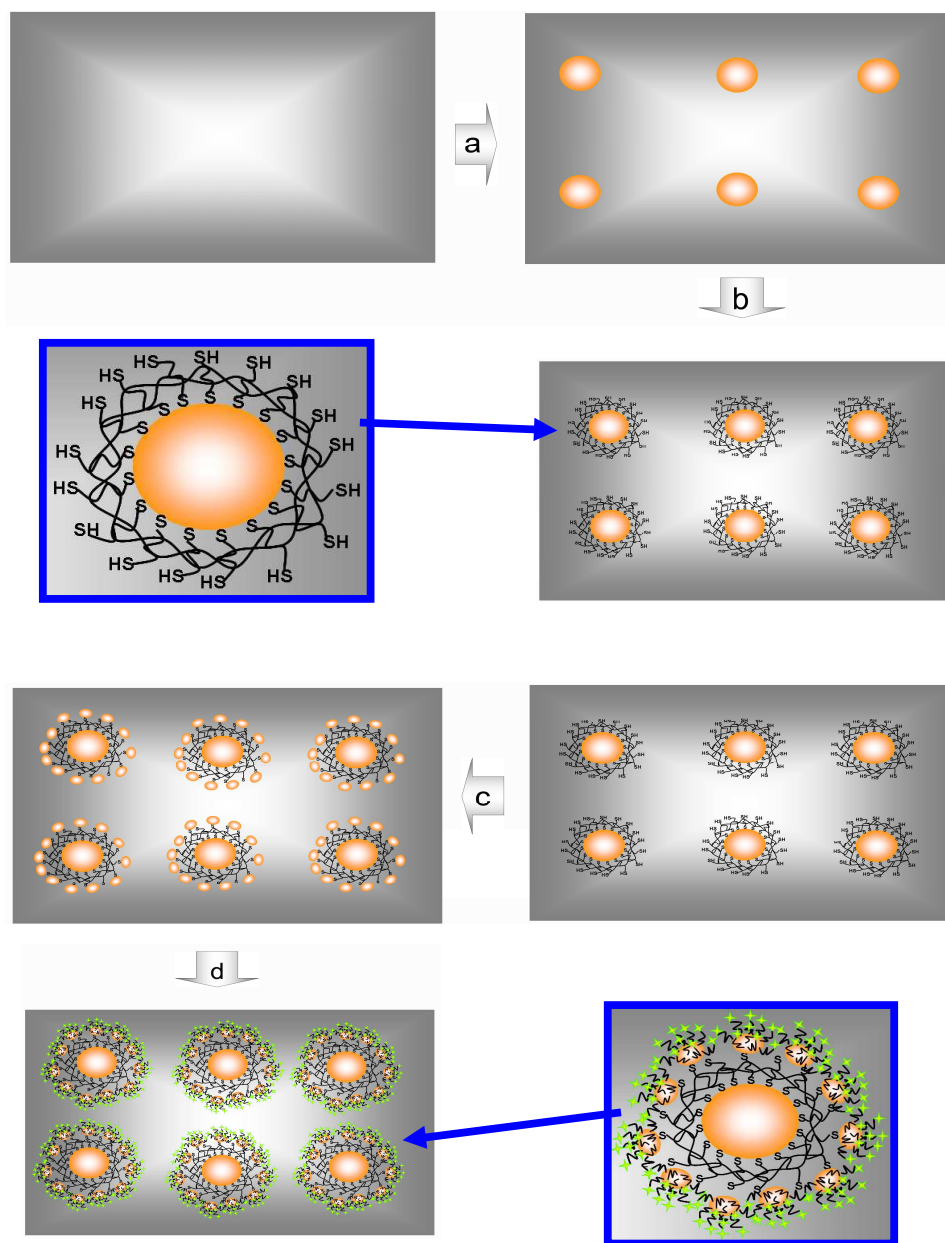


Figure 4.1. Schematic illustration of the electrode fabrication: (a) electrochemical deposition of AuNPs; (b) self-assembling of MPTS on the surface of the first AuNPs layer; (c) chemisorption of the second AuNPs layer on the thiol sol-gel overcoating; (d) attaching of PET onto the surface of the second AuNPs layer.

like self-assembly of AuNPs. The fabrication leads to a significantly enlarged Au electrode surface and expands the use of AuNPs to the GCE surface. To our knowledge, such a double-AuNPs-layer approach on the GCE surface has not been reported.

When positively charged pyridinium functional groups were attached to the AuNPs surface through Au←S bonds, ultratrace negatively charged HCrO_4^- was successfully accumulated on the electrode surface, leading to a low detection limit (2.9 ng L^{-1}) and a concentration range ($10\text{--}1,200 \text{ ng L}^{-1}$) that is much larger than those by other related electrodes.¹⁹⁻²¹ This electrode was successfully used for the Cr(VI) detection in a pretreated blood sample.

4.2. Experimental

4.2.1. Reagents and Materials

The following chemicals were used as received: chromium standard solution (1000 mg L^{-1} , Fluka), MPTS (Gelest), 4-pyridineethanethiol hydrochloride (PET, TCI-EP, Japan), chloroauric acid (HAuCl_4 , 99.99%, Aldrich), trisodium citrate (Aldrich), $\text{K}_3\text{Fe}(\text{CN})_6$ (Aldrich), $\text{K}_4\text{Fe}(\text{CN})_6$ (Aldrich), NaF (Certified ACS, Fisher), KCl (Certified ACS, Mallinckrodt). Ultrapure water from a Millipore water purified system ($\geq 18 \text{ M}\Omega \text{ cm}$, Barnstead) was used in all assays. Other reagents were of analytical grade and used as received. All glassware were soaked in 1 M nitric acid bath and thoroughly rinsed with deionized (DI) water before use.

4.2.2. Instrumentation

Electrochemical measurements were carried out on a modulated potentiostat (CHI 650a, CH Instruments). A three-electrode configuration consisted of a modified GCE (3 mm in diameter, BAS Inc.), Ag/AgCl (saturated KCl solution, CH Instruments) and a platinum wire (CH Instruments) as working, reference and counter electrodes, respectively. Electrochemical impedance spectroscopy (EIS) was performed in 0.1 M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Scanning electronic microscopic (SEM) images were obtained with a LEO 1525 field emission scanning electron microscope. Reflection-transmittance FT infrared spectroscopy (IR) was measured using a Thermo Scientific NicoletTM iSTM 10 spectrometer.

4.2.3. Preparation of Colloidal AuNPs

Colloidal AuNPs were prepared by the reported method.⁴⁰ The size effect of metallic nanoparticles was studied over the range of 1-80 nm. According to the reported method, it is possible to synthesize monodispersed colloidal AuNPs over a wide size range by varying the $[\text{Au(III)}]/[\text{citrate}]$ ratio during the reduction step as listed in Table B.1 in Appendix B. A standard procedure for the preparation of 20 nm AuNPs is set as a model. A 25 mL aqueous solution of HAuCl_4 (0.25 mM) was added to a three neck round bottom flask, heated to boiling with vigorous stirring and 438 μL of trisodium citrate (1%) was added. The color of the solution changed from a faint blue (nucleation) to dark blue and

finally to red, which was an indication of the formulation of the AuNPs. The nanoparticle solution was allowed to cool to room temperature with continuous stirring and stand for ~24 h before use.

4.2.4. Preparation of Pyridinium-Functionalized Flower-Like Self-Assembly of AuNPs

Prior to use, GCEs were polished carefully to a mirror-like surface on a standard electrode polishing kit (CH Instrument) including a 1200 grit Carbimet disk, 1.0 and 0.3 μm alumina slurry on a nylon cloth, and 0.05 μm alumina slurry on a microcloth polishing pad. After polishing, GCEs were successively sonicated with deionized (DI) water, ethanol and DI water for 5 min each. The deposition of the first AuNPs layer was carried out by the method reported in the literature.⁴¹ In brief, the GCE was put in 0.01 M $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution containing 0.1 M KCl and scanned by cyclic voltammetry in the range from -0.2 to 0.6 V until a pair of well-defined redox peaks was observed. AuNPs were then electroplated on the GCE surface by immersing the electrode into a 0.2 g L^{-1} HAuCl_4 solution and applying a constant potential of -0.2 V for 100 s. After being rinsed with acetone, ethanol and DI water, respectively, the AuNPs coated GCE was treated in a fresh piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, 7:3) for 5 min and then rinsed again with DI water. Finally, the AuNPs coated/modified GCE was kept under N_2 gas flow for subsequent use. This first AuNPs layer acts as anchors for binding to MPTS in the next step. *Caution: The piranha solution is corrosive and*

should be used with extreme caution and handled only in a small quantity.

MPTS modification was carried out by soaking the electrode in a 21 mM solution of MPTS in ethanol for 10 min. The MPTS self-assembled on the surface of the AuNPs by forming Au←S bonds and, after hydrolysis of the sol-gel precursor, existed as a three dimensional (3D) porous silicate network with thiol tail groups (-SH) (Figure 4.1).⁴³ The MPTS-modified GCE was then immersed into a solution containing colloidal AuNPs for 24 h to form a self-assembly of the second AuNPs layer. The resulting electrode was then dipped into a 5 mM solution of 4-pyridineethanethiol (PET) containing 0.5 M H₂SO₄ for 10 min to attach the pyridinium group to the electrode surface. The electrode is ready to accumulate hydrogen chromate (HCrO₄⁻) and analyze it. It should be pointed out that in the acidic solution, HCrO₄⁻ is the dominant species.¹⁹

Various stages of the modification were prepared under the optimized conditions.

4.2.5. Measurement Procedure

To carry out the voltammetric measurements for Cr(VI), the AuNPs self-assembled electrode was first put in 20 mL of 0.15 M fluoride buffered solution (pH 4.5) and stirred for 5 min under open-circuit potential.¹⁹ After the accumulation, the determination of Cr(VI) was carried out by transferring the electrode to a Cr(VI) free solution (0.15 M NaF, pH 7.8) and performing cathodic stripping square wave voltammetry (CSSWV) from 0.35 to -0.2 V with frequency

of 15 Hz, step potential of 4 mV and amplitude of 25 mV. Stirring at a high speed was required during the accumulation process but not during the stripping step. The electrode surface was regenerated between measurements by electrocycling in the potential range from 0.35 to -0.2 V in 0.1 M HClO₄ for the elimination of Cr(VI) from the electrode surface.¹⁹

A porcine blood sample was pretreated using the advanced oxidation process (AOP) as described in Part 2, and Cr in the sample was converted to Cr(VI) in the pretreatment. Prior to the analysis, the blood sample was diluted using 0.15 M NaF buffer (pH 4.5). Standard additions of 100 and 200 ng L⁻¹ Cr(VI) were added to the blood sample to a total volume of 20 mL.

4.3. Results and Discussion

4.3.1. Characterization of Flower-Like Self-Assembly of AuNPs on GCE

The preparation of the pyridinium-functionalized flower-like surface self-assembly of AuNPs on GCE consists of four steps, as shown in Figure 4.1. EIS and SEM measurements were used to characterize the modification from each step. EIS is an effective method for probing the features of a surface modified electrode. In the terms of EIS, the semicircle portion at higher frequencies corresponds to the electron-transfer limited process and the linear portion at lower frequencies may be attributed to diffusion. The semicircle diameter equals the electron-transfer resistance, which depends on the dielectric and insulating

features at the electrode/electrolyte interface.⁴¹ Figure 4.2 shows the EIS measurements of different electrodes including bare GCE, AuNPs/GCE, MPTS/AuNPs/GCE, AuNPs/MPTS/AuNPs/GCE, and PET/AuNPs/MPTS/AuNPs/GCE. The small semicircle diameter of the bare GCE shows a relatively small electron-transfer resistance (curve a). An almost straight line observed for AuNPs/GCE (curve b) indicates faster electron transfer kinetics of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ on AuNPs/GCE. However, the presence of MPTS sol-gel overcoating on AuNPs/GCE surface remarkably increases the electron transfer resistance (curve c), indicating the larger obstruction effect of MPTS sol-gel layer on the flow of electrons. The secondary AuNPs are therefore introduced to the electrode surface and self-assembled onto the 3D silicate network. In comparison with MPTS/AuNPs/GCE, the semicircle diameter of AuNPs/MPTS/AuNPs/GCE was much smaller (curve d). This is contributed to the high electrical conductivity of secondary AuNPs, facilitating the electron transfer between the electrode and the self-assembled layers. As PET can partly resist the electron transfer of the electrode, a slight increase of the impedance was observed after the attachment of PET (curve e) to the surface of secondary AuNPs, which consequently does not affect the detection since the attachment of PET will facilitate the attraction of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ions and a similar effect should be at play for Cr(VI) through the self-assembled layer.

The SEM images of various stages of the modification under the optimized conditions are shown in Figure 4.3. After the electrochemical deposition, AuNPs

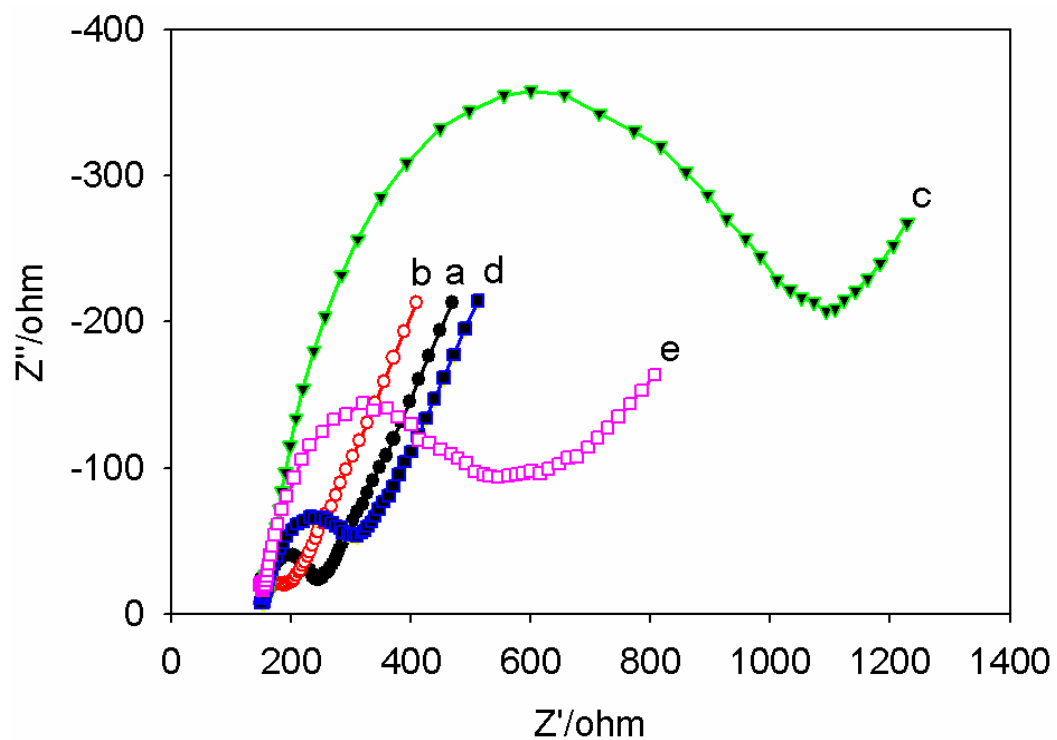


Figure 4.2. EIS of 0.01 M $\text{Fe}(\text{CN})_6^{3-/4-}$ and 0.1 M KCl at: (a) bare GCE; (b) AuNPs/GCE; (c) MPTS/AuNPs/GCE; (d) AuNPs/MPTS/AuNPs/GCE; (e) PET/AuNPs/MPTS/AuNPs/GCE.

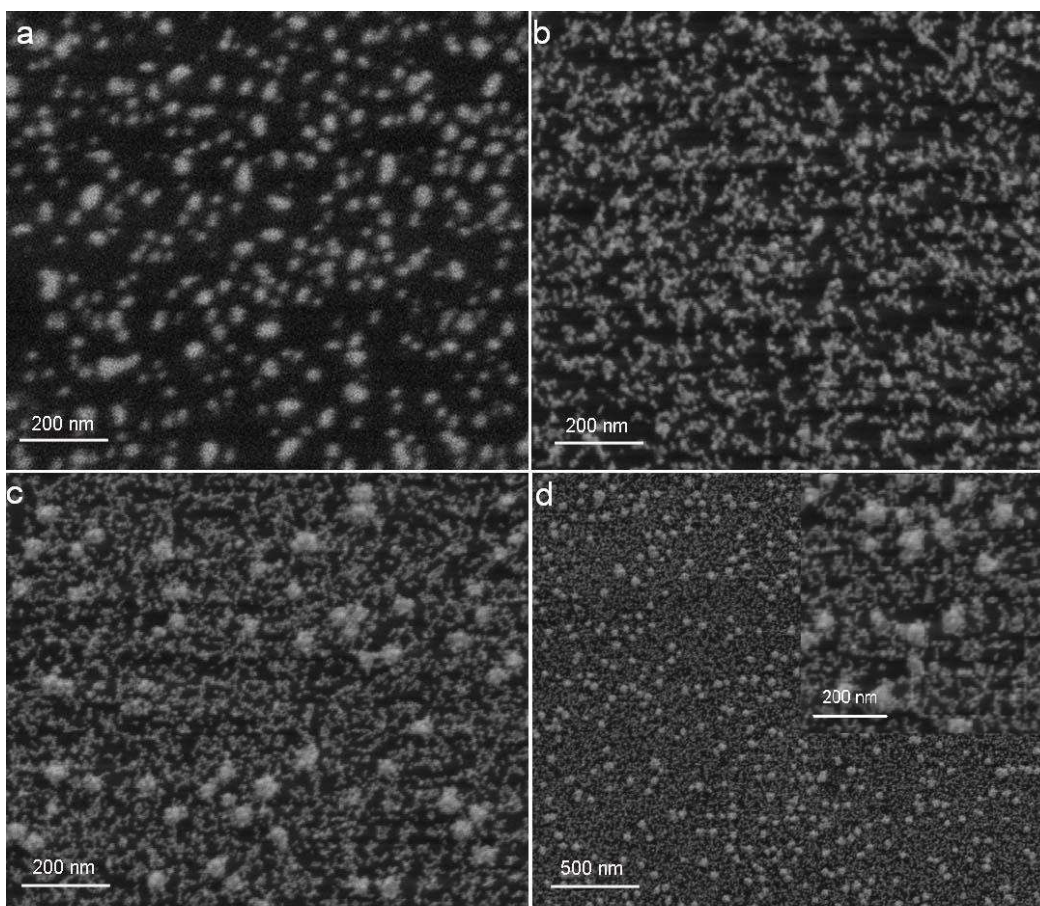


Figure 4.3. SEM images of: (a) AuNPs/GCE; (b) MPTS/AuNPs/GCE; (c) AuNPs/MPTS/AuNPs/GCE – The term “flower-like” is used to describe the distribution of AuNPs on the thiol MPTS sol-gel film; (d) PET/AuNPs/MPTS/AuNPs/GCE. The insert in (d) is in the same magnification as that in (c).

with diameters of 25-40 nm were formed and well distributed on the GCE surface (Figure 4.3a). Compared to a gold disk electrode, AuNPs/GCE provides more surface area to chemisorb the thiol sol-gel precursor MPTS. A 3D porous structure indicated the successful formation of the MPTS sol-gel overcoating, which was full of thiol tail groups (-SH), on the first AuNPs layer (Figure 4.3b). Then the 20-nm colloidal AuNPs were attached to the thiol groups present inside and on the surface of the silicate network, which was verified by both EIS (Figure 4.2, curve c) and SEM (Figure 4.3c). The second, flower-like AuNPs layer was used to improve the electron transfer and immobilize the pyridinium group. The diameter of the second AuNPs layer was slightly increased after being coated by the thiol-pyridinium PET (Figure 4.3d), but there was no obvious difference in the surface morphology.

IR spectra of the modified electrodes further confirmed the success of the step-by-step modification. Figures 4.4a and b show the reflection-transmittance FT-IR spectra of the MPTS precursor and its monolayer on the surface of AuNPs. Bands at 802 and 1075 cm^{-1} originating from Si-O-C modes of the MPTS precursor are replaced by stretching absorption of broad Si-O-Si bands positioned at ca. 1100 cm^{-1} in the monolayer spectrum. Furthermore, the disappearance of the 1189 cm^{-1} band was observed due to OCH_3 groups corroborating hydrolysis of the precursor in the sol-gel process. The existence of free -SH tail groups in the monolayer was verified by the S-H stretching band around 2600-2630 cm^{-1} in Figures 4.4a and b. The peak frequencies for +

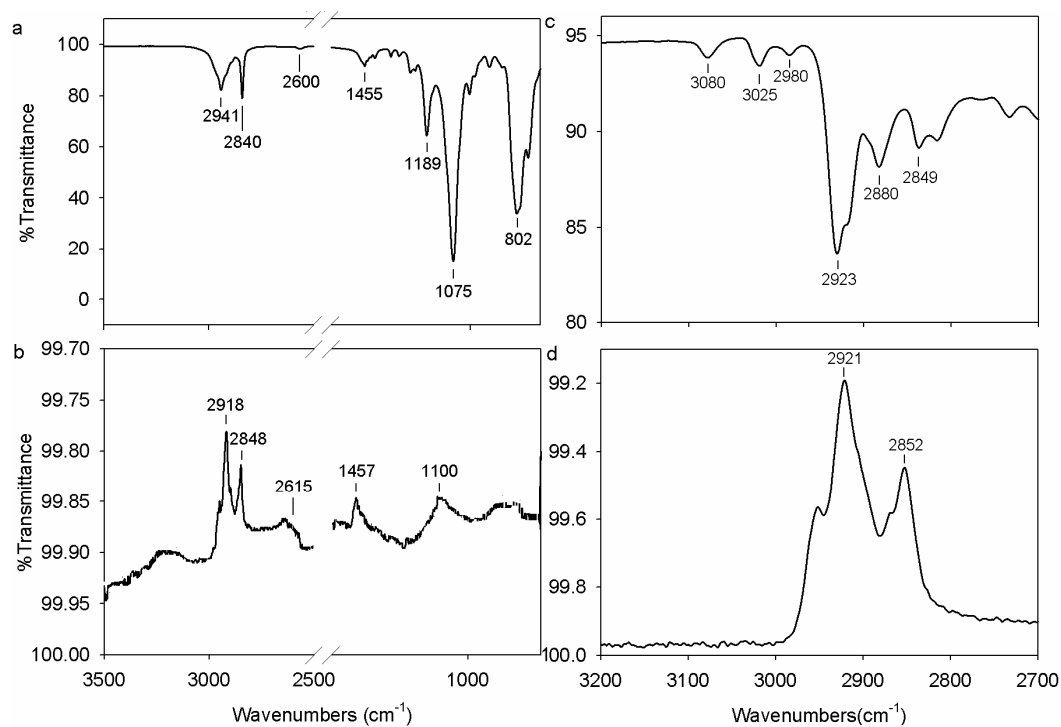


Figure 4.4. Reflection–transmittance IR spectra of (a) MPTS; (b) MPTS chemisorbed on AuNPs/GCE; (c) PET; and (d) PET attached on AuNPs/MPTS/AuPs/GCE.

asymmetric and symmetric methylene (CH_2) stretching vibrations appear at 2918 and 2848 cm^{-1} , respectively (Figure 4.4b). These results indicated the formation of a cross-linked silicate structure on the AuNPs surface.⁴⁵ IR spectra of pure PET and its monolayer on the surface of the second AuNPs layer were studied as well (Figures 4.4c and d). After PET was self-assembled onto the AuNPs surface, the bands at 2980, 3025 and 3080 cm^{-1} assigned to the C-H stretching of the ring disappeared. However, the bands at 2852 and 2921 cm^{-1} corresponding to the symmetric and asymmetric alkyl C-H stretching were observed before and after the self-assembly. These results indicate the chain disorder similar to the chains of short alkanethiols on gold due to the formation of Au←S bonds¹⁹ and accordingly imply the successful attachment of PET onto the surface of AuNPs.

4.3.2. Voltammetric Behavior of Different Modified Electrodes

It is worthwhile to compare the electroactivity of bare GCE (a, Figure 4.5) and electrodes modified with (b) MPTS/AuNPs, (d) PET/AuNPs, (e) AuNPs/MPTS/AuNPs, (f) AuNPs, (g) PET/AuNPs/MPTS/AuNPs, and (c) the gold disk electrode modified with PET (Figure 4.5). Bare GCE and MPTS/AuNPs/GCE did not show obvious electrochemical response to 1500 ng L^{-1} Cr(VI), probably because there are no active sites on the GCE surface to adsorb Cr(VI) and the MPTS network can largely suppress the electron transfer for Cr(VI) reduction on the electrode. A small current was found for Cr(VI)

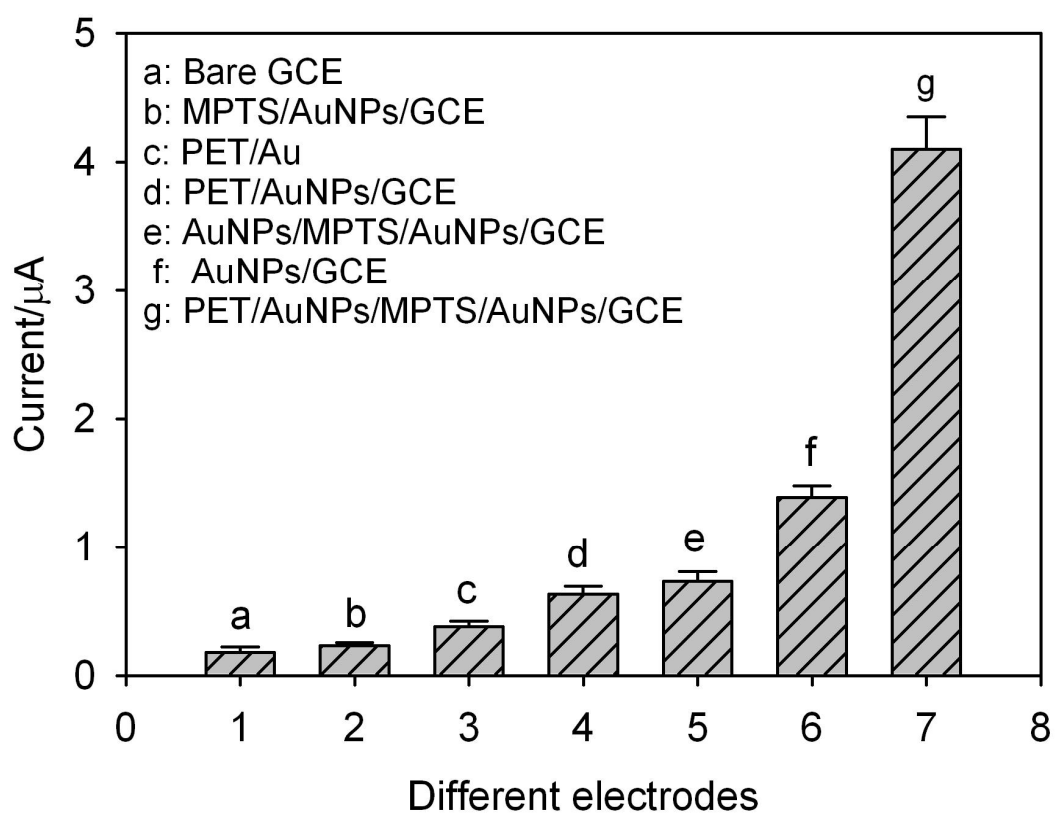


Figure 4.5. Graphical comparison of (a) bare GCE; (b) MPTS/AuNPs/GCE; (c) PET/Au; (d) PET/AuNPs/GCE; (e) AuNPs/MPTS/AuNPs/GCE; (f) AuNPs/GCE; and (g) PET/AuNPs/MPTS/AuNPs/GCE toward 1500 ng L⁻¹ Cr(VI) detection.

reduction on either PET/AuNPs/GCE or AuNPs/MPTS/AuNPs/GCE. The pyridinium group chemisorbs HCrO_4^- by ion exchange, and it is possible that AuNPs can also show some electrocatalytic activity towards the reduction of Cr(VI),⁴⁵ providing a good reason for the small response to Cr(VI). Even though the detection performance of AuNPs/GCE was improved to some extent, the electrode designed for Cr(VI) detection in this investigation displayed the overall best behavior and in particular, a great improvement in the response to Cr(VI) reduction in comparison to PET/Au.

Two layers of AuNPs were successfully self-assembled on the GCE surface. Compared to the Au disk electrode, the first layer of AuNPs increased the surface roughness and greatly enlarged the effective surface area of AuNPs coated electrode, leading to the chemisorption of more MPTS. The more MPTS adsorbed, the more thiol tail groups are introduced to the electrode surface, increasing the number of secondary AuNPs on the 3D silicate network. Finally, the functional pyridinium groups are attached onto the surface of secondary AuNPs, playing a very important role in the accumulation of Cr(VI). More AuNPs lead to more pyridinium groups present on the electrode surface, and consequently result in the best responsive detection of Cr(VI) among the various electrodes. It appears that the two layers of AuNPs greatly contribute to the improved performance of the designed electrode for Cr(VI) detection at low levels.

4.3.3. Analytical Performance

The peak current and potential for the Cr(VI) reduction depend on the modification of the electrode. The pyridinium-functionalized, AuNPs-based electrode exhibited a typical voltammetric response for Cr(VI) reduction, presumably from Cr(VI) to Cr(III). It is interesting that the peak potential for Cr(VI) reduction was found to be at 0.08 V, negatively shifted from 0.18 V when the Au disk electrode was modified using only PET.¹⁹

The optimization of other experimental conditions is discussed in detail in Appendix B. Afterward the performance of PET/AuNPs/MPTS/AuNPs/GCE toward the detection of Cr(VI) was tested by CSSWV. Figure 4.6 displays the current response of the designed electrode towards Cr(VI) in the concentration range of 10-1,200 ng L⁻¹. PET/AuNPs/MPTS/AuNPs/GCE showed a well-defined voltammetric peak at ~0.08 V and the peak current proportionally increased with the increasing concentration of Cr(VI). It should be noted that a slight positive shift of the peak potential was observed for higher Cr(VI) concentration, which also occurred in the previous report.¹⁹ The reason for this shift is not understood, but may be related to the exchange of fluoride by HCrO₄⁻ ions. The calibration plot showed a good linear relationship by using the peak current as a function of the Cr(VI) concentration with a correlation coefficient of 0.999 (Figure 4.6 *inset*). The detection limit (LOD) of 2.9 ng L⁻¹ was calculated according to a signal-to-noise ratio of 3, which was comparable with those of previously reported Cr(VI) detection using electrochemical techniques.^{19,46,47} Standard deviations of 3.1%

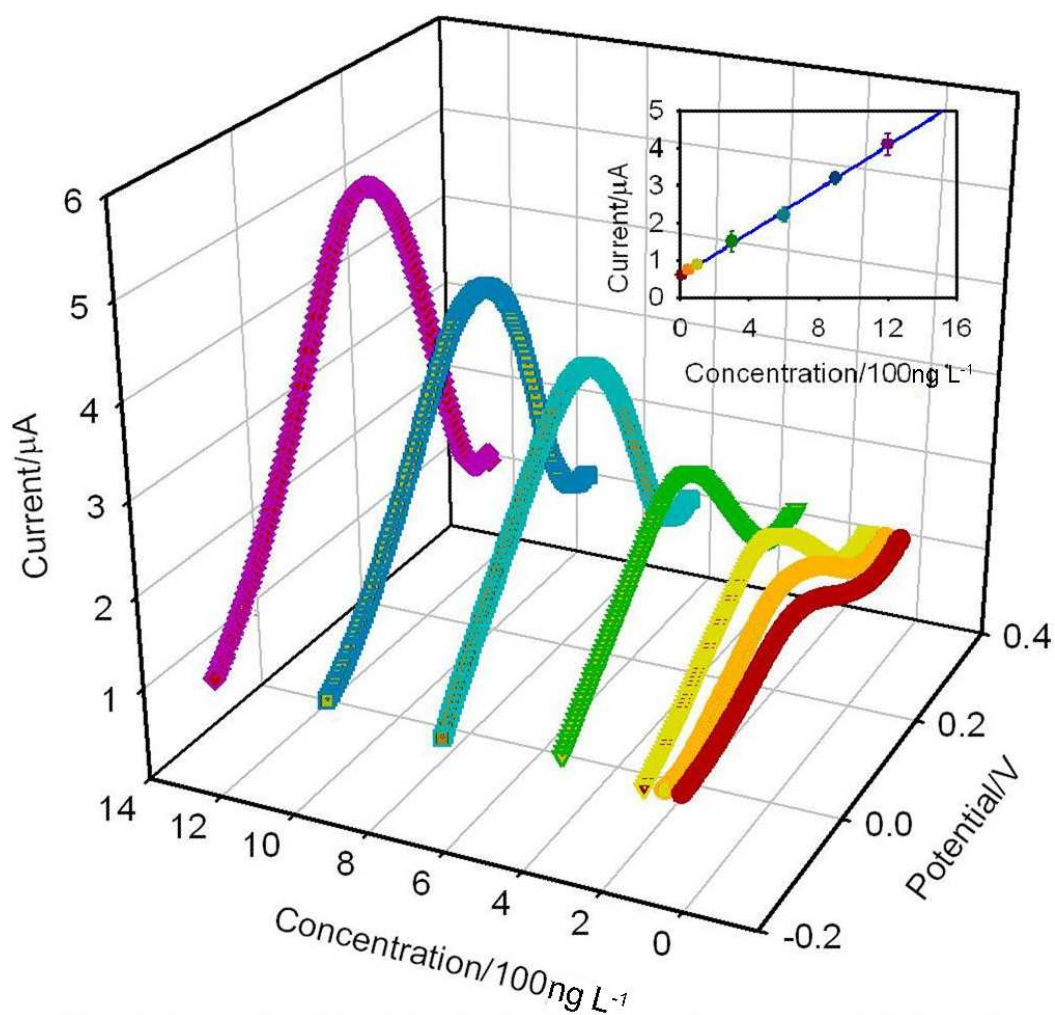


Figure 4.6. Voltammetric performance and the corresponding calibration plot (inset) of PET/AuNPs/MPTS/AuNPs/GCE toward Cr(VI) reduction in the concentration range from 10 to 1200 ng L^{-1} . Error bar: $n = 3$.

($n = 3$) for inter-group detection and 4.2% ($n = 3$) for outer-group detection, respectively, were obtained, indicating high sensitivity, better stability and reproducibility of the method for Cr(VI) reduction. Such a high sensitivity could be ascribed to the synergistic effect of the pyridinium-functionalized, flower-like self-assembly of AuNPs and the flower-like gold nanoelectrode. Moreover, this method showed a wide detection range of Cr(VI), probably as a result of the large AuNPs surface area. The detection here demonstrates that the current method could be used for reliable and accurate Cr(VI) measurement at ng L^{-1} level in real samples. The electrode, once made, was used for at least fifteen measurements with no obvious decrease in peak current.

4.3.4. Interference Study

A major challenge for Cr(VI) detection in real samples is the elimination of interferences. In order to evaluate the performance of PET/AuNPs/MPTS/AuNPs/GCE toward Cr(VI) reduction in the presence of coexisting anions or cations including ClO_4^- , NO_3^- , BrO_3^- , MoO_4^{2-} , SO_4^{2-} , Cr^{3+} , Pb^{2+} , Al^{3+} , Cd^{2+} , Fe^{3+} , Cu^{2+} , and Zn^{2+} , the effect of these ions on the cathodic peak current of Cr(VI) reduction was examined (Figure 4.7). The voltammetric response of the designed electrode towards 300 ng L^{-1} Cr(VI) was first measured, and 500-fold excess of the coexisting ions was subsequently added into the supporting electrolyte. At pH 4.5, HCrO_4^- is the main form of Cr(VI) in the aqueous solution. As expected, no change in the voltammetric response of

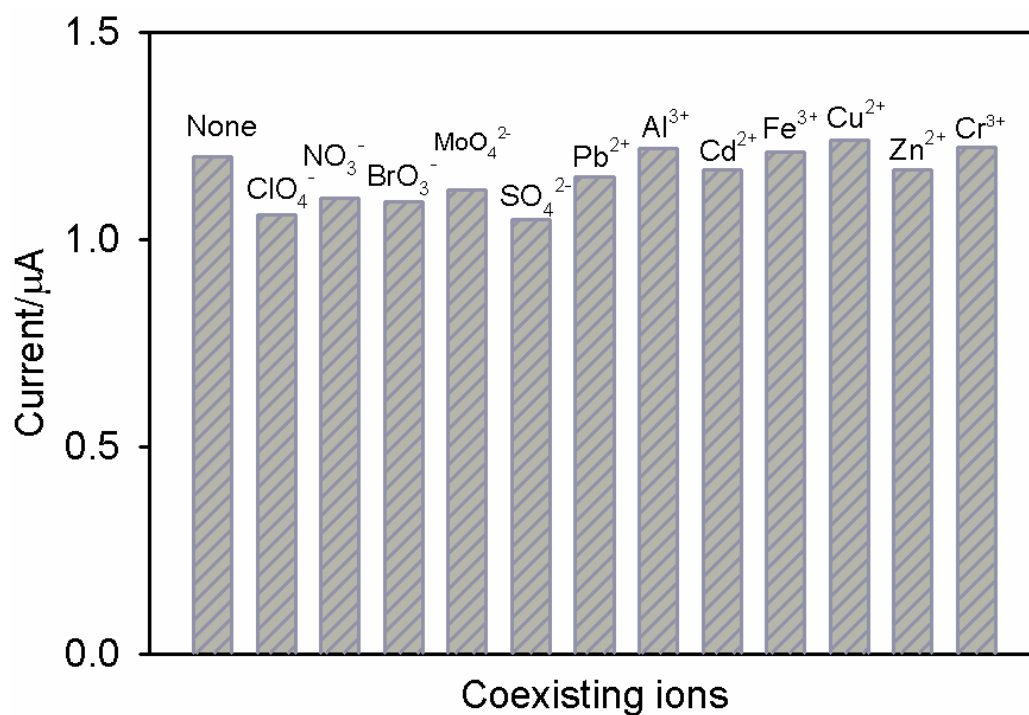


Figure 4.7. Voltammetric response of PET/AuNPs/MPTS/AuNPs/GCE toward 300 ng mL⁻¹ Cr(VI) with and without 500-fold excess of coexisting ions in 0.15 M fluoride solution (pH 7.8).

Cr(VI) was observed in the presence of various cations, as the positively charged ions such as Cr^{3+} and Cu^{2+} are repelled by the positive charge of pyridinium group on the electrode surface. It is interesting that the presence of negatively charged ions only showed minute interference toward Cr(VI) reduction, implying that the preconcentration of Cr(VI) was not a simple ion-exchange process. Moreover, even MoO_4^{2-} with the similar structure and size did not exhibit obvious interference.

4.3.5. Blood Sample Analysis

The analytical reliability and practical application potential of the new electrode was evaluated by measuring Cr(VI) in a blood sample. Chromium is an essential trace element for mammals (3-10 ppb in the blood), and it is bound to peptides.⁴⁸ Prior to the analysis, the blood sample was first pretreated by AOP in order to decompose the organic and biological species in the sample and to free chromium. As a result, Cr in the blood was converted to Cr(VI) in the pretreatment. Standard addition method was employed and the CSSWV measurements were performed before and after the addition of 100 and 200 ng L^{-1} of Cr(VI) to the sample (Figure 4.8). A well defined peak around 0.0 V was observed and increased proportionally with the Cr(VI) addition. A slight, negative shift of the peak potential might be caused by the background of the AOP-treated blood sample. The original concentration of Cr(VI) in the blood sample was found to be $6.9(0.5) \mu\text{g L}^{-1}$, which is consistent with the previously reported value

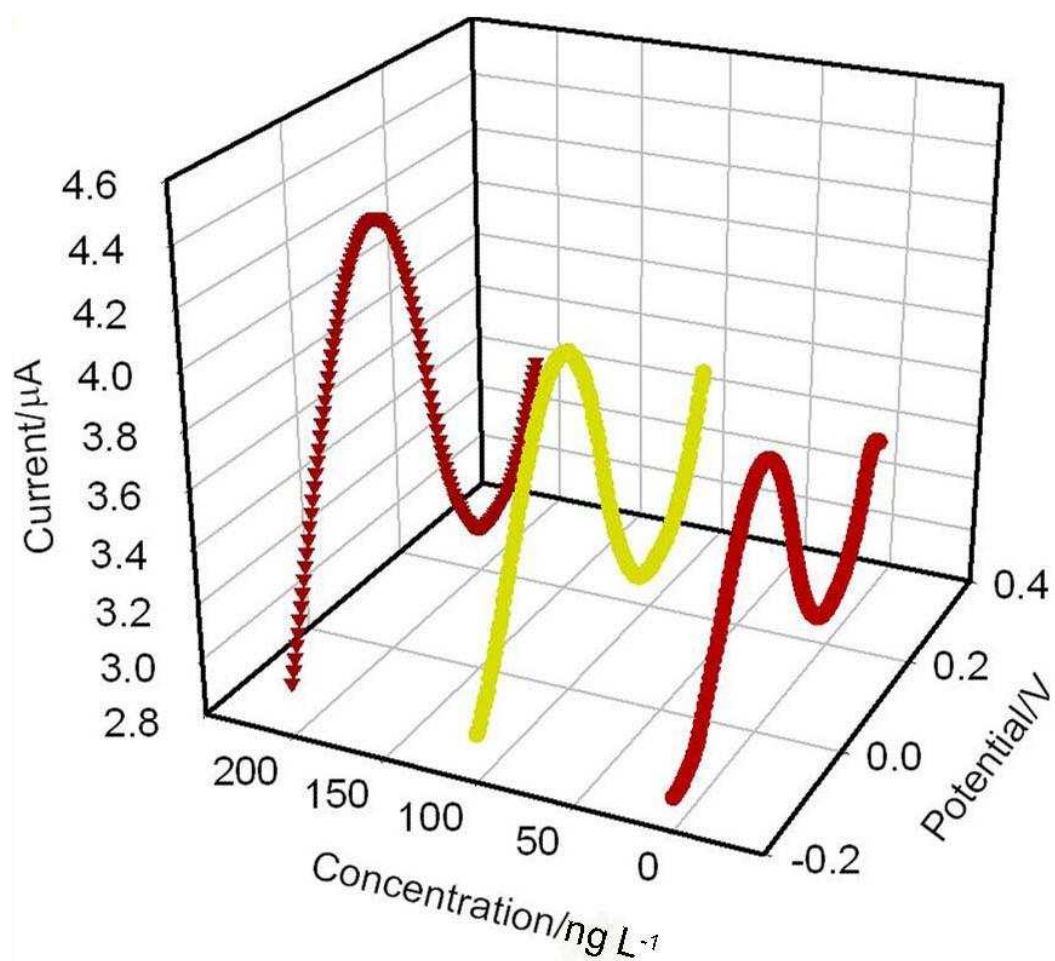


Figure 4.8. CSSWVs of the blood samples spiked with: (a) 0; (b) 100; and (c) 200 ng L⁻¹ Cr(VI).

in Part 3, with a recovery of more than 93%, and with a relative error less than 5.3%. This result demonstrates the successful use of the new electrode for the detection of Cr(VI) in the blood samples with acceptable accuracy. More importantly, the residual H_2O_2 from the blood pretreatment did not cause obvious interference in the performance of the current electrode. This is an evidence of the accuracy and reliability of the new electrode for the applications in real samples.

4.4. Conclusions

The fabrication of a new flower-like self-assembly of AuNPs-modified electrode is reported here. After functionalization by a thiol pyridinium, the electrode was used successfully toward Cr(VI) preconcentration and subsequent analysis. At the electrodes, Cr(VI) was efficiently reduced at 0.08 V in the absence of a complexing ligand, which is ~ 100 mV less positive than that for conventional Au disk electrodes. The introduction of two layers of AuNPs significantly enhances the detection behavior of the electrode and makes measurement simple, highly sensitive and selective, and stable. It provides a very low detection limit and a large, linear response comparable with that based on a bismuth film electrode that requires the use of a complexing ligand to exhibit high sensitivity for Cr(VI) reduction. No interference was found for aqueous Cr(VI) detection with the coexisting ions. The AuNPs-based electrode was used

successfully for the detection of Cr(VI) in AOP treated blood sample without interference by residual H₂O₂. The current work provides both a new flower-like AuNPs electrode on the GCE surface and a method for highly sensitive and selective Cr(VI) detection.

References

1. Acharya, U.R.; Mishra, M.; Tripathy, R.R.; Mishra, I. *Reprod. Toxicol.* **2006**, *22*, 87-91.
2. Kotas, J.; Stasicka, Z. *Environ. Pollut.* **2000**, *107*, 263-283.
3. Norseth, T. *Environ. Health Perspect.* **1981**, *40*, 121-130.
4. Stohs, S.J.; Bagchi, D. *Free Radic. Biol. Med.* **1995**, *18*, 321-326.
5. Bag, H.; Turker, A.R.; Lale, M.; Tunceli, A. *Talanta* **2000**, *51*, 895-902.
6. Hirata, S.; Honda, K.; Shikino, O.; Maekawa, N.; Aihara, M. *Spectrochim. Acta, Part B* **2000**, *55*, 1089-1099.
7. Tang, B.; Yue, T.; Wu, J.; Dong, Y.; Ding, Y.; Wang, H. *Talanta* **2004**, *64*, 955-960.
8. Kaneko, M.; Kurihara, M.; Nakano, S.; Kawashima, T. *Anal. Chim. Acta* **2002**, *474*, 167-176.
9. Muto, K.; Ohno, K.; Lin, J. M.; Yamada, M. *Jpn. J. Iron Steel* **2003**, *89*, 68-74.
10. Marguí, E.; Fontàs, C.; Toribio, M.; Guillem, M.; Hidalgo, M.; Queralt, I. *Appl. Spectrosc.* **2010**, *64*, 547-551.
11. Han, Z.Q.; Qi, L.; Shen, G.Y.; Liu, W.; Chen, Y. *Anal. Chem.* **2007**, *79*, 5862-5868.
12. Chen, H.; Du, P.; Chen, J.; Hu, S.H.; Li, S.Q.; Liu, H.L. *Talanta* **2010**, *81*, 176-179.

13. Sasaki, K.; Oguma, S.; Namiki, Y.; Ohmura, N. *Anal. Chem.* **2009**, *81*, 4005-4009.
14. Carrington, N.A.; Thomas, G.H.; Rodman, D.L.; Beach, D.B.; Xue, Z.-L. *Anal. Chim. Acta* **2007**, *581*, 232-240.
15. Carrington, N.A.; Xue, Z.-L. *Acc. Chem. Res.* **2007**, *40*, 343-350.
16. Dansby-Sparks, R.N.; Ouyang, R.Z.; Xue, Z.-L. *Sci. China Ser. B. Chem.* **2009**, *52*, 1777-1788.
17. Cox, J.A.; Kulesza, P.J. *Anal. Chim. Acta* **1983**, *154*, 71-78.
18. Yang, Y.J.; Huang, H.J. *Anal. Chem.* **2001**, *73*, 1377-1388.
19. Turyan, I.; Mandler, D. *Anal. Chem.* **1997**, *69*, 894-897.
20. Carrington, N.A.; Yong, L.; Xue, Z.-L. *Anal. Chim. Acta* **2006**, *572*, 17-24.
21. Carrington, N.A.; Qiu, H.; Xue, Z.-L. *Am. Lab.* **2007**, *39*, 37-41.
22. Carrington, N.A.; Rodman, D.L.; Xue, Z.-L. *Anal. Chim. Acta* **2006**, *572*, 303-308.
23. Carrington, N.A.; Rodman, D.L.; Goswami, K.; Xue, Z.-L. *J. Appl. Polym. Sci.* **2007**, *104*, 1043-1048.
24. Crosmun, S.T.; Mueller, T.R. *Anal. Chim. Acta* **1975**, *75*, 199-205.
25. Boussemart, M.; Van den Berg, C.M.G.; Ghaddaf, M. *Anal. Chim. Acta* **1992**, *262*, 103-105.
26. Lin, L.; Lawrence, N.S.; Thongngamdee, S.; Wang, J.; Lin, Y. *Talanta* **2005**, *65*, 144-148.
27. Wang, J.; Lu, J.M.; Hocevar, S.B.; Farias, P.A.M.; Ogorevc, B. *Anal.*

- Chem.* **2000**, 72, 3218-3222.
28. Wang, J.J.; Lu, M.; Kirgoz, U.A.; Hocevar, S.B.; Ogorevc, B. *Anal. Chim. Acta* **2001**, 434, 29-34.
29. Armstrong, K.C.; Tatum, C.E. Ph.D. dissertations, The University of Tennessee, Knoxville, 2007 and 2011, respectively.
30. Guo, S.J.; Wang, E.K. *Anal. Chim. Acta* **2007**, 598, 181-192.
31. Yun, Y.H.; Dong, Z.; Shanov, V.N.; Doepke, A.; Heineman, W.R.; Halsall, H.B.; Bhattacharya, A.; Wong, D.K.Y.; Schulz, M.J. *Sens. Actuators B* **2008**, 133, 208-212.
32. Sohn, K.; Kim, F.; Pradel, K.C.; Wu, J.; Peng, Y.; Zhou, F.; Huang, J. *ACS Nano* **2009**, 3, 2191-2198.
33. Vidotti, M.; Carvalhal, R.F.; Mendes, R.K.; Ferreira, D.C.M.; Kubota, L.T.; *J. Braz. Chem. Soc.* **2011**, 22, 3-20.
34. Daniel, M.C.; Astruc, D. *Chem. Rev.* **2004**, 104, 293-346.
35. Kohli, P.; Harrell, C.C.; Cao, Z.; Gasparac, R.; Tan, W.; Martin, C.R. *Science* **2004**, 305, 984-986.
36. Cheng, W.; Dong, S.; Wang, E. *Anal. Chem.* **2002**, 74, 3599-3604.
37. Jia, J.; Wang, B.; Wu, A.; Cheng, G.; Li, Z.; Dong, S. *Anal. Chem.* **2002**, 74, 2217-2223.
38. Cao, X.D.; Ye, Y.K.; Liu, S.Q. *Anal. Biochem.* **2011**, 417, 1-16.
39. Pingarrón, J.M.; Yáñez-Sedeño, P.; González-Cortés, A. *Electrochim. Acta* **2008**, 53, 5848-5866.

40. Ghosh, S.K.; Pal, A.; Kundu, S.B.; Nath, S.; Pal, T. *Chem. Phy. Lett.* **2004**, 395, 366-372.
41. Xie, C.G.; Li, H.F.; Li, S.Q.; Wu, J.; Zhang, A.P. *Anal. Chem.* **2010**, 82, 241-249.
42. Jena, B.K.; Raj, C.R. *Talanta* **2008**, 76, 161-165
43. Jena, B.K.; Raj, C.R. *Chem. Eur. J.* **2006**, 12, 2702-2708.
44. Yong, L.; Armstrong, K.C.; Dansby-Sparks, R.N.; Carrington, N.A.; Chambers, J.Q.; Xue, Z.-L. *Anal. Chem.* **2006**, 78, 7582-7587.
45. Zhao, J.; Chen, M.; Yan, F.Y. *Colloid Surface A* **2009**, 346, 75-82.
46. Tsai, M.C.; Chen, P.Y. *Talanta* **2008**, 76, 533-539.
47. Li, F.M.; Liu, J.M.; Wang, X.X.; Lin, L.P.; Cai, W.L.; Lin, X.; Zeng, Y.N.; Li, Z.M.; Lin, S.Q. *Sens. Actuators B.* **2011**, 155, 817-822.
48. Vincent, J. *The Nutritional Biochemistry of Chromium(III)*, Elsevier Science, Amsterdam, The Netherlands, 2007.

Part 5

Electrodeposition of Sol-gel Using an Ethylenediamine Compound for Chromium(VI) Detection

Abstract

The novel method of modifying a glassy carbon electrode with physical deposition of single-walled carbon nanotubes (SWNTs) followed by electrochemical deposition of a sol-gel using base-catalyzed hydrolysis is reported here. The thin sol-gel film fabricated from a new ethylenediamine derivative is believed to preconcentrate Cr(VI) anions. The SWNTs allow for greater conductivity and increased surface area on the electrode surface which in turn gives greater sensitivity. Our studies have shown detection of Cr in the low ppb and ppt range.

5.1. Introduction

As mentioned previously, hexavalent chromium is a toxic, strong oxidizing agent. The wide use of chromium in industry has inevitably led to ecological impacts from Cr(VI) contamination, causing allergic dermatitis, as well as toxic, carcinogenic effects and mutagenesis in animals and humans.¹⁻⁴ There has thus been a growing interest in highly sensitive and selective assays for trace Cr determination. A number of sensitive techniques have been employed, such as atomic absorption spectrophotometry,⁵ plasma mass spectrometry,⁶ spectrofluorimetry,⁷ spectrophotometry,⁸ and chemiluminescence,⁹ but each of these processes is time consuming and necessitates expensive equipments.

Various sensor systems have also been reported.¹⁰⁻¹⁶ Most of these systems, however, have either limitations in sensitivity, simplicity or the high cost of equipments as well.

Electrochemical methods have been considered desirable for the Cr(VI) determination, and they have demonstrated advantages including speed of analysis, good selectivity, and sensitivity.¹⁷⁻³¹ Mercury electrodes have also been used for the electrochemical detection of Cr(VI).^{17,18} While extremely sensitive, their applications are limited due to the toxicity of mercury. In an effort to find alternative electrodes to mercury, bismuth-film electrodes (BiFEs) have been developed for Cr(VI) detection through catalytic adsorption stripping voltammetry (CAAdSV).¹⁹⁻²² However, the poor reproducibility of BiFEs is challenging for Cr detection at a low concentration, and a complexing ligand is needed for the process, adding to the complexity of the detection process. A poly(4-vinylpyridinium)-coated platinum electrode was developed by Cox and Kulesza for Cr(VI) preconcentration in the polymer films, followed by determination with the lack of interference by metal cations.²³ In a flow injection analysis system, a polyaniline/polystyrene composite electrode was used as a detector to monitor Cr(VI) with a detection limit of 4 ng L⁻¹.²⁴ A self-assembled monolayer of pyridinium on the Au electrode surface for sensitive Cr(VI) detection has been developed by Turyan and Mandler.²⁵ Our group has developed 2- and 4-pyridinium-functionalized sol-gel films through electrodeposition at a glassy carbon electrode (GCE) surface for Cr(VI) detection in aqueous solution.^{26,27} The

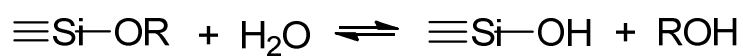
narrow concentration ranges, however, significantly limit the practical applications. It is thus highly desirable to develop a system that is not only sensitive, selective, and reliable but also simple, practical, and economical in its operation.

Sol-gels have become very popular for electrochemical sensing.²⁶⁻⁴¹ Due to the unique and compact structures created using sol-gel materials, they are advantageous for Cr(VI) detection. Spin coating has been the primary deposition technique for many years,^{30,34,38,39} usually incorporating acid catalysis.^{30,32} These films, however, are not very porous. Using base catalysis reactions, as shown in Figure 5.1, give a higher porosity^{41,42} which is beneficial in most sol-gel sensing applications.

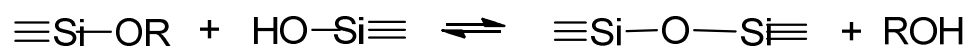
Work in recent years has established highly porous sol-gel films using electrodeposition at the electrode surface,^{31-33,35,36} including publications by previous members of this research group.²⁶⁻²⁹ First investigated by Shacham et al.³⁶ and later elaborated on by Collinson et al.^{31,32} and Walcarius and coworkers,^{33,34} this technique relies on the application of a negative potential to increase the pH at the electrode surface, causing the immediate condensation of the sol-gel. Because of this, gel formation and drying occur independently of each other, allowing more porous films to form.³²

As mentioned before, electrodeposition of sol-gel films has been performed by a previous group member.²⁶⁻²⁹ A sol-gel precursor containing

Hydrolysis



Alcohol Condensation



Water Condensation

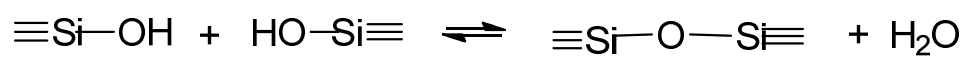


Figure 5.1. The basic sol-gel reactions.

pyridinium was used to preconcentrate and detect Cr(VI) on a glassy carbon electrode. While this method was found to be successful, the film was often irreproducible, thick, and the limit of detection remained in the ppb range. Since chromium concentration is often less than 10 ppb, it is advantageous to have sub-ppb detection available. The focus of this research was to develop a new sol-gel electrochemical detection method.

One major change to the electrode fabrication is the addition of single walled carbon nanotubes (SWNTs). SWNTs have been shown to possess potential for heavy metal analysis due to their strong sorption properties, high electrical conductivity, high surface area, significant mechanical strength, and good chemical stability.⁴³ In this work, the SWNTs are functionalized prior to use. The carboxylic groups on the SWNTs improve the adhesion of not only SWNTs to the GCE but also the sol-gel to the electrode. Additionally, as sol-gels are made from a combination of alkoxides that ultimately create a thin glass-like film, they are not highly conductive as a result. Therefore the presence of SWNTs on the electrode surface further enhances the electrochemical activity of the electrode.

In addition to SWNTs, the use of a different sol-gel precursor was implemented. Since a pyridinium containing compound was found to preconcentrate Cr(VI) successfully, the idea of using a similar amine-containing compound is presented. It is hypothesized that the use of a dication derived from a diamine allows for additional binding sites to preconcentrate Cr(VI), a

significant portion of which exists as dianions, thereby increasing the concentration range and potentially lowering the detection limit. Unlike pyridinium, this compound, $(\text{MeO})_3\text{Si}(\text{CH}_2)_3\text{NHCH}_2\text{CH}_2\text{NH}_2$ (TMSen), has not been previously used for Cr(VI) detection. The drawback of directly using a compound containing ethylenediamine is the increase in pH. As electrodeposition of the sol-gel is base catalyzed, a basic solution will cause immediate hydrolysis, even prior to applying a negative potential. In order to counteract such a basic pH, the compound is acidified first by HCl, forming $(\text{MeO})_3\text{Si}(\text{CH}_2)_3(\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_3)\text{Cl}_2$ ($\text{H}_2\text{TMSenCl}_2$). This step will not only prevent early hydrolysis but may also slightly inhibit hydrolysis at the electrode surface, thereby generating a thinner film. Previous work with pyridinium containing compounds required protonation as well, allowing for a better preconcentration.^{26,27}

In this work, the electrodeposition technique is used in conjunction with a previously modified glassy carbon electrode containing a film of SWNTs. The SWNTs allow for greater conductivity and increased surface area on the electrode surface which in turn gives greater sensitivity. This work surrounds the use of the new sol-gel precursor not previously used for the preconcentration of Cr(VI) anions, leading to Cr(VI) detection with enhanced sensitivity (Figure 5.2). The approach is based on the electrostatic interaction between the positively charged ethylenediamine groups in the sol-gel matrix and the negatively charged Cr(VI) anions.⁴⁴⁻⁴⁷ These anions are then reduced to Cr^{3+} cations, regenerating

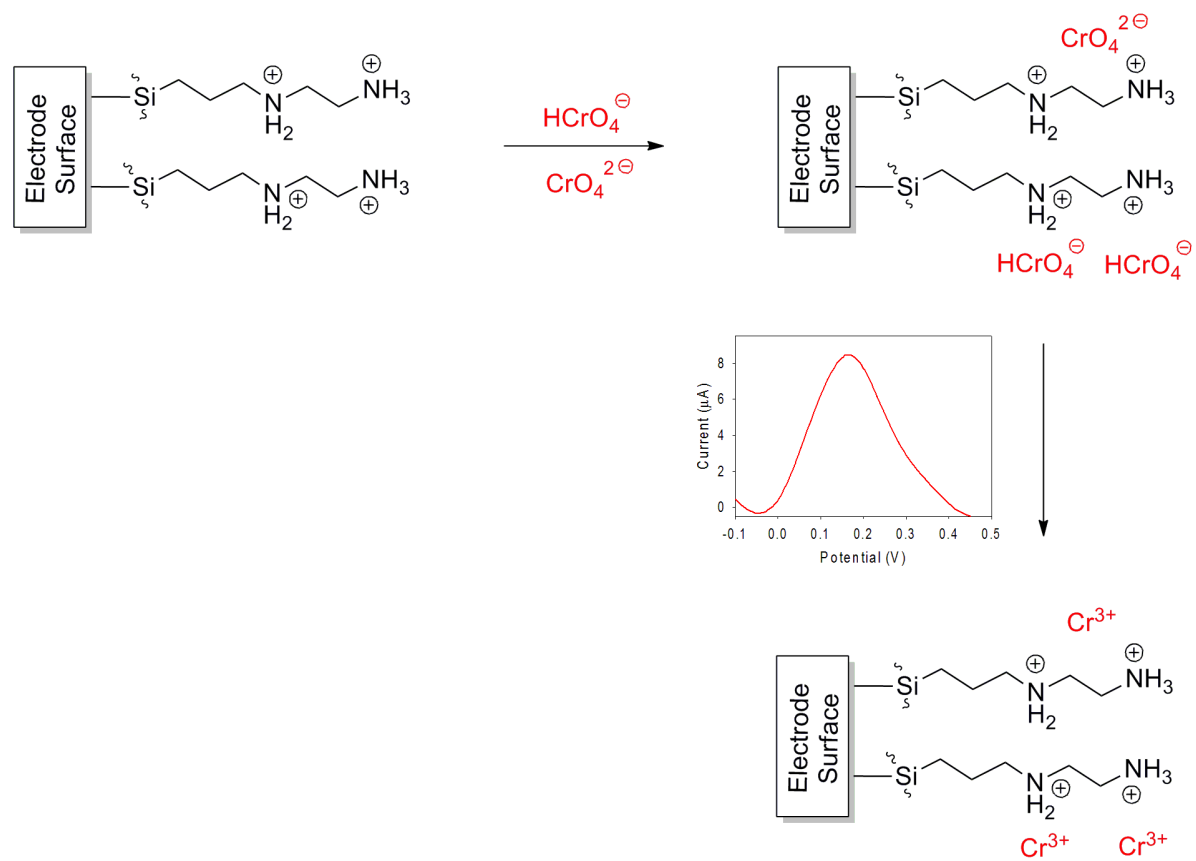


Figure 5.2. Illustration of Cr(VI) accumulation, reduction, and detection. Cl^- ions are omitted for clarity.

the electrode surface for subsequent preconcentration and analysis. The results are reported here.

5.2. Experimental

5.2.1. Chemical Reagents and Materials

Tetramethyl orthosilicate (TMOS, $\text{Si}(\text{OMe})_4$, 98%, Sigma-Aldrich), N-[3-(trimethoxysilyl)propyl]ethylenediamine (TMSen, 97%, Sigma-Aldrich), hydrochloric acid (HCl, certified A.C.S., Fisher), hydrofluoric acid (HF, certified A.C.S., Fisher), diethyl ether (Et_2O , Sigma-Aldrich), HCl in diethyl ether (1.0 M, Sigma-Aldrich), sodium fluoride (NaF, Fisher), methanol (MeOH, HPLC Grade, Fisher), and potassium chloride (KCl, certified A.C.S., Mallinckrodt) were used as received. Single walled carbon nanotubes were obtained from Shenzhen Nanotech Port Co., Ltd. (<http://www.nanotubes.com.cn/doce/default.html>), China. Standard solutions of Cr(VI) were prepared by serial dilution of a 1000 $\mu\text{g/mL}$ AA standard (Sigma-Aldrich). Solutions and standards were prepared using deionized (DI) water ($18\text{M}\Omega\text{ cm}$) from a Barnstead International E-pure 4-holder deionization system.

For analysis procedures, a three-electrode configuration consisted of a modified GCE, Ag/AgCl (saturated KCl solution, CH Instruments) and a platinum wire (CH Instruments) as working, reference and counter electrodes, respectively. For the electrodeposition experiments, a Ag/AgCl wire electrode,

prepared by soaking Ag wire in bleach, was used. The working electrodes typically used during the experiments were Teflon-encased glassy carbon electrodes (3 mm diameter, BAS Inc.). Before each coating process GCEs were polished carefully to a mirror-like surface on a standard electrode polishing kit (CH Instrument) including a 1200 grit Carbimet disk, 1.0 and 0.3 mm alumina slurry on a nylon cloth, and 0.05 μm alumina slurry on a microcloth polishing pad. After polishing, GCEs were successively sonicated with DI water, ethanol, and DI water for 5 min each. Electrodes were then soaked in piranha solution for an additional 15 min.

5.2.1.1. Preparation of Acidified TMSen

All manipulations were carried out under a dry nitrogen atmosphere with the use of standard Schlenk techniques. Diethyl ether was purified by distillation from a potassium benzophenone ketyl and stored under a nitrogen atmosphere. TMSen (1.997 g, 1.9 mL, 8.98 mmol) was dissolved in Et_2O (10 mL) in a flamed dried 125 mL Schlenk flask. HCl in diethyl ether (1.0 M, 18 mL, 18.0 mmol) was added to a Schlenk graduated cylinder and was added dropwise to the vigorously stirred TMSen/ Et_2O solution at 0 $^\circ\text{C}$. The slurry solution was allowed to stir for 18 h and volatiles were removed in vacuo to afford a white solid of $\text{H}_2\text{TMSenCl}_2$ (2.351 g, 7.96 mmol, 88.73% yield).

5.2.2. Instrumentation

All electrochemical measurements were carried out on a modulated potentiostat (CHI 440a/650a, CH Instruments). All microscopic images were obtained using a Leica S8APO StereoZoom optical microscope.

5.2.3. Preparation of Single-Walled Carbon Nanotubes

SWNTs of a certain mass (40 mg) were dispersed in 30% HNO₃ and then refluxed for 24 h at 140 °C to obtain carboxylic group-functionalized SWNTs. The resulting suspension was centrifuged, and the sediment was washed with deionized water until the pH reached 7.0. Then, the oxidized SWNTs were dispersed in deionized water to a concentration of 0.5 mg/mL for storage purposes but were further diluted prior to deposition. SWNTs were then physically deposited and allowed to dry overnight.

5.2.4. Coating Procedure

A sol solution consisting of 8 mL of 0.2 M KCl, 8 mL of MeOH, 500 µL of TMOS, and 1.34 g of H₂TMSenCl₂ was prepared and stirred thoroughly for several minutes to ensure a homogeneous mixture. After mixing, the working electrode was exposed to the solution, and a potential of -1.2 V was applied for 70 s. The working electrode was then rinsed with several aliquots of a 1:1 mixture of MeOH and DI water. It was then allowed to dry at 60 °C for 5 h followed by further drying under nitrogen flow for 18 h prior to use.

5.2.5. Analysis Procedure

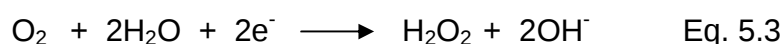
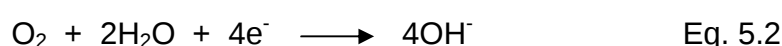
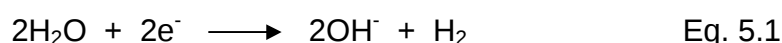
The electrodes were exposed to a solution consisting of 0.1 M HCl, 0.1 M KCl, and a variable amount of Cr(VI). After 5 min of stirring, the electrode was placed in a solution of 0.15 M NaF (pH 7.8). A square wave voltammogram was then collected, typically from 0.6 V to -0.3 V with a frequency of 15 Hz, amplitude of 0.025 V, and incremental potential of 0.004 V. The electrodes were then rinsed with DI water and a cleaning step was performed by holding the potential at -0.2 V for 60 s in 0.1 M HCl/KCl to ensure that any residual Cr(VI) present was eliminated. Subsequent analyses of various Cr(VI) concentrations could then be carried out.

5.3. Results and Discussion

5.3.1. Electrode Fabrication and Characterization

The electrodeposition process described is similar to a process reported previously.^{26-29,31,32} By applying a negative potential to the working electrode, hydroxide ions are generated and increase the pH directly at the electrode surface (Eq. 5.1-5.3). When TMOS and H₂TMSenCl₂ are present, the influx of OH⁻ base catalyzes the hydrolysis and condensation (Figure 5.1) of the sol at the electrode surface and produces a film functionalized with H₂TMSenCl₂. The use of SWNTs allows for increased surface area, greater conductivity, and better adhesion of the film to the electrode. Exposing the glassy carbon electrode to

piranha solution prior to deposition of the SWNTs and subsequent electrodeposition also enables adhesion of the nanotubes and the film to the electrode by producing surface oxides. An illustration summarizing the fabrication of the sol-gel electrode is shown in Figure 5.2.



Sol-gels allow for a thin film, and are often transparent. Carbon nanotubes are also not readily visible on the electrode surface, as shown in Figure 5.3. Thus it may be difficult to see a condensed sol-gel on the surface, particularly when the sol solution does not readily gel (Figure 5.4). In order to affirm the electrodeposition of the film, a platinum electrode (CH Instruments) was used to replicate the electrode fabrication, allowing for a better visual confirmation.⁴⁸ In this instance, however, Coumarin-153 (Aldrich) dye was added to the sol-gel solution prior to electrodeposition. The images are shown in Figure 5.5. It is very clear that SWNTs alone do not visibly alter the look of the electrode surface, but after electrodeposition of the doped sol-gel, it is very obvious that a film is on the surface. This confirms the film readily electrodeposits, despite its transparency.⁴⁸

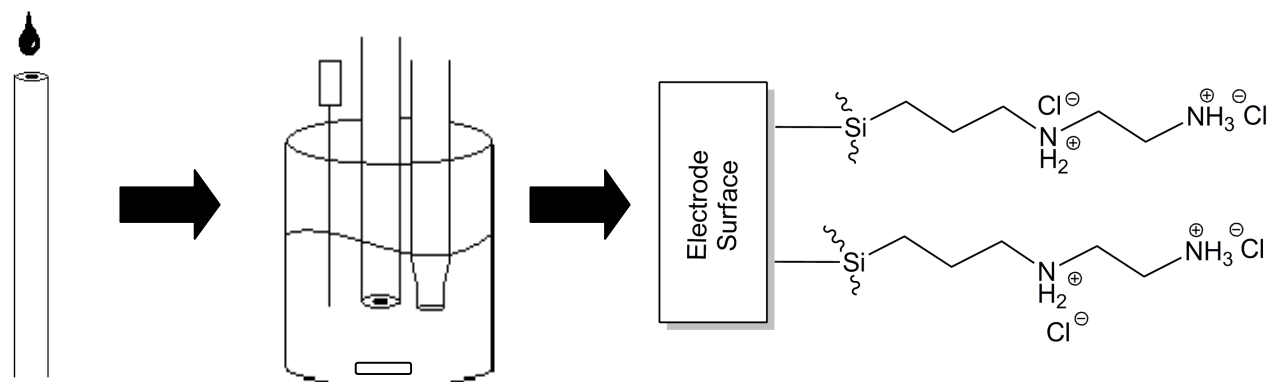


Figure 5.3. Illustration of electrode fabrication: (a) physical deposition of SWNTs onto GCE; (b) electrodeposition of sol-gel; (c) condensation of sol-gel onto GCE/SWNT.

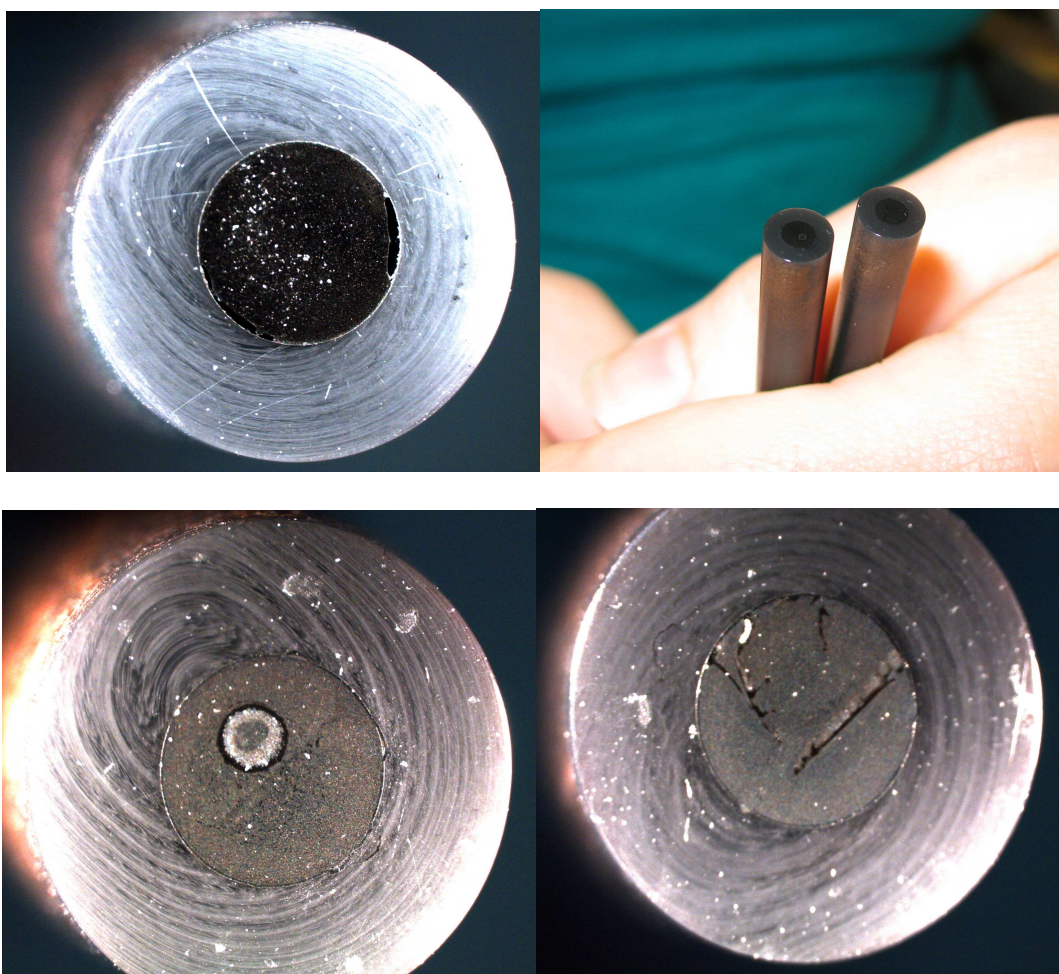


Figure 5.4. GCE/SWNT (top left); GCE/SWNT/H₂TMSenCl₂ (top right and bottom).



Figure 5.5. Images of a platinum electrode modified with only SWNTs (left), and after electrodeposition with a Coumarin-153 doped sol-gel.

5.3.2. Cr(VI) Analysis

The general process occurring at the glassy carbon electrode modified with carbon nanotubes during Cr(VI) analysis is illustrated in Figure 5.2. The ethylenediamine groups present in the sol-gel are protonated prior using HCl in diethyl ether before the sol-gel solution is prepared and electrodeposited (Figure 5.3). The use of HCl in Et₂O, which is free of water, is an important feature of the protonation step. Thus the product H₂TMSenCl₂ would not hydrolyze before the electrochemical deposition. The electrode is then exposed to a Cr(VI) solution where the analyte anions are preconcentrated at the electrode surface through the electrostatic interaction between the positively-charged ethylenediamine cations and the negatively-charged Cr(VI) ions. After a period of time has passed to allow the analyte species sufficient time to diffuse to the electrode surface, the electrode is placed into a stripping solution containing 0.15 M NaF and a square wave voltammogram is collected. A peak occurs at approximately 0.17 V corresponding to the reduction of Cr(VI) to Cr(III), as has been reported previously.^{23,25,26,27} The produced Cr(III) cations are then expelled from the sol-gel film. To ensure that the Cr species are removed, however, the electrodes are rinsed and a cleaning step was performed by holding the potential at -0.2 V for 60 s in 0.1 M HCl/KCl to ensure that any residual Cr(VI) present was eliminated.

Chromate exists in both monoanionic HCrO₄⁻ and dianionic CrO₄²⁻ forms. In addition, dichromate Cr₂O₇²⁻ may also be present as an equilibrium mixture with HCrO₄⁻ and CrO₄²⁻.⁴⁹ While each cationic site of the diammonium group

may attract HCrO_4^- , the dication of the diammonium group may help attract dianionic CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$. It is known that large cations bind more strongly with large anions stabilize large cations.⁵⁰ Thus the large diammonium group may be particularly suitable for $\text{Cr}_2\text{O}_7^{2-}$. In other words, the use of the diammonium-functionalized sol gel films may lead to the preconcentration of all forms of Cr(VI).

5.3.3. $\text{Fe}(\text{CN})_6^{4-}$ as a Redox Probe

$\text{Fe}(\text{CN})_6^{4-}$ is a well-established redox system⁵¹ that was used to investigate the anion-exchange capability of the sol-gel film. It is ideal for demonstrating the film's ability due to its negative charge, and previous work has demonstrated its effectiveness.^{52-54,26,27} The Heineman group⁵²⁻⁵⁴ as well as our group^{26,27} showed that sol-gel derived films were effective in demonstrating the use of anion preconcentration via the $\text{Fe}(\text{CN})_6^{4-}$ redox probe.

In past work using a pyridinium compound, it was noted that there is a decrease in the peak current, attributed to the increased resistance to mass transport through the sol-gel.^{26,27} Thicker films cause an even greater resistance. However, in the current study, the film is relatively thin which is easily observed by the naked eye (Figure 5.3), and the redox probe further confirms this as the current does not substantially decrease in comparison to a bare GCE. In order to best compare the redox probe to Cr(VI) detection, the electrode was allowed to stir in the $\text{Fe}(\text{CN})_6^{4-}$ solution for 5 min. With the incorporation of

SWNTs, the protonated thin sol-gel film successfully preconcentrates the negatively-charged analyte species better than a bare GCE, as shown in Figure 5.6. Protonating the electrode prior to exposure to the $\text{Fe}(\text{CN})_6^{4-}$ solution did not improve the peak current, which is reasonable since the sol-gel precursor was acidified initially and the sol-gel solution remained acidic. An electrode consisting of SWNTs on a GCE was also tested. However, the CV did not stabilize and later it was observed that the carbon nanotubes were in the $\text{Fe}(\text{CN})_6^{4-}$ solution.

This correlates well with previous reports showing enhanced sensitivity when anionic-exchange films are used for the determination of $\text{Fe}(\text{CN})_6^{4-}$.^{26,27,52-55} Although the mechanisms of analyte transport for the reversible $\text{Fe}(\text{CN})_6^{4-}$ system (diffusion and charge-transport processes) and irreversible Cr system (mass transport) are very different, the results imply that the functionalized sol-gel films are adequate for the preconcentration and analysis of Cr(VI) anions.

5.3.4. Cr(VI) Quantification

Studies were carried out in which the electrode response was monitored as a function of Cr(VI) concentration. The square wave voltammograms and the corresponding calibration plots are given in Figures 5.7-5.10. There is a linear response between the concentrations of Cr(VI) and the peak currents at the electrode.

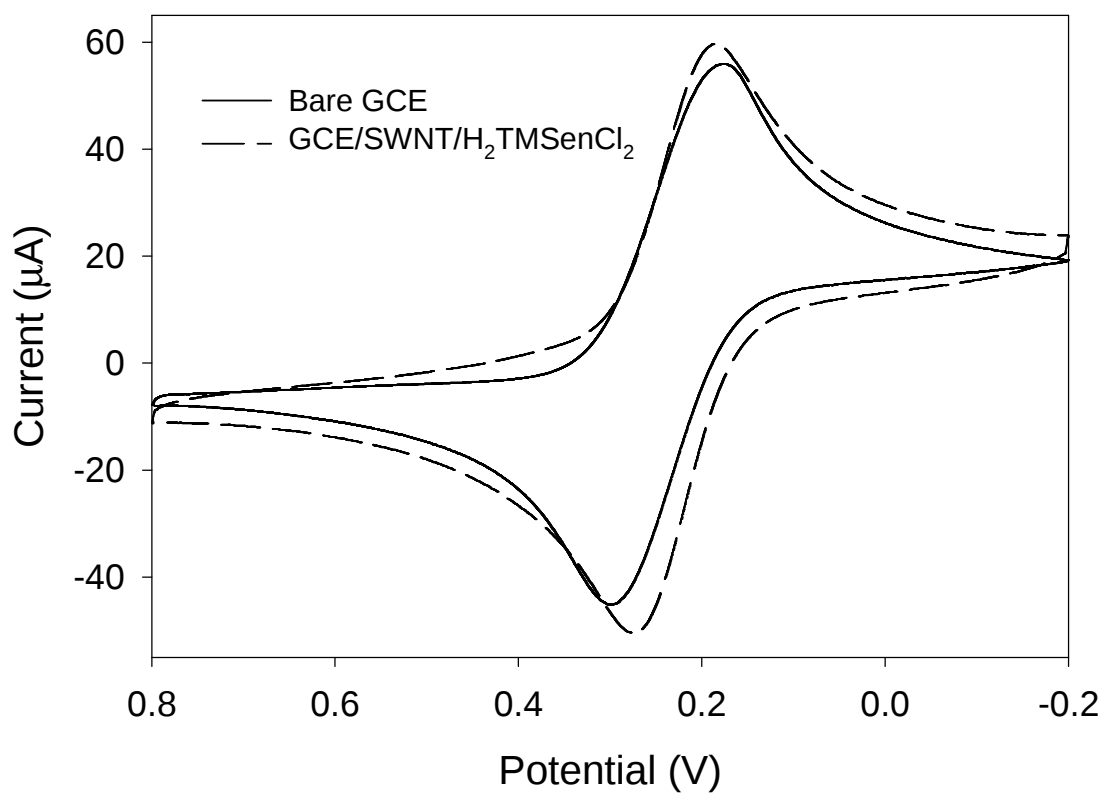


Figure 5.6. Cyclic voltammograms of 5mM $\text{Fe}(\text{CN})_6^{4-}$ at a glassy carbon electrode before and after modification with the SWNTs and $\text{H}_2\text{TMSenCl}_2$ functionalized sol-gel.

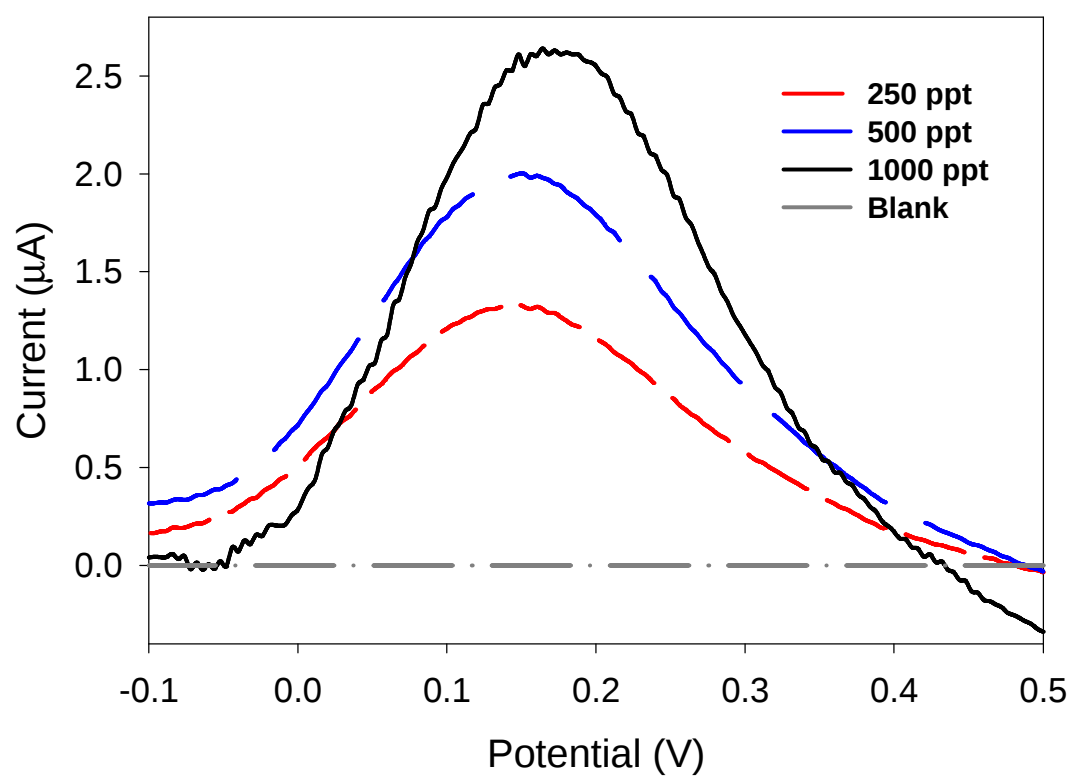


Figure 5.7. Square-wave voltammograms of various Cr(VI) concentrations collected at a GCE/SWNT/ $\text{H}_2\text{TMSenCl}_2$.

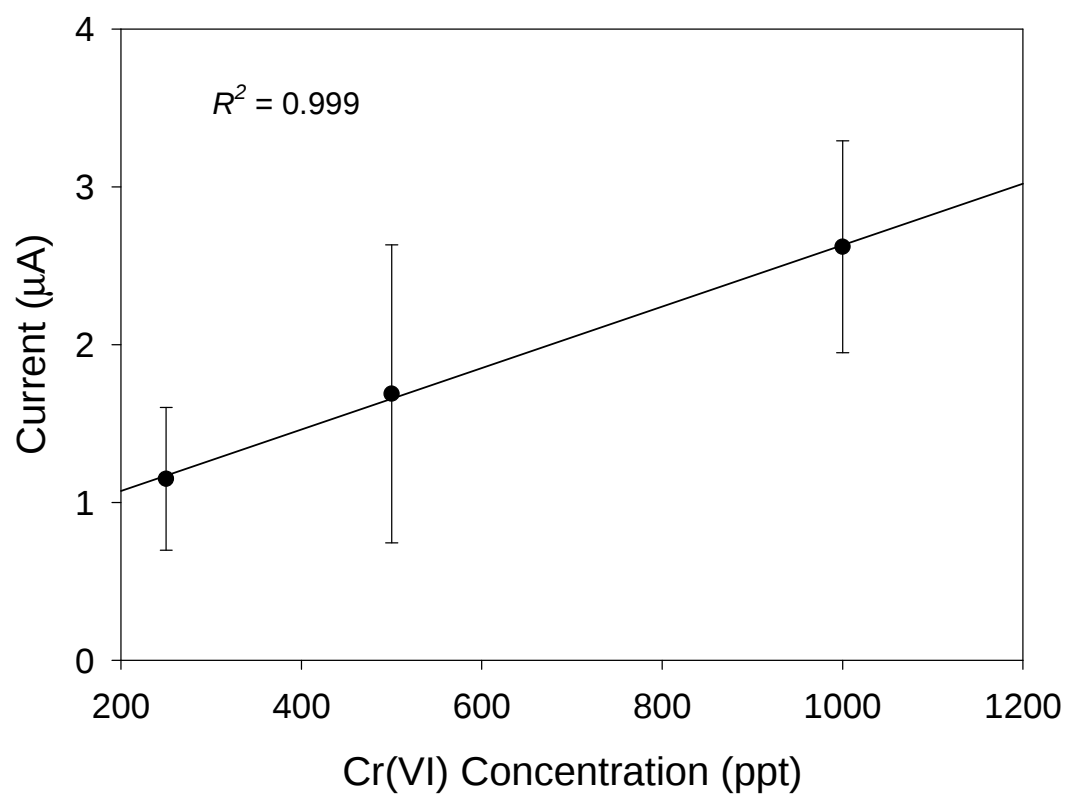


Figure 5.8. Calibration plots for the measurements in Figure 5.7.

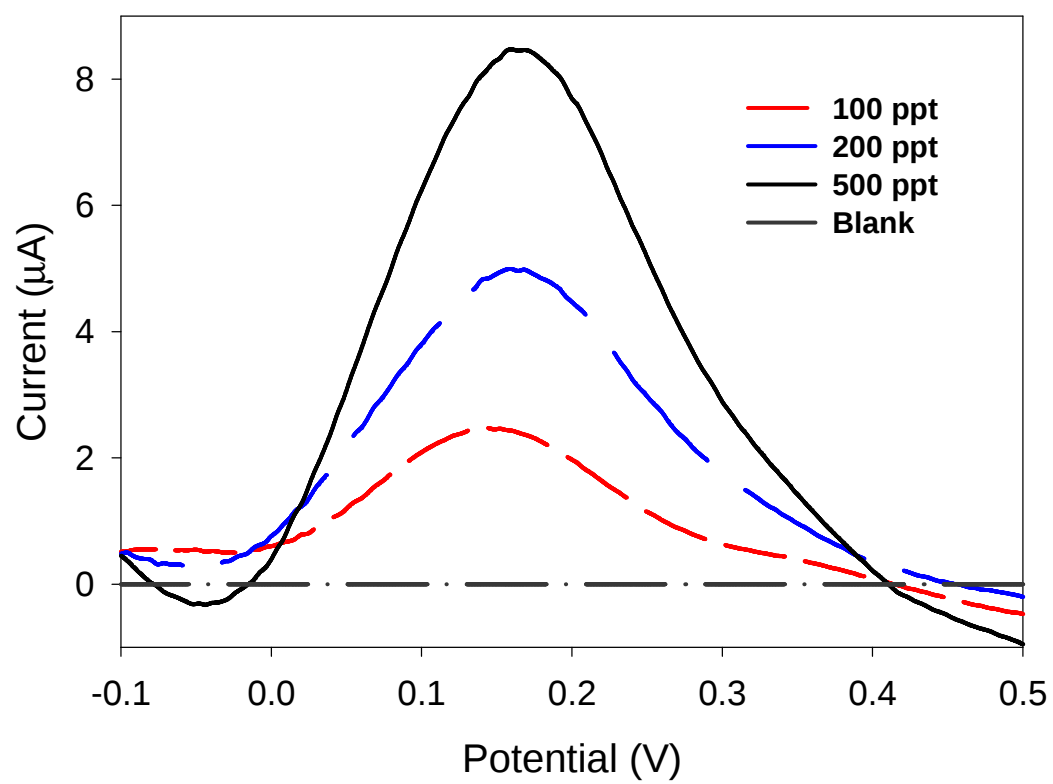


Figure 5.9. Square-wave voltammograms of various Cr(VI) concentrations collected at a GCE/SWNT/ $\text{H}_2\text{TMSenCl}_2$.

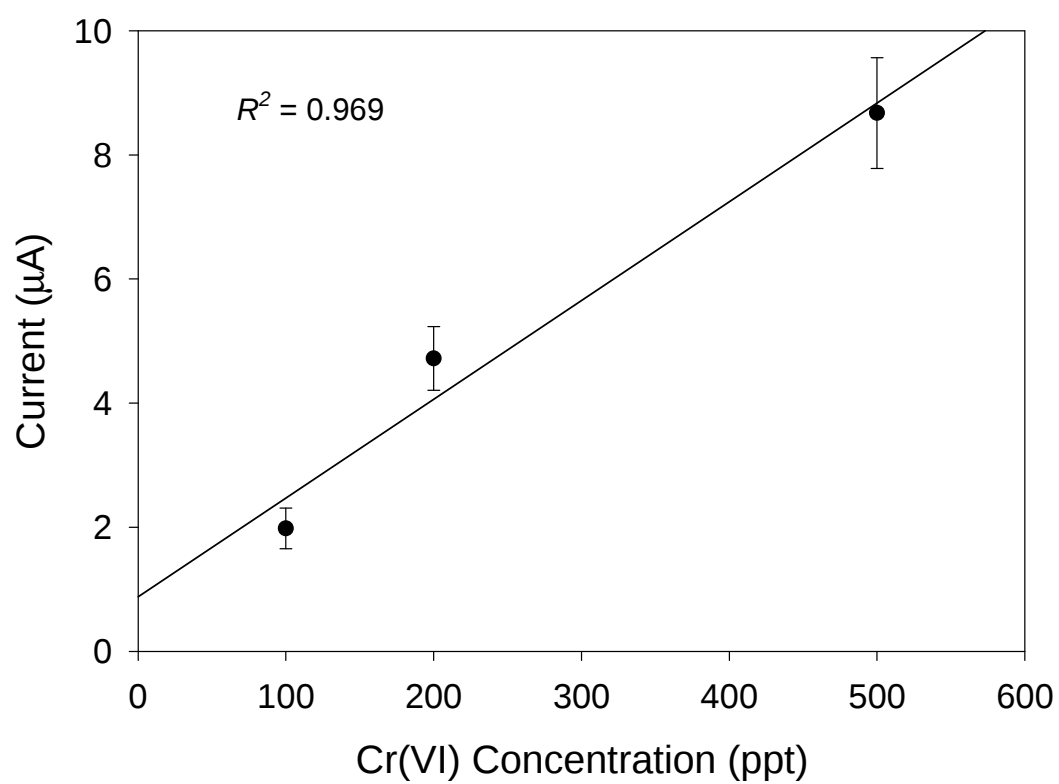


Figure 5.10. Calibration plots for the measurements in Figure 5.9.

5.3.5. Effect of Preconcentration Time

In a typical analysis procedure, the functionalized electrode is exposed to the Cr(VI) solution for 5 min while stirring. Past work using the pyridinium precursor required a 10 min accumulation step, but this was found to be unnecessary in the experiments described here. Multiple reasons are believed to cause this occurrence, including the implementation of carbon nanotubes, a thinner film deposition, and a shorter drying time. A 10 min preconcentration time likely causes a saturation of the electrode surface.

5.4. Conclusions

Ethylenediamine-functionalized sol-gel films have been electrodeposited onto a SWNT-modified GCE for the detection of Cr(VI). The technique uses commercially available reagents and can be performed easily and reproducibly. The film deposition has been confirmed and characterized using a $\text{Fe}(\text{CN})_6^{4-}$ redox probe. The resulting electrode is very sensitive, showing detection in the ppt range. This work demonstrates the success of electrodeposition for an ethylenediamine-functionalized sol-gel film and exhibits its potential in Cr(VI) applications.

References

1. Acharya, U.R.; Mishra, M.; Tripathy, R.R.; Mishra, I. *Reprod. Toxicol.* **2006**, *22*, 87-91.
2. Kotas, J.; Stasicka, Z. *Environ. Pollut.* **2000**, *107*, 263-283.
3. Norseth, T. *Environ. Health Perspect.* **1981**, *40*, 121-130.
4. Stohs, S.J.; Bagchi, D. *Free Radic. Biol. Med.* **1995**, *18*, 321-326.
5. Bag, H.; Turker, A.R.; Lale, M.; Tunceli, A. *Talanta* **2000**, *51*, 895-902.
6. Hirata, S.; Honda, K.; Shikino, O.; Maekawa, N.; Aihara, M. *Spectrochim. Acta, Part B* **2000**, *55*, 1089-1099.
7. Tang, B.; Yue, T.; Wu, J.; Dong, Y.; Ding, Y.; Wang, H. *Talanta* **2004**, *64*, 955-960.
8. Kaneko, M.; Kurihara, M.; Nakano, S.; Kawashima, T. *Anal. Chim. Acta* **2002**, *474*, 167-176.
9. Muto, K.; Ohno, K.; Lin, J. M.; Yamada, M. *Jpn. J. Iron Steel* **2003**, *89*, 68-74.
10. Marguí, E.; Fontàs, C.; Toribio, M.; Guillem, M.; Hidalgo, M.; Queralt, I. *Appl. Spectrosc.* **2010**, *64*, 547-551.
11. Han, Z.Q.; Qi, L.; Shen, G.Y.; Liu, W.; Chen, Y. *Anal. Chem.* **2007**, *79*, 5862-5868.
12. Chen, H.; Du, P.; Chen, J.; Hu, S.H.; Li, S.Q.; Liu, H.L. *Talanta* **2010**, *81*, 176-179.

13. Sasaki, K.; Oguma, S.; Namiki, Y.; Ohmura, N. *Anal. Chem.* **2009**, *81*, 4005-4009.
14. Carrington, N.A.; Thomas, G.H.; Rodman, D.L.; Beach, D.B.; Xue, Z.-L. *Anal. Chim. Acta* **2007**, *581*, 232-240.
15. Carrington, N.A.; Xue, Z.-L. *Acc. Chem. Res.* **2007**, *40*, 343-350.
16. Dansby-Sparks, R.N.; Ouyang, R.Z.; Xue, Z.-L. *Sci. China Ser. B. Chem.* **2009**, *52*, 1777-1788.
17. Crosmun, S.T.; Mueller, T.R. *Anal. Chim. Acta* **1975**, *75*, 199-205.
18. Boussemart, M.; Van den Berg, C.M.G.; Ghaddaf, M. *Anal. Chim. Acta* **1992**, *262*, 103-105.
19. Lin, L.; Lawrence, N.S.; Thongngamdee, S.; Wang, J.; Lin, Y. *Talanta* **2005**, *65*, 144-148.
20. Wang, J.; Lu, J.M.; Hocevar, S.B.; Farias, P.A.M.; Ogorevc, B. *Anal. Chem.* **2000**, *72*, 3218-3222.
21. Wang, J.J.; Lu, M.; Kirgoz, U.A.; Hocevar, S.B.; Ogorevc, B. *Anal. Chim. Acta* **2001**, *434*, 29-34.
22. Armstrong, K.C.; Tatum, C.E. Ph.D. dissertations, The University of Tennessee, Knoxville, 2007 and 2011, respectively.
23. Cox, J.A.; Kulesza, P.J. *Anal. Chim. Acta* **1983**, *154*, 71-78.
24. Yang, Y.J.; Huang, H.J. *Anal. Chem.* **2001**, *73*, 1377-1388.
25. Turyan, I.; Mandler, D. *Anal. Chem.* **1997**, *69*, 894-897.
26. Carrington, N.A.; Yong, L.; Xue, Z.-L. *Anal. Chim. Acta* **2006**, *572*, 17-24.

27. Carrington, N.A.; Qiu, H.; Xue, Z.-L. *Am. Lab.* **2007**, *39*, 37-41.
28. Carrington, N.A.; Rodman, D.L.; Xue, Z.-L. *Anal. Chim. Acta* **2006**, *572*, 303-308.
29. Carrington, N.A.; Rodman, D.L.; Goswami, K.; Xue, Z.-L. *J. Appl. Polym. Sci.* **2007**, *104*, 1043-1048.
30. Collinson, M.M.; Rausch, C.G.; Voigt, A. *Langmuir* **1997**, *13*, 7245-7251.
31. Collinson, M.M.; Moore, N.; Deepa, P.N.; Kanungo, M. *Langmuir* **2003**, *19*, 7669-7672.
32. Deepa, P.N.; Kanungo, M.; Claycomb, G.; Sherwood, P.M.A.; Collinson, M.M. *Anal. Chem.* **2003**, *75*, 5399-5405.
33. Sayen, S.; Walcarius, A. *Electrochem. Comm.* **2003**, *5*, 341-348.
34. Etienne, M.; Walcarius, A. *Electrochem. Comm.* **2005**, *7*, 1449-1456.
35. Walcarius, A.; Sibottier, E. *Electroanal.* **2005**, *17*, 1716-1726.
36. Shacham, R.; Avnir, D.; Mandler, D. *Adv. Mater.* **1999**, *11*, 384-388.
37. Marx, S.; Zaltsman, A.; Turyan, I. Mandler, D. *Anal. Chem.* **2004**, *76*, 120-126.
38. Fireman-Shoresh, S.; Turyan, I.; Mandler, D.; Avnir, D.; Marx, S. *Langmuir* **2005**, *21*, 7842-7847.
39. Kane, S.A.; Iwuoha, E.I.; Smyth, M.R. *Analyst* **1998**, *123*, 2001-2006.
40. Walcarius, A.; Mandler, D.; Cox, J.A.; Collinson, M.; Lev, O. *J. Mater. Chem.* **2005**, *15*, 3663-3689.
41. Buckley, A.M.; Greenblat, M. *J. Chem. Ed.* **1994**, *71*, 599-602.

42. Brinker, C.J.; Scherer, G.W. *Sol-Gel Science*, Academic Press, San Diego, 1990.
43. Trojanowicz, M. *Trends Anal. Chem.* **2006**, 25, 480-489.
44. Camelot, M. *Rev. Chim. Miner.* **1969**, 6, 853-884.
45. Corey, E.J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 5, 399-402.
46. Martin-Zarza, P.; Gili, P. Rodriguez-Romero, F.V.; Ruiz-Perez, C.; Solans, X. *Polyhedron* **1995**, 14, 2907-2917.
47. Sisler, H.; Ming, W.C.L.; Metter, E.; Hurley, F.R. *J. Am. Chem. Soc.* **1953**, 75, 446-448.
48. Niedziolka-Jonsson, J.; Jonsson-Niedziolka, M.; Nogala, W.; Palys, B. *Electrochim. Acta.* **2011**, 56, 3311-3316.
49. Ramsey, J.D.; Xia, L.; Kendig, M.W.; McCreery, R.L. *Corros. Sci.* **2001**, 43, 1557-1572.
50. Shriver, D.F.; Atkins, P.W.; Langford, C. H. *Inorganic Chemistry*, W. F. Freeman and Co., New York, 1990, p. 131.
51. Pauliukaite, R.; Hocevar, S.B.; Ogorevc, B.; Wang, J. *Electroanal.* **2004**, 16, 719-723.
52. Petit-Dominguez, M.D.; Shen, H.; Heineman, W.R.; Seliskar, C.J. *Anal. Chem.* **1997**, 69, 703-710.
53. Shi, Y.; Slaterback, A.F.; Seliskar, C.J.; Heineman, W.R. *Anal. Chem.* **1997**, 69, 3679-3686.
54. Ross, S.E.; Shi, Y.; Seliskar, C.J.; Heineman, W.R. *Electrochim. Acta*

2003, 48, 3313-3323.

55. Harrison, D.J.; Daube, K.A.; Wrighton, M.S. *J. Electroanal. Chem.* **1984**, 163, 93-115.

Part 6
Error Analysis

With any experiment come errors, whether they are random or systematic. Systematic errors come from limitations in the instrumentation, while random errors occur due to reproducible inaccuracies during an experimental procedure. While random errors can statistically affect the data either positively or negatively, systematic errors are consistently in the same direction.¹ It is advantageous to minimize errors, but it is just as important to identify the sources. This part provides an analysis of errors in the studies reported in the previous parts.

6.1. Part Two

Blood is a complex mixture of many chemical and biological components, and there is currently no good method to follow the decomposition of every component in blood. We monitored the degradation of blood at 380 nm, which is the absorbance of hemoglobin. We attempted to obtain random error at this wavelength by using a sample of pure porcine hemoglobin and performed identical experiments numerous times. Not only was the peak confirmed, but this also showed that degradation at this particular wavelength could be contributed to hemoglobin specifically. Hemoglobin is a major component of blood, and its decomposition provides a simple, direct means to follow the blood decomposition. However, using one component in the blood (hemoglobin) to represent the whole blood containing many chemical and biological components

introduces one major systematic error.

When studying the AOP of a particular sample, monitoring the kinetics provides an understanding of the pretreatment process. Studying basic kinetic parameters such as rates and orders with respect to reactants often helps to better design the process itself. The rates and orders were determined with respect to whole blood AOP and other experiments were performed in order to identify the reactions leading to the degradation, such as Fenton and photo-Fenton processes. The process involves many steps, and we do not know which is the rate-determining step. Fitting the overall AOP reaction, pretreating the blood samples using first-order kinetics, is perhaps a major source of systematic error in the kinetic order as well the rate constants.

Another systematic error in the kinetic order and rate constants is treating the kinetic order with respect to $C_{\text{H}_2\text{O}_2,t=0}$ to be zero. This assumption works for $C_{\text{H}_2\text{O}_2,t=0} \geq 3.0 \text{ g L}^{-1}$, as shown in Figure 2.4, but is expected to introduce an error. In addition, H_2O_2 is consumed in the AOP process, and its concentration changes during the reaction. When $C_{\text{H}_2\text{O}_2,t=0}$ is sufficiently large, this change does not significantly affect the kinetic studies. But it is expected to introduce an error, although it might be a small one.

While there may be some variability among different blood samples, the degradation should be relatively the same based on the reported protocol. AOP has been performed numerous times at multiple H_2O_2 concentrations and the confidence level of consistency is greater than 99%. In addition, the deviation

among results is less than 10^{-4} . Random errors in the rate constants are given in Table 2.1. The analysis here indicates that the random error is small in the current AOP pretreatment of the blood samples.

6.2. Part Three

One source of error and perhaps the most common during dry ashing is sample loss. Attempts were made to minimize any loss by heating the sample in stages, gradually increasing the flame temperature and closely monitoring the blood sample itself so that bumping and boiling of the sample was prevented.

Acid digestion of the ashes is expected to completely dissolve metal oxides into the solution. If ashes is not completed dissolved, metals inside the ashes may not be digested, introducing another systematic error, although our current work suggests that this is a small contribution.

The determination of metal concentrations in the blood (and ashes) relies on ICP-OES and a set of standards. The systematic errors in ICP-OES and the method itself will be carried over here as a systematic error. Errors in the standards will be propagated as a random error here.

Random errors are given in Table 3.2.

6.3. Part Four

A possible source of error for this method is inconsistencies in the electrode surface due to the fabrication of the layers. For example, if PET does not adhere such that all binding sites are available to accumulate Cr(VI), this may affect the analysis and overall reproducibility of the method.

The size of the Au nanoparticles (AuNPs), especially the second layer, is not uniform. The size ranges from 25 to 40 nm. Although the size range is small and nanoparticles are typically known to have a size distribution, the non-uniformed sizes probably introduce a systematic error. The thickness of the MPTS sol gel was not measured. If the thickness is not uniformed, this may introduce another systematic error.

Interferences provide another source of systematic error. In order to evaluate the performance of the electrode toward Cr(VI) reduction in the presence of coexisting anions or cations, the effect of several ions on the cathodic peak current of Cr(VI) reduction was examined (Figure 4.7). As described in Part 4, no change in the voltammetric response of Cr(VI) was observed in the presence of various cations, as the positively charged ions are repelled by the positive charge of the pyridinium groups on the electrode surface. Negatively charged ions only showed minute interference toward Cr(VI) reduction. Using the new method, standard deviations of 3.1% ($n = 3$) for inter-group detection and 4.2% ($n = 3$) for outer-group detection were obtained

respectively. On the other hand, the very similar results obtained by the second person in our research group were obtained with a standard deviation of 4.4% ($n = 3$) for outer-group detection. Moreover, four electrodes were used to detect Cr(VI) in the AOP treated blood samples, and standard deviations of 4.7% for outer-group detection and 2.5% for inter-group detection were obtained, indicating the good stability, reproducibility and repeatability of the method towards Cr(VI) reduction.

6.4. Part Five

A possible source of systematic error for this electrode is in the fabrication of the electrode surface. These inconsistencies may lead to possible variations in the electrochemical deposition of the film and as a result, the current response of the blank. Due to variation of the current, the background and, in turn, the standard deviation can vary from electrode to electrode.

The size of the commercial carbon nanotubes may not be uniform. Although nanomaterials are typically known to have a size distribution, the non-uniformed sizes probably introduce a systematic error.

Another systematic error can arise during the electrochemical detection of Cr(VI). The use of an accumulation time of 5 min may cause saturation of the electrode surface, particularly at higher Cr(VI) concentrations. Additionally, there

is a possibility of alteration of the electrode surface due to the stripping or cleaning solution used between accumulations and electrochemical scans.

To demonstrate the reproducibility of the film fabrication, five prepared electrode surfaces were examined by measuring the blank for each. For each of these, a small peak is present in the blank around 0.08 V. The average current was found to be $15.3 \pm 4.1 \mu\text{A}$. During Cr(VI) analysis, the current continued to increase, but reproducibility declines after consecutive measurements. For optimal measurements, background subtraction was used for Figures 5.7 and 5.9.

While interferences have not been studied extensively with regards to this electrode, the positive charge of the ethylenediamine groups on the electrode surface should repel positively charged cations. Additionally, the presence of other interferences can be minimal based on the potential window, such that these interferences will not be detected in the same potential range where Cr(VI) is present.

References

1. Harris, D.C. *Quantitative Chemical Analysis*, 8th ed.; W. H. Freeman, 2010.

Part 7
Concluding Remarks

This work centered on the detection of trace metals in biological samples. More specifically, an original process to pretreat whole blood was developed and optimized, followed by fabrication of novel electrochemical sensors for the detection of various species. As interactions between the human body and its surrounding environment become more complex, it is imperative that society find ways to monitor the elements in which there is continuous contact, whether malignant or benign. As the idiom “too much of a good thing” implies, there is often a point when something well meaning can be in excess. Thus it is important to be able to examine a wide range of concentrations easily and efficiently so that the line between benevolent and toxic is acknowledged.

The first portion of this dissertation, Part 2, described a new process to pretreat blood samples. This process combines the advanced oxidation process (AOP) treatment (using aqueous H_2O_2 and UV irradiation) with acid deactivation of the enzyme catalase in blood. The kinetics of whole blood AOP in a single-cell reactor was studied, showing that at $C_{\text{H}_2\text{O}_2, t=0} = 3.0, 4.0, \text{ and } 5.0 \text{ g L}^{-1}$, the reaction is zeroth order with respect to $C_{\text{H}_2\text{O}_2}$ and first order with respect to C_{blood} . The rate limiting process is photon flux from the UV lamp. The effect of pH on the AOP process has been investigated. Additionally, the degradation of whole blood was compared with that of pure hemoglobin samples. A four-cell reactor has been designed and built in house that uses 80% less blood samples in comparison to the single-cell reactor. This procedure leads to the successful decomposition of biological/organic species in blood.

The next part described an optimized dry ashing method. This method was crucial in confirming metal concentrations in whole blood as they pertained to both AOP treated samples as well as electrochemical detection. Ashing whole blood samples are, however, often difficult to carry out with significant sample loss, and the procedure was not well documented previously. A new procedure has been developed and optimized to dry ash whole blood samples for trace metal analyses. The procedure reduces both sample loss and the dry ashing time by more than two thirds. The ashed sample can be readily used in subsequent, simultaneous or individual analysis of several metals by ICP-OES, as demonstrated in the analysis of a whole blood sample. The new procedure is simple, inexpensive, and faster than the established method.

The remaining two parts focused on electrochemical sensing of chromium using nanotechnology and sol-gel chemistry. Part 4 described the fabrication of a flower-like self-assembly of gold nanoparticles (AuNPs) on a glassy carbon electrode (GCE) as a highly sensitive platform for ultratrace Cr(VI) detection. Two AuNP layers were used in the current approach, in which the first is electroplated on the GCE surface as anchors for binding to an overcoated thiol sol-gel film derived from 3-mercaptopropyltrimethoxysilane (MPTS). The second AuNP layer is then self-assembled on the surface of the sol-gel film, forming flower-like gold nanoelectrodes enlarging the electrode surface. When functionalized by a thiol pyridinium, the fabricated electrode displays a well-defined peak for selective Cr(VI) reduction with an unusually large, linear concentration range of 10–1200

ng L⁻¹ and a low detection limit of 2.9 ng L⁻¹. In comparison to previous approaches using MPTS and AuNPs on Au electrodes, the current work expands the use of AuNPs to the GCE. Subsequent functionalization of the secondary AuNPs by a thiol pyridinium and adsorption/preconcentration of Cr(VI) lead to the unusually large detection range and high sensitivity. The stepwise preparation of the electrode was characterized by EIS, SEM, and IR. The newly designed electrode exhibits good stability, and was successfully employed to measure chromium in an AOP treated blood sample. The method demonstrates acceptable fabrication reproducibility and accuracy.

In addition to this electrode, this dissertation also includes the novel fabrication of a modified glassy carbon electrode with physical deposition of single-walled carbon nanotubes followed by electrochemical deposition of a sol-gel using base-catalyzed hydrolysis. In Part 5, the thin sol-gel film fabricated from a novel molecule is believed to preconcentrate Cr(VI) anions. The SWNTs allow for greater conductivity and increased surface area on the electrode surface which in turn gives greater sensitivity. Our studies have shown detection of Cr in the low ppb and ppt range.

Future work will focus on identifying inconsistencies causing irreproducibility in the sol-gel electrodeposition followed by the use of this electrode in an AOP blood sample.

The aim of the work in this dissertation focused on developing an efficient pretreatment method for whole blood and reliable electrochemical sensors for the

detection of biologically and environmentally relevant substances. Both the pretreatment and detection was achieved. Additionally, the use of nanotechnology provided enhanced sensitivity not previously accomplished in this research group. The development of highly selective and sensitive methods for the detection of biologically meaningful substances is always essential. As a result of the projects described in this dissertation, these methods could be used to aid in relevant medical and environmental research.

Appendices

Appendix A

A.1. Experimental: Exp. 2–4 Fenton Processes with or without UV Irradiation

Exp. 2 - Fenton Processes without UV Irradiation.

This experiment was conducted as in Exp. 1, except that the Pen-Ray UV lamp was turned off during the test. The data collected using the apparatus shown in Figure 2.1 was compared to samples using no UV light exposure but identical H₂O₂ concentrations. The UV-Vis spectrum was recorded every minute for the first 10 min and then every 5 min for the remaining 50 min (60 min total) and is shown in Figure A.1. Due to the high absorbance of the solution at 380 nm a twelve-fold dilution of the sample was used during UV-Vis analysis.

Exp. 3 - Fenton Processes with Added FeSO₄ and No UV Irradiation.

Red blood cells have an abundance of iron, but Fenton processes typically use free iron during the catalysis. The difference in free and bound iron was tested here by adding 4 mg of ferrous sulfate hydrate to the solution, followed by performing the Fenton process. The data collected using the apparatus shown in Figure 2.1 was compared to samples with added FeSO₄ using no UV light exposure but identical H₂O₂ concentrations. The UV-Vis spectrum was recorded every minute for the first 10 min and then every 5 min for the remaining 50 min (60 min total) and is shown in Figure A.2. Due to the high absorbance of the

solution at 380 nm a twelve-fold dilution of the sample was used during UV-Vis analysis.

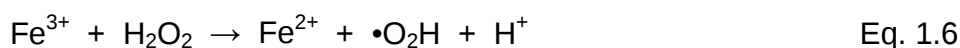
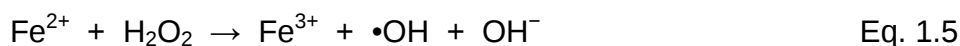
Exp. 4 - Effect of Added FeSO₄ in the Photo-Fenton Process

The difference between free and bound iron in the photo-Fenton process was studied here. The AOP process of whole blood was therefore examined in comparison to the same solution with additional free iron added. The data collected using the apparatus shown in Figure 2.1 was compared to samples using no UV light exposure but identical H₂O₂ concentrations and an additional 4 mg of ferrous sulfate hydrate. The UV-Vis spectra were recorded every minute for the first 10 min and then every 5 min for the remaining 50 min (60 min total) and is shown in Figure A.3. Due to the high absorbance of the solution at 380 nm a twelve-fold dilution of the sample was used during UV-Vis analysis.

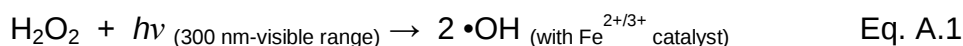
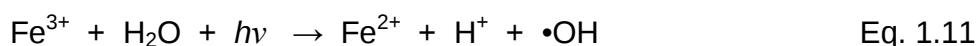
This setup was the same as in Exp. 1, except that the UV lamp was turned off during the test and only one sample was used. A small portion of the sample was removed every minute for the first 10 min and then every 5 min for the remaining 50 min before being diluted by 12 fold for UV-visible analysis. Experiment 3 was conducted as in Exp. 2, except that FeSO₄•7H₂O (4 mg) was added to the solution. Experiment 4 was conducted as in Exp 1, except that FeSO₄•7H₂O (4 mg) was added to the solution.

A.2. Results and Discussion of the Fenton Processes

In the presence of $\text{Fe}^{2/3+}$ ions as catalyst under acidic conditions, H_2O_2 may be converted to $\bullet\text{OH}$ radicals, *without UV irradiation*, in the Fenton process [A.1-A.2].^{1,2} Since Fe-containing proteins are present in the blood of mammals, we have studied whether the Fenton process operates in the current system. Two key reactions in the Fenton process are listed in Eqs. 1.5 and 1.6. One major advantage is that the Fenton process is simple requiring no irradiation.



In the presence of photons, Fe^{3+} ions may be reduced by water (Eq. 1.11) in the photo-Fenton process with Eq. A.1 as the net reaction between Eqs. 1.5 and 1.11.



¹ Koppenol, W. *Redox Rep.* **2001**, 6, 229-234.

² Bautista, P.; Mohedano, A.F.; Gilarranz, M.A.; Casas, J.A.; Rodriguez, J.J. *J. Hazard. Mater.* **2007**, 143 128-134.

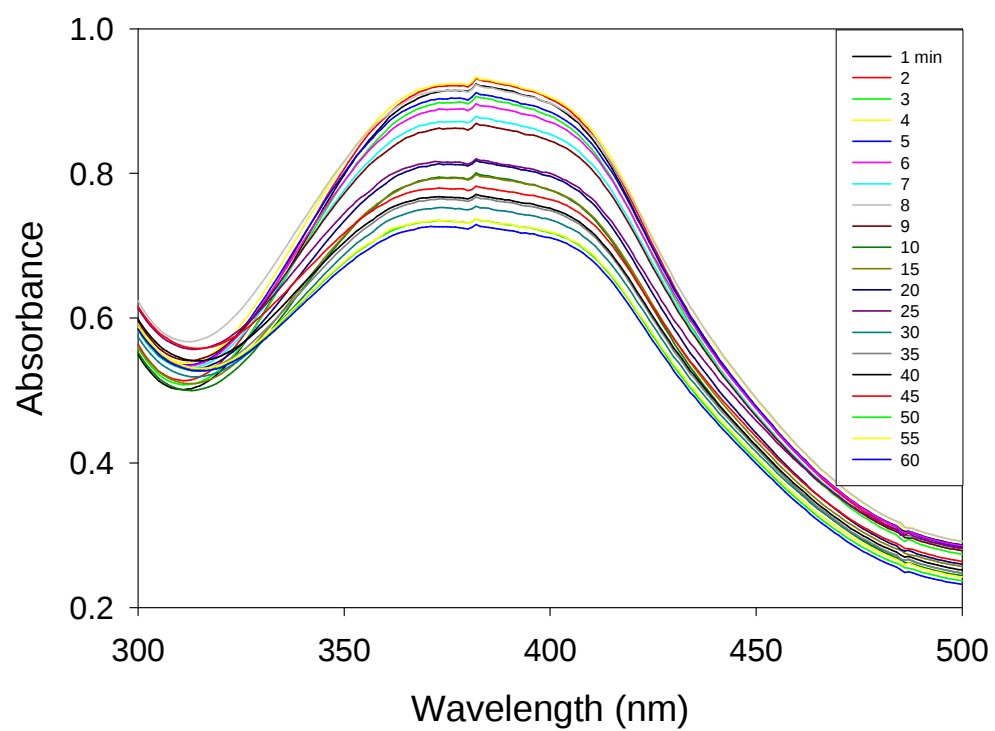


Figure A.1. UV-visible spectra of blood in the AOP pretreatment in Exp. 2, as shown by the peak at 380 nm. $C_{H_2O_2} = 1.5 \text{ g L}^{-1}$.

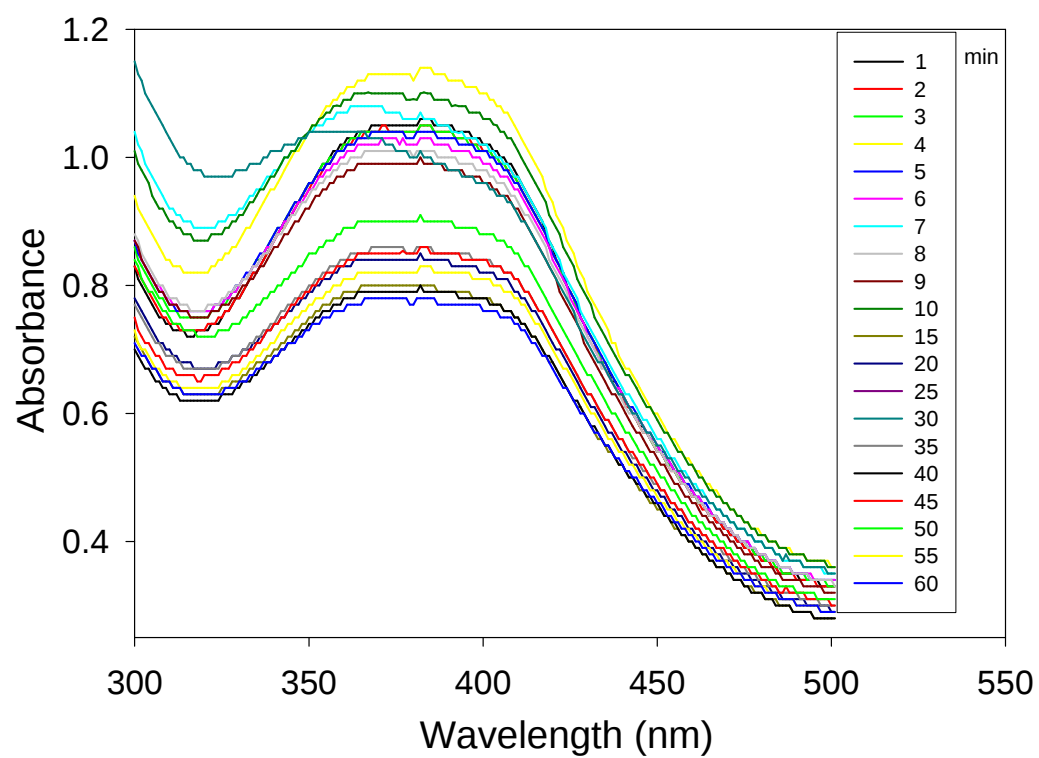


Figure A.2. UV-visible spectra of blood in the AOP pretreatment in Exp. 3.

$C_{\text{H}_2\text{O}_2} = 1.5 \text{ g L}^{-1}$.

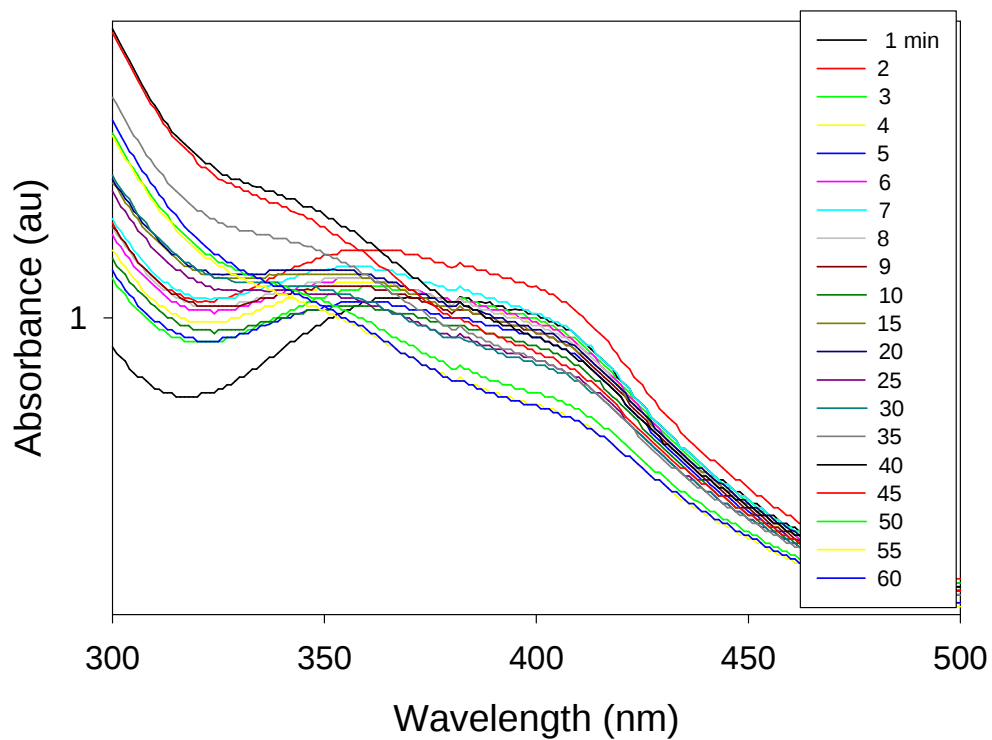


Figure A.3. UV-visible spectra of blood in the AOP pretreatment in Exp. 4, as shown by the peak at 380 nm. $C_{H_2O_2} = 1.5 \text{ g L}^{-1}$.

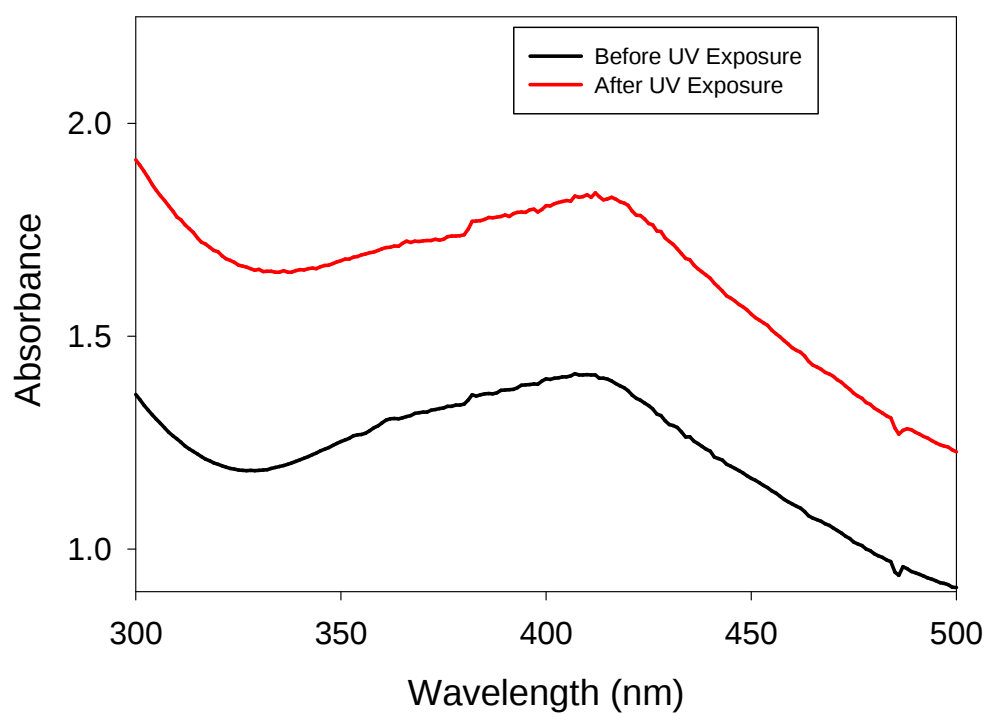


Figure A.4. UV-visible spectra of blood in the AOP pretreatment in Exp. 5, as shown by the peak at 380 nm. $C_{H_2O_2} = 1.5 \text{ g L}^{-1}$.

Although H_2O_2 dissociates into $\bullet\text{OH}$ radicals by photons in both Eqs. 1.1 and A.1, Eq. A.1 uses $\text{Fe}^{2/3+}$ as catalyst, while direct H_2O_2 photodissociation in Eq. 1.1 requires none. Another difference is that direct photodissociation requires short UV wavelength (254 nm), while the photo-Fenton process works with 300 nm up to the visible range.³

A.3. Kinetics of Photolytic Reactions

In general, when a solution of photolyte A is irradiated at a given wavelength, the amount of light *absorbed* by A per unit time, b is defined by Beer-Lambert's law, as shown in Eq. A.2.

$$b = I_0 - I_0 \exp(-2.303 \epsilon / C_A) = I_0 [1 - \exp(-2.303 \epsilon / C_A)] \quad \text{Eq. A.2}$$

where I_0 = incident light intensity (in einstein $\text{L}^{-1} \text{s}^{-1}$), ϵ = molar absorptivity of A (in $\text{L mol}^{-1} \text{cm}^{-1}$), b = path length (in cm), C_A = concentration of A , and $b_0 \exp(-2.303 \epsilon / C_A)$ = amount of light transmitted according to Beer-Lambert's law.

³ Litter, M. In *Environmental Photochemistry Part II*; Boule, P.; Bahnemann, D.W.; Robertson, P.K.J. Eds.; Springer-Verlag: Berlin/Heidelberg, 2005; Vol. 2M, pp. 325-366.

The rate of photolysis of A (or consumption of A), $-(dC_A / dt)$, is directly proportional to the rate of light absorption by A per unit volume (Eq. A.3).^{4,5}

$$-(dC_A / dt) = b\Phi = b_0 \Phi [1 - \exp(-2.303 \epsilon / C_A)] \quad (\text{Eq. A.3})$$

where Φ = quantum efficiency (in mole of A einstein⁻¹).

Since Eq. A.6 is wavelength-dependent, the rate of photolysis of A is the integral over all wavelengths that are emitted by the light source and absorbed by A. Molar extinction coefficient ϵ of H₂O₂ decreases significantly at higher wavelengths.⁶

There are two limiting cases. If absorbance condition for the compound A is high [$\epsilon / C_A > 1$, and $\exp(-2.303 \epsilon / C_A) \approx 0$], all incoming light is essentially absorbed by A. Eq. A.3 becomes

⁴Wang, F.; Haag, W. 1999 *Proceedings of the 4th International Conference on Atomic and Molecular Pulsed Lasers*, Tomsk, Russia, September 13-17, 1999; UCRL-JC-135610.

⁵Zepp, R.G. ; Cline, D.M. *Environ. Sci. Technol.* **1977**, *11*, 359-366.

⁶Schumb, W.C.; Satterfield, C.N.; Wentworth R.L. *Hydrogen Peroxide*, Reinhold Publishing: New York, 1955; p. 291.

$$-(dC_A / dt) \approx b_0 \Phi = k_0 \quad (\text{Eq. A.4})$$

The consumption of the photolyte in this case is zero order.

Thus, if the concentration of H_2O_2 is high, and the absorption by water in the UV region is ignored, the limiting reagent is the photon flux of the UV light source (rate of photon emission). Such photochemical dissociation of H_2O_2 follows zeroth-order kinetics.

When the absorbance ($2.303 \epsilon / C_A$) of the photolyte A is low, the use of the Taylor series for the exponential function [$\exp(-x) = 1 - (x / 1!) + (x^2 / 2!) - (x^3 / 3!) \dots \approx 1 - x$] for Eq. A.3 gives:

$$-(dC_A / dt) \approx b_0 \Phi (2.303 \epsilon / C_A) = k_1 C_A \quad (\text{Eq. A.5})$$

The consumption of the photolyte in this case is first order.

If the concentration of H_2O_2 ($C_{\text{H}_2\text{O}_2}$) is not high, the appropriation for high absorbance [$\epsilon / C_{\text{H}_2\text{O}_2} > 1$, and $\exp(-2.303 \epsilon / C_{\text{H}_2\text{O}_2}) \approx 0$ for Eq. A.5] may not be valid. Such photochemical dissociation of H_2O_2 follows first-order kinetics. Thus the limiting kinetic orders for the H_2O_2 photolysis itself are zero when $C_{\text{H}_2\text{O}_2}$ is high and first when $C_{\text{H}_2\text{O}_2}$ is not high.

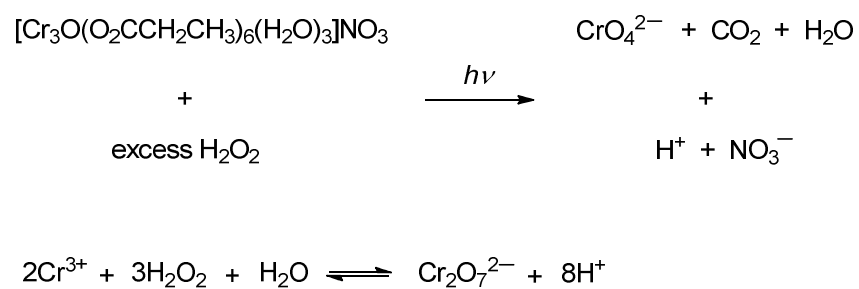


Figure A.5. The generation of protons from the AOP treatment of chromium(III) propionate $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]\text{NO}_3$, a biomimetic chromium species, and the $\text{Cr(III)} \rightarrow \text{Cr(VI)}$ conversion.

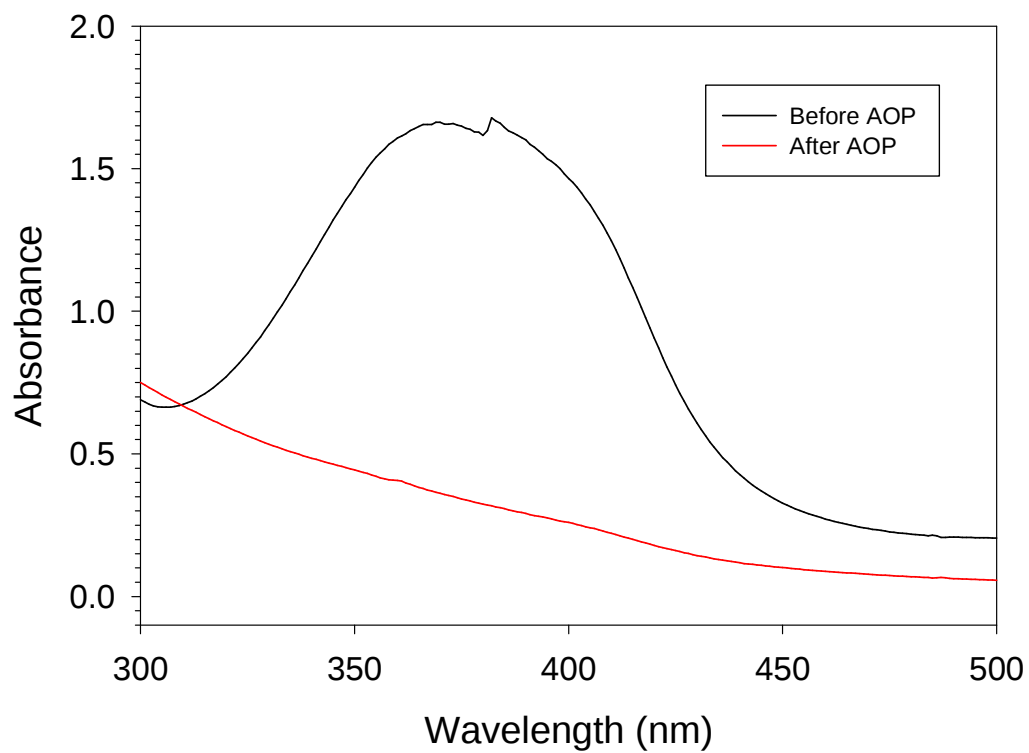


Figure A.6. UV-visible spectra of hemoglobin degradation by AOP over 60 min (Exp. 6). $C_{H_2O_2} = 1.5 \text{ g L}^{-1}$.

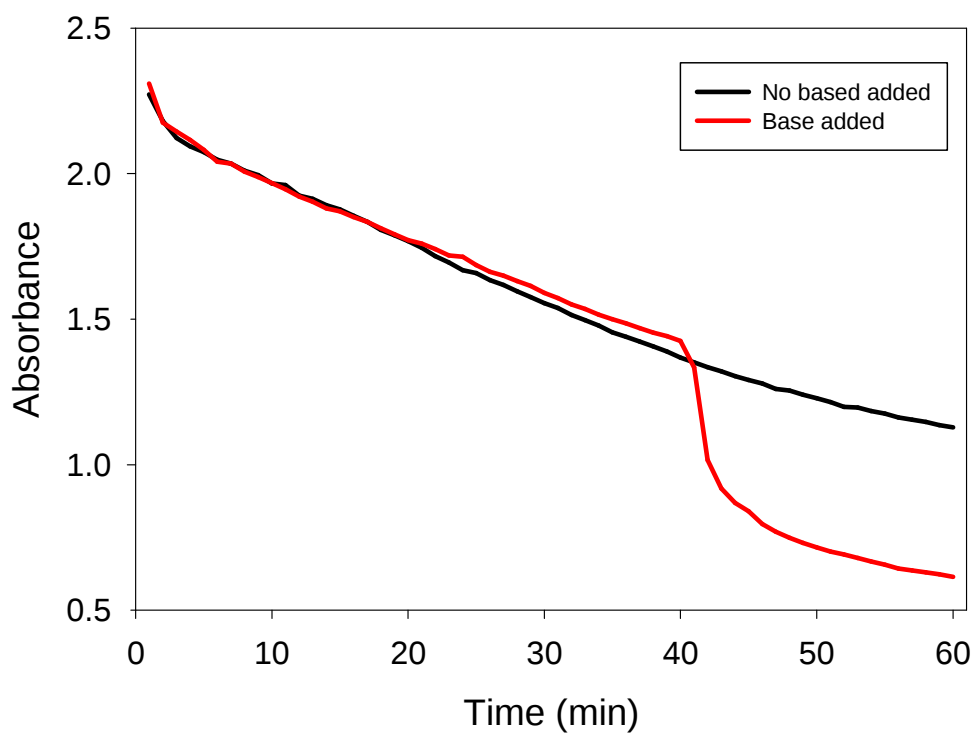


Figure A.7. A comparison of AOP treatments of a blood sample with and without the addition of base at 40 min. The experiment was conducted using the single-cell reactor. $C_{H_2O_2, t=0} = 5.0 \text{ g L}^{-1}$.

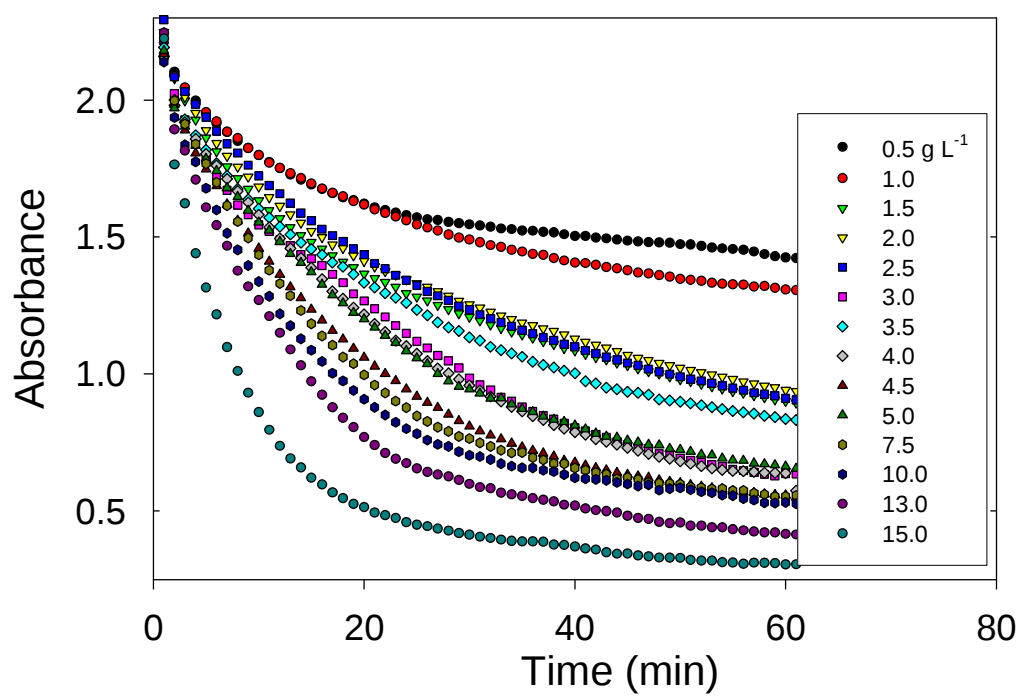


Figure A.8. Absorbance of blood samples at 380 nm during the AOP by Exp. 1.

Fourteen initial concentrations of H_2O_2 ($C_{\text{H}_2\text{O}_2, t=0}$) were used, as listed in the plots.



PenRay Spectrum

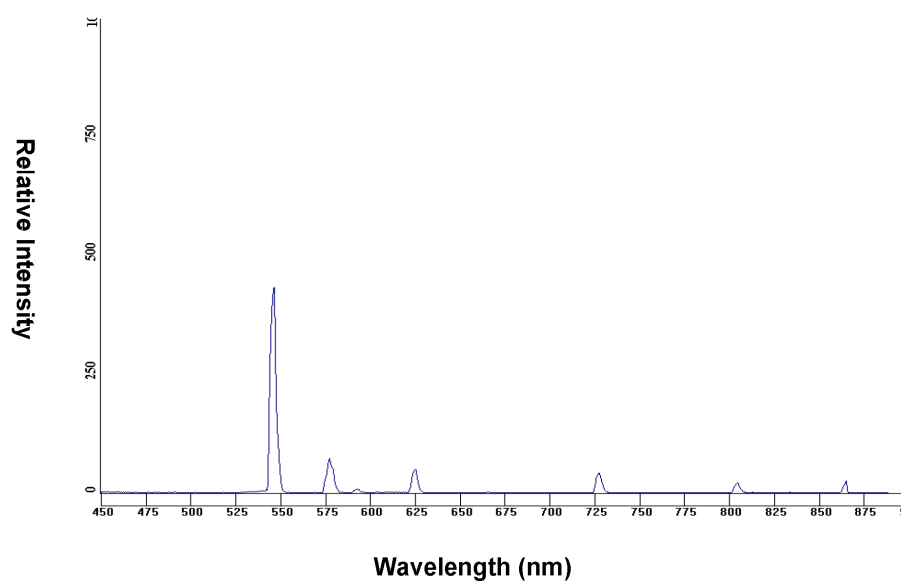
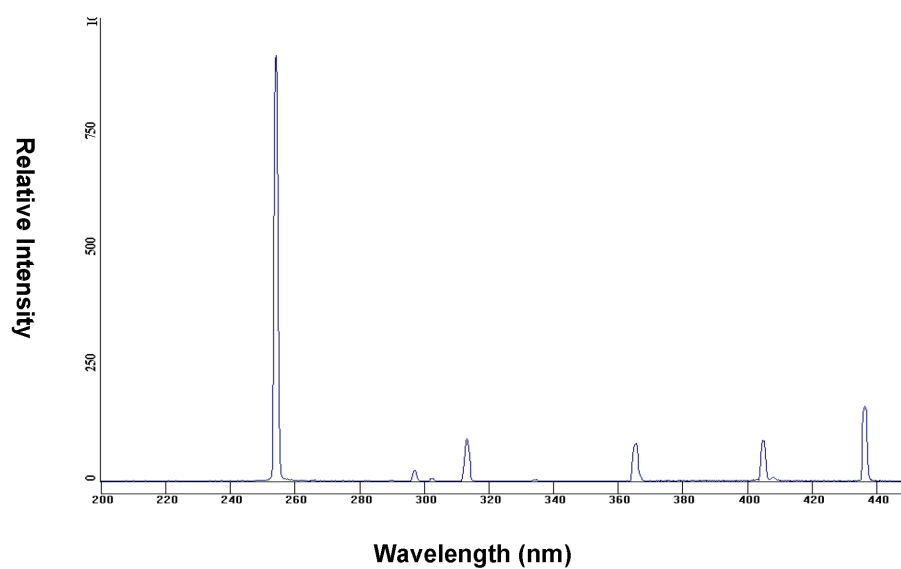


Figure A.9. Spectra of the 5.5-W PenRay UV lamp used in the single-cell reactor. Courtesy of UVP, LLC, Upland, CA.

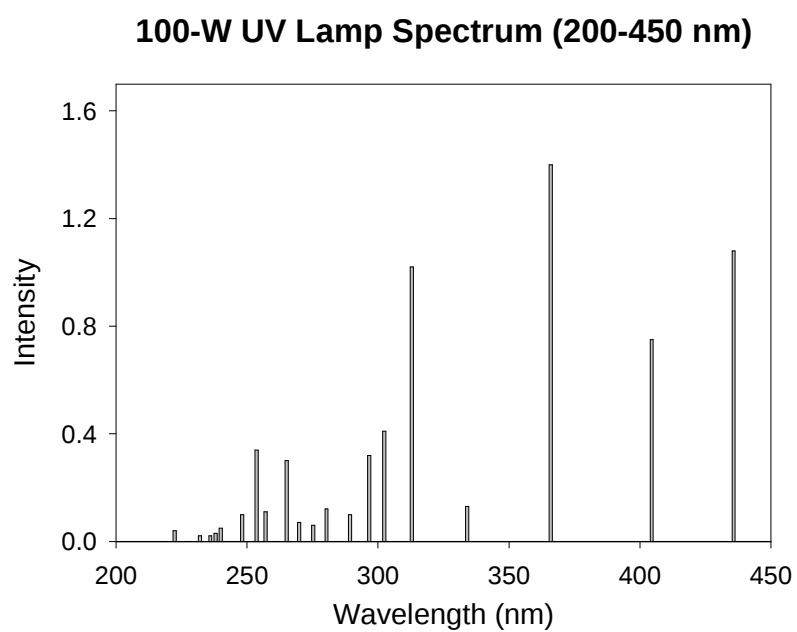
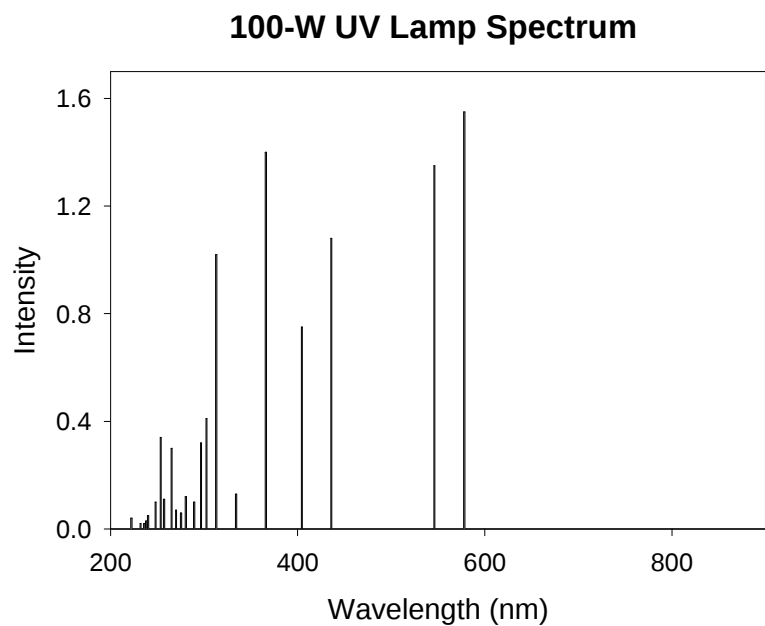


Figure A.10. Spectra of the 100-W Ace-Hanovia UV lamp used in the four-cell reactor. The plots were made based the spectral energy distribution of radiated mercury lines in the lamp provided by Ace Glass, Inc., Vineland, NJ.

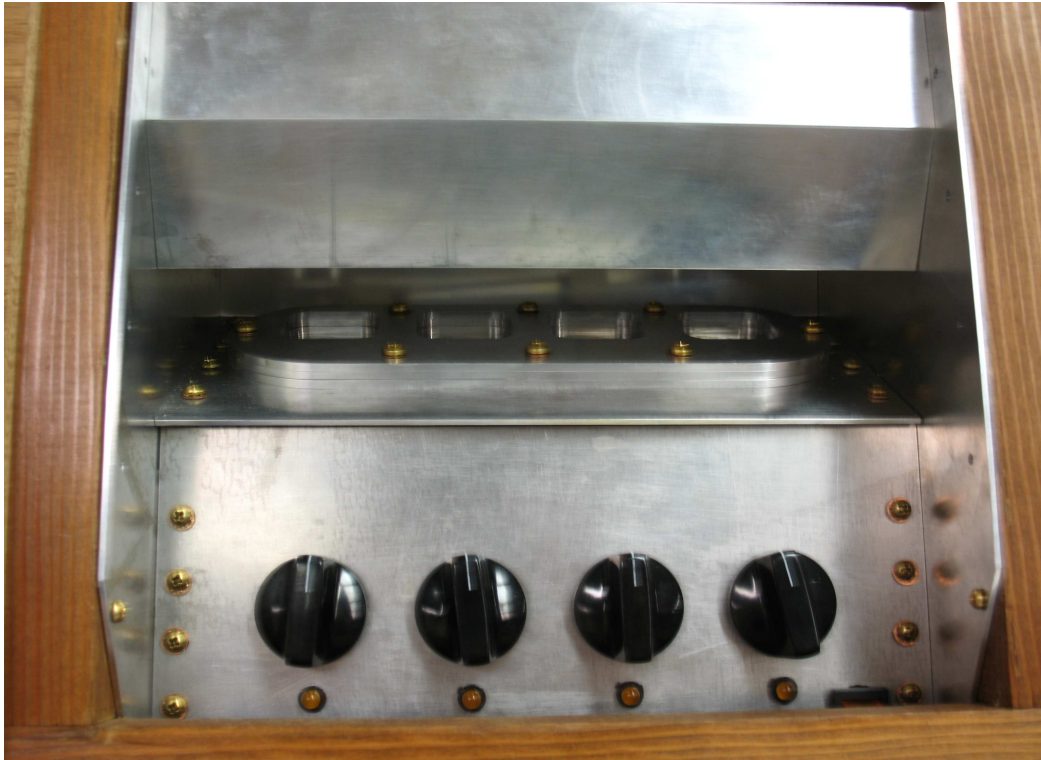


Figure A.11. Photo of a portion of the four-cell reactor.

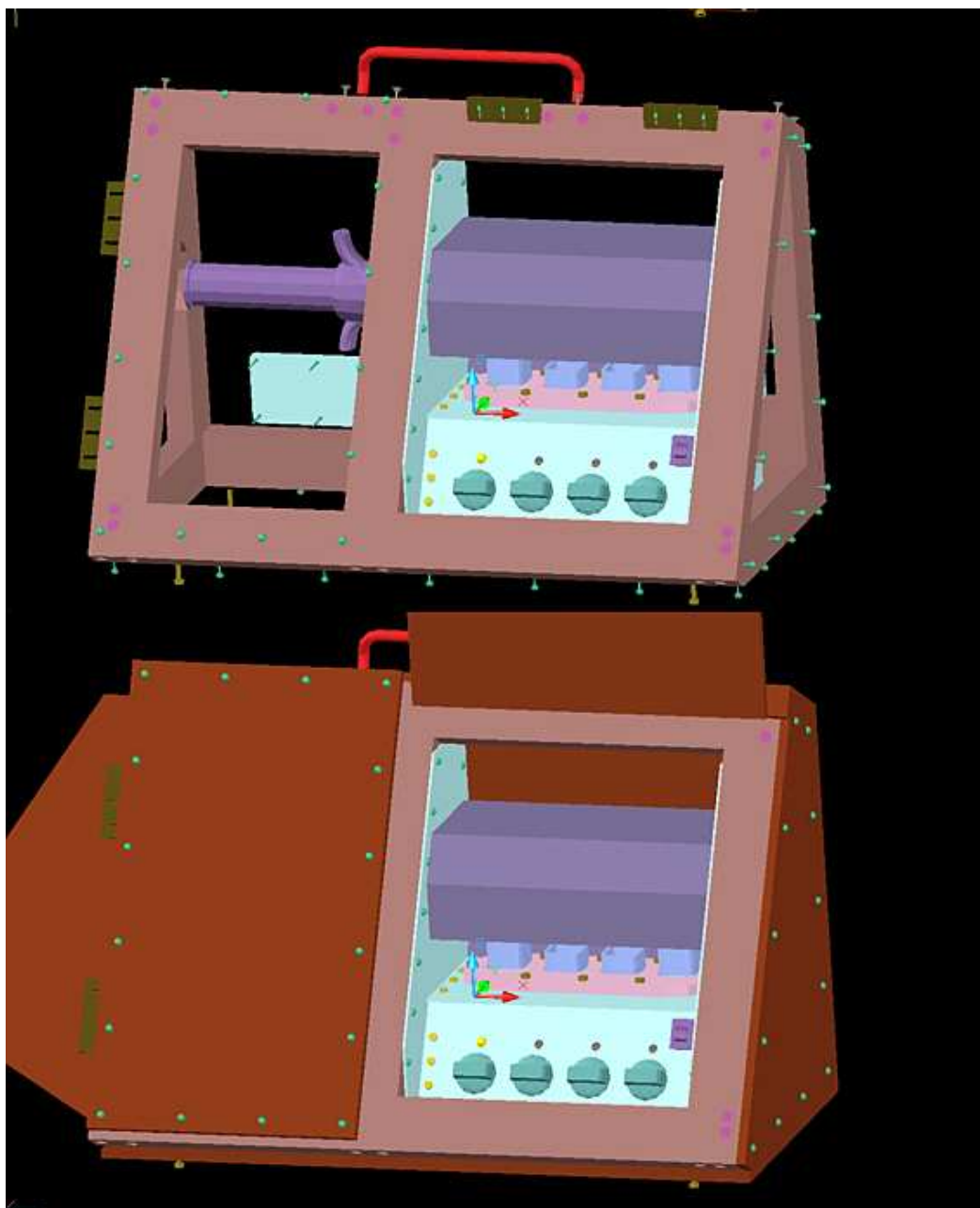


Figure A.12. Drawings of the four-cell reactor.

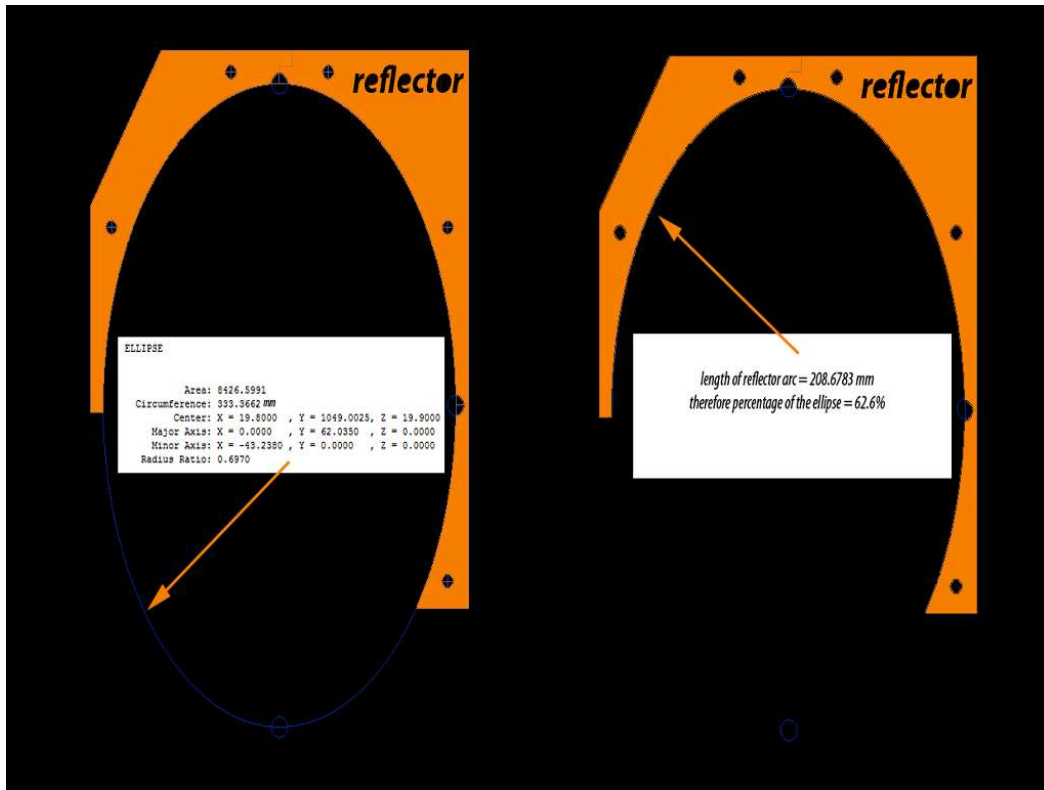


Figure A.13. A drawing of the elliptical cylinder in the four-cell reactor.

Appendix B

B.1. Optimization of the Experimental Conditions

In order to get the best performance of the proposed electrode toward Cr(VI) detection, the experimental parameters were optimized (Figure B.1). The deposition time was used to control the number of AuNPs on GCE surface. The ideal deposition time was determined by measuring the voltammetric response of corresponding electrodes towards Cr(VI). As shown in Figure B.1a, the peak current of Cr(VI) reduction was the strongest with 100 s deposition and then decreased slowly with increasing deposition time. Although the longer deposition time could increase the number of AuNPs on GCE surface, aggregations of AuNPs on GCE surface decrease the surface area of AuNPs, leading to a decreased thiol concentration. On the other hand, the peak current increased with increasing deposition time until 100 s, which could be ascribed to the increase of AuNPs effective surface area, chemisorbing more thiol tail groups onto GCE. As illustrated in Figure B.1, when the AuNPs/GCE was immersed into a 21 mM MPTS ethanol solution for 10 min at room temperature, a self-assembled monolayer (SAM) of MPTS network with a lot of thiol tail groups was formed on AuNPs surface by Au-S bonds. This MPTS monolayer can serve as a matrix to adsorb the secondary AuNPs layer. Therefore, the optimization of

Table B.1. Characteristic parameters on the preparation and characterization of the colloid AuNPs.

<i>Set</i>	V_{HAuCl_4} (0.25 mM, mL)	$V_{trisodium\ citrate}$ (1%, mL)	<i>Color</i>	<i>Average</i> <i>diameter</i> (nm)
1	25	0.8	Red	10
2	25	0.65	Red	13
3	25	0.5	Red	16
4	25	0.438	Red	20
5	25	0.375	Red	25
6	25	0.313	Pinkish red	32
7	25	0.25	Pink	41
8	25	0.2	Pink	55
9	25	0.15	Orange	73

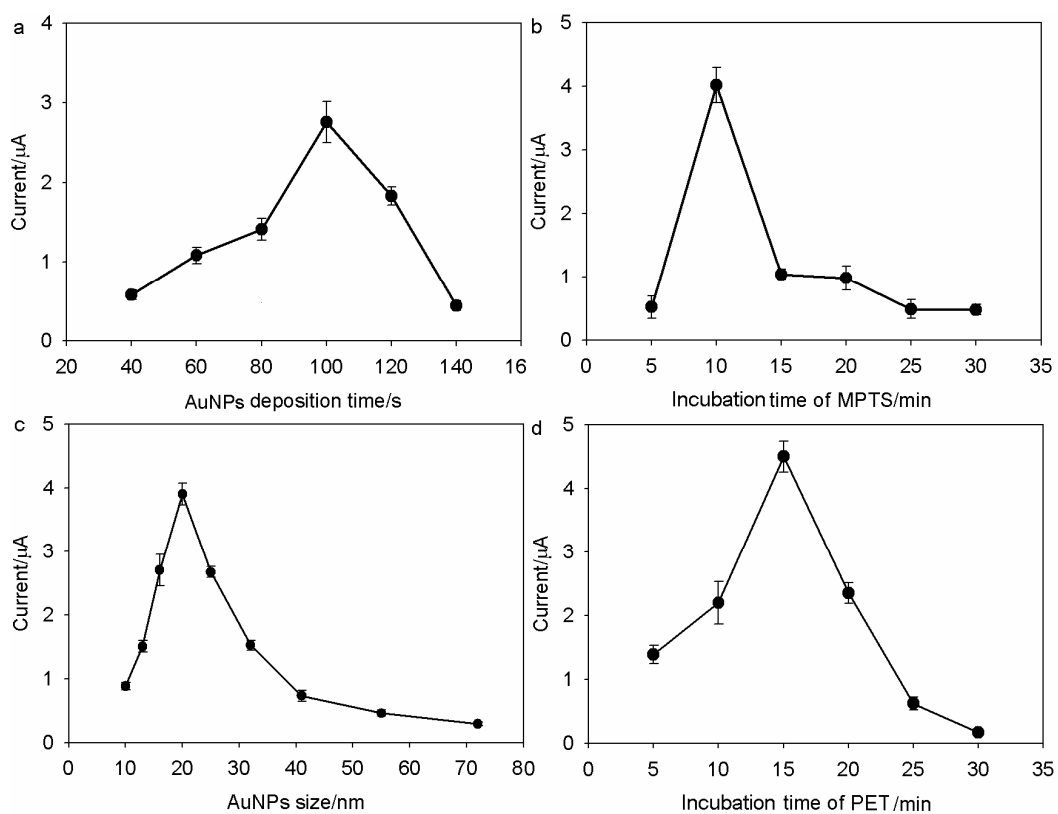


Figure B.1. Effect of (a) AuNPs deposition; (b) immersing time of electrode in MPTS solution; (c) size of the chemically prepared AuNPs; and (d) immersing time of electrode in PET solution on the detection of 1.5 μg mL⁻¹ Cr(VI). Error bar: n = 3.

Table B.2. Experimental conditions for Figure B.1.

	<i>AuNPs</i>	<i>Incubation</i>		<i>Incubation</i>
<i>Optimized</i>	<i>deposition</i>	<i>time of</i>	<i>AuNP size/nm</i>	<i>time of</i>
<i>parameters</i>	<i>time/s</i>	<i>MPTS/min</i>		<i>PET/min</i>
AuNPs	40-140	10	16	10
deposition				
time				
Incubation	100	5-30	16	10
time of MPTS				
AuNP size	100	10	10-73	10
Incubation	100	10	20	5-30
time of				
PET/min				

MPTS modification is important to get the best performance of the proposed electrode toward Cr(VI) detection (Figure B.1b). The detection performance was remarkably enhanced with a longer preincubation time of the electrode in MPTS solution; the highest current was obtained when the preincubation time was 10 min. This could be due to an increase in the number of thiol tail groups on the electrode surface, eventually leading to more active sites for Cr(VI) accumulation. A preincubation time longer than 10 min caused a current decrease such that the longer the preincubation time, the more MPTS molecules were self-assembled onto the electrode surface, which likely suppressed the electron transfer of the electrode.

The electroactivity of the designed electrode toward Cr(VI) reduction also depends significantly on the size of secondary AuNPs. The effect of particle size from 10 to 73 nm was thus studied (Figure B.1c). As the diameter of AuNPs was increased up to 20 nm, the peak current was gradually enhanced. The larger particle has greater efficient surface area, which may increase the number of pyridinium groups on the electrode surface. Accordingly, more Cr(VI) may be accumulated and reduced. However, the decrease in the peak current when the AuNPs particle size was larger than 20 nm might result from the decrease in the spatial area for Cr(VI) reduction. Therefore, it was concluded that Cr(VI) reduction on the designed electrode was very sensitive to the AuNPs particle size. In this work, AuNPs with diameter of 20 nm were used to produce the secondary AuNPs on the electrode. Finally, the effect of the incubation time of

AuNPs/MPTS/GCE in pyridinium thiol solution was examined from 5 to 30 min.

The results in Figure B.1d indicated that the best detection behavior of the electrode was found at 15 min and then became worse with the longer incubation time. Although longer incubation time increases the number of pyridinium thiol self-assembled on electrode surface, it appears to weaken the electron transfer of the electrode and decrease peak current.

VITA

Stefanie Anne Bragg was born on June 8, 1985 in Glasgow, Kentucky. She was raised in nearby Etoile and graduated from Barren County High School in May 2003. The following fall she enrolled at Western Kentucky University in Bowling Green, KY. She graduated with honors in May 2007 with a major in chemistry and a minor in biology.

Stefanie began her graduate studies in chemistry at the University of Tennessee in August 2007. She joined Dr. Zi-Ling (Ben) Xue's research group with a primary focus on trace metal detection in biological samples using sample pretreatment and electrochemical techniques. Her work resulted in three publications by the time she received her Ph.D in the Summer of 2012 with more to follow.